

## **Book of Abstracts**

# **11<sup>th</sup> International Phytotechnologies Conference**

### **Organized by:**

International Phytotechnology Society

### **Hosted by:**

Technical University of Crete,  
Technological Educational Institute of Crete

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Th. Manios

**Heraklion, Crete, Greece, Sept. 30 - Oct. 3, 2014**

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## PREFACE

On behalf of the hosting institutions (TU-Crete and TEI-Crete), we would like to welcome you to Heraklion for the 11th International Phytotechnologies Conference (Sept 30 to Oct 3, 2014).

The recent tradition of the IPS conferences to rotate on a biannual basis between North America and another continent continues. It started with Nanjing, China in 2008; St. Louis, MO in 2009; Parma, Italy in 2010; Portland, OR in 2011; Hasselt, Belgium in 2012; Syracuse, NY in 2013 and this year in Heraklion, Greece for the 11th IPS conference. This conference provides the opportunity to scientists, engineers, consultants, policy regulators and other interested individuals to explore and discuss how recent developments in phytotechnologies address current and emerging environmental challenges. We received a total of 420 abstracts from 49 countries. After several cancelations due to the very limited funds available for travel assistance, the final program is comprised of 129 full platform presentations, 63 short platform presentations and 147 posters – a total of 342.

Phytotechnologies refer to plant-based technologies to clean water, soil, air and provide ecosystem services including energy from biomass. The Conference will feature a wide range of topics. Selected papers will be published in the *International Journal of Phytoremediation* following peer review.

As in previous IPS conferences, besides three plenary lectures, several mini plenary speakers will introduce each session topic and the oral presentations to be presented in each session. In addition, post-conference tours will provide an opportunity to experience what Crete has to offer as a famous tourist destination.

Enjoy the conference and have a wonderful stay in Crete!

Conference co-chairs:



**Nicolas Kalogerakis**  
TU-Crete



**Thrassyvoulos Manios**  
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## 2014 IPS PHYTOSCHOLARS



National Institute of  
Environmental Health Sciences  
*Superfund Research Program*

Congratulations to the 2014 scholars!

NAME	INSTITUTION
<b>Paul Vaugh Manley</b>	Virginia Commonwealth University
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<b>Andrew McEachran</b>	North Carolina State University

Special thanks to **Prof. Stephen Ebbs** who on behalf of IPS prepared and submitted the Phyto-Scholars proposal which was quite more detailed as new rules by National Institutes of Health came in effect. The proposal was successful and a grant was awarded by the National Institution of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) enabling the above named scholars from several universities across USA to attend the 11<sup>th</sup> International Phytotechnologies Conference in Heraklion, Crete, Greece.

The Phyto-Scholars program is well suited to SRP mission “to seek solutions to the complex health and environmental issues associated with the nation's hazardous waste sites.” By promoting remediation and sustainability, phytotechnologies directly protect public health, promote environmental stewardship, and improve ecosystem function.



## THE GORDON AWARD



In 2005, the Phytoremediation community lost one of the pioneers of the field. **Milton P. Gordon**, Professor of Biochemistry at the University of Washington, had a life-long love of science, and his research passions allowed him to have an impact in many fields, from red-tide toxins, the development of *Agrobacterium* as a tool for plant genetic engineering, plant pathogenesis-related proteins, astrobiology, and his love, phytoremediation.

Milt loved all aspects of this work, from understanding, on a molecular and genetic level, how plants are able to degrade a wide range of organic pollutants, the genetic engineering of plants to increase their ability to do this degradative task, field studies and final application of the technology to hazardous waste sites. In this capacity, Milt was the driving force behind the formation of the remediation and consulting firm Verdant Technologies.

Milt was also an outstanding teacher, and taught not only the courses required in his department, but also was involved in courses in scientific ethics and astrobiology. His love of science came through to the students, and he was always available beyond the classroom to discuss anything. This also highlights one of his other traits, that he would never turn away a student in need. He was always available to help students understand material and expand their knowledge, but he was also there for his students if they had problems outside the classroom.

In 2007, Steve McCutcheon, a friend a colleague, proposed to the International Phytotechnology Society that an award be presented at the annual meetings to honor Milt. Thus the Gordon Award was formed. The award honors people in the field of phytoremediation that exemplify the traits that made Milt the person he was, and outstanding researcher, a strong proponent of the development of phytoremediation as an applied technology, a teacher and dedicated supporter of students. Every year since 2007, the IPS has presented the Gordon award, to both honor Milt and the current year's recipient, and to serve as an incentive to up and coming researchers in the field of phytoremediation to strive for excellence in these important areas.









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## **PLENARY PRESENTATIONS**





## GORDON AWARD PLENARY PRESENTATION

### FROM PHYTOREMEDIATION TO PHYTOMANAGEMENT

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#### **ABSTRACT**

Studies on the behavior of trace elements in the plant-soil systems date back to the 60' at the INRA Bordeaux-Aquitaine Research Centre. Molecular mechanisms in the rhizosphere (notably the pivotal role of root exudates) were explored in the 80-90'. Since 1991, we have been investigating the potential of soil conditioners, native and naturalized colonizer species, annual crops, microorganisms, cultural practices, and post-harvest treatments to build up relevant phytoremediation options. The new paradigm of phytomanagement includes to build tools for assessing initial and residual risk assessments, to deeply investigate the molecular mechanisms driving plant tolerance to excess metal(loid)s (with emphasis here for Cu) for selecting relevant genotypes, to test the effectiveness of plant and endophyte assemblages, to long-term monitor the phytomanagement options implemented at various sites (for effectiveness and life cycle assessment). Milestones where for instance obtained for the use of zerovalent iron grit and Linz-donawitz slags, phytoremediation of Cu-contaminated soils, hydrothermal treatment of phytoremediation-borne biomass, and changes in the soluble proteome of *Agrostis capillaris* exposed to excess Cu. Both terrestrial options and constructed wetlands must be sometime implemented at several sites to address the pollutant linkages. The crop production on metal(loid)-contaminated soils under phytoremediation and its use for notably plant-borne feedstock in local conversion chains (trying to generate financial opportunities) as well as the restoration of ecosystem services are both exciting challenges, which are in particular explored within the EU FP7 Greenland project (<http://www.greenland-project.eu/>).



## PLANT TRANSFORMATIONS OF HORMONE CHEMICALS

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### ABSTRACT

Hormones are used widely in the cattle industry to improve weight-gain and prevent disease. Manure from the animals is frequently applied on agricultural land as fertilizer, and hormonally active compounds can move with runoff into surface waters where they pose a risk to human and environmental health. However, riparian zone plants and associated bacteria in the root zone could uptake and biodegrade hormonally active compounds from agricultural fields. Hydroponic uptake, translocation, and transformation of three estrogens (17 $\beta$ -estradiol, 17 $\alpha$ -ethinylestradiol, and zeranolZAL) and one androgen (trenbolone acetate, TBA) were assessed in this laboratory study by poplar trees (*Populus deltoides* x *nigra*, DN-34). Treatments included dead poplar controls (dead roots and bacteria only), living poplars excised to minimize evapotranspiration, and whole living poplars in hydroponic solution. Aqueous concentrations of target hormones decreased by more than 97% (from initial concentrations of 2 mg L<sup>-1</sup>) after 10 days of exposure to whole or excised poplars, and by less than 84% with exposure to dead poplars. Target hormones and major transformation products (estrone and estriol for estradiol, zearalanone for zeranolZAL, and trendione for trenbolone acetate) were detected in the tissues of all three poplar treatments, with concentrations peaking after 1 to 5 days and then decreasing in full and excised poplars. Concentrations increased slowly in the roots of dead poplars and degraded more slowly. Taken together, these data show that poplar trees may be effective in controlling the movement of hormonally active compounds from agricultural runoff to streams. This is the first report of plant transformation of TBA, a widely used hormone in the cattle industry.



## USING NATURAL FUNCTIONAL BIOTA SOLVES FARMLAND POLLUTION IN A SUSTAINABLE WAY

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### ABSTRACT

With the rapid development of industrialization, urbanization, and intensive agriculture in China, more and more agricultural soils have been contaminated with persistent toxic substances such as cadmium (Cd), polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), and this has threatened the security of agricultural production, the natural environment, and human health. Therefore, a better understanding of pollution characteristics has always been the scientific frontier in the field of soil environmental science. How to achieve the green, economic, and sustainable remediation of contaminated agricultural soils is still a topic of global concern. The key to solving the problem is to provide insight into the contamination processes and bioremediation mechanisms of pollutants in the soil. Since the last decade the research group has selected typical agricultural soils with low-to-moderate pollution levels representing different regions and has investigated contamination processes and bioremediation mechanisms. These studies have provided a theoretical and methodological framework for the green and sustainable remediation of contaminated agricultural soils worldwide.

The primary discoveries are as follows:

1. Identifying the sources and bonding mechanisms of heavy metals (HMs) and persistent organic pollutants (POPs) in agricultural soils; Predicting the spatial distribution of HMs in edible crops based on bioavailability; Proposing a set of soil ecotoxicity and ecological risk assessment methods based on different scales from genes to communities.
2. Clarification of the accumulation and detoxification mechanisms of a newly found cadmium/zinc hyperaccumulator, *Sedum plumbizincicola*, indicating that *S. plumbizincicola* can rapidly absorb the soluble Cd and Zn from the rhizosphere soil and transfer them to the shoots; Certification of the solubilization and migration effects on adsorbed HMs by organic chelate; Elucidation of the principles for organic chelate-enhanced phytoremediation.
3. Isolating microbial strains with high biodegradation ability for high-molecular-weight PAHs; Revealing the mechanism of metabolic degradation of toxic B[a]P by a bacterial strain; Filling in the gap in knowledge of fungal laccase oxidation remediation of PAH contaminated soils; Elucidating synergistic remediation mechanisms under reductive dechlorination of PCBs and symbiotic nitrogen fixation by legume-rhizobium associations.

In conclusion, the research group has made some breakthroughs in basic scientific problems including source identification and bonding mechanisms, chemical speciation and bioavailability, hyperaccumulation and detoxification mechanisms, and microbe enhanced phytoremediation principles.







## **FIELD PROJECTS**



## LARGE SCALE DEMONSTRATION OF TREE-BASED PHYTOSTABILIZATION ASSISTED BY SYMBIOTIC FUNGI OF METAL-CONTAMINATED SITES

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### ABSTRACT

We developed an integrated bioremediation strategy that combines two poplar clones with rhizospheric microorganisms, exploiting the complex interactions evolved for the mutual benefit of both organisms, in which plant roots provide habitat, nutrients and exudates to microbial populations, whereas microbes facilitate mineral nutrition of plants. The rationale behind combining tree species and mycorrhizal fungi resides in the fact that poplar species selected are fast growing, producing large amount of harvestable biomass and capable of forming arbuscular mycorrhizal and ectomycorrhizal symbiosis with fungi. Hosting different mycorrhizal types might be of functional importance for plant nutrition and has been shown to contribute to metal tolerance of host plants as fungi can reduce the metal uptake by the plant by sequestration, extracellular precipitation and biosorption to the cell walls. Indeed, a number of recent data support the hypothesis that mycorrhizal fungi play a filtering/sequestering role on plant roots enhancing root to shoot metal ratio and increasing survival rate in harsh conditions.

Implementations of large scale field trials of poplars (SRC) inoculated with symbiotic microorganisms have been set up. Three sites of about 1 ha have been selected on the basis of (i) contaminants pollution level/history, (ii) hydrological/soil conditions, (iii) ongoing soil remediation research. The first site is a former agricultural area contaminated for nearly a century by metals due to the deposit of waste waters from the city of Paris. The second site is an industrial tailing pond contaminated by mercury and arsenic. The third site is a former agricultural soil that received large amounts of metal-enriched sediments. Poplars were planted and inoculated or not with a consortium of mycorrhizal fungi. Mycorrhizal species were chosen from results of molecular analyses of the mycorrhizal diversity of poplar roots collected from several metal-polluted sites. Genomic analyses revealed a predominance of *Glomus* and *Hebeloma* species. Two year after planting, woody biomasses were estimated using dendrometric parameters. Mycorrhizal poplars produced significantly more biomass than non inoculated ones. Height, diameter, and basal area of inoculated trees varied among the three studied sites and were generally between 15 and 35% higher when compared to non inoculated trees.

From these experimental sites, we have also developed a strategy for isolating fungal strains of interest. We have therefore isolated a set of ectomycorrhizal and endomycorrhizal isolates, as well as fungal endophytes from poplar roots. The isolated strains were first characterized and tested in metal tolerance assays and the most tolerant strains were used in a second set of experiments for their capacity to improve plant growth under controlled conditions. Four fungal strains were able to stimulate growth of their host plants. The two most promising strains, belonging to the group of dark-septate endophytes, were found to be tolerant to a set of metals and significantly improve plant growth. We also succeeded in isolating and producing *in vitro* large scale inocula of several endomycorrhizal isolates.

The usefulness of symbiotic inocula for biomass production on metal-polluted sites, as well as their potential for improving the efficiency of phytotechnologies in the remediation process will be further discussed.

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## GENTLE SOIL REMEDIATION OF TRACE ELEMENT-CONTAMINATED SOILS – SUCCESS STORIES FROM THE GREENLAND PROJECT

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### ABSTRACT

Contamination of soils with trace elements (TE) is worldwide still one of the major environmental problems. Conventional technologies for soil remediation are usually very expensive and may negatively affect or destroy soil structure and functions. Phytoremediation technologies, however, include a set of gentle remediation options (GRO) which are approaches based on plants and associated microbes as well as soil amendments for remediating trace element-contaminated soils at low cost and without significant negative effects for the environment. The main technologies are phytoextraction, in situ immobilization and assisted phytostabilization. Although GRO comprise very innovative and efficient technologies, they are still not widely used as practical site solution due to several reasons of hindrance.

Although major progress has been achieved on the lab scale, success stories obtained in the field are still limited, in particular regarding the long-term efficiency. Also, the issue of valorization of the potentially contaminated plant biomass has insufficiently been addressed so far. Furthermore, additional development is needed regarding the adequate determination of end-points of GRO. Finally, the application of GRO as practical site solution may be hindered by legal frameworks and by insufficient knowledge of the decision makers. Long term and large-scale field experiments may provide data required for the overall assessment of GRO efficiency, effectiveness and sustainability. Therefore, the EU-FP7-project "Gentle remediation of trace element-contaminated land – GREENLAND; [www.greenland-project.eu](http://www.greenland-project.eu)) with 17 partners from 11 countries has been launched on January 1 2011 to address these issues and to make GRO ready for use as practical site solution.

The project has the following main objectives:

- \* Sustainable management adapted to TE-contaminated sites and deployment of GRO at field scale, using 16 case studies on different sites across Europe
- \* Valorisation of plant biomass produced on TE contaminated sites where GRO are implemented
- \* Harmonization of methods to assess the bioavailability of TE and development of a tool set to monitor the sustainability of GRO
- \* Improving GRO through plant and microbe selection and modifications in soil TE bioavailability
- \* Appraisal of current GRO practice, and development of implementation guidance and decision support frameworks

An overview on the major outcomes and implications of the Greenland project will be presented.

*Acknowledgements:* The financial supporting of the FP-7 project GREENLAND, contract No. 266124, by the European Commission is greatly appreciated.





## FIELD DEMONSTRATIONS OF PHYTOREMEDIATION OPTIONS IN THE EU FP7 GREENLAND NETWORK OF TRACE ELEMENT-CONTAMINATED SITES

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### ABSTRACT

Performances of the most promising gentle remediation options (GRO) for trace element-contaminated soils (TECS), i.e. (aided) phytostabilisation, phytoextraction, and in situ stabilization/phytoexclusion, are assessed in a European network of 14 large field trials, within the EU FP7 GREENLAND project (<http://www.greenland-project.eu/>). The GRO efficiency is evaluated regarding various (a)biotic stresses, climatic conditions, pollutant linkages, (phyto)remediation strategies and sustainable land management. Investigated field sites cover a range of contamination scenarios (e.g. agricultural soils contaminated by dust fallout, sludged soils, mine tailings, landfills, dredged sediments, and post-industrial soils).

Harmonized datasets are built up on metal(loid) exposure, plant parameters and yields (notably for plant parts converted into feedstock), mineral and biochemical composition of plant parts, ecosystem services, financial return and costs. Soils are sampled to monitor changes in metal(loid) exposure (e.g. labile contaminant pools), transfer to environmental compartments and bioaccessibility, ecotoxicological risks, and soil (multi)functionality and biodiversity. Transfer and bioconcentration factors, shoot metal(loid) removal, contaminant fluxes, and tolerance indices are computed. Dose (exposure) – plant response relationships are modelled.

Data are summarized for various plant covers including poplar and willow short rotation coppices, annual crops of secondary metal accumulators (sunflower and tobacco), and metal-excluders (e.g. perennial grasses, barley and maize cultivars). The long-term efficiency and sustainability of GRO, progresses in remediation objectives (in compliance with national and best procedures), timescale management, maintenance, uncertainty and limitations (including spatial variation of contaminants, water requirements, global changes, etc.), potential flexibility and deployment at other sites are discussed as well as new deployed GRO and cultural practices (e.g. bioaugmentation).

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## PHYTOTOXICITY ARISING VIA SOIL POLLUTION FROM GOLD MINE WASTES IN SOUTH AFRICA – A SITUATION ANALYSIS

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### ABSTRACT

Phytotoxicity is the term generally used to describe the toxic effects that organic and inorganic compounds have on plant growth. In the case of mining activities, high levels of inorganic compounds (such as trace elements) and salinity commonly cause phytotoxic effects. Degraded landscapes adjacent to gold mine wastes typically suffer loss of efficiency as a result. Restoration of these areas has become a primary concern in the fields of environmental science and management in recent decades.

Assessments and detailed monitoring of pollution took place over a period of four years on farmland adjacent to the Mine Waste Solutions (MWS) No. 5 gold tailings disposal facility (TDF). Very little surface rehabilitation was conducted on the MWS-5 TDF prior to 1992 due to the lack of environmental legislation in South Africa at the time. The adjacent land north of the MWS-5 TDF has therefore been subjected to soil pollution and vegetation degradation from this TDF. During dry periods, significant amounts of sulphate salts accumulate on the soil surface of the farm, extending over a distance of at least 3.5 km from the TDF in a north-eastern direction.

In this study, Landscape Function Analysis (LFA) and Ecosystem/Extended Function Analysis (EFA) were combined with vegetation and soil analyses. The LFA/EFA monitoring procedure was developed by David Tongway for the Australian rangelands to assess the impact of pollution by phytotoxic trace elements on the phytostability of natural vegetation within a study area. The LFA was used in combination with the Descending-Point method to assess changes in vegetation in order to ascertain the effects of phytotoxic trace elements on the natural vegetation during the time period 2010 to 2014.

The primary objective of this study was to determine/identify LFA indicators in order to assess which of the twelve fixed points could be considered functional or dysfunctional. Additionally, the species composition and soil quality indicators of the different fixed points were determined to evaluate the effects of the pollution. Phytotoxic trace elements such as Fe, Mn, Cr, Co, Ni, Cu, Cd, Zn, As, Pb and U were extracted from the soil and plant tissue using the standard method described by the EPA 3050B by means of total acid digestion. Plant-availability of these trace elements in the soil solution was assessed via the ammonium nitrate extraction method. The concentrations of the extracted trace elements were quantitatively determined by ICP-MS analysis.

Over the extent of the study area there were marked changes in species composition, richness and diversity, with *Cynodon Dactylon*, *Setaria sphacelata* var. *torta* and *Eragrostis chloromelas* being the dominant grass species. Several sites showed encroachment of *Seriphium plumosum* which has a negative effect on grazing qualities. The different phytotoxic trace elements that exceed the toxic threshold values or that pose a threat to the three dominant grass species are discussed in detail. The change in phytotoxic trace element concentrations over the four years of this study is profiled. Furthermore, explanations relating to the ongoing effects of the phytotoxic trace elements, as reflected in the LFA at the fixed-point sites, are provided.

**Keywords:** phytotoxicity, soil quality indicators, gold TDF, pollution, LFA/EFA

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## PHYTOEXTRACTION OF LEAD CONTAMINATED SOILS WITH *FAGOPYRUM ESCULENTUM*: A FIELD AND LABORATORY SCALE STUDY

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### ABSTRACT

Soil contamination has been widely studied and the development of more sustainable and cheaper remediation techniques (bioremediation, phytoextraction) is increasingly gaining interest, especially for diffuse contamination of trace elements as in agricultural contaminated soils. Numerous studies have already tested Pb removal from soils using phytoextraction and several hyperaccumulators have been identified, such as buckwheat (*Fagopyrum esculentum* Moench) [1,3], whose interest relies on its hardiness and its low consumption of fertilizers. Despite the high amount of papers about Pb phytoextraction, very few studies have tested in the field the efficiency of the selected plants [4].

Our research project is part of a national program for monitoring urban garden soils (JASSUR) taking place in seven French cities. Concentrations of Pb exceeding the threshold value for human consumption have been found in vegetables, and resulted in the closure of several garden plots by the city council, [2]. A costly rehabilitation management (i.e 60 k€ for 2000 m<sup>2</sup>) was undertaken by bringing clean arable soil, not to mention the consumption of soil, a non-sustainable resource.

As an alternative, *in situ* phytoextraction with buckwheat, combined with a 5 mmol/kg citric acid application was tested to enhance the phytoextraction rate and hence to reduce the time needed for the soil rehabilitation (main limiting factor of this technique). A field experiment was conducted during 5 months on a contaminated garden parcel, with a Pb gradient from 97 to 314 mg/kg of soil. The parcel was divided in 32 squares: 8 without plant, 16 with *Fagopyrum esculentum* and 8 with *Fagopyrum esculentum* and citric acid. Several sampling times were performed during each stage of the plant growth and the mobile form of Pb (CaCl<sub>2</sub> extracted), as well as Pb in the roots and shoots of the plant were analyzed.

The first results showed that Pb uptake by buckwheat was far below our expectations with a bioconcentration factor (BCF) of 0.008 while Tamura *et al.* showed a BCF of 0.63 with the same plant, in laboratory conditions. To explain these results, several tests have been started: (i) the characterization of Pb mobility and speciation in naturally-contaminated (situation of the garden plots here studied) vs. anthropogenic-contaminated soils (same Pb content but originated from an atmospheric contamination), (ii) a selection of different extractants at different concentrations to enhance the phytoextraction rate, (iii) a comparison of different species of buckwheat.

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## THE USE OF RECLAIMED WATER IN SILVICULTURE FOR WOOD PRODUCTION IN SMALL COMMUNITIES AND RURAL ZONES

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### ABSTRACT

In recent years in the south of Spain has been a change in land use. This change is a result of various factors such as the low value of agricultural products on the market, the abandonment of rural areas and therefore farmland and a trend towards urbanization of small towns. In relation to the above, there has been an abandonment of agriculture on marginal lands and in contrast intensification of agriculture in those suitable land for it. In this context new forest areas such as forest plantations are created. These plantations can have different purposes such as intensive forestry for wood production, afforestation of marginal agricultural land for use as carbon sinks for achieving the Kyoto Protocol targets or, in other cases, as established Green Filters for wastewater treatment in small communities or rural areas.

At small-scale application, wastewater treatment requires simple techniques with low operating and maintenance costs. This approach increasingly promotes the use of so-called 'non-conventional' technologies, due mainly to the fact that they have many advantages when it comes to debugging the wastewater. Likewise, in the context of wastewater reuse, the use of low-cost water reclamation technologies is also necessary, provided it is done with sufficient safeguards for public health.

The study has been conducted in the Experimental Center of Carrión de los Céspedes, Seville, Spain; center on R&D&I for urban wastewater treatment and reuse (<http://www.centa.es>). The aim of the present work is to assess the suitability of a Green Filter of 2,000 m<sup>2</sup> as a water reclamation system and the end wood production of two plantations of trees (*Populus euroamericana* and *Eucaliptus camaldulensis*), irrigated with treated urban wastewater by Lagooning system during a period of ten years (Figure1).

Fortnightly sampling of raw wastewater (preliminary treated by means a bar screen of 3 cm and sieve of 3 mm) was carried out, as well as monthly sampling from the final effluents of a maturation pond and six lysimeters (catching treated effluents at 30, 60 and 90 cm deep), subsequently replaced by three piezometers at 10 m deep.

The results of the study have been assessed based on physic-chemical effluents quality regarding BOD<sub>5</sub>, COD and TSS concentrations and microbiological concentration on *E. coli* and intestinal helminthes eggs. Some operational parameters as flow (m<sup>3</sup>/d) and hydraulic retention time- HRT- (d), as well as meteorological values (precipitation, temperature and evaporation) have been included, in order to complement the performance analysis of wastewater treatment technologies.

Regarding to the evolution of forest biomass and the wood production, five sampling have been done: 2005, 2006 and 2014. During each sampling period, were done measurements of the height and diameter of trees at a height of 1.30 m above the ground. For analysis of biomass, were selected and extracted 10 trees of each plot, which were extracted fractions of aerial and underground parts for the calculation of wet and dry weights, and humidity. Data reveal that most of the effluents met BOD<sub>5</sub>, COD and TSS removal of 80-95%. As for bacteriological characterization, in all cases the *E. coli* and total coliforms concentration found are below the ranges specified in Annex IA of Spanish regulation for wastewater reuse. Regarding the eggs of intestinal helminths, although in raw wastewater has been found the following species: *Toxocara sp.* of animal origin, *Trichuris sp.* and *Himenolepys sp.*, in analyzed effluents have not been detected the presence of them.

Regarding the evolution of biomass and wood production, final results after 10 years of monitoring will be presented at the congress, due to the final cutting down has been done two days before abstract's writing.

Results and the experience of several years of study, can allow ensure that from the environmental standpoint, Green Filters are systems involving reclamation of treated wastewater, reuse the same for obtaining biomass and recharge underlying aquifers and the improvement of the landscape (by incorporating forested areas). This can promote the socio-economic integration of the environment and improve the development degree of small and rural areas as other marginal agricultural lands.

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Figure 1. Overview of the studied Green Filter, planted with *P. euroamericana* and *E. camaldulensis*.



## PHYTOREMEDIATION OF AN ORGANIC AND INORGANIC POLLUTED SOIL: REAL SCALE EXPERIENCE

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### ABSTRACT

An increasingly industrialized global economy over the last century has led to dramatically elevated release of anthropogenic chemicals into the environment, in particular petroleum hydrocarbons, polycyclic aromatic hydrocarbons, halogenated hydrocarbons, pesticides, and metals. As a result, much recent research activity has focused on the application of phytoremediation technologies (the use of plants and their associated microorganisms) as an environmentally friendly and economically feasible means for decontamination of polluted soil. Phytoremediation is, in fact, cost-effective and less disruptive for the environment and, other than remove the contamination, it can improve the overall soil quality and texture through the incorporation of organic materials, nutrients and oxygen released by plants and microbial metabolic processes.

In the present investigation, a phytoremediation approach made up of native plants and natural growing vegetation has been proposed at real-scale to bioremediate and functionally recover a soil historically contaminated by metals, hydrocarbons and polychlorobiphenyls (PCB). The soil (about 0.5 ha), located in an industrial area of San Giuliano Terme Municipality (Pisa, Italy), was divided in four plots and planted with four different plant species: *Paulownia tomentosa*, *Cytisus scoparius*, *Populus nigra*, *Populus alba*.

Soil samples were periodically (every six months) collected from the experimental area at 0-30 and 30-60 cm depth. In the attempts to assess both efficiency and evolution of the remediation system towards a natural soil ecosystem, two classes of marker parameters were selected: those describing the nutrient status (total and soluble forms of C, N, P) and efficiency of the microbiological components (enzyme activities) and those describing the capability of the system to efficiently remove pollutants (hydrocarbon, heavy metals and polychlorobiphenyls).

After about three years of phytoremediation process, all plant species were successfully adapted to the substrate, showing a regular growth and reaching 60 cm depth with their root system.

In all the planted plots, a general improvement in the nutritional-chemical soil properties and in the biological activities (dehydrogenase, b-glucosidase and phosphatase) were observed over time, indicating the activation of microbial metabolism favored by plant root-microorganism interaction.

All the plant species used were effective in the reclamation of the polluted soil from both organic and inorganic contaminants, showing a reduction of about 20%, 50%, and 90% in heavy metals, total hydrocarbons and PCB content, respectively. Considering the different plants used in the study, the poplar is likely to have contributed more to organic removal, in fact, its ability to take up and detoxify several organic pollutants is well known. Conversely, the *Cytisus scoparius* was generally the less effective plant in soil decontamination.

Therefore, the selected phytoremediation technology can be effectively used to remediate and recover soil from agronomical and functional point of view.

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## PHYTOTECHNOLOGY RESEARCH AT MANY SCALES: HOW DO WE INTEGRATE LABORATORY AND FIELD STUDIES?

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### ABSTRACT

The scientific merits of laboratory and field studies are complementary: while laboratory studies permit control of variables to elucidate fundamental mechanisms, field studies are required to capture the variability and unknowns associated with real-world conditions. Consequently, the applicability of laboratory study results to real systems is often questioned. The aim of this presentation is to closely compare and contrast conclusions that can be drawn from laboratory and field studies. The objectives are to (1) examine the processes contributing to poplar plantation treatment of food processing wastewater treatment in small columns, large columns, and a field plot and (2) compare the effects of species on treatment of stormwater through bioretention in small columns and a field site.

*Case 1: Poplar treatment during land application of food processing wastewater.* Land application of large volumes of high-strength wastewaters can create saturated, moderately-reducing conditions that mobilize metals, including manganese, iron, and arsenic. Poplar trees have the potential to decrease reducing conditions during land application of wastewaters through many processes, including (1) decreasing saturation through evapotranspiration, (2) phytoaccumulation, (3) phytosequestration, and (4) phytostimulation. For three years, we have examined the potential of these processes to decrease leaching of metals during land application of wastewaters in small (6-inch diameter) columns, large (3-foot diameter) columns, and a 1-acre field site. Mass balances completed in both column studies indicate that decreases in leachate volume, due to evapotranspiration, was primarily responsible for decrease in mass of metals leached. Additionally, evapotranspiration decreased soil moisture content at all three scales. Uptake of metals, including manganese, iron, and arsenic, and nitrate was also observed at multiple scales. However, decreases in metal concentrations in leachate were not statistically significant in all three scales. Further analyses are underway to quantify relationships between the three experimental scales.

*Case 2: Bioretention of stormwater.* Most design manuals for bioretention require use of native plants; however, the studies justifying this requirements are limited. Column- and field- scale studies were conducted to directly compare the benefits of native versus ornamental species of four plant genera commonly used for bioretention, including two herbaceous perennials (*Rudbeckia* and *Pycnanthemum*), one grass (*Calamagrostis*), and one sedge (*Carex*). No statistical differences in performance were detected between native and ornamental species, even though differences in performance were quantified between genera. These conclusions were independent of whether evaluation was completed at laboratory- or field- scale. For example, the native species of *Rudbeckia* showed poor growth and nutrient uptake in columns and the field. Extensive data was collected on plant biomass, which is currently being analysed to develop relationships between biomass growth in columns and in the field.

In addition to closely examining these two case studies, the presentation will include a comprehensive analysis of current literature on approaches to relate results from column and field studies. Additionally, an evaluation of the advantages, disadvantages, and caveats of both approaches will be presented.

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## MULTIFACETED MONITORING OF A LARGE PHYTOREMEDIATION SYSTEM DEMONSTRATES LONG-TERM EFFECTIVENESS

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### ABSTRACT

Throughout the 1950s and 1960s, carbon tetrachloride was in widespread use as a state-of-the-art fumigant for grain preservation in the midwestern United States. Entering the environment either because of spillage or methods of application, the fumigant migrated through the near-surface and unsaturated vadose zone soils into groundwater. To remove carbon tetrachloride from impacted groundwater and surface water, a large-scale, integrated, multi-species phytoremediation system including 2,000 trees and constructed wetlands was installed in 2005 at a former U.S. Department of Agriculture grain storage facility in Nebraska. This paper focuses on the development of a comprehensive monitoring system and an assessment of the remedial system's performance during the past nine years.

Specific monitoring elements were integrated into the system's design from project inception to achieve a multifaceted understanding of the remedial action's performance over time. The well-instrumented monitoring network includes an automated water level monitoring system, a weather station, runoff gauges, and a sap flow measurement system. Extensive seasonal sampling and analyses are conducted for carbon tetrachloride in groundwater and plant tissue, in conjunction with growth and biomass measurements. The monitoring system is designed to (1) determine water use by the plant system, as indicated by sap flow rates and groundwater and surface water responses; (2) estimate contaminant depletion via the uptake of carbon tetrachloride by plant tissues and other natural removal processes; (3) monitor plant growth and biomass development and their relation to carbon tetrachloride removal; (4) track changes in chemical parameters with time in groundwater and surface water; (5) measure ambient air concentrations of carbon tetrachloride in the phytoremediation area; and (6) quantify the overall mass reduction of carbon tetrachloride as contaminated water flows through the phytoremediation area and wetlands.

Comprehensive analyses of various data sets recovered from the monitoring network indicate that the phytoremediation system is having a measurable effect on the hydrologic regime and the fate of the contaminant plume. Fluctuations in groundwater levels and reduced surface water flow rates identified at multiple monitoring locations during the growing season appear linked to the diurnal effects of groundwater uptake by the trees. Analysis of the monitoring data further demonstrates a localized, targeted reduction in the maximum concentrations of carbon tetrachloride in groundwater in the phytoremediation area, in response to the seasonal plant growth cycle; the results indicate that the phytoremediation system is achieving significant carbon tetrachloride mass removal. The system's performance has been validated repeatedly in accord with the designated remediation targets and meets compliance requirements established by the U.S. Environmental Protection Agency.

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## RECENT FINDINGS ON THE PHYTOREMEDIATION OF POLLUTED SOILS BY THE GENOREM MULTIDISCIPLINARY RESEARCH TEAM

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### ABSTRACT

A multidisciplinary project (GenoRem) was undertaken to develop innovative phytoremediation procedures for polluted soils based on the use of various willow cultivars and their associated fungal and bacterial symbionts. Field and greenhouse experiments were set up to facilitate data integration and collaboration at all levels of this research project. Combinations of 'omics' tools and bioinformatic approaches are being used to study plant responses, fungal and bacterial community structures and functions, and soil-plant-microbe interactions in relation to soil contaminants. Two years after plantation establishment, the presence of willows was found to correlate with the degradation of PAHs and petroleum hydrocarbons in soil. The capacity of the tested willow cultivars to uptake trace elements (TE) was also estimated. TE were found in different willow tissues: Cd and Zn translocated to aboveground parts while As, Cu and Pb were preferentially sequestered in roots. Some willow cultivars were found to be more tolerant to soil contaminants and showed little or no difference in their physiological and growth parameters whether grown in polluted or in control soils. Differential gene expression analyses showed a cross-tissue pollution effect, with significant differential expression of 3969 transcripts, thereby highlighting potential genes and pathways involved in phytoremediation. Metagenomics of the soil microbiome showed that the microbial communities are affected by the presence of contaminants but also by the presence of willows (rhizosphere). Moreover, we found that the abundance of some specific fungal classes in highly contaminated soils was directly related to willow phylogeny. These findings, combined with the isolation and characterization of indigenous selected candidate microbes that could potentially be used on extremely polluted sites (petroleum hydrocarbons), are very promising for the commercial development of this approach to phytoremediation as well as the social acceptability and normative requirements for its application.

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## A MONITORING OF A LONG TERM FIELD STUDY OF SEWAGE SLUDGE APPLICATION ON SOIL CONTAMINATED WITH TRACE ELEMENTS

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### ABSTRACT

Sewage sludge, in particular from the food industry, is characterized by fertilizing properties, due to the high content of organic matter and nutrients. Furthermore, the application of sewage sludge causes an improvement of soil parameters as well as increase in cation exchange capacity, and thus stronger binding of cations in the soil environment, which involves the immobilization of nutrients and greater resistance to contamination.

In a field experiment sewage sludge has been used as an additive to the soil supporting the phytoremediation process of land contaminated with heavy metals (Cd, Zn and Pb) using trees species: Scots pine (*Pinus silvestris* L.), Norway spruce (*Picea abies*) and oak (*Quercus robur* L.). The aim of the research was to determine how the application of sewage sludge into the soil surface improves the phytoremediation process after five years of the experiment.

In the field experiment located in the zone of influence zinc smelter (18°54'E, 50°30'N) in Poland's Silesia Region, soil parameters and trees biomass were determined during five years of the research. The investigated area is dominated mainly by podsollic soil, characterized by low humidity and a low pH value. This type of soil poses a poorly formed soil profile, with high permeability and a very low content of humus. These soils are also poor in nutrients and are distinguished by a low sorption capacity and small capacity buffer. The contamination of study area is a result of the impact of particulate emissions from the zinc smelter and is characterized by high accumulation of heavy metals such as Cd, Pb and Zn. In a field experiment sewage sludge was used to support the phytoremediation process of contaminated soil. Sewage sludge used in the study was collected from the sewage treatment plant at the factory of mineral waters. Used sewage sludge was characterized by a low content of heavy metals and eggs of parasites and, for this reason, can be used with both the restoration of degraded land and agriculture, having a positive effect on the increase in of biomass and number of soil microorganisms. The field study was conducted in 2010. Over the five years (2010 – 2014) soil samples and samples of plant material were collected twice a year (spring and autumn).

On the plot fertilized with sewage sludge the proper growth of plants and large increase in biomass were noted. Sewage sludge used in this experiment is of considerable importance in the phytoremediation process of soils contaminated with heavy metals (Cd, Zn and Pb). Sewage sludge supplied to the soil environment some biogenic elements necessary for the growth and development of trees used in the study. During the phytoremediation process of degraded land with support of sewage sludge and with used trees species: Scots pine, Norway spruce, oak, the improvement of many soil properties has been noted. Application of sewage sludge from the food industry to soil contributed to the increase in humic acids and the sorption capacity of the soil, and improvement of the protective buffering functions of the soil. Moreover, the used amendment showed the beneficial fertilizing properties and entered mineral compounds stimulated the plants growth and development. Conducted studies have shown that the addition of sludge caused the sorption of metals in the soil, mainly the addition of sewage sludge to contaminated soil contributed to the sorption of heavy metals within organic matter. The application of sewage sludge to the soil creates conditions for plant cover restoration, which protects against sudden metals migrating into the soil profile as well as wind erosion, and this phenomena is confirmed by the absence of growth of roots on untreated soil. The monitoring of soil solution confirmed, that the content and mobility of biogenic elements and heavy metals in the soil and from soil to soil solution is dependent on the seasonal processes of humification and mineralization of organic matter. Conducted field experiment demonstrated that selected trees like Scots pine and Norway spruce, because of its excellent adaptability can be used in the remediation of soil and of soilless devastated areas, as pioneering plants.

**Acknowledgements:** The project was supported by Polish- Norwegian Research Programme Core 2012 (decision No DZP/POL-NOR/1885/2013).



## DEPLOYMENT OF AIDED PHYTOSTABILISATION AT FIELD SCALE: SET UP AND MONITORING LESSONS

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### ABSTRACT

In the project GREENLAND (FP7, KBBE-2010-4, 266124) project, one work package aims at testing different phytomanagement options at large field scale to gain information on practical deployment and long term efficiency. Among the network of 13 large trace element (TE) contaminated sites, one located in the Nord-Pas-de-Calais region (France), was implemented in September 2011 with aided phytostabilisation, i.e. the combination of plants and soil amendment to reduce the risks associated to the presence of soil TE. The site (1 ha) is part of a large sediment disposal site which was affected by intensive industrial activities. As local authorities are required to manage contaminated landfill sites, they developed a management strategy based on implementation of an environmental management system which aims to meet best practices and comply with environmental regulation in the field of human health and environment. In this context, the objective was to combine aided phytostabilisation with bioenergy production based on *Salix* cultivation to reduce the environmental risk posed by these sediments and allow the economic valorization of the contaminated sediments via the sale of the produced biomass. Another objective was to test aided phytostabilisation as a strategy to avoid the propagation of the Japanese knotweed, an invasive species that occurred at the sediment landfill site.

In a first step, *Deschampsia cespitosa*, a grass, and Thomas basic slag (TBS), a basic mineral amendment, already tested at field site on an experimental TE contaminated landfill site [1], were used to stabilise the TE of the top sediment. In a second step, a SRC composed of two *Salix* cultivars ('Inger' and Tordis') was deployed to produce biomass.

First results show the success of the plant cover that rapidly reached 100% and the success of the *Salix* plantation although phytotoxicity signs appeared after few months. Several hypotheses were studied of which the grass competition for water and essential nutrients. As the sediment landfill site is highly contaminated with metals, mainly Zn and Cd, TBS is expected to decrease the metal shoot transfer towards the grass and the two planted *Salix* clones and to decrease the metal labile pool [1]. At this stage of the project monitoring the effects of TBS on soil metal mobility or the *Salix* leaf metal concentrations have not yet been measured. The grass showed very little Zn and Cd concentrations in aerial parts that confirm the choice of this plant as a candidate for phytostabilisation. Conversely, the two *Salix* cultivars showed very high Cd and Zn leaf concentrations that might not be compatible with a phytostabilisation strategy. The Cd and Zn concentrations in leaves and wood at harvest might direct the conversion process of the produced biomass (thermal treatment, metal recovery). A decrease in Japanese Knotweed was visible after one year of monitoring and corresponded to a reduction in coverage of 27% of the surface area showing that the Japanese Knotweed is less competitive and its growth decrease represents a beneficial effect of phytostabilization in terms of ecological services.

The whole chain of the aided phytostabilisation, i.e. from field preparation to the conversion process of the produced biomass, is addressed by this project. Results will be presented step by step taking into account practical experiences and scientific knowledge as well as regulation and economical aspects.

#### Reference:

1. Bert V, et al. (2012). Environmental Pollution 169, 1-11.

**Acknowledgements:** This work was financially supported by European funds through GREENLAND (FP7, KBBE-2010-4, 26124).



## REMEDIATION OF UNSATURATED SOILS BY PHYTOREMEDIATION AT VENICE TECHNOLOGIES SPA, PORTO MARGHERA (VE)

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### ABSTRACT

The remediation by phytoremediation has been applied to the unsaturated portion of the soil of the two areas, called SP3 and FS4 and having a total area of 720 mq, located inside Venice Technologies Research Centre in Porto Marghera (VE), Italy. The analyses carried out in the areas of study showed an increase in the amount of metals (SP3: Cd 74 mg/Kg, Hg 20,9 mg/Kg and Zn 1547 mg/Kg; FS4: Hg 31,8 mg/Kg) with respect to limit concentrations (CLA) indicated by the ex DM 471/99 all. 1 tab. 1 col. B (Cd 15, Hg 5 and Zn 1500 mg/Kg). The remediation has the goal of achieving the "objective values" as defined by the minutes of Conference Services (CS) 6-31-2006 therefore the concentrations of metals in unsaturated soils to reach are the following: Cd 50 mg/Kg, Hg 10,5 mg/Kg and Zn 1500 mg/Kg.

The National Research Council (CNR) in Pisa by studies in meso- and microcosm chose to use for phytoremediation project these two species: *Helianthus annuus* L. and *Brassica juncea* (L.) Czern. To increase the removal of metals have been used the chelating agents: ammonium thiosulphate for Hg and EDTA for Zn and Cd.

Before planting the plants it was necessary to prune woody plants already in the area and remove those found in the study area to reduce shadows. Finally, the soils were processed with motor cultivator and was built the irrigation system. The plants have been used by short rotation; the first crop cycle was done with the *H. annuus* while the second cycle with the *B. juncea*. During and after the process of phytoremediation have been made both chemical and physical analyses of the soil and analyzes on water to verify that pollutants did not pollute the aquifer. Also the biomass and the amount of metals in the soils was analyzed before and after treatment with the chelating; the metals were evaluated also at the end of the cycle.

The results showed that FS4 area already after the second crop cycle had reached the remediation of soil instead a part of the SP3 area still showed concentrations of Zn greater than the "target values"; for which it was made a further cycle with *H. annuus*. SP3 area reached the goal after the third crop cycle.

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## LOCAL COMMUNITY PERCEPTIONS OF MINE SITE RESTORATION USING PHYTOREMEDIATION IN ABITIBI-TEMISCAMINGUE (QUEBEC)

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### ABSTRACT

Canada is foremost among the world's nations that are actively engaged in mining and has numerous ongoing projects focused on mineral exploration, mine development, and mining. In Quebec, one-third of mining projects are linked to gold, while the remainder is associated with polymetallic or diamond extraction. As shown in previous studies, ore extraction can drastically affect receiving ecosystems. Many restoration strategies have been developed throughout the world to reduce or counteract the long-term negative effects that the mining industry may impose on the environment. In the province of Quebec phytoremediation is one of the most eco-friendly restoration strategy emerged since the last two decades but studies on local people perception on this restoration strategy are scarce. Given the importance of local community acceptance in the success of mine site restoration strategies, there is a need to fill in this knowledge gap and provide all stakeholders with useful information.

This work assesses people's perceptions of and expectations for mine site restoration, particularly phytoremediation strategies, and analyses their preferences for species that were used in phytoremediation. Data were collected from mining stakeholders using a structured questionnaire administered through snowball sampling method. Participants were selected from different categories, and included industrial workers, mine employees, local communities, environmentalists, and government actors. They were asked to respond to 35 questions, of which 28 were closed-ended questions. We used Multiple Correspondence Analysis (MCA) as implemented in the software XLSTAT to visualize relationship between participants' characteristics, their view on mine site restoration and phytoremediation.

Results on participants' characteristics show out that the research sample included more men (58% of respondents) than women (42%). Globally, 75% worked in a field that was related either directly or indirectly to the mining industry. Twenty-five percent worked directly for mining companies, while 29% of respondents had previously worked in the mining industry, generally in their early years. MCA results clearly show out that people perception on mine site restoration is influenced by mining activities effects on health and region attractiveness. Among restoration methods phytoremediation (65.21%) was rated positively with regard to its environment potential, aesthetic and consideration for future generation followed by fillings and excavating. Restoration strategy costs have no effect on people choice. Indeed, independently to the considered method, costs required to implement the technology have the lowest effect on participant's choice. According to them, mine sites need to be restored at any cost and mining managers being the biggest beneficiaries should have the obligation to restore them. Results also revealed that participants prefer use of shrubs as vegetation component of phytoremediation to reach their restoration objective. Their preference for shrubs is mainly supported by its quick growth when compared to trees. Participants' opposition to the use of trees to restore mine site could also be justified by their economic value. As Abitibi-Temiscamingue is a forested area, they are particularly worried about the potential to see the forest industry logging before site rehabilitation is completed.

With increasing importance of social acceptability in Quebec, local communities have the opportunity to convince the mining industry to use sustainable methods for site restoration. Therefore, their consideration for environmental sustainability and future generation will play a very important role in mining sustainability.

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## PHYTOREMEDIATION OF SEWAGE AND INDUSTRIAL WASTEWATER USING HIGH RATE TRANSPIRATION SYSTEM

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### ABSTRACT

Industrial wastewater generated in India is less compared to the sewage but the strength of pollutants is very high compared to the sewage. The ground and surface water contamination caused due to wastewater is increasing day by day. The operation and maintenance and capital cost for the conventional treatment is reaching sky high such that the developing countries are not able meet the expenses. Hence an imperative requirement of cost effective and eco-friendly technology is required for pollution abatement and also treatment and reuse of wastewater.

The objective of the study was to develop and demonstrate a phytoremediation technology - High Rate Transpiration System (HRTS), i.e. phytohydraulics – based on the principle of ability of plants to capture, transport and transpire wastewater (w/w) containing pollutant which in turn controls the hydrology of the environment. This technology is used for treatment and utilization of wastewater (colored) without causing soil and groundwater contamination. The laboratory studies were carried out at CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), Nagpur to optimize hydraulic loading and materials for filter bed media (saw dust, lime sludge and gypsum for industrial w/w and coconut husk, soil and gypsum in case of sewage). The filter media helps in removal of organics by aerobic fixed-film biological treatment (soluble BOD) and filtration of organic solids by vegetation and soil (particulate BOD). Removal of nitrogen by plant uptake, nitrification and denitrification processes and phosphorous by precipitation, adsorption onto soil particles and also uptake by the plants. The field studies were carried out for paper mill wastewater at Amlai, Madhya Pradesh & for domestic sewage at Puri Sea Beach, Orissa. The schematic representation of HRTS for treatment and disposal of wastewater is given in Fig 1. Length of ridge varies from 5-20m depending upon the type of soil and plant species.

The present study describes long-term operating experience of zero discharge of pulp and paper mill wastewater and sewage, which is characterized by high BOD/COD, TDS, nutrient and color (pulp and paper mill). The result of field trials showed that there was no appreciable build-up of salt in the furrows even at the wastewater (both) application rate of  $250 \text{ m}^3 \text{ ha}^{-1} \text{ day}^{-1}$ . The BOD, Nitrogen, Phosphorous removal was estimated at 80-94%, 25-32 %, 20-28% respectively under laboratory conditions for sewage whereas in case of paper mill wastewater COD, BOD and color removal was found to be 95%, 99% , and 100% respectively.

The results show that under scientific design and standardized management, wastewater could be used in HRTS. The irrigation is safe and reliable with a certain pre-treatment process, which has no adverse impact on the soil and ground water. HRTS is a cost-effective and alternative to conventional treatment processes. The quality of groundwater was monitored within and outside the HRTS through well water sampling and it was found to be well within the permissible limits. The findings showed that HRTS is environmentally acceptable phytoremediation solution to manage the pollutant problem of wastewater and also minimize the impact on groundwater quality (Fig. 2).

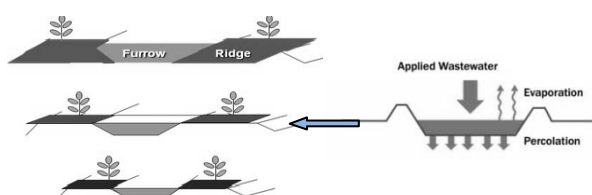


Fig 1: Schematic of HRTS

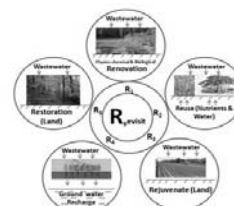


Fig. 2: 6-R Concept of Wastewater Management



## PHYTOREMEDIATION OF MANGANESE MINE SPOIL DUMP

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### ABSTRACT

Landscapes created as a result of heaping and peeling of mine spoil in open cast mines are devoid of nutritive and supportive value to anchorage the plants due to destruction of original soil ecosystem, horizonation, structure, texture, organic matter and microbial activity and it takes several decades to restore. This paper envisages the long term monitoring of phytoremediation studies over a time span of 25 years on a reclaimed manganese mine spoil site at Central India.

The objective of the study was to develop and demonstrate a phytoremediation technology for the restoration and rejuvenation of manganese mine spoil dumps. An ecofriendly technology was employed to restore an area of 6ha of barren mine spoil dump at central India. The site characteristics are such that it receives an annual precipitation of 1100mm and min temp. of 10°C and max temp. 47°C. The employed technology used the wastes generated from industries and few microbial isolates (*Rhizobium*, VAM spores & *Azotobacter*) to restore the mine spoil. Laboratory experiments were done for analyzing the survivability of plants in such conditions using the additive material (Press mud from sugar mill). The experiments suggested that the optimal pressmud concentration to be added as 100T/ha. Various plant species (*Tectona grandis*, *Cassia siamea*, *Acacia nilotica*, *Dendrocalamus strictus*, *Azadirachta indica*, *Ficus religiosa*, *Eugenia jamolana*, *Ailanthus exelsa*, *Gmelina arborea* and *Pongamia pinnat*) were screened and planted on the mine spoil dump by pitting technique. Mine spoil consist of 40-60 % gravel and stone and higher concentration of available manganese (1.3-6.3%). The physico chemical and microbiological properties of mine spoil and restored mine spoil (Table 1). The long term field demonstration project supported with over 1,50,000 trees of economically and ecological values grown on a manganese mine spoil dump having natural succession of plants, reestablishment of grass canopy and ecological cycles proved that this technology is a most proven approach in reclamation of mine spoil dumps (Fig 1).

Parameter	Mine spoil	After 25 Years
<b>Bulk density (Mg m<sup>-3</sup>)</b>	1.4±0.05	1.13±0.1
<b>Max. Water Holding Capacity (%)</b>	29.8±2.8	53.2±5.2
<b>Porosity (%)</b>	32.1±5.2	65.82±7.3
<b>pH</b>	6.9±0.2	7.2±0.2
<b>Available Nitrogen*</b>	12±2.2	148±15.6
<b>Available phosphorous*</b>	9.2±0.3	34±2.5
<b>Available potassium*</b>	1.8±0.1	47±0.1
<b>Organic carbon (gKg<sup>-1</sup>)</b>	0.994±0.05	31±1.8
<b>Available Mn concentration*</b>	215±38.2	38.7±9.3
<b>Fungi (CFU/g)</b>	0.1X10 <sup>1</sup>	9X10 <sup>4</sup>
<b>Bacteria (CFU/g)</b>	0.6X10 <sup>1</sup>	5X10 <sup>9</sup>
<b><i>Rhizobium</i> (CFU/g)</b>	0	5X10 <sup>5</sup>
<b><i>Azobacter</i> (CFU/g)</b>	0	4.1X10 <sup>5</sup>
<b>VAM/10 g</b>	0	52

\*mgkg<sup>-1</sup>



Fig 1: Ecosystem Development in Mine spoil dump



## STUDY OF LONGTIME SOIL FORMATION ON OVERGROWN QUARRY ON THE SOUTH OF KAZAKHSTAN

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### ABSTRACT

Soils formed on loesses along with floodplain soils are the cradle of agriculture. Origin of agriculture in Central Asia also occurred on soils formed on loesses. Despite these advantages, loesses are more exposed to erosion and are increasingly being destroyed by technogenic causes.

Overgrown loessial quarry of Shymkent Cement Plant which in accordance with natural zone distribution belongs to ephemeral short-grass semisavanna, has been chosen for research. On the territory of this quarry areas, which under the influence of natural vegetation were on the initial stages of young soils formations, were allocated (22,32,41 years).

The quarry had big cup-shaped form with low-sloped and steep slopes in some places. The depth of Shymkent quarry is 15-20 meters. The quarry has irregular bottom with knolls and deepings, formed during the period of the development of loesses.

20-45 years ago, for trial establishment, mine technical recultivation was conducted; it included leveling the bottom of quarry, cutting the irrigators and fertilization; agricultural recultivation.

In our opinion, replantozem has already formed in these loessial quarries, however, separation of fully developed soil profile which is distinctive for soft ground has not occurred yet.

Nowadays, Shymkent quarry is intensively used as natural pasture as it is located close to the settlement. In this connection, the recovery of grass stand is slow. A large area is occupied by weed-ephemeral (ruderalherbae - ephemerae) sub-climax plant community. Besides, alhagae - ephemerae plant communities are widely widespread.

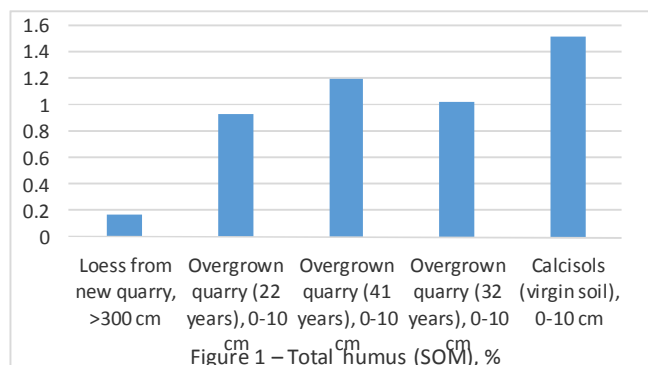
The increase in content of physical clay has been also observed in loesses of overgrown quarry of Shymkent cement plant. However, its values hardly reach ones of newly formed loesses in new quarry. The content of physical clay in the upper (0-10 cm) layer of loesses was decreasing as a result of their illimerization to sublayer by rainwater and melt-water. Different minerals show different capacity to transfer in the process of illimerization.

Humus condition of replantozem from overgrown quarry of Shymkent cement plant is also dependent on time factor (duration of soil formation). Freshly exposed loess of Shymkent cement plant contains 0,17% of humus. In the loess of Shymkent cement plant (22, 31 and 41 years) the content of humus increased by 0.94; 1.02 and 1.2% respectively. Fulvic acid, the share of which is gradually decreasing, dominate in humus group content.

Initially, loesses contained only decimal places of total humus (Figure 1). The content of nonhydrolyzed residue in humus of Shymkent loesses accounts to 18%. This is due to the relatively low content of organic matter on this depth, which is mainly represented by labile fulvic acids (72%) and microbial protein.

Thus, the analysis of humus condition of replantozem has shown that joint effect of natural, ecological (climate, biota, soil formations, landscape and time) and antropogenic factors of soil formations (recultivation) leads to marked accumulation of organic substance of humate type in it. Most actively the processes of humus accumulation runs in the upper part of humus horizon of replantozem that is due to the character of inflow and mineralization of plant residues in it. The time (duration of soil formation), among all studied ecological factors, plays the main part in changing the quantitative and qualitative composition of humus in recultivated loesses.

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## PHYTOREMEDIATION OF SULFENTRAZONE-CONTAMINATED SOILS USING GREEN MANURE UNDER FIELD CONDITIONS

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### ABSTRACT

In Brazil, the phytoremediation of herbicides has received increased attention in recent years because of the use of compounds with long residual activity in large agricultural fields, particularly those used in the cultivation of sugarcane and soybeans, and in pasture. This technique has had good results with the use of green manure. In addition to environmental considerations, phytoremediation has agricultural importance, as it enables the reuse of agricultural fields even in the presence of high levels of xenobiotics and favors the introduction of crop rotation. The decrease in the time required for the introduction of crops susceptible to sulfentrazone may represent an important economic advantage for rural entrepreneurs.

The objective of this study was to evaluate the performance of two species of green manure: *Canavalia ensiformis* (jack bean) and *Crotalaria juncea* (sunn hemp) for the phytoremediation of sulfentrazone in a field, thereby enabling the validation of the results obtained under controlled conditions.

The field experiments were performed in the experimental farm of CEUNES/Federal University of Espírito Santo in São Mateus, the state of Espírito Santo, Brazil. The soil of the experimental field was classified as Haplic Acrisol, with a slope of 2%.

Treatments consisted of a combination of *C. ensiformis* and *C. juncea*, control treatment (an uncultivated plot that was weeded manually), and four doses of sulfentrazone (0, 200, 400, and 800 g ha<sup>-1</sup>). The experimental design was of complete randomized blocks with split plots, in which the factor in the main plot was the plant species and the factor in the subplot was the herbicide dose, with four replicates.

Fifteen days after the application of sulfentrazone, as a simulation of soil contamination, the phytoremediation species *C. ensiformis* and *C. juncea* were sown. Plant spacing used was 0.50 m at a density of 20 *C. ensiformis* plants/m<sup>2</sup> and 120 *C. juncea* plants/m<sup>2</sup>.

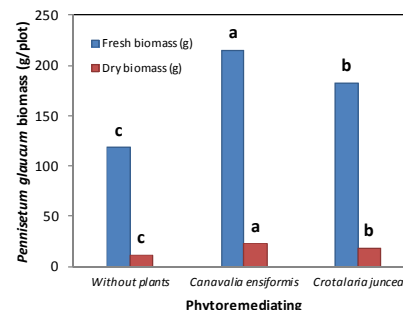
Seventy-five days after sowing (DAS), these plants were cut close to the ground. During this same period, the control treatment (uncultivated plot) was maintained. After a week, *Pennisetum glaucum* (pearl millet) was tilled on the cultural remains of the phytoremediation species and in the weeded control plot. *P. glaucum* is susceptible to sulfentrazone and can serve as an indicator of its presence in soil.

Thirty-four DAS of the indicator species, phytotoxicity was visually assessed, and fresh and dry weights of the aerial parts were evaluated. Eighty-four DAS (end of the cycle), the following parameters of *P. glaucum* were assessed: height, stem diameter, length between nodes, number of leaves and panicles, fresh and dry weight of panicles, and dry matter of the whole plant.

Apparently, the three types of management were effective in removing the residual effect of sulfentrazone, because all the variables measured for the millet remained constant with increasing doses of sulfentrazone. This may have been caused by herbicide leaching (data not shown), considering that the soil is sandy. However, the growth of the millet increased when cultivated in soil receiving *C. ensiformis* and *C. juncea* treatment compared with when cultivated in soil receiving the control treatment. In addition to the phytoremediation action, this result can also be attributed to the beneficial effects of cover crops.

Therefore, prior cultivation of the phytoremediation species *C. ensiformis* and *C. juncea* promoted the remediation of sulfentrazone and enabled the growth of pearl millet grass under normal conditions. In addition, *C. ensiformis* was the most effective species for herbicide decontamination in the field.

**Acknowledgments:** This study was funded by the Edital Universal from the 2011 CNPq and by the Institutional PNPd Program from the 2011 CAPES.





## A DECADE OF PHYTOREMEDIATION PROJECTIONS BY EUCALYPTUS TREES PREVENTING CONTAMINATED PLUME MIGRATION

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### ABSTRACT

At a former tin Smelter Site in Texas City, Texas (USA), phytoremediation evaluation studies started in 2002 and continued. Primary contaminants at the Site are heavy metals (barium, cadmium, chromium, copper, lead, and magnesium). In 2013, the same stand of trees was evaluated using identical methods, allowing a comparison of the ability of the matured eucalyptus trees to hydraulically control the migration of the impacted groundwater, and make more refined projections on actual transpiration measured by sap flow sensors. This data comparison presents a unique opportunity to generate a eucalyptus crop specific Kc (crop coefficient), which in turn provides a well-accepted factor to apply to weather-derived evapotranspiration potential index (ETo) based transpiration algorithms. The transpiration compared to rainfall provides hydraulic models with a water balance approach to contaminated plume remediation and migration prevention.

In the 2002 evaluation of a selected stand of three-year-old *Eucalyptus camaldulensis*, the analysis of transpiration and water consumption projection was based on measurements by Dynamax TDP sap flow sensors, the leaf area index, the weather based ETo and rainfall. By projection of maximum leaf coverage at maturity, and the stand density of a large tree population, the total water consumption was projected based on the Kc in 2002 as well as the annual ETo estimate for the area. Based on a 2000 trees per hectare density, the total water draw down was 400-800 mm per year (deficit), depending on rainfall. With a new set of sap flow readings for the 14 year old trees, we expected a reduced sap flow per basal area, a reduced sap velocity, but an overall increase in water flux consumption due to increased tree height, cross section and larger leaf area. We expected a reduced sap flow per basal area, a reduced sap velocity, but an overall increase in water flux consumption due to increased tree height, cross section and larger leaf area. We present the latest Kc factors for 2013, and the results of ETo projections, as well as the expected water deficit created by the mature stand at the projected density. The presentation summarizes the differences in transpiration rates, the Kc changes over time, the expected annual draw down in both dry and wet years. With the refined sap flow and Kc factors for modelling, we improve the long term projections of plume migration prevention off the site, and enable a refined a projection of plume remediation by transpiration and plant – root activity.



## PHYTOSCREENING OF TREES RECOLONISING A DISUSED POLLUTED INDUSTRIAL LANDFILL

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### ABSTRACT

Old industrial sites have often a complex story due to the changes of technologies, of economic frame and environmental awareness, occurred during their life-time. In these cases, the old landfills record the changes in term of an elevated spatial heterogeneity of pollutants, difficult to assess and normally requiring an improved sampling strategy integrating different technological approaches. Among the different technologies, the phytoscreening approach could provide a low cost tool to analyse the spatial distribution of soil contaminants and to obtain preliminary information about the suitability of the phytoremediation approach for the reclamation of the site.

The aim of this work was to use the phytoscreening to identify the superficial contamination (0-2 m depth) in an old industrial landfill of an electrochemical factory in Papigno (Terni, Italy), not more active. For that purpose we sampled leaf and woody tissue of trees naturally recolonising the landfill area.

The surface of the landfill has an extension of about 3,5 hectares and has been preliminarily characterized using soil cores, sampled according to a reference grid 50 x 50 m wide. As supposed, the results of the soil cores analysis showed a typical hot-spot contamination pattern, with several independent spots of contamination spread over the entire area. The pollutants found were: Polychlorinated biphenyl (PCB), heavy hydrocarbons (C>12), lead (Pb) and chrome (Cr tot).

The population of trees growing on the landfill surface was preliminarily characterised in term of: species composition, trees abundance, tree dimensions and age (by tree coring analysis). The results showed that the recolonisation started about 20 years ago and continuing until present, starting from the pioneer species already surrounding the area. Furthermore, the growth rates of the different species were in line with the climate of the area, without any evidence of phyto-toxicity caused by the pollutants. On the other hand, significant reductions of radial growth occurred during hot and dry years.

The sampling strategy for the chemical analyses of the plant tissues was related to the specific objectives of: evaluate the dimension of the hot-spots, confirm the pattern of the pollutant distribution, evaluate the species specific plant-pollutant interaction. Furthermore, additional soil cores were collected around the sampled trees, to determine the true BCF.

The leaf tissues showed higher values of heavy hydrocarbons (C>12) compared to the woody ones, with an average concentration, on leaf tissues, of 254.5 mg/kg and 675.1 mg/kg in Robinia spp. and Populus spp. (the most abundant species), respectively. The lack of correlation between the C>12 concentration of the hot-spots and the surrounding plants, as well as between the distance of the trees from the hot-spots and their level of contamination, demonstrated the limited spatial (horizontal and vertical) diffusion of the contamination which remain confined probably in the places where the contaminated matrix was placed.

Around to the few hot-spots contaminated with heavy metals (Pb and Cr) the plants resulted lightly polluted, without any significant difference between leaf and woody tissues. Similarly, the PCB was detected in the plants growing close to the contaminated hot-spot.

In conclusion, using the phytoscreening in an old and abandoned industrial landfill resulted to be a valid support to identify the diffusion of contamination in the top-soil (important also for the risk analysis) and to validate phytoremediation projects. On the other hand the use of phytoscreening on natural recolonising trees, should be integrated with other direct analyses when the vertical profile of contamination is unknown.



## SWINE WASTE PHYTOREMEDIATION USING DUCKWEED (*LANDOLTIA PUNCTATA*, LES & CRAWFORD) IN A FULL SCALE PLANT

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### ABSTRACT

The fast growth of world pork production has caused major environmental impacts, especially in developing countries. The large amount of nitrogen and phosphorous compounds found in pig manure has caused ecological imbalances, with eutrophication of major river basins in the producing regions. Moreover, much of the pig production in developing countries occurs on small farms, and therefore causes diffuse pollution. Therefore, duckweeds have been successfully used for phytoextraction of nutrient and heavy metals (Zn and Cu) from swine waste, generating further a biomass with high protein content. The aquatic macrophyte *Landoltia punctata* is promising specie to be used in agro- wastes phytoremediation considering their high growth rate and nutrient requirements.

The present study evaluated the phytoextraction of nitrogen and phosphorus from swine waste using two full scale duckweed ponds (called DP1 and DP2) in a small pig farm. Duckweed ponds series received the effluent from a biodigester-storage pond, with a flow rate of  $1 \text{ m}^3 \cdot \text{day}^{-1}$  produced by 300 animals. Even after anaerobic stabilization a high nutrient concentration was found in effluent reaching  $832 \text{ mgNTK} \cdot \text{L}^{-1}$  and  $92 \text{ mgPT} \cdot \text{L}^{-1}$ , considering average values. Ammonia was the predominant form of nitrogen compost representing 2/3 of total nitrogen, however, the suitable pH values (6,8 on average) reduce the  $\text{NH}_3$  toxicity for duckweeds. During experimental period approximately 28.5 kg of phosphorus and 260 kg of nitrogen was recovered from the water (or  $0.47 \text{ gPT} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$  and  $4.4 \text{ gNTK} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ ), however, direct phytoextraction plays a partial role mainly for nitrogen removed. Analysis of the biomass nitrogen content demonstrated a percentage of  $6.6\% \pm 0.8$  of total nitrogen (dw), in average. This nitrogen content found is related with the high protein content in duckweed biomass reaching 41,25%.

Therefore, knowing total biomass produced was possible to calculate that 28% of the nitrogen removal in DP1, that is 81 kg of TKN (or  $1.2 \text{ g TKN} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ ) was due direct phytoextraction by duckweeds. Additionally, (72%) was removed by nitrification and denitrification processes. Strong denitrification can be justified by several factors including aerobic and anoxic zones ( $2.1 \text{ mg DO} \cdot \text{L}^{-1}$  on the surface and  $0.5 \text{ mg DO} \cdot \text{L}^{-1}$  on the bottom) always being present, a large area for a biofilm to attach, optimal pH and temperature ranges, and availability of food (BOD) for heterotrophic microorganisms (denitrificants). Also, nitrate ( $\text{NO}_3$ ) was detected sometimes, usually during the summer, reaching  $32 \text{ mg/L}$ . However, because nitrate can be used by both duckweeds and denitrificant bacteria, the nitrate concentration ranged widely. By contrast, in DP2 a different proportion was found; 96% of the total removed nitrogen was due to duckweed phytoextraction and only 4% was caused by denitrification. The applied nitrogen load was larger in DP1 than DP2, so it is possible that almost all of the nitrogen applied to DP2 was required for duckweed growth.

Unlike nitrogen, the main route for phosphorus removal was the phytoextraction process. The large difference in removal rate between N and P may be due to several factors such as nutritional requirements, initial concentrations of P and N and pH variations which interferes in nutrients solubility. However, the present data showed that nitrifications/denitrification processes were improved by duckweed mat and strongly affect the difference in removal rate between N and P, mainly in DP1.

Due to the high rate of nutrient removal, and also the high protein biomass production, duckweed ponds revealed, under the presented condition, a great potential for phytoremediation of swine waste. Nevertheless, this technology should be better exploited to improve the sustainability of small pig farms in order to minimize the impacts of this activity on the environment.

*Acknowledgements:* The authors would like to thank the team from the Laboratory of Effluents at the Federal University of Santa Catarina, the Petrobrás Environmental Program, FAPESC, CNPq and CAPES/PNPD.







## **CONSTRUCTED WETLANDS**



# OLIVE OIL MILL WASTEWATER TREATMENT IN CONSTRUCTED WETLANDS COMBINED WITH ADVANCED OXIDATION PROCESSES

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## ABSTRACT

The disposal of the olive oil mill wastewater (OMW) is a main issue at the olive oil producing countries. OMW is characterized by very high organic load, dark color and ecotoxicity, while it is produced seasonally. The aim of this work was to examine the treatment of olive oil mill wastewater in a hybrid system, consisting of a vertical flow subsurface constructed wetland (CW) combined with advanced oxidation process (AOP). Three AOP's were studied, photocatalytic oxidation with titanium dioxide, photo-Fenton oxidation and electrochemical oxidation.

OMW were diluted with raw wastewater taken from the treatment plant of the Technical University of Crete campus. The constructed wetland pilot unit was a stainless steel square tank with an area of 0.64m<sup>2</sup> and depth 1.20m, consisting of two successive layers of certain granulometry. The surface layer was 30cm fine gravel (4-8 mm) and the drainage layer was 30cm coarse gravel (8-18 mm).

The CW was operated under intermittent flow (every 8 hours, three times daily), without aeration and at ambient temperature. Two different operating periods were carried out with variations in the organic loading rate. During Period I the organic loading rate (OLR) was 102 g COD/(m<sup>2</sup>d) and the COD concentration 1450 mg/L, while during Period II the OLR was 247 g COD/(m<sup>2</sup>d) and the COD concentration 3540 mg/L. Monitoring of the system took place by composite sampling every four days by mixing samples taken three times during sampling day. Chemical oxygen demand (COD), dissolved organic carbon (DOC), total suspended solids (TSS), total nitrogen (TN), phosphorus and color (TCU) were monitored systematically throughout the operating periods.

Subsequently, the effluent of the CW was treated in parallel by three lab-scale AOP's units. In order to succeed homogenous and comparable results, all the experiments were held with the same day CW effluent. Photocatalytic oxidation experiments were carried out at TiO<sub>2</sub> concentrations of 0,5 g/L, 1 g/L and 1,5 g/L and H<sub>2</sub>O<sub>2</sub> concentrations of 500 mg/L and 1000 mg/L for the 1,5 g/L TiO<sub>2</sub>. Photo-Fenton oxidation experiments were carried out at Fe<sup>2+</sup> concentrations of 5 mg/L and 20 mg/L and H<sub>2</sub>O<sub>2</sub> concentrations of 100mg/L, 300 mg/L and 500mg/L. Electrochemical oxidation experiments were carried out at current of 10A and 18A and Na<sub>2</sub>SO<sub>4</sub> concentration of 1% and 2% w/v.

During Period I the mean COD reduction at the CW pilot unit expressed as mass rate (mg/d) was 83%, while for Period II the equivalent reduction was 79%. The respective reduction in DOC was 59% and 67%, in TSS 89% and 85%, and in TN 76% and 61% respectively. The color removal was limited during the first period and zero at the second.

Photocatalytic oxidation experiments achieved 36% DOC reduction for Period I and 0% for Period II. The respective DOC reduction for photo-Fenton experiments was 64% and 23% for the two periods, while the equivalent removal rates for electrochemical oxidation were 58% and 27% respectively. The optimum COD reduction for electrochemical oxidation was 56% and 62% for the two periods, while the TCU reduction was 87% and 94% respectively.

Even though during the second period the organic load had been doubled as applied COD mass rate, the mean COD removal at the CW unit was stable or even increased in some cases. Photo-Fenton oxidation and electrochemical oxidation showed similar removal rates, while photocatalytic oxidation had clearly the lowest removal rates.

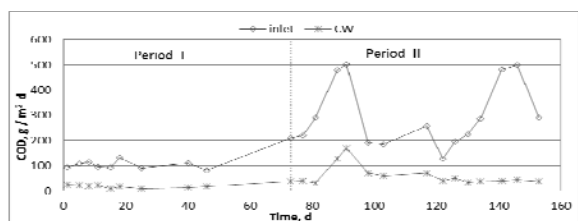


Figure 1: Performance of the CW as COD mass rate

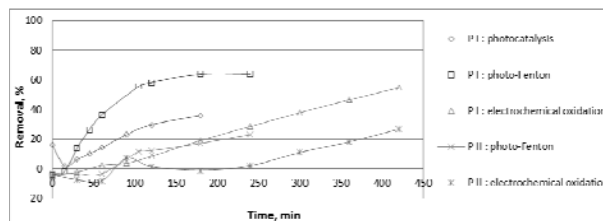


Figure 2: DOC removal rate of the AOP's units for the two periods



## REUSING CONSTRUCTED WETLAND EFFLUENTS FOR VEGETABLE CROP IRRIGATION

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### ABSTRACT

The study focus on wastewater reuse practice potential for irrigated agriculture. Tertiary treated effluents coming from a horizontal sub-surface flow constructed wetland system were reused for research purposes to irrigated vegetable crops. The effects of the combination of wastewater reuse with microirrigation techniques were faced during the trial. The water-soil-plant system was analysed in terms of microbial contamination and physical-chemical modification. The experiment was conducted in Eastern Sicily during 2013.

**METHODS:** The experimental site for wastewater reuse was equipped with a microirrigation system, consisting of surface and sub-surface laterals, supplying tertiary treated wastewater. Wastewater of the small community of S. Michele di Ganzaria (Eastern Sicily, Italy) were conveyed to a Constructed Wetland (CW) system working as tertiary treatment; the CW effluents, after a sand filter, were supplied to the experimental irrigation systems. Vegetable crops, planting in row, like lettuce, aubergines and zucchini were alternated during the trial, accordingly to their growth cycle; the contamination effects on the products, in terms of *E.coli*, was analysed at the light of both the Italian wastewater reuse legislation and WHO guidelines (2006). Crop production features were evaluated by comparing the vegetable crops irrigated by reclaimed water (RW) with the analogous irrigated by fresh water. The soil was analysed to detect any eventual microbial contamination, along the profile, and changes on soil hydraulic and physical properties due to the low quality water supplied. The irrigation system performance was evaluated in terms of emission uniformity (EU) and emitter flow reduction. Preliminary analyses were carried out on the microbial biofilm detection within the irrigation pipelines.

**RESULTS:** RW showed a mean *E.coli* contamination of  $2.4 \cdot 10^4$  CFU/100 ml, ranging between  $10^3$  CFU/100 ml and  $8.5 \cdot 10^4$  CFU/100 ml, during August-October 2013. Results on *E.coli* can be analysed with regard to both the Italian wastewater reuse standards (M.D. 185/03), indicating a threshold for 80% of samples of 50 CFU/100 ml and the WHO guidelines (2006), evidencing a potential risk for rotavirus detection when the concentrations of *E.coli* is higher than  $10^3$  CFU/100 ml. Analyses on soil sampled at different depths within the experimental field revealed a certain microbial contamination, with an average *E.coli* of  $1 \cdot 10^1$  CFU/g. Soil retention and permeability at saturation were not significantly altered during the experiment. Among the different crops irrigated by RW, lettuce revealed the worst symptoms due to the low quality water supplied; in particular, microbial contamination measured in terms of *E. coli* reached a maximum of  $8 \cdot 10^4$  CFU/g only on one sample. The overall production of the vegetable crops showed some significant differences among the treatments (i.e. RW and fresh water supplying). The microirrigation system evidenced a certain reduction of emission uniformity (EU) during the trial; values of EU decreased significantly in both surface and sub-surface systems, up to about 70%. This reduction was most likely caused by a microbial film growth within the pipelines, as suggested by recent literature. The presence of the microbial biofilm was confirmed in our study.

**CONCLUSIONS:** WW reuse for irrigation should be practiced in the agricultural context of the Mediterranean basin. This due to both the ever more limited conventional water resources for irrigation uses and the need to reduce the environmental impact of WW discharge into natural water bodies. The analysis of the potential impact of WW reuse on soil and plant contamination and the technological features of the irrigation systems should be supported by numerous research activities. The study herein presented was focused on these objectives. The overall results of the study, even if required further research materials.

**Acknowledgements:** The research was carried out within Project PON SIBAR financed by Italian Ministry of University and Research.





## BIOREMEDIATION OF BENZENE-CONTAMINATED GROUNDWATER WITH PILOT-SCALE CONSTRUCTED WETLANDS

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### ABSTRACT

Remediation of groundwater contaminated with benzene was tested in different pilot-scale horizontal subsurface-flow constructed wetland (CW) types (gravel CWs with different filter composition and a hydroponic plant root mat) at a field site. The performance evaluation comprised of (i) the determination of removal efficiency based on season-dependent concentration decline, (ii) investigations of the seasonal chemical's fate based on a mass balance approach taking into account the main removal pathways volatilization, plant uptake and microbial degradation, (iii) detailed investigations on the microbial community involved in benzene transformations, and (iv) hydraulic characterization of the CWs.

Overall, contaminant decline was greatly affected by the wetland type, and was season-dependent. The hydroponic plant root mat and the planted gravel CW efficiently improved the water quality as indicated by the achieved concentration decline during summer of  $99 \pm 1 \%$  and  $81 \pm 7 \%$ . The filter composition did not enhance treatment efficiency. Mass balancing revealed that the main benzene sink during summer was microbial degradation, with minor removal via plant uptake and volatilization. Assessment of microbial *in situ* activities highlighted several key players involved in benzene transformations. The hydraulic characterizations of the CWs evinced that good hydraulic flow behavior with low dispersion is not the only decisive factor for high contaminant removal, but high actual water-root contact, root density and a sufficient residence time are also important.

To conclude, both CW types represent a promising approach for the remediation of benzene-contaminated groundwater during spring/summer time.

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## USE OF CONSTRUCTED WETLAND PLANTED WITH HALOPHYTE FOR DOMESTIC WASTEWATER TREATMENT

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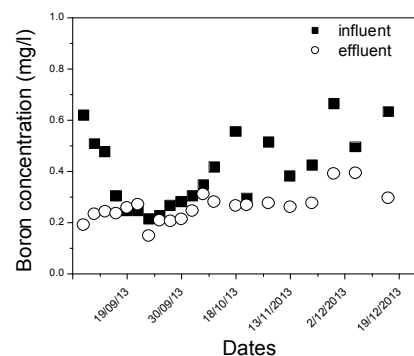
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### ABSTRACT

Constructed wetlands (CW) are low cost systems used worldwide to treat municipal wastewater. These systems are based on natural ecosystems and treatment processes involve complex interactions between soil, water, plants, microorganisms and prevailing flow patterns. There is growing interest into the potential of constructed wetlands planted with facultative or obligate halophytes for the remediation of domestic wastewaters. Since plant roots are the major source of oxygen in subsurface flow (SSF) wetlands, aside from atmospheric diffusion the role of higher plants is crucial in establishing a successful CW. The study took place at an experimental pilot plant located in Heraklion, Crete, South Greece (N 35°, 19"; E 25°, 10"). The plant was constructed in summer 2006. The experimental plant include among others a subsurface flow wetland vegetated with halophyte. The SSF wetland (with a total surface of 45.36 m<sup>2</sup>) was filled with two layers of different diameter gravel. The front and the effluent end of the bed contained coarse gravel (60-100mm) and included the drainage system. Over this layer the main gravel media (30mm) was positioned with a depth of 0.45 m. The constructed wetland was planted with four species of halophytes, *Tamarix parviflora* (5 plants), *Limoniastrum monopetalum* (8 plants), *Junkus sp* (8 plants) and *Sarcocornia perennis* (15 plants). The system operates receiving partially treated wastewater (sedimentation) from the City of Heraklion, Greece sewage treatment plant. A timer-controlled pump distributed the wastewater onto the surface of the wetland as described in Picture 3. The overall hydraulic loading is 1.4 m<sup>3</sup>/d. Water samples were taken as grab samples from the influent and effluent of constructed wetland and analyzed for chemical oxygen demand (COD), total nitrogen, total phosphorus, nitrate, ammonium and boron using standard test kits (Hach-Lange GmbH) and DR 2800 spectrophotometer (Hach-Lange GmbH). In addition, pH was measured with a pH-meter (WTW, 3110) and electrical conductivity (EC) with a conductimeter (Hanna, 8333) in accordance with Standard Methods (APHA, 1995). Unfiltered 5-day biochemical oxygen demand (BOD<sub>5</sub>) was analysed using WTW OxiTop meters.



Low removal efficiencies were observed for almost all examined parameters. COD mean concentration in the effluent was 101.3 mg/l while BOD value was 46.4 mg/l. Average removal between 10-25% was observed for nitrogen and phosphorus. On the other hand boron concentration (Figure 1) was decreased from 0.40 mg/l in the influent to 0.26 mg/l in the effluent.

**Acknowledgements:** This research has been financed by the European Union (Work Program 2012 "COOPERATION": project acronym: "Water4crops")



## BIOREMEDIATION OF GROUNDWATER CONTAMINATED WITH ORGANIC MICRO-POLLUTANTS USING HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS

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### ABSTRACT

Constructed Wetlands are considered an effective remediation technology for groundwater contaminated with organic micro-pollutants like benzene and MTBE. Aerobic biodegradation appears to be the dominant removal mechanism. However, their efficiency in the removal of phenolic compounds is still not well investigated and respective removal processes not fully understood. To this, possible interactions due to simultaneous presence with other contaminants have not been examined. This project aims at clarifying these knowledge gaps.

Three pilot-scale Horizontal Subsurface Flow CWs (HSFCWs) were used in the experimental facility in Leuna, Germany. Each bed is a steel basin (L:W:D = 5.9:1.1:1.2 m); two beds were plants with *Phragmites australis* and one was kept unplanted. All beds were fed with contaminated groundwater pumped from the local aquifer, containing benzene and MTBE (inflow 11 L/h, HRT 10 days). A solution of phenol (15 mg/L) and *m*-cresol (2 mg/L) was injected to the groundwater influent loaded to two of the beds (planted and the control). The experimental period covered the full growth season from April to October 2013. Samples were taken from influent and effluent points every two weeks, as also from three points along each wetland length (0.5, 1.9 and 4.1 m) and depth (0-5, 30, 80 cm) for spatial distribution observation. All samples were analyzed for concentrations determination of the various compounds (phenol, *m*-cresol, MTBE, benzene, etc). Microbial community patterns were observed using flow cytometry technique and DNA analyses.

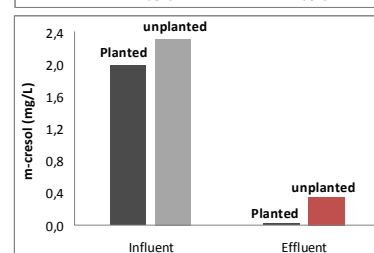
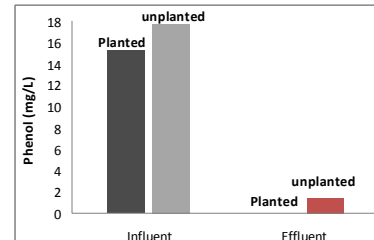
Overall results showed that planted HSFCWs are capable of completely removing phenolic compounds (as figure shows). Removal in the unplanted bed was incomplete. Spatial analysis revealed a gradual removal of the compounds, while the major portion is removed at the first part of bed length. Higher concentrations are detected at the bottom of the beds, especially in the unplanted bed, indicating the absence of oxygen in deeper parts and the positive role of plants.

A shift in microbial community patterns was detected during the first two months after the injections with phenols started. This implies a first response of the microbial community towards its adaptation to the compounds added. This initial stress was thereafter limited and the system recovered with time, obtaining similar characteristics with the planted bed without receiving the phenolic compounds. DNA analyses revealed that different communities are present in the CW beds compared to the groundwater inflow. MTBE and benzene removal rates were not affected by the addition of the two phenolic compounds in the influent groundwater and were adequately removed, while higher rates observed in the planted beds (53% for MTBE and 82% for benzene).

Results from this study show that the tested HSFCWs are an appropriate technology for the removal of phenols and organic hydrocarbons from contaminated groundwater. Biodegradation appears to be the dominant removal mechanism and the presence of plants acts beneficially for the system performance. Vertical concentration profiles show that in deeper parts the lack of oxygen limits but does not stop the contaminant removal, especially in the unplanted bed. Oxygen input via the CW surface affects the aerobic removal rate but also anaerobic removal takes place.

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**Acknowledgements:** The financial support by BP International is greatly appreciated. Further funding was provided by the Helmholtz Centre for Environmental Research in the scope of the SAFIRA II Research Programme: Revitalization of Contaminated Land and Groundwater at Megsites, project "Compartment Transfer."



## NUTRIENT REMOVAL FROM EUTROPHIC WATERS USING FLOATING TREATMENT WETLANDS

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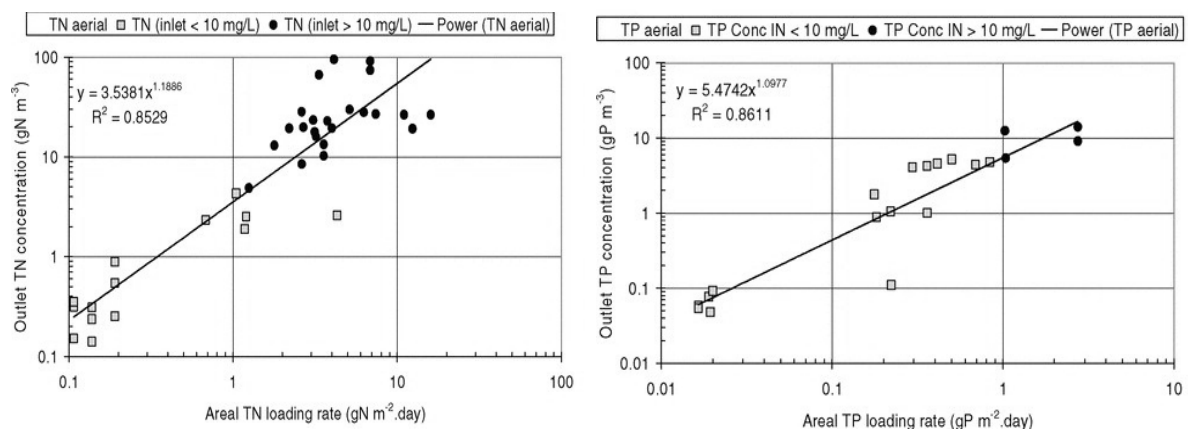
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### ABSTRACT

Floating treatment wetlands (FTWs) are an innovative new constructed wetland format that expands the range of situations where wetland treatment can be applied for water quality enhancement. Floating treatment wetlands (FTWs) involve emergent wetland plants growing on tethered buoyant mats or rafts on the water surface. The plant roots grow through the floating mat and into the water below. Although the key nutrient removal processes operating are similar to those operating in surface-flow wetlands, FTWs have a number of unique attributes which can provide advantages over conventional systems in some situations. In particular, tolerance of deep and fluctuating water levels enables FTWs to be retrofitted into ponds, lakes, slow-flowing and tidally-influenced waters. The plants growing in FTWs assimilate nutrients directly from the water column, in contrast to bottom-rooted emergent macrophytes which take them predominantly from the bottom sediments. The roots also provide a large surface area for adsorption and biofilm attachment within the water column, encourage aggregation and settling of fine particulates and promote quiescent anoxic zones beneath the floating mats. Until recently there has been little reliable quantitative information available on their nutrient removal performance. This paper briefly introduces the key elements of this novel new ecotechnology and reports nutrient removal rates from recent trials in static and flowing waters. Areal TN removal rates of 500–800 mg/m<sup>2</sup>/d were recorded in static batch trials, whilst 160–240 mg/m<sup>2</sup>/d were recorded in flow-through field trials. Areal TP removal rates of 40–60 mg/m<sup>2</sup>/d were recorded in static batch trials and 2-5 mg/m<sup>2</sup>/d in flow-through field trials. Comparison is made with published results from other studies around the world to discern performance trends (see Figures below) and identify potential niches for this treatment wetland type.



**Acknowledgements:** The financial support for this work from the Ministry of Research, Business and Employment through the AgResearch-led *Clean Water: Productive Land Programme*, Auckland Council and Bay of Plenty Regional Council is greatly appreciated.



## FLOATING WETLANDS FOR WATER TREATMENT IN A EUTROPHIZED URBAN LAKE LOCATED IN XALAPA CITY, MEXICO

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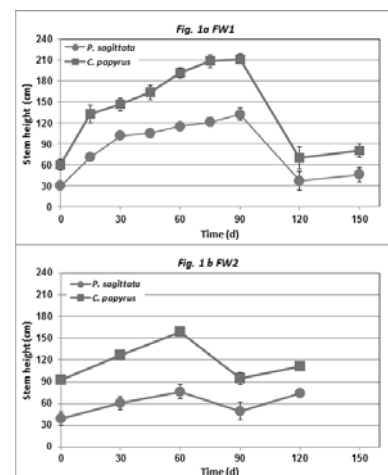
### ABSTRACT

Floating Wetlands (FW) represent a novel water treatment in which emergent plants grow forming a mat floating on the water surface. The extensive surface area of the roots is a key factor for the attached biofilm growth and the entrapment of small suspended particles. FWs have been proposed for the water treatment of lakes, lagoons, rivers and stormwaters due to their ability to tolerate wide fluctuations in water depth [1,2]. "Los Lagos" (three artificial lakes) of Xalapa City in Mexico represents a recreational place for the community; however, they are polluted due to non-punctual urban discharges, resulting in the presence of phytoplankton mats.

The objective of this work was to assess the use of floating wetlands for the water treatment in a eutrophized lake located in Xalapa City, México (sub-tropical weather). Two linear FWs of 17.5 (17.5m x 1m) (FW1) and 33 m<sup>2</sup> (33m x1m) (FW2) were built with a combination of *Pontederia sagittata* and *Cyperus papyrus* and using light plastic structures to enhance the buoyancy of the system. FW1 and FW2 were placed in the most eutrophized zone of Lake #1 in August (summer) and November (autumn) of 2013, respectively. Growth monitoring was carried out every 15 days and 30 days for FW1 and FW2, respectively, determining the stems height. Biomass was harvested after 90 days in FW1 and after 60 days in FW2 to determine productivity. Water analysis was carried out at different points around the lake and FWs determining the following parameters: BOD, COD, TKN, NH<sub>4</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P, SO<sub>4</sub>, pH and TDS. The presence of phytoplankton was assessed through chlorophyll and microcystin contents.

The results showed that the values of BOD, COD, NH<sub>4</sub>-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P, Chl-A during January and May of 2013 were very high compared to the maximum values recommended by USEPA for the aquatic life protection indicating eutrophication. Microcystin level resulted in the range recommended by WHO for water human consumption.

After 3 months (November), the quality of water treated by FW1 improved mainly in terms of COD (71.72%) and NH<sub>4</sub>-N (27.27%), while the maximum NO<sub>3</sub>-N removal was found during winter (January) (61.42%). TDS was removed in an average of 39.75±5.27% during autumn (October and November) and winter (January). In the case of the water treated by FW2, the content of COD was reduced 52.22% during autumn while in winter it was reduced in a range from 5.29 to 24.76%. BOD, NO<sub>3</sub>-N and NH<sub>4</sub>-N contents in the water lake were below the maximum level recommended by USEPA before and after FW2 during November and December. *P. sagittata* and *C. papyrus* adapted well to the water lake; both grew along 90 and 60 days in FW1 and FW2, respectively (Fig. 1a and 1b). The productivity obtained for the mix of both plants was 4.14 and 2.33 Kg<sub>dw</sub>/m<sup>2</sup>-month for FW1 and FW2, respectively. A smaller growth was observed in *C. papyrus* in FW2, compared to that found in FW1 at day 60, most probably due to this plant was more sensitive to the cold climatic conditions prevailing in winter (December and January).



It was concluded that the use of FWs using a combination of *P. sagittata* and *C. papyrus* is a novel and suitable system for the improvement of the water quality of a eutrophized urban lake. Work is in progress to evaluate the effect of the FW design on water quality.

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**Acknowledgements:** The financial support by the local Government of the Xalapa City is greatly appreciated.



## HIGH CAPABILITY OF *PTERIS VITTATA* IN ARSENIC REMOVAL FROM CONTAMINATED WATER USING NOVEL HYDROPONICS CULTIVATION SYSTEM

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### ABSTRACT

*Pteris vittata*, the first identified arsenic-hyperaccumulator land plant, can accumulate more than 22,000 mg/kg arsenic in fronds also it can produce 1-2 kg/m<sup>2</sup> biomass [1]. Utilization of *P. vittata* for the remediation of arsenic contaminated soil is effective and has been widely applied. However, treatment of the arsenic water pollution using this arsenic hyper-accumulator plant has not been fully investigated. Most of research for arsenic phytoextraction by using *P. vittata* still treated it as the land plant. In our research, we tried to cultivate *P. vittata* as a water plant, and develop the novel cultivation system for *P. vittata* hydroponics to get the optimum performance of arsenic phytofiltration.

To confirm the best water level condition for *P. vittata* for hydroponic cultivation, we set up 3 different hydroponic cultivations of *P. vittata*. ①: fronds, rhizomes and parts of roots were above the water, ②: fronds and rhizomes above the water, ③: only fronds above the water. No aeration was applied in all 3 cultivation experiments, and 2ml of 0.2 X HG solution was add directly to rhizomes every 2 days. After one month, ferns grew very well in ① and ② conditions, the fern did not grow and died in ③ condition. These results indicate that keeping rhizomes above the water surface level was needed, aeration was not necessary and also low nutritional condition was required for *P. vittata* in hydroponic cultivation.

For arsenic phytofiltration experiment, two types of *P. vittata* were used. After 2 months growth in soil, the ferns were transplanted to the hydroponics (Fern L), and the fern seedlings were directly used for the hydroponics (Fern S). During 4 months hydroponics cultivation of the both type ferns, no aeration and very low nutrition were applied to the hydroponic cultivation. The roots elongated to 40-50 cm and the biomasses of roots were 5-10 times more than the fronds. After the roots elongation, the arsenic phytofiltration experiments were started using tree serial vessels continuous recirculation hydroponics systems. The recirculation flow rate was controlled 48 Lpd.

At first 50 µg/L arsenate (AsV) was added to water, after the AsV concentration reached below measurement limitation, the As concentration conditions were shifted to 500 µg/L AsV and then 1000 µg/L AsV water. The ferns (Fern L & Fern S) were living during this whole experiment. Water samples were analysed by ICP-MS. Arsenic concentration in water reduced to below 10 µg/L (WHO As water standard) within 1 day (50 µg/L AsV), 6 days (500 µg/L AsV), 10 days (1000 µg/L AsV). The arsenic concentrations in water were still kept at under the measurement limitation. These results demonstrated that the ferns have not only high-efficiency of arsenic removal but also hyperaffinity of arsenic uptake from water.

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**Acknowledgements:** The financial support by Grant-in-Aids for Scientific Research from the Japan Society for the Promotion of Science (no. 24404018), the Project for Promotion of Strategic Establishment of Research Foundation in Private Universities from the Ministry of Education, Culture, Sports, Science and Technology-Japan (MEXT), a and the Project from the Mitsui & Co., Ltd. Environment Fund.



# MICROALGAE BIOTECHNOLOGY FOR WASTEWATER TREATMENT. OPPORTUNITIES AND CHALLENGES

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## ABSTRACT

There are a number of wastewater nutrient removal processes available. Some systems are designed to remove only nitrogen (i.e. Bardenpho) or phosphorous (i.e. AO), while others remove both (i.e. A2O). Although these technologies have well a proven efficiency removing nutrients from wastewater, they consume significant amounts of energy and chemicals and therefore are costly.

More than 50 years ago, several authors [5] suggested the use of microalgae for wastewater treatment; because of they are highly effective in reducing simultaneously nitrogen and phosphorous concentration in wastewater as well as raising the oxygen concentration in wastewater. Nevertheless the use of microalgae biotechnology in wastewater treatment is still not a technological alternative to conventional processes, Why?. In this work, advantages and disadvantages of microalgae biotechnology compared with conventional chemical and biological nutrient removal processes are analysed. Finally, some of the main gaps to be solved for the viability of microalgae biotechnology in wastewater treatment are outlined.

### *Results and Conclusions:*

The main disadvantages of conventional nutrient removal technologies could be resumed as follows

The complexity of the processes, particularly biological processes: Conventional Technology: for nitrogen and phosphorous removal, many key factors influence nutrient removal efficiency; an adequate supply of carbon, sufficient alkalinity, sludge age and maintenance of a deep sludge blanket in the secondary clarifier, an adequate supply of VFAs etc. Microalgae photobioreactors are less complex processes as they can remove simultaneously nitrogen and phosphorous by means of a single type of reactor [1].

The high consumables costs associated with chemicals, especially in the case of conventional physicochemical processes. In the case of microalgae the chemicals used include inorganic carbon supply (CO<sub>2</sub> which source could be flue gas), and flocculants-coagulants needed to harvest algal biomass [6].

Sludge production. Conventional biological nutrient removal treatments, as well as microalgae biotechnology, produces sludges to be handled. In the case of microalgae the range of possibilities for biomass reuse seems to be much broader [4].

WWTP retrofitting. In the case of conventional biological nutrient removal processes, WWTP retrofit implies deep changes in the layout and design of the existing treatment processes. In the case of microalgae photobioreactors as well as some conventional chemical processes, they are end-of pipe technology that could be implemented after secondary treatment with minimal modifications in the original WWTP flowchart [2].

Greenhouse Gases. As primary producers, the retrofit of a WWTP with microalgae biotechnology could reduce significantly the ecological footprint of the whole process [3].

Nevertheless, despite these microalgae biotechnology advantages, there are two aspects to be optimized for this technology to be considered competitive: increase the photosynthetic efficiency of the photobioreactors in order to reduce the surface requirements and improve the harvesting technology towards a less energy consuming process.

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## SEASONAL DYNAMICS OF Cd AND Cr IN ABOVEGROUND BIOMASS OF *PHALARIS ARUNDINACEA* IN A CONSTRUCTED WETLAND

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### ABSTRACT

Constructed wetlands are very complex systems, which are used for wastewater treatment, especially for domestic and municipal wastewater. Currently, constructed wetlands are monitored with focus on the removal of organics, suspended solids, nutrients or microorganisms, whereas sufficient information about the heavy metals removal is quite limited. Only few studies on the accumulation of heavy metals in plants of constructed wetlands have been completed. Information about the seasonal dynamics of heavy metals in the vegetation in constructed wetlands is entirely missing.

The aim of this work was to evaluate the seasonal dynamics of Cd and Cr in the biomass of *Phalaris arundinacea* in a constructed wetland in South Bohemia. The plant biomass from the constructed wetland designed for 400 inhabitants was harvested in one or two-month intervals during the period May 2011 - March 2012. Biomass was separated into stems, leaves and flowers and dried at 60 °C into constant weight. In samples of biomass and also wastewater Cd and Cr were determined using ICP-OES. Total amounts of both metals in biomass per square meter (standing stock) in each month were evaluated (Fig. 1).

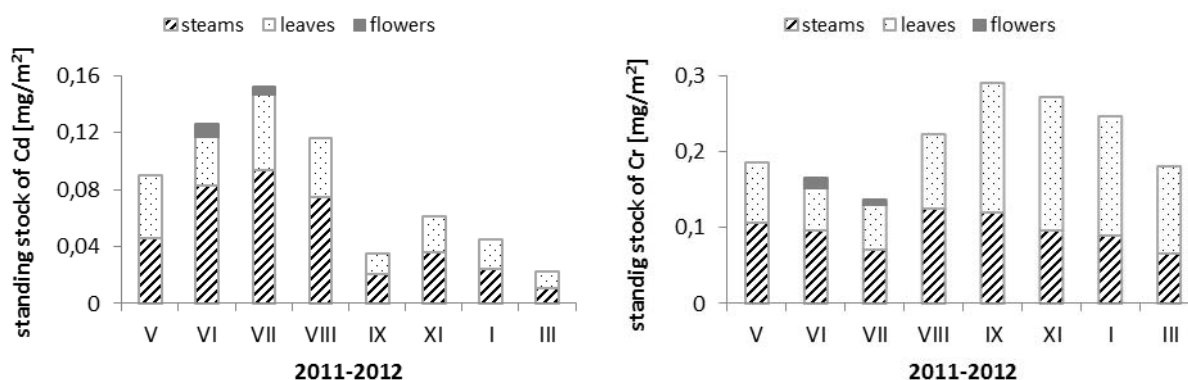


Fig. 1: Average standing stocks for Cd and Cr in the aboveground biomass of *Phalaris arundinacea* depending on the period of the year.

The results should revealed the maximum accumulation of Cr and Cd in the biomass and consequently the optimal time for vegetation harvesting in order to achieve maximum removal when heavy metals are the treatment target. The obtained results show that elements are probably transported to aboveground biomass of *Phalaris* during different periods of the year. Whereas maximum amount of Cd in the biomass was determined at the top of growing season, the highest amount of Cr was captured at the end of the growing season. In addition concentrations of Cr were highest at the end of growing season in leaves. It seems that *Phalaris* may have special mechanisms for detoxification typical for each metal. This knowledge of the seasonal dynamics of heavy metals in the aboveground biomass may contribute to more effective removal of heavy metals from wastewater.

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## SELECTION OF SUITABLE PLANT SPECIES IN SEMI ARID CLIMATIC CONDITIONS FOR QUALITY IMPROVEMENT OF SECONDARY TREATED EFFLUENT BY USING VERTICAL CONSTRUCTED WETLAND

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### ABSTRACT

Constructed wetlands are becoming popular as decentralized wastewater treatment for smaller communities, where land is easily available and frequent power tripping prohibits the advocacy of highly mechanized plants. Constructed wetlands have been proved for their treatment performance for physico-chemical and microbiological contaminants removal. The wetland plant species play the most critical role in determining the performances of the wetland systems. The wetland plants govern multiple roles in constructed wetlands during biological treatment of wastewater. They acts as biological pumps controlling the oxygen transfer, provide shelter to micro fauna (microbes) governing the pollutants biodegradation, uptake of recalcitrant contaminates etc. Thus selection of suitable plant species for vegetation in treatment wetland units is of great importance to enhance the efficiency of system. The present research aims to identify the suitable plant species for constructed wetlands in the dry/warm climates of Rajasthan (India). The performance of two widely used Indian wetland plants, *Phragmitis australis* and *Canna indica* were evaluated in vertical up-flow constructed wetland using secondary treated effluents. Performance efficiency of both the plants species was evaluated for physico-chemical and microbial contaminants removal. The study highlighted the comparative as well as significant suitability of *Canna indica* plantation over the *Phragmitis australis* under semi arid climatic conditions.

The unit planted with *Canna indica* showed 37.33% and 54.08% removal for total kjeldhal nitrogen (TKN) and ammonia-nitrogen respectively. Nitrate nitrogen in the treated effluents has a significant increment of 3.8 times higher than influents. Importantly, the indicator organism coliform reduction was observed as 1.87 log (MPN/100ml) in the effluent of *Canna indica* planted unit as against 1.01 log (MPN/100ml) in the effluent of *Phragmitis australis* planted wetland.



## PERFORMANCE OF YOUNG CONSTRUCTED WETLAND SYSTEM AT DIFFERENT SUBSTRATE CONFIGURATIONS

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### ABSTRACT

Engineered constructed wetlands (ECWs) are designed with greater control to improve the water quality. Numerous research has already been done across the globe to understand the processes occurring in the ECWs. Yet, ECWs pose challenges to the researchers in quantifying the processes. The degradation of BOD, COD and removal of Nitrate, Phosphate and TSS from the domestic wastewater has been studied with respect to the time at two different substrate configurations. One with a mix of coarse sand (<2 mm) and marble chips (10-15 mm) and another with a mix of marble chips and gravel (50-100 mm). Each treatment is tested with and without the plant *Typha latifolia*. The experiments were carried out in batches, in specially designed columns having filled with the designated substrate media. The results indicate that, the effect of substrate is significant ( $p < 0.01$ ) than the presence of vegetation in the removal of Phosphate and TSS. Whereas, for the removal of BOD and COD; the contribution of both substrate and vegetation is found to be significant ( $p < 0.05$ ). Further, the treatment having coarse sand, marble chips, and vegetation is found to have higher degradation rate-constants for the pollutants, and the removal percentages of BOD, COD, Phosphate and TSS are measured to be 91%, 98 %, 96.7 % and 96.2 % respectively, after 3 days of retention time.

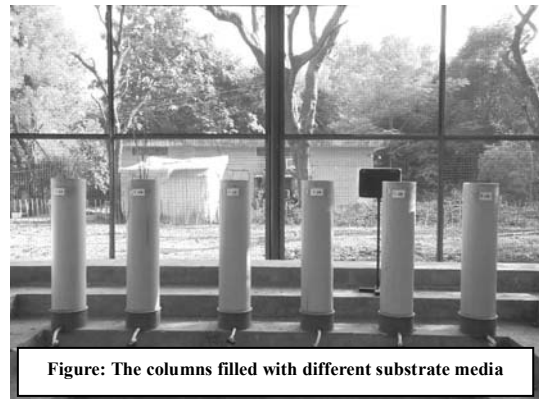


Figure: The columns filled with different substrate media

**Keywords:** engineered constructed wetlands, substrate, *Typha latifolia*, rate kinetics, domestic wastewater

### Abbreviations:

BOD : Biological Oxygen Demand

COD : Chemical Oxygen Demand

ECWs : Engineered Constructed Wetland system

TSS : Total Suspended Solids



## HYDRAULICS OF HORIZONTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS

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## ABSTRACT

The aims of this study was to analyze the hydraulic aspects of horizontal subsurface flow (H-SSF) constructed wetlands (CWs), and, in particular, to investigate the clogging phenomena. Experiments were carried out in three full-scale CW beds, functioning in parallel with different operational life, that receive secondary effluent from a conventional wastewater treatment plant (WWTP) in Eastern Sicily. One CW has been in operation since 2006, while other two have been in operation since summer 2013. Two beds have an almost equal surface area (about 2,000 m<sup>2</sup> each bed), treat an equal wastewater flow (about 2 L s<sup>-1</sup> per bed) and were planted with *Phragmites australis*. *Typha* was used in the smallest one, that has a surface area of about 1,000 m<sup>2</sup>.

Clogging indicators, such as accumulated solids, hydraulic conductivity and drainable porosity were determined and compared among CW systems. Synthetic informations on hydraulic properties of the CWs both in terms of the efficient use of its volume and of mixing patterns were extracted from the wastewater residence time distributions (RTDs). These were obtained by field tracer tests, as a breakthrough curve of a non-reactive tracer (NaCl) instantaneously injected into the entrance of H-SSF CWs and observed in the outflow from the treatment systems (Figure 1). Tracer analyses were also conducted inside the wetlands to determine flow patterns. Preliminary correlations were highlighted among the solids accumulated and both saturated hydraulic conductivity (Ks) and drainable porosity (DP) reduction over time. The saturated hydraulic conductivity of H-SSF CWs was estimated by using three different in situ methods: Darcy's Law, Drainage Equation and Falling Head (FH) method. Darcy's Law and Drainage Equation were applied during the same drainage experiments; the mean hydraulic conductivity value of each bed, obtained by the drainage equation was not significantly different from the value obtained with the Darcy's Law method (4,000-6,500 m day<sup>-1</sup> in H-SSF3 bed). To apply the FH method, in each constructed wetland, hydraulic conductivity was measured in several points along three longitudinal transects of the wetland. During the FH tests negative exponential curves were produced, which represent a decrease in the water height inside sampling points over time; the slope of each curve is linked to hydraulic conductivity (Figure 2). The mean hydraulic conductivity of H-SSF2 gravel is lower because the constructed wetland is in operation by 6 years earlier than the other two beds.

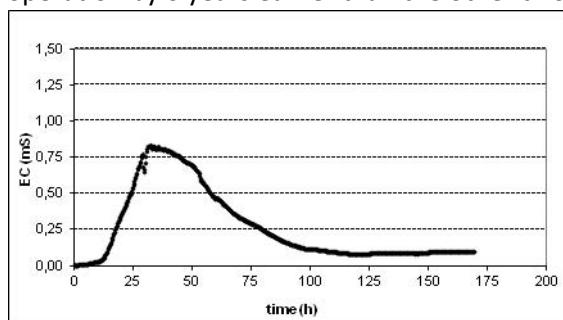


Figure 1. Effluent tracer measurements (Electrical Conductivity, EC) over time in tracer test

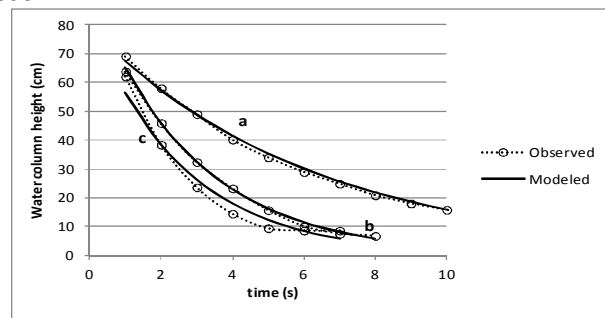


Figure 2. Decrease in water height column over time in three sampling points at H-SSF2 (a), H-SSF3 (b) and H-SSF4 (c).

Evapotranspiration (ET) rates and removal efficiencies of chemical and physical contaminants were also assessed in the H-SSF CWs.

**Acknowledgements:** The financial support by FP-7 project Water4Crops contract No. 311933 is greatly appreciated.



## INNOVATION IN CONSTRUCTED WETLAND DESIGN - NOVEL SYSTEM OF FLOATING PLANT MATS

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### ABSTRACT

Artificial floating treatment wetlands are an innovative variant of the more traditional constructed wetland and pond technologies. For intentional designs floating matrices are introduced to enable non-floating plants moving freely at the water surface. In this way, certain characteristics of plants in environmental services could be combined for waste water quality improvement [1].

The task embedded in the project “Water4Crops” is designated for developing innovative biotechnologies for the treatment of municipal waste water to be reused in agriculture, e.g. for irrigation. The solutions will be particularly suited for decentralized application at small communities. In context to plant-based systems one of the project objectives is aimed at testing the efficiency in pathogen reduction of novel floating plant mats.

In preliminary tests different plant species will be investigated for their potential to reduce coliform bacteria, e.g. acute sedge (*Carex acuta*), hare’s-tail cottongrass (*Eriophorum vaginatum*), yellow flag (*Iris pseudacorus*), soft rush (*Juncus effuses*), common reed (*Phragmites australis*). These plants are promising to have an effect in reduction of coliforms based on root exudates, oxygen enrichment and formation of root associated biofilms [2,3,4]. In greenhouse experiments slightly moving water bodies based on hydroponic systems will be simulated to show an effect according to the planting density (Figure 1). The contact time of the plants with pre-treated municipal waste water will be varied according to the plants behaviour as some of them are known to have longer adjustment period to have an antibacterial effect. Mixture of best performing plants will be tested in the same way.

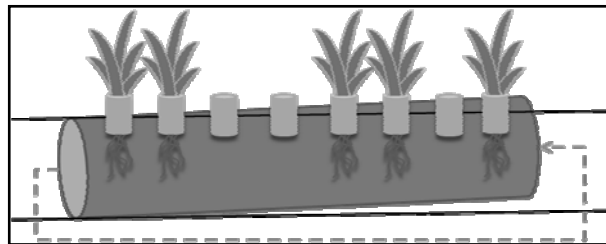


Figure 1: Scheme of experimental setup

An important fact while developing an intentional floating plant mat is the application of suitable floating matrices. There are several variants present on the market all with more or less advantages and disadvantages. A novel system might be established.

During the 11<sup>th</sup> International Conference in Phytotechnologies we will present the results and conclusion of the experiments done during summer 2014. The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2012-2016) under the grant agreement number 311933.

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## PHYTO PROCESS PREDICTABILITY TREATS FOOD PROCESSING WASTEWATER YEAR-ROUND

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### ABSTRACT

A phyto waste water reactor is designed to be a rhizosphere with a predictable volume, pollutant dose, reaction rate and dwell time. Regulatory agencies understand this approach when approving conventional lagoons or in-tank treatment systems for industry and towns.

Port of Morrow (POM) at Boardman OR, USA is in the fourth year of a waste water phyto treatment research program. POM discharges 21 million liters (5.8 million gallons) waste water per day from food processors averaging 114 mg nitrogen per liter. Current effluent treatment during the growing season requires 5,000 acres of irrigated food & feed crop. Controllable variables include waste water dose, pretreatment, crop variety, irrigation equipment, and cropping techniques. Uncontrollable variables include native soils, past farming practices and weather.

Effluent irrigation water chemistry is analyzed and tabulated. Ground water recharge is estimated using a water balance analysis, with inputs from both soil moisture sampling and lysimeters. Soil moisture content is monitored by neutron probe and real-time sensors

Winter waste water potentially can be irrigated onto deep-rooted poplar. Demonstration has started using 23 hectares (60 acres) of poplar trees with an alfalfa understory irrigated with effluent. Phyto nitrogen treatment will be managed using Irrigation Management ~ Online (IMO), an irrigation management program that explicitly models the spatial distribution of soil water movement and percolation in irrigated fields. IMO has potential for operating daily effluent irrigation at full-scale. At full-scale, 500 acres of deep-rooted poplar will be irrigated during the dormant season. The University of Iowa is determining nitrogen removal mechanisms from a poplar rhizosphere during tree dormancy. The field and laboratory data combine to better quantify nitrogen transport and cycling dynamics as POM irrigates waste water.

*Keywords:* food processing, waste water, irrigation, rhizosphere, poplar



## SCREENING OF SIX *SALIX* SPP. (WILLOW) GENOTYPES FOR THE BIOREMEDIATION OF MUNICIPAL EFFLUENT

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### ABSTRACT

There is a growing interest in using Short Rotation Coppice (SRC) willows as a means of treating municipal wastewater. Willows have many advantages over other plants included: longer growing season; reducing soil losses; large root system; wide genetic diversity. SRC willow may represent an ideal solution to clean-up polluted water and reduce emission of greenhouse gases. Current studies suggest that the recommended rate of nitrogen (N) application to willow should not exceed 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This is to minimize potential leaching of N to groundwater.

The aim of this study was to evaluate the response of six *Salix* spp. genotypes when irrigated with high levels of wastewater. Plants were treated using four concentrations (0, 300, 450, 600 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Plant height, above ground biomass and root weight were used to assess the effect of prolonged use of wastewater on growth, and chlorophyll content was used as an indication of stress levels in plants. Wastewater irrigation at all levels resulted in increased height. All the genotypes showed the maximum height when irrigated with the highest dosage, with the exception of "Anki 832502" which was tallest when treated with 300 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Plants used as control (0 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were significantly ( $p < 0.01$ ) smaller than those treated. Above ground weight was significantly ( $p < 0.01$ ) greater in plants receiving wastewater (300, 450, 600 kg N ha<sup>-1</sup> yr<sup>-1</sup>) than control, although there was no significant difference between treatments. Root weight showed a different trend. The highest biomass was obtained when genotypes were treated with 300 and 450 kg N ha<sup>-1</sup> yr<sup>-1</sup> but produced significantly ( $p < 0.01$ ) less biomass at the highest dosage. Chlorophyll content increased significantly in plants both used as control and treated with 300 kg N ha<sup>-1</sup> yr<sup>-1</sup> while showed a diminution in chlorophyll content at the highest wastewater applications ( $p < 0.01$ ).

Wastewater irrigation resulted in increased height, and biomass production (above and below ground) suggesting that these genotypes studied had a beneficial effect from it. However, most of genotypes treated with the highest wastewater dosages (450, 600 kg N ha<sup>-1</sup> yr<sup>-1</sup>) showed severe toxic effects by the end of the treatment period, which is consistent with data obtained from root weight and chlorophyll content. At the highest dosages (450-600 kg N ha<sup>-1</sup> yr<sup>-1</sup> for chlorophyll content and 600 kg N ha<sup>-1</sup> yr<sup>-1</sup> for root weight), willows showed a significant reduction of these two values. Wastewater application estimated at 300 kg N ha<sup>-1</sup> yr<sup>-1</sup> did not have negative effect on plants health and consequently may not pose a risk for the environment, although further studies are required to confirm this study.

*Funding support:* This ANSWER (Agricultural Need for Sustainable Willow Effluent Recycling) project is part-financed by the European Union's European Regional Development Fund (ERDF) through the INTERREG IVA Cross-border Programme, managed by the Special EU Programmes Body (SEUPB).



## ECO-ENGINEERING PROCESS FOR THE TREATMENT OF CONTAMINANTS FROM SALTED HIGHWAY RUNOFF: ADAPTED WETLAND AND ACTIVE FILTER

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### ABSTRACT

Highway and road de-icing operations provide safe driving conditions during winter in Canada, north of US and Europe. However, the runoff from salted roads adversely affects aquatic and terrestrial ecosystems by increasing salts and other contaminants (heavy metals and hydrocarbons) concentrations. Salt may accelerate eutrophication in water bodies and may reduce biodiversity of flora and fauna or encourage dominance of salt-tolerant species. Therefore, salted road runoff should be treated before discharge into water bodies in order to protect natural resources and to benefit from ecosystem services. A series of laboratory and greenhouse assays were conducted as means to develop two treatment methods. Based on these results a pilot treatment station was constructed near Quebec City on a heavily urbanized watershed. This compact, innovative, economically viable and ecologically sustainable treatment system includes 2 unit eco-processes: an adapted constructed wetland (ACW) that uses salt-tolerant and halophytes plants, and an active filter bed (AFB) that uses calcite as reactive filter media. This paper presents the research findings that resulted in the design and construction of this innovative system.

**Table 1: Contaminants found in used snow and highway trenches - 1: Acceptable quality criteria for fresh water**

Contaminants	Concentrations (mg/l)	Thresholds for surface water (mg/l) <sup>(1)</sup>
Suspended matter	1209	< 30
Oil and Grease	105	5
Sodium (Na <sup>+</sup> )	350	200
Calcium (Ca <sup>2+</sup> )	-	-
Chloride (Cl <sup>-</sup> )	3851	250
Lead (Pb <sup>2+</sup> )	85	0,01
Cadmium (Cd <sup>2+</sup> )	-	0,005
Zinc (Zn <sup>2+</sup> )	-	7,4
Iron (Fe <sup>3+</sup> )	913	0,3
Phosphorous (PO <sub>4</sub> <sup>2-</sup> )	-	0,02-0,03

This study used one salt-tolerant and three halophytic plants natives of the south shore of the St. Lawrence River estuary (natural saline wetlands of Kamouraska, Quebec): *Atriplex patula*, *Salicornia europaea* and *Spergularia Canadensis*. Some results are presented in figure 1 below.

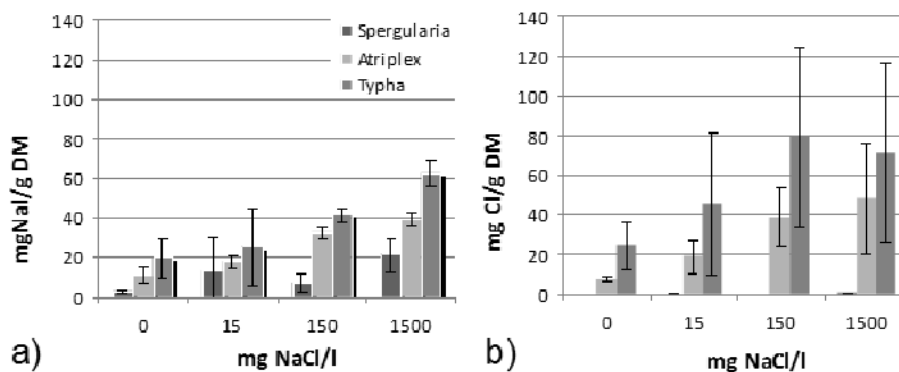


Figure 1. Bioaccumulation of Na and Cl by halophyte and salt resistant plants



## COMPARISON OF ENERGY AND WATER BALANCE METHODS TO ESTIMATE EVAPOTRANSPIRATION IN A CONSTRUCTED WETLAND

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### ABSTRACT

**Introduction:** The analysis of soil-plant-atmosphere (SPA) interactions within a constructed wetland may allow the comprehension of complex mechanisms acting within the system and indispensable for reliable design procedures and hydraulic modelling of pollutant removal. In this study, the fluxes of energy (solar radiation) exchanged within the complex SPA environment of a full-scale Horizontal Subsurface Flow Constructed Wetland (H-SSF CW) were monitored through the energy balance approach; the results of the method in terms of evapotranspiration (ET) fluxes were compared with the ET estimated by the water balance of the CW working as a lysimeter.

**Methods:** The CW, located in San Michele di Ganzaria (Eastern Sicily), has been operating since December 2012 and receives part of secondary effluent (about 2 L/s) of the conventional wastewater treatment plant (Imhoff tank, trickling filter and a secondary sedimentation tank) of the village. The wetland, with a surface of about 2,000 m<sup>2</sup>, was planted in autumn 2012 with *Phragmites australis* (Cav.) Trin. at density of four rhizomes m<sup>-2</sup> and it was completely covered in summer 2013. Electronic flow meters were installed at the CW inlet and outlet and connected to a control panel for recording the measured volumes. Rainfall data were measured by a weather station located at the experimental site. Data monitoring allowed to directly determining the amount of water loss by ET from the constructed wetland through a simple water balance method. Infiltration losses were neglected since the wetland is lined with an impermeable membrane and the CW water storage capacity was considered, when relevant, by measuring the water level inside the bed. The outputs of the water balance method were compared with the ET fluxes estimated as residual term of the energy balance through the SPA system. To solve the energy balance, the CW was equipped by a micrometeorological station, located inside the CW, allowing adequate fetch conditions. The station was provided by one net radiometer for directly measuring the net radiation ( $R_n$ ), three heat flux plates to measure the heat flux (G) coming from the substrate of the CW, and two fine-wire thermocouples for estimating the sensible heat flux (H) via the Surface Renewal technique [2]. Data on H from Surface Renewal were calibrated by H data directly measured by one Eddy Covariance sonic anemometer. ET rates (or latent heat fluxes, LE) were finally obtained as residual term of the energy balance equation as:  $ET=R_n-G-H$ .

**First Results:** The preliminary assessment of the research is referred to the first months of 2014, when both the methods (water balance and energy balance) worked simultaneously. ET rates obtained by the energy balance were in the order of 3.0 mm d<sup>-1</sup>, around 27 mm on ten-day periods (i.e. time step more reliable for the water balance application). The comparison between the two methods in terms of ET losses was reasonably good, with differences lower than 10% during the whole monitoring. The crop coefficient of the *Phragmites australis* (Cav.) Trin., calculated by the *mean K<sub>c</sub> approach* as suggested by the FAO-56 paper, had a mean of 1.4, with peaks of 4.5, as confirmed by similar studies [1]. Plants inside the CW were able to use more than 80% of the available energy ( $R_n-G$ ) for evapotranspiring; this rate is quite higher than that of evergreen crops.

**Conclusions:** First results of the study have proved the reliability of micrometeorological methods based on energy balance to estimate ET fluxes from CW systems. However, effective conclusions regarding the performance of the techniques will require a wider data set.

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## CAPABILITY OF HIGH RATE ALGAL PONDS FOR REMOVING EMERGING CONTAMINANTS FROM DOMESTIC WASTEWATER

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### ABSTRACT

High rate algal ponds (HRAPs) are a promising wastewater technology that have receiving a lot of interest during the last years due to the resource recovery of algal biomass, for use as fertilizer, protein-rich feed or biofuel, and an effluent treated water of high quality [1]. HRAPs are shallow raceway reactors, where microalgae and bacteria grow in symbiosis. In these systems, organic matter is degraded by heterotrophic bacteria, which consume oxygen provided by microalgal photosynthesis and, therefore, no aeration is needed [3]. Although the capability of microalgal systems for wastewater treatment has already been studied for removing nutrients, heavy metals, bacteria, helminth eggs, phenolic compounds, surfactants, biocides and polycyclic aromatic hydrocarbons, little attention has been paid to its effectiveness for removing emerging contaminants (ECs). ECs are comprised of a broad spectrum of compounds belonging to different types of chemical products such as pharmaceuticals, personal care products, plasticizers, flame retardants, surfactants, and certain pesticides among others, and whose ecotoxicological effects are relatively unknown. Therefore, the occurrence of ECs in the aquatic environment is nowadays a well established issue and has become a matter of both scientific and public concern.

Our aim in this study was to evaluate for the first time the effect of hydraulic residence time (HRT) and temperature on the removal efficiency of 25 ECs in two HRAP pilot plants feed with real domestic wastewater. The HRAP pilot plants were located at the NE of Spain (see picture). Samples from the influent and effluent of both systems were collected daily over a week in summer (July 2013) and winter (December 2013). Samples were analyzed for ECs as described previously [2]. Finally, hazard quotients (HQs) were calculated for ecotoxicological risk assessment.



The removal efficiency of ECs ranged from no removal to up to 90%. HRAPs performed slightly better removal efficiencies of ECs than conventional activated sludge wastewater treatment plants. ECs were classified in three groups in accordance with their overall average removal efficiency in HRAPs: high removal (>90%, caffeine, acetaminophen, ibuprofen, methyl dihydrojasmonate and hydrocinnamic acid), moderate-high removal (from 60% to 90%, oxybenzone, ketoprofen, 5-methyl benzotriazole, naproxen, galaxolide, tonalide, tributylphosphate, triclosan, bisphenol A and octylphenol), moderate-low removal (from 40 to 60%, diclofenac, benzotriazole, triphenyl phosphate, cashmeran, diazinone, celestolide and atrazine) and poor or no removal (<30%, carbamazepine, benzothiazole, methyl paraben, OH-benzotriazole, tris(2-chloroethyl) phosphate, and 2,4-D). The elimination of ECs in HRAPs was only affected by the HRT during cold season, whereas no removal differences were observed in warm season. Cold season decreased the removal efficiency of most of the compounds due to the lower temperature and sun-light irradiation. The compounds with the highest occurrence (caffeine, acetaminophen and ibuprofen) presented removal efficiencies up to 90% with low seasonally and HRT effect. The ecotoxicological risk assessment study revealed that the HQ at the affluent wastewater was removed by up to 90%, which indicates no environmental risk of water effluents.

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## TRANSFORMATION OF CHLOROFORM IN SUBSURFACE-FLOW CONSTRUCTED WETLANDS: A MASS BALANCE STUDY

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### ABSTRACT

Chlorination of water has been reported to produce disinfection byproducts (DBPs), some of which are carcinogenic and are consequently of health and regulatory concern. Chloroform was the most frequently detected DBPs in drinking water treatment plants, and it is urgent priority of regulation by the government of many countries due to its potential human-health concerns. Thus, how to efficiently remove chloroform in water is potentially of much practical value. Our previous studies showed that the use of planted or litter added CWs as tertiary treatment systems resulted in a good removal efficiency for chloroform, and its half-lives in CWs are much shorter than those in control CWs and other systems. However, the main mechanism for chloroform removal in CWs is still not clear, because the contributions of biodegradation, sorption and other elimination processes are scarce to date. The objective of this study was to evaluate the fate of chloroform in SSF CWs, including planted and unplanted CWs with or without litters.

In this study, Six SSF CW microcosms (0.3 m×0.3 m×0.5 m) were located in a controlled greenhouse, and the wetlands were W1 (control), W2 (100 g cattail litter), W3 (200 g cattail litter), W4 (22 plants m<sup>-2</sup>), W5 (40 plants m<sup>-2</sup>) and W6 (22 plants m<sup>-2</sup>, 100 g cattail litter). All the microcosms were filled with gravel (8–13 mm, porosity = 0.4), and W4, W5 and W6 were planted with cattail (*Typha latifolia*). The feed water of the CWs was the secondary effluent from a wastewater treatment plant with chlorine disinfection, and the concentration of the chloroform was 60 ± 18 µg L<sup>-1</sup>. The wetlands operated as a batch system with pulse loading, which were filled with wastewater at the start of each batch and were gravity drained within 1 h prior to the next batch. All the treatments were triplicated, and there were 18 batches (each batch lasted for 5d).

As shown in Fig 1, sorption is the dominant pathway for chloroform removal in litter added wetlands, accounting for 73.5-81.2% of the total removal. Sorption to litters and biofilms might be an important mechanism that contributes to the removal of chloroform. Furthermore, biodegradation was another important removal pathway in litter added CWs, which accounted for 17.6%-26.2% of the chloroform removal. In the planted CWs, sorption on gravel was the primary route for chloroform removal (53.6%-66.1%), and plant uptake was the second route for chloroform removal (25.3%-36.2%), and densely planted unit more efficient than the sparsely planted one through plant uptake. As shown in Fig 1, biodegradation and volatilization only accounted for 6.5%-7.6% and 2.0%-2.5% of the chloroform removal in planted CWs. The large contribution of plant uptake (25.3-36.2%) but the marginal volatilization (2.0%-2.5%) for chloroform removal suggested that most of the chloroform have accumulated in the plant tissues after the uptake.

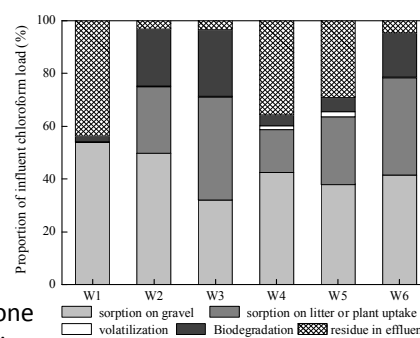


Fig 1 The fate of chloroform in W1-W6 wetlands

Further study showed that the removed chloroform was stored in both belowground and aboveground of plant, and chloroform content in roots (1079-1750 µg) was significantly higher than that in shoots (292-530 µg) and leaves (284-486 µg). This suggested that about 40% of the chloroform transported from the roots to shoots and leaves, higher than the ratio of other organic pollutants reported in previous study. Considering the low hydrophobicity of chloroform (log K<sub>ow</sub> = 1.97), it might be bound loosely to the roots surface that it can be easily translocated within the plant via the transpiration stream. The high plant transpiration (31-35% of inflow) positively correlated to the efficient uptake (25-36% of chloroform removal) in planted CWs, suggesting that transpiration could be the main driver for plant uptake of chloroform in wetlands.

In conclusion, our mass balance study of chloroform phytoremediation suggests that both the active and senescent cattail (*Typha latifolia*) litter can effectively remove the chloroform. In litter added CWs, sorption was the primary route for chloroform removal, and biodegradation was another important removal pathway; in the planted CWs, sorption and plant uptake was the main route for chloroform removal, and the volatilization was negligible.

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## FACTORS INFLUENCING BACTERIA REMOVAL IN CONSTRUCTED WETLANDS

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### ABSTRACT

Treated domestic wastewater is an important water resource for irrigation purposes. A major challenge for its reuse is the reduction of pathogen counts to safe levels. Constructed wetlands (CWs) are able to lower the concentration of pathogens in domestic wastewater by several orders of magnitudes but perform best in two-stage plants. However, despite already extensive research activities the CW's inherent pathogen removal processes are still not sufficiently understood.

Here we report on several laboratory-scale and pilot-scale experiments targeting factors that may influence bacteria removal from model- and domestic wastewater. In laboratory-scale simulated vertical down-flow CWs loaded with *Escherichia coli* cell suspensions or domestic sewage, the presence of particular macrophytes (*Juncus effusus* or *Phragmites australis*) had no significant influence on bacteria cell removal. Likewise, bacterial cell adsorption to the sand filter material was of low significance. In contrast, disinfection of plant roots with sodium hypochlorite hampered *E. coli* removal, and a negative correlation of protozoa and *E. coli* cell numbers was observed. Beside protozoa also predatory bacteria of the group *Bdellovibrio* showed an increased number while bacteria cell number of *E. coli* suspension or domestic sewage decreased. The relationship between bacterial pathogen removal and community structures of microbial predators was further investigated in two types of horizontal flow CWs. The research results strongly support the assumption that microbial predatory activities play an important role in wastewater hygienization in CWs. The role of bacteriophages should be characterized in future investigations.

In general, a better understanding of the microbial predatory activities in CWs could be helpful for a more directed optimization of their running conditions and design for wastewater hygienization.



## UNEXPECTED NET EXPORT OF FAECAL INDICATOR BACTERIA FROM A *TYPHA* WETLAND INTERCEPTING AGRICULTURAL DRAINAGE

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### ABSTRACT

This paper reports on studies showing that a constructed wetland treating episodic (rain event driven) dairy pasture subsurface drainage can be a *net exporter* of the faecal indicator bacterium, *Escherichia coli*. Monthly monitoring of farm drainage to the wetland during the wetter months of the year indicated a relatively low median inflow concentration of 23 *E. coli*/100mL increasing to 98 *E. coli* per 100mL in the outflow. This is a surprising finding given that constructed wetlands treating steady-flow wastewaters usually achieve net *removal* of *E. coli*. A cautious approach to this unexpected finding was undertaken by investigating if the increase in *E. coli* might be an artefact of a sampling regime biased towards flow recession after rain events. Testing this “hypothesis” entailed considerable flood-chasing work using time-based and flow-proportional sampling with calculation of inflow and outflow fluxes (cfu/s) and loads (cfu/event and cfu/yr) – culminating in the eventual conclusion that the wetland was indeed a net exporter of *E. coli* with increases in *E. coli* export ranging from 2 to 34 fold. The increase in *E. coli* is probably due partly to wildlife deposition, but genetic and other evidence suggests that the main source is growth of this bacterium as environmental ‘naturalised’ populations within the wetland. This raises interesting questions regarding the microbial ecology of *E. coli* and interpretation of *E. coli*-based water quality in relation to waterborne disease and health risk downstream from wetland environments.



## THE EFFECT OF SWINE WASTEWATER ON THE ECOPHYSIOLOGY AND STOICHIOMETRY OF TWO OPPORTUNISTIC MACROALGAE

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### ABSTRACT

The effects of swine wastewater stream of high salinity, ammonia, and organic nutrients collected from a pig farm located in Cyprus on the growth, photosynthetic performance and tissue content (C, N, and P) on two macroalgae (*Cladophora* sp. and *Gracilariopsis longissima*) were studied. Two functional indices, the effective quantum yield of photosystem II ( $\Delta F/F_m$ ) and the relative growth rate (RGR, day<sup>-1</sup>), under constant laboratory conditions (21°C ±1°C, 33.2-34.5 PSU, 70 μmol photons m<sup>-2</sup>s<sup>-1</sup>, 14h light per day), after 9 days cultivation were examined. Various experiments with media renewal and measurements on a daily basis were performed using dilutions from 1/20 to 1/500 (sewage:seawater).

The results were analyzed performing factorial or repeated two-way ANOVA. The two species responded significantly different to the effluents with *Cladophora* sp. showing a better performance than *Gracilariopsis longissima* in higher effluent concentrations. The limit to perform was on dilution 1/40 for *Cladophora* sp. and 1/100 for *G. longissima*. Mean tissue nitrogen content ranged from 7 to 17% (dry weight) and from 7 to 10 % N for *Cladophora* sp. and *G. longissima*, respectively. Maximum N values estimated for *Cladophora* sp. (17%) at dilution 1:80 and for *G. longissima* (10%) at dilution 1:400. The N:P ratio between the species' tissue had no statistical differences, whereas, it was significantly different based on their interaction with the different dilutions. Tissue C content showed no statistical difference amongst the two species and the different dilutions.

**Keywords:** seaweeds, effective quantum yield, relative growth rate, tissue content, N:P ratio

**Acknowledgements:** The financial support by the Cyprus Research Promotion foundation (ΠΕΝΕΚ/0311/21) is greatly appreciated.



## REMOVAL OF Cr(III) AND Cr(VI) IONS FROM AQUEOUS SOLUTION BY *CALLITRICHE COPHOCARPA*

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### ABSTRACT

The aim of this study was to determine the adsorption capacity of the dry mass of *Callitriche cophocarpa* for Cr(III) and Cr(VI) ions. Additionally, the influence of pH in initial solution (3.0 and 5.0) and the kind of anion (chloride and sulfate) on the amount of bonded ions were investigated. The Cr was applied as Cr(III) in the form of  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$  or  $\text{KCr}(\text{SO}_4)_2$ , whereas Cr(VI) was used in the form of  $\text{K}_2\text{CrO}_4$ . This particular plant species was chosen due to its excellent Cr(VI)/Cr(III) accumulation capacity in an aquatic environment, that was proved in our earlier works. The sorption process was performed under static conditions contacting the solid phase:solution (batch method), at initial Cr concentration in solution from 0.1 mg/L to 700 mg/L. Solid phase and the solution ratio was 1:100 (i.e. 0.5 g of plant sample + 50 ml of Cr solution at the adequate concentration), and the shaking time was 24 hours.

The adsorption capacity of the *Callitriche* biomass was different for studied Cr ions. It depended on the initial concentration of Cr, kind of an ion and the value of pH in solution. The amount of Cr(III) adsorbed increased in order: Cr(III) – Cl pH 3.0 (22.0 mg/g) < Cr(III) –  $\text{SO}_4$  pH 3.0 (27.1 mg/g) < Cr(III) –  $\text{SO}_4$  pH 5.0 (35.8 mg/g) < Cr(III) – Cl pH 5.0 (51.2 mg/g). The amount of Cr(VI) increased in order: Cr(VI) pH 3.0 (6.58 mg/g) < Cr(VI) pH 5.0 (17.61 mg/g). The coefficients in the adsorption isotherms were estimated with the linear and non-linear regression methods. The linear regression method was found that the Freundlich and Dubinin-Radushkevich isotherms described the element sorption much better than the Langmuir model. On the other hand, all three models described well the experimental data in the non-linear regression method. Furthermore, the  $1/n$  value (<1) obtained from the Freundlich equation for all the Cr-sorbent systems indicated the favorable sorption.

Interpretation of experimental results should take into consideration *i.a.* acid-base equilibrium for Cr(VI) and Cr(III), excellent complexation ability for Cr(III) and redox properties for Cr(VI). Formation of sulphato-complexes with Cr(III) may explain better sorption potential at lower pH (preferable adsorption of greater molecule for complex) and worse sorption potential at higher pH (delayed formation of polymeric hydroxide from hydrolysis of sulphato-complexes). On the other hand, for Cr(VI) at lower pH it is not possible to neglect the equilibrium between chromate  $\text{CrO}_4^{2-}$  and dichromate  $\text{Cr}_2\text{O}_7^{2-}$ . Dichromate which is present at greater quantities at lower pH and due to its larger size than chromate, may exhibit limited diffusion to the deeper parts of the plant tissue. Another yet possibility is related to increasing oxidative power of Cr(VI) in acidic solution, sufficient enough to oxidize primary alkoxy- or phenoxy or aldehyde functional groups, resulting with formation of stable, soluble Cr(III) complexes, staying in solution rather than in an adsorbed state onto biomass.

Experimental constants determined for Freundlich and Dubinin-Radushkevich isotherms indicate, that biomass of *Callitriche cophocarpa* reveals remarkable heterogeneity and that adsorption of chromium ions can proceed as a multilayer process, governed rather by the chemisorption mechanism than the physisorption one.

Sorption capacity found for dry biomass of *Callitriche cophocarpa* is in case of Cr(III) better than other adsorbing agents – including activated carbon, examined earlier by the others and comparable to majority of chromate natural adsorbents in case of Cr(VI).

**Acknowledgements:** The financial support by NCN project DEC-2011/03/B/NZ9/00952 is greatly appreciated.



## HARNESSING THE INTERACTION OF PLANTS AND BACTERIA IN WETLAND SYSTEMS TO REMEDIATE TRICHLOROETHYLENE CONTAMINATED GROUNDWATER

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### ABSTRACT

The Environmental Protection Agency (EPA) has classified trichloroethylene (TCE), a common groundwater contaminant, as a carcinogen. Exposure has detrimental effects on mammalian liver, kidneys, central nervous system and reproductive system. Phytoremediation is the preferred method for remediation in lieu of traditional removal methods that are invasive and expensive. The utility of wetland plants for phytoremediation of TCE has not been extensively researched as is the case for poplar trees and larger plants. To fill that void, this research focuses on wetland plants such as *Carex crinita*, *Iris versicolor*, and *Scirpus atrovirens*. Post exposure, the plant tissue will be analyzed by gas chromatography for TCE and its metabolites. Serial dilutions and Denaturing Gradient Gel Electrophoresis (DGGE) will be employed to detect shifts in the soil microbial community population. This research will shed light on the mechanisms underlying the degradation of TCE in wetland systems.

*Acknowledgements:* The financial support by Roux Associates is greatly appreciated.



## AQUATIC MACROPHYTES *AZOLLA* AND *SALVINIA* AS BIOFILTERS FOR SURFACTANT DECONTAMINATION

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### ABSTRACT

The wide range of domestic and industrial applications of surfactants can arise ecological problems when these molecules are present in the aquatic environment in relatively high concentration, exhibiting toxic effects and affecting at different levels the ecosystems. Conventional wastewater treatment plants are not always perfectly designed to remove these contaminants, thus many of them can occur at different concentrations in surface waters and soils. In previous works two species of floating macrophytes, *Azolla filiculoides* Lam. and *Lemna minor* L., have been exploited for phytoremediation of sodium dodecyl sulphate (SDS), a commercially important anionic surfactant, that is the core component of several detergent and cosmetic product formulations, contributing significantly to the pollution profile of sewage and wastewater of all kinds. The uptake rates of SDS by duckweed and water velvet showed a potential and remarkably efficient phytoremediation system [1]. Besides the use of living plants, dried macrophyte biomasses can be used to biosorption of pollutants like a wide range of heavy metals, as demonstrated by Tel-Or and coworkers [2, 3 and references therein]; this biosorption procedure offers a different approach for reclamation of metals from mining waste and industrial discharges.

The aim of this work was to verify the applicability of this technology for SDS removal from polluted water in order to offer a possible alternative solution to the use of wetlands and to the burning problem of our industrial society, i.e. to reduce water consumption trying to recycle waste water.

For this purpose, biomasses of *Azolla filiculoides* Lam. and *Salvinia natans* (L.) All. were dried in oven (70-80° C for 48 hrs) or in the sun and stored at room temperatures until use. 100 grams of mildly crushed dried biomass were rewetted in 500 ml distilled water for 1 hr, and packed in the column (Biorad), according to the procedure of Cohen- Shoel et al. [2]. Solutions, containing 40 ppm SDS were passed through the dried biomass by hydrostatic pressure at a flow rate of 5 to 10 mL min<sup>-1</sup>. The effluent was collected in 250 mL fractions. pH effect on SDS biosorption was tested at pH 2, 7 and 10. The pH of the solution was adjusted by addition of HCl or NaOH. Before starting the filtration, each biofilter was rinsed with double distilled water (DDW) at the same pH of the experiments. The amount of SDS in the eluates was determined according to Forni et al. [1]

The different pH of SDS solution influenced the binding ability of the biofilters, which biosorption rates enhanced along with the increase in pH, being higher at pH 7 than at pH 2. The binding of SDS to insoluble constituents in the fern matrix most probably involves cell wall charged groups, therefore low pH influenced the binding of the anionic surfactant probably because of the negative charged groups of the cell walls. In fact, the dry biomass of both ferns contains very little cellulose and higher amount of pectins, a major component in *Azolla* (5-7%), and lignin [2]. These molecules together with polyphenols and phytates may play a role in biosorption.

Good binding performances were obtained at pH 7 and 10. *Azolla* biofilter was better than *Salvinia*, in some cases 90 -100 % of SDS removal could be obtained. A good rate of the binding already occurred in the first fractions, suggesting that the initial rinsing with DDW at different pH was necessary to adjust the charges of binding groups and a progressive saturation of the binding sites.

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## Cr(VI)-CONTAMINATED GROUNDWATER REMEDIATION BY CONSTRUCTED WETLANDS PLANTED WITH FOUR HALOPHYTIC PLANTS

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### ABSTRACT

Chromium being widely used in the metal and chemical industries is considered one of the world's most useful materials; however, hexavalent chromium is a heavy metal that causes serious environmental pollution to soils, sediments, groundwater and surface waters. In central Greece, groundwater contamination with Cr(VI) due to former industrial activity in the area has been identified and requires immediate attention. The use of constructed wetlands (CWs) as an alternative wastewater or contaminated groundwater remediation method has increased over the past years and in this study, Cr(VI)-contaminated groundwater remediation by constructed wetlands is investigated with the use of halophytes. Halophytic plants thrive in excessive toxic ion environments and are specially considered for this ability in the scientific community while research efforts have revealed that some halophytes through common physiological mechanisms may also tolerate more stresses than salty environments such as heavy metals.

The specific aims of this study are to investigate the potential of halophyte-based CWs for the removal of Cr(VI) from contaminated groundwater and evaluate the Cr(VI) rhizofiltration ability and tolerance of the halophytes *Juncus acutus*, *Tamarix gallica*, *Halimione portulacoides* and *Sarcocornia perennis*. For that purpose, four horizontal, continuous flow and with complete water recirculation wetlands were constructed and planted with each plant species. All experiments were conducted with initial Cr(VI) concentration in the range of 60–160 µg/L and inlet-outlet samples were collected daily and analyzed for total and hexavalent Cr. Furthermore, the plants were examined for their tolerance towards Cr(VI) and finally plant tissues were analysed for Cr accumulation.

The data of the above experiments revealed that all four plants were able to rhizofiltrate Cr(VI) with the highest and fastest efficiency performed by *J. acutus* and the lowest by *T. gallica*. Especially for *J. acutus* it was found that after five days maximum of re-circulating flow, the concentration of hexavalent chromium in the water medium was below the detection limit (Figure). Moreover, Cr removed from the system was found to be accumulated in the plants tissues.

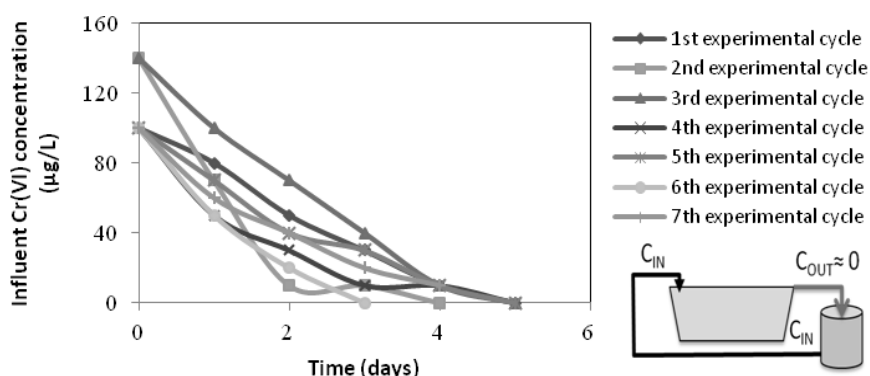


Figure. Concentration of Cr(VI) in the influent of the CW in which *J. acutus* plants are grown.

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## MONITORING OF IRON ABSORPTION USING A CONSTRUCTED WETLAND PILOT PLANT WITH AN HYBRID LAYOUT

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### ABSTRACT

The main purpose of this study was strictly focused on the treatment of iron pollution present in an artificial wastewater (obtained starting from iron sulphate heptahydrate) by the use of constructed wetland system.

The effect of different tanks technologies (vertical and horizontal subsurface flow), bedding material, and plant species was also investigated. A pilot-scale study was conducted on an hybrid layout CW system operated in vertical and horizontal mode. Two vertical systems differed from each other with their bedding material and planted with different species (*Juncus* and *Typha Latifolia*). Also one horizontal system, filled with different substrate and planted with Papyrus, was used after the two vertical tanks.

The CW was designed to work as an hybrid system where the flow, of 50 l/day, crossed alternately from the two vertical tanks and then to the horizontal one. The hydraulic retention time was set equal to about 4 days and the Iron concentration in the inlet was 50mg/l. The test was designed to use batch feeding inside the system as a tank of 1000l was initially filled with 490l of water and 25g of iron sulphate diluted in 10l of water. After ten days of operation the inlet tank was refill again to restart another feeding phase.

The test was conducted during two years of efficiency monitoring. Both physical and chemical parameters were collected with a multiparameters probe three times a week at three different sites of the system, in order to had sufficient and representative data. To determine the abatement of the initial concentration of iron in the CW system were done different samplings at the exit of each tanks (VF1, VF2 and HF).

The target to the CW system was provided from the Italian legislation (Legislative Decree 152/2006) for wastewater discharges into surface water bodies and drains. Concentration based on the best removal efficiencies for VF1, VF2 and HF were Iron 87,9%, 91%, 12,2% and a total removal efficiency of 90,7%.



## INSIGHTS ON THE ROLE OF VEGETATION ON NITROGEN REMOVAL IN SUBSURFACE FLOW CONSTRUCTED WETLANDS

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### ABSTRACT

In this study we investigate the role of vegetation on nitrogen cycling in Constructed Wetlands (CWs) subsurface flow fed with synthetic municipal wastewater. Six pilot CWs were set, duplicates of the following treatments, without vegetation and planted with *Typha latifolia* and *Arundo donax*. Analyses of COD, TKN,  $\text{NO}_3^-$ -N, and  $\text{NH}_4^+$ -N were carried out at the inlet and the outlet of CWs in a weekly basis to investigate their treatment efficiency in terms of N removal. We also monitored the abundance of ammonia oxidizing organisms and denitrifiers through qPCR to provide insights on the pathways operating. Our findings revealed a seasonal impact of the vegetation on N removal rates between 67<sup>th</sup> and the 178<sup>th</sup> day of the experiment, when CWs planted with *T. latifolia* showed higher removal rates for TNK and  $\text{NH}_4^+$ -N compared to the unplanted or planted with *A. donax* CWs. Before and after this interval there was no effect of vegetation on the treatment performance of CWs. Analysis of variance revealed that there was not any significant effect of vegetation on the abundance of gene copies of ammonia oxidizers and denitrifiers. Ammonia oxidizing bacteria (AOB) dominated over ammonia oxidizing archaea throughout the study period implying that they had a higher contribution on nitrification. This hypothesis is strengthened by the significant correlation between AOB and  $\text{NH}_4^+$ -N removal rate and denitrification genes. Finally, denitrification genes found to be highly abundant indicating denitrification as the major pathway of N removal.

*Acknowledgements:* The financial support by FP-7 project WATER4CROPS Grant No. 311933 is greatly appreciated.



## EVALUATION OF A PILOT-SCALE CONSTRUCTED WETLAND WITH *JUNCUS ACUTUS* L. FOR THE REMOVAL OF BISPHENOL A FROM SECONDARY-TREATED WASTEWATER

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### ABSTRACT

Previous studies have shown the ability of halophytes to grow in soil polluted by heavy metals and toxic ions and also the tolerance of *J. acutus* endophytic bacteria to heavy metals and bisphenol A [BPA, 4-[1-(4-hydroxyphenyl)-1-methylethyl]phenol]. Moreover, several endophytic strains of *J. acutus* have the potential to enhance not only bioremediation of bisphenol A but also plant growth. Thus *J. acutus* was demonstrated as an appropriate species for implementing phytoremediation strategies in areas with contaminated groundwater. Application of the phytoremediation strategy in secondary treated municipal wastewater is now investigated.

The aim of this study was to evaluate the removal of the phenolic estrogen BPA, in a small pilot-scale constructed wetland system fed with secondary-treated municipal wastewater, spiked with known amounts of BPA. For that reason a constructed wetland system, planted with *J. acutus*, was designed and operated in the WWTP of city of Chania, Crete, Greece. The unit was running in a horizontal flow with a surface area of 1m<sup>2</sup>. In the evaluation of the CW, removal efficiencies of BPA was monitored and several parameters were measured; Chemical Oxygen Demand (COD), Biochemical oxygen demand (BOD), pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Organic Carbon (TOC), Total Phosphorus (TP) and Dissolved Nitrogen. Experiments were conducted at different hydraulic residence times (HRTs) and robustness of the system was also tested by changing the BPA concentration of the influent stream from 20ppb to 200ppb. The results indicated significant removals of BPA, while plants did not show any toxicity symptoms.

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## CONSTRUCTED WETLAND WITH HALOPHYTES FOR THE DEGRADATION OF BISPHENOL A IN PRIMARY-TREATED MUNICIPAL WASTEWATER

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### ABSTRACT

Evaluation of the removal of the phenolic estrogen bisphenol A (BPA, 4-[1-(4-hydroxyphenyl)-1-methylethyl]phenol) in a constructed wetland system fed with primary-treated municipal wastewater and spiked with known amounts of BPA, is investigated in pilot-scale. The influents of the CW are artificially spiked, primary-treated municipal wastewater from the wastewater treatment plant of the city of Heraklion, Crete. The halophyte-based rhizo-degradation CW, planted with four Mediterranean wetland halophytic species (*Tamarix parviflora*, *Limoniastrum monopetalum*, *Sarcocornia perennis* and *Juncus inflexus*), is operated as a Horizontal Subsurface Flow system (HSF). In the evaluation of the CW several process parameters were measured; namely, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), pH, Electrical Conductivity (EC), Total Nitrogen (TN), Total Phosphorus (TP) and Total Boron (Bo). Three different hydraulic residence times (HRTs) were applied to the system in order to evaluate the dependency of treatment efficiency on HRT with and without bioaugmentation with BPA degrading strain *Sphingomonas* sp. strain TTNP3.

The study was conducted during a 7 month period. The average removal efficiency for BPA was 98%, under nominal conditions during summer period. However significant sensitivity in the system was observed by changing HRT. Degradation rate decreased when HRT was switched to half and started to increase again, after increasing HRT, as expected from the literature. Bioaugmentation with *Sphingomonas* sp. strain TTNP3 showed a slight increase on the removal of BPA. For almost all the other examined parameters, low removal efficiencies were observed.



## THE PLANTS THAT PURIFY THE WATER (LE PIANTE CHE DEPURANO L'ACQUA)

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### ABSTRACT

The book *LE PIANTE CHE DEPURANO L'ACQUA*, published by IL CAMPO, BOLOGNA (ITALY) in February 2014, with a preface of Paola Rossi Pisa (Department of Environmental Science and Technology, University of Bologna, Italy), describes the botany and the ecology of native and non-native aquatic plants and several hygrophytes that can be used in applications of constructed wetlands (HF, VF, FWS) for wastewater treatments, phytoremediation and natural swimming pools. It is the very first book in Italy that illuminates and joins scientific aspects and technical applications of aquatic plants. In-depth knowledge of aquatic macrophytes and hygrophytes is offered in this book as a foundation for their appropriate and informed application in constructed wetlands with the purpose of water purification.



The first chapter of the book contains information about ecology, cultivation and maintenance of aquatic plants; the following three chapters describe their applications. The fifth chapter is organized in 60 plant profiles, regarding the botanical, ecological and phenological aspects of native aquatic plants, non-natives and hygrophytes. Each profile provides descriptive photos of the main parts of the individual plant. The description of every species is completed by its possible application in constructed wetlands, in phytoremediation and in regeneration areas of a natural pools. The aim was to create a work-book that provides hands-on experiences and tested knowledge from different fields of phytoremediation and water purification: the goal is to investigate and to encourage a wide range of possibilities in the use of aquatic species and hygrophytes, emphasizing that each plant must be seen and respected in its ecological characteristics, in order to ensure a long life in an demanding environment. Preference is given to native species rather than non-natives, as they seem to proof more resistant and naturally adapted to the climatic parameters of the environment in which they will live. This is illustrated in graphs with Ellenberg indicators and other tables. Another goal is to mimic nature in its biodiversity, so the authors are going to suggest plant communities that create ecosystems as close to natural example as possible, plant communities that can successfully thrive in semi-natural environments. In addition, the book describes in detail the cultivation and maintenance of all plants, as we wanted to show how to manage them and how to keep them healthy for many years, once the installation has come to life and begun to fulfill its purpose. The book is also supported by a glossary and a complete plant list.



## PELLICE RIVER BASIN: ECO-RESTORATION WITH A SMALL RIVERINE WETLAND AND OLD FRUIT SEED BANK CREATION

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### ABSTRACT

From the IPS website the *“Phytotechnology is the strategic use of plant ..... and by restoring ecosystem services in managed landscapes”*. The aim of this abstract is to describe a case study of eco-restoration and habitat recreation with a small riverine wetland. The result is the valorisation of the entire river basin, with the population, local authorities and stakeholder’s involvement. The river basin is an ecosystem service and for this reason, our goal is to increase its value.

The paper carried out in the frame an ALCOTRA Project between Italy and France still on going TT:Co:Co focused on river management.

The River basin is characterized by an unstable balance between conflicting aspects which represents two sides of the same system. On one side, the fluvial ecosystem constitutes an great ecological and recreational value to be conserved in order to protect the autochthone flora and fauna; on the other side the maintenance of the quantity and the quality of the water needs to be improved to make better use of this natural resource for human activities.

The study area is located in Pellice Valley, which belongs to the Piedmont Region in North-western Italy. The alpine valley coincides with the catchment of the Pellice River, which is the first left tributary of the Po River the major Italian river. This area is characterized by an important vegetation coverage which makes the Pellice valley one of the most wooded Italian valleys. The presence of a rich forest landscape and of two Sites of European Community Importance (SCI) (European Commission, 2001) gives the territory a particular natural and environmental value. Furthermore, presence of the river gives the area a great ecological value, because it represents an ecological corridor characterized by linearity and organized into complex networks, that need to be protected (European Commission, 2001).

The paper presents the restoration project of a polluted area in the community of Villar Pellice. Old train wagons used as “caravan” in a non-authorized campsite will be removed and the area will be returned to the population. A new designed small wetland will be the natural link between two existing artificial ponds, while another part of the area will planted with ancient autochthons fruit trees in order to create a seed bank for the entire valley. The creation of a specific didactical structure will improve the two important described ecological services.

*Acknowledgements:* The authors thanks the French partners of the TT:CoCo. Project and the financial support by ALCOTRA









## **METALS**



## EFFECT OF HYPERACCUMULATOR PLANTS AND ASSOCIATED RHIZOBACTERIA ON THE EFFICIENCY OF NICKEL EXTRACTION

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### ABSTRACT

Many works have attempted to relate the association of different plants on the efficiency of inorganic pollutants extraction, with the hypothesis that these multi-species covers promoted the development and the activity of rhizosphere microorganisms, such as PGPR. Up to now, the focus has been on crop associations (maize, tobacco, *Brassica* species). Only few studies have concerned the effect of the combination of metal hyperaccumulator plants with other species non-hyperaccumulators. These experiments showed that co-cropping with nonhyperaccumulator plants could enhance the growth of the hyperaccumulator and metal accumulation.

The objective of this work was to study the effect of species richness in vegetation cover (mono- vs co-cropping), which only consisted of four hyperaccumulator plant species (*Brassicaceae*), on the efficiency of Nickel (Ni) extraction from an ultramafic soil containing significant nickel concentrations (Ni = 1480 mg.kg<sup>-1</sup>). The effects on some soil physicochemical properties and on microbial communities colonizing the rhizosphere were also evaluated.

An experiment was set up with four hyperaccumulator species (*Leptoplax emarginata*, *Noccaea tymphaea*, *Alyssum murale* and *Bornmuellera tymphaea*). Six treatments had been realised (one mixed cover, four monospecies covers and unplanted soil) with 7 replicates for each. After four months of culture in controlled conditions, the estimation of plant biomass and Ni concentrations in shoots and roots were evaluated. In the meanwhile, microbial biomass carbon, size of cultivable rhizosphere bacterial community (UFC), as well as the potential production of auxin compounds (AIA), were evaluated for each treatment. Bacterial communities were also characterized by genetic (SSCP) and metabolic (Biolog Ecoplate™) structures. Moreover, different microbial enzymes (ACCd - 1-Aminocyclopropane-1-carboxylate deaminase, urease, acid phosphatase,  $\alpha$ -glucosidase, arylsulphatase and FDA-fluorescein diacetate hydrolysis) were measured in rhizosphere soil samples.

The presence of a cover, whether single or multi-species, caused a reduction in the concentration of extractable Ni from the soil. In our case, this effect was most pronounced in the presence of mesocosms planted with *B. tymphaea* and *N. tymphaea*. Similarly, the Ni bioconcentration factor showed a good correlation with shoot biomass and Ni concentration in shoot, especially for *B. tymphaea* and *N. tymphaea* and in a less extent for cocropping species. Moreover, the treatment with *N. tymphaea* showed the lowest pH value – which favours Ni solubility in soil. A strong correlation between pH, microbial enzyme activities and the size of the bacterial community was also observed. No significant change in enzyme activities was observed with covers, except in the case of arylsulphatase. The characterization of bacterial communities from soil samples by genetic (SSCP) and metabolic (Biolog Ecoplate™) structures revealed differences between mesocosms.

The co-cropping of the four hyperaccumulator species did not significantly improve the process of phytoextraction. However, the biomass produced by *B. tymphaea* was in the same range as that of *N. tymphaea* and *L. emarginata* but *B. tymphaea* bioconcentration factor was the highest among all four species (i.e. more than 1% Ni in its dried shoots). Therefore, *B. tymphaea*, and to a lesser extent *N. tymphaea*, were the two species with the greatest potential of phytoextraction of Ni in co-cropping systems. A combination of different hyperaccumulator plants appears promising in phytoremediation practices, but further research is needed to unravel the links between aboveground hyperaccumulating plants, the belowground rhizosphere microbial communities in metaliferous soils and the implication of these microbial communities in the survival of plants and their ability to extract Ni. In particular, associations of plants should be tested in pairs, to define the plant cover providing the best phytoextraction.

**Acknowledgements:** This work was supported by the French National Research Agency through the national program "Investissements d'avenir" with the reference ANR-10-LABX-21 - RESSOURCES21 and by the project LORVER, financed by the FEDER and the "Région Lorraine".



## REGULATION OF COPPER DELIVERY TO PLASTOCYANIN VIA THE PAA2/HMA8 TRANSPORTER

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### ABSTRACT

Copper (Cu) is an essential micronutrient for plants, as it is a cofactor for enzymes that are involved in housekeeping processes such as respiration (cytochrome-c oxidase) and photosynthesis (plastocyanin). Cu is thus essential for plant biomass formation. Under Cu limiting conditions, several mRNAs encoding seemingly non-essential cuproproteins such as Cu/ZnSOD are subject to Cu-microRNA-mediated down-regulation. The concerted expression of these Cu-microRNAs is mediated by the Cu-responsive transcription factor SPL7. We have proposed that the Cu-microRNAs serve in order to economize the available cellular Cu for use in plastocyanin (PC), thus allowing to maintain photosynthesis under Cu deficient conditions (Burkhead et al., 2009, *New Phytol.* 182, 799-816). Indeed PC is a preferred target for Cu delivery when previously Cu-depleted plants are re-supplied with Cu. Studies in Poplar confirmed and extended this Cu economy model (Ravet et al., 2011, *Plant Physiol.* 157:1300-1312). How is the prioritization of Cu delivery to PC achieved? PC mRNA is not a target of a microRNA. In order for Cu to reach PC in the thylakoid lumen, cytosolic Cu is first transported over the inner chloroplast envelope and subsequently the thylakoid membrane by two P<sub>1B</sub>-type ATPases, PAA1/HMA6 (inner chloroplast envelope) and PAA2/HMA8 (thylakoid membrane). We confirmed the subcellular localization of these proteins by direct biochemical approaches and analyzed their targeting to the correct chloroplast membrane systems. Since PC seems to be the primary target in Cu deficiency, we investigated if PAA1 or PAA2 regulation are involved in sub-cellular Cu distribution. We found that PAA2 protein is most stable at low Cu concentrations and its abundance decreases significantly with Cu addition (Tapken et al., 2012, *J. Biol. Chem.* 287:18544-18550). This regulation occurs post-translationally in an SPL7-independent manner via turnover mediated by the CLP protease system.

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## ANALYSIS OF SELENIUM ACCUMULATION, SPECIATION AND TOLERANCE OF POTENTIAL SELENIUM HYPERACCUMULATOR *SYMPHYOTRICHUM ERICOIDES*

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### ABSTRACT

Selenium (Se) hyperaccumulator plants can accumulate Se to extraordinary levels (0.1-1.5%) of their dry weight, levels toxic to most other organisms. Selenium promotes hyperaccumulator growth and offers the plant several ecological advantages through negative effects on Se-sensitive partners (for a review see [1]). High tissue Se levels can reduce herbivory, and high-Se litter deposition can inhibit neighboring plants [2]. *Symphyotrichum ericoides* was found to contain hyperaccumulator levels of Se (up to 4,000 mg kg<sup>-1</sup> DW) on a seleniferous field site. It often grew next to other Se hyperaccumulators *Astragalus bisulcatus* and *Stanleya pinnata*, where it was on average 2-fold larger and suffered less herbivory than when growing next to non-hyperaccumulators [3]. This raised two questions, whether *S. ericoides* is capable of hyperaccumulation without neighbor assistance, and whether its Se-derived benefit is merely ecological or also physiological. In a comparative greenhouse study, Se accumulation and tolerance of *S. ericoides* were analyzed in parallel with hyperaccumulator *A. bisulcatus*, Se accumulator *Brassica juncea* and related Asteracea *Machaeranthera tanacetifolia* [4]. *Symphyotrichum ericoides* and *M. tanacetifolia* accumulated Se to hyperaccumulator levels: up to 3,000 and 1,500 mg Se kg<sup>-1</sup> DW, respectively. They were completely tolerant to these Se levels and even grew 1.5-2.5 fold larger with Se than without. *Symphyotrichum ericoides* showed very high leaf Se/S and shoot/root Se concentration ratios, similar to *A. bisulcatus* and higher than *M. tanacetifolia* and *B. juncea*. Se X-ray absorption near-edge structure spectroscopy showed that *S. ericoides* accumulated Se predominantly (86%) as C-Se-C compounds indistinguishable from methyl-selenocysteine, which may explain its Se tolerance; the remainder was inorganic Se. Thus, in the greenhouse study *S. ericoides* displayed all of the characteristics of a hyperaccumulator; whether it can hyperaccumulate independently in the field requires more investigation. The bigger size displayed by the high-Se *S. ericoides* growing next to hyperaccumulators may be explained by a Se-related physiological benefit, in addition to the ecological benefit demonstrated earlier. These findings are useful for the management of seleniferous areas and cultivation of Se-rich crops for phytoremediation or biofortification.

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## TOLERANCE LEVEL OF DOUGLAS FIRS (*PSEUDOTSUGA MENZIESII*) PLANTLETS TO METALLIC TRACE ELEMENTS

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### ABSTRACT

Tree species can be good tools for phytoaccumulation because of their high biomass and their deep root system. Gymnosperms are not widely used although they can be as efficient as angiosperms [1,2,3]. The aim of this study was to evaluate the tolerance of Douglas firs to metallic trace elements (MTE) when soils are contaminated by several MTE instead of a single one [4]. Indeed, polluted soils are generally the result of multiple contaminants.

Douglas fir seeds were stored for 2 months at 4°C in plastic bags containing moist sterile sand for stratification. They were then transferred in greenhouse in pots with soils contaminated with several MTE. Soil samples (the upper 15-20 cm) were collected on two sites in France: the first is a former mine of lead and silver in Pontgibaud (Puy-De-Dôme, Auvergne) and the second is a former mine of gold in La Petite Faye (Creuse, Limousin). The MTE in soils of Pontgibaud (mean content in mg. kg<sup>-1</sup> dry soil) are Pb (11270), As (910), Zn (460) and Cd (3). The soils of La Petite Faye contained Pb, As, Sb and Cd but were sampled in two zones (Z1 and Z2) due to the gradient of MTE concentrations of soils [4]. Mean concentrations (in mg.kg<sup>-1</sup> dry soil) of Pb, As, Sb and Cd were 119900, 21300, 1400 and 2.2 in Z1 and 14500, 68100, 930 and 1.7 in Z2, respectively.

Preliminary experiments showed that the rate of Douglas fir germination was close to 70% on uncontaminated soil. Thus, approximately 50 seeds per pot were sowed in order to obtain at least 30-35 plantlets per pot. Germination was performed directly on contaminated soils or on a mixture of contaminated soil and horticultural compost / sand (75/25, v/v) with a final content of contaminated soil of 25 or 50%. Germinations on 100% horticultural compost / sand were performed in parallel as a control of optimal growth for Douglas fir. All pots were watered with demineralized water for 10 weeks after seed sowing (the first two weeks corresponded to the time of germination) and plantlets were then harvested. Root system was rinsed in demineralized water and then incubated in 20 mM EDTA to remove metallic cations adsorbed on root surface. Roots, stem and needles were then weighted and divided into several batches for analyses: dry weight, MTE quantification, photosynthetic pigment and soluble carbohydrate contents, oxidoreduction status and thiol-rich oligopeptide content (often involved in Cd and other MTE scavenging). Moreover, MTE concentrations measured in the different organs will be discussed as a function of MTE speciation in soils. Analyses are actually in progress.

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## ARSENIC PHYTOEXTRACTION BY AN INDIAN ECOTYPE *PTERIS VITTATA*: MOLECULAR EVIDENCE OF ITS GENETIC POTENTIALITY

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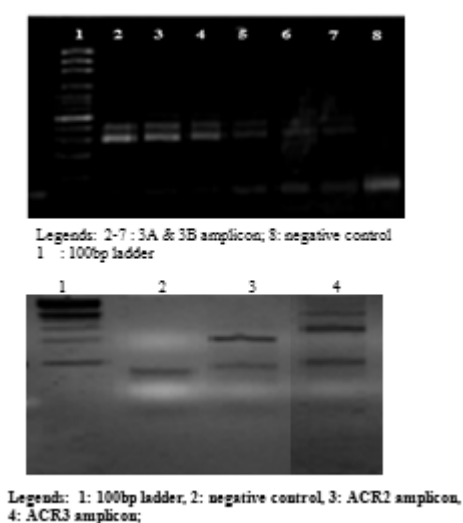
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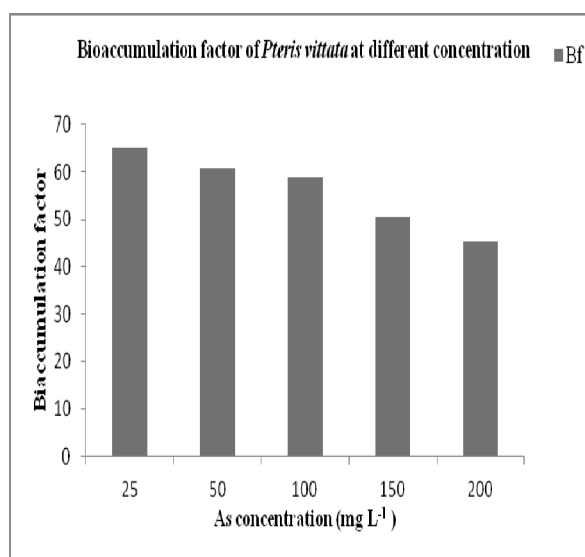
### ABSTRACT

Arsenic(As) is increasingly enriched in soil and water due to unsustainable industrial activities such as; mining, coal burning, arsenical pesticide, geogenic formation due to disproportionate underground water harvest, indiscriminate discharge of As waste and natural contamination. Due to its non-degradability, As concentration in the environment is exceeding the safe level, and its acute toxicity is posing a great threat to mankind. Remediation of As pollution through phytoextraction by hyperaccumulator plants is being increasingly investigated to develop an alternative, and sustainable method over the existing conventional *ex situ* methods. The inherent ability of a plant for metal accumulation with high Bioaccumulation Factor (BF) is the primary requisite to make such proposition a reality. In this study, As remediation potentiality of an Indian ecotype *Pteris vittata* is determined and the mechanism underneath its tolerance and accumulation has been investigated. The plant was treated with different concentration of As (25, 50, 100, 150, 200 mg L<sup>-1</sup>) in hydroponic condition. This ecotype accumulates up to 9000 mg As Kg<sup>-1</sup> dry weight of biomass with BF up to 65. There were no visible toxicity symptoms of the plant after treatment with 50 mg L<sup>-1</sup> As for one month indicating its high tolerance for As. Molecular study confirmed presence of orthologs of *arsC* gene of the *ars* operon, which encodes arsenate reductase (AR) that mediates conversion of AsV to AsIII. Arsenate reductase activity was increased by two fold in As treated plant in comparison to control, and more activity was observed in frond to that of root. Occurrence of the molecular determinant for arsenate reduction similar to other hyperaccumulator for detoxification and accumulation in the plant biomass, confirms this plant as As hyperaccumulator. The present study establish this *Pteris vittata* Indian ecotype is a As hyperaccumulator and a suitable plant species for As remediation application.

**Figure:** *arsC* amplification using gene specific primers in *Pteris vittata* genomic DNA



**Figure:** Arsenic accumulation in plant biomass



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## THE STUDY OF THE PROCESS OF METALS ACCUMULATION BY FLOATING MACROPHYTES UNDER WATER POLLUTION CONDITIONS

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### ABSTRACT

Phytoremediation technology is an effective sustainable, energy and cost economic method of environment clearing due to the plants ability to hyper-accumulation of the pollutants. The floating macrophytes water hyacinth, *Eichhornia crassipes* (EC) and *Pistia Stratiotes* (PS) are applied most often to sewage treatment of the industrial enterprises and may consider as an efficient tool for contaminated waters cleaning up.

Unfortunately in contemporary literature the studies are mainly focused on the actual metals uptake by plants but sufficiently less is known about the process of their transport and transformation inside the plant's tissue and practically nothing is known about the role of microorganisms in the process of metals bioaccumulation. At the moment there is a great need in strengthening of such knowledge as well as those of the mechanism of the process which would help to manage the pollutants extraction technology for its practical application.

In frames of present investigation the following questions were considered on the base of natural modelling approach with cadmium as a model element:

- an estimation of the contribution of the different ways of pollutants removal from contaminated water;
- the study of the mechanism of penetration of metals within the plant and its fate during accumulation act.

As it is known there are at least two channels of metals removal from contaminated natural water: the sedimentation process and accumulation by plankton organisms. We found that these channels provide water purification at the level of about 40%, however, in the presence of the floating plants the plankton channel doesn't work. As a result of microorganisms species diversity investigation in water and in plant's rhizosphere the microorganisms-destructors resistant to pollutant were identified. It was shown that the efficiency of metal extraction by microorganisms may reach about 50% of the total value of those accumulated by plant. Herewith the rhizosphere microorganisms demonstrate the highest accumulative capacity. It has been assumed that their possible role consists in the destruction of dying plankton and heavy metals transformation into the forms which may be accumulated by the plants more effectively.

It was also shown that at the first stage the pollutant sorption on the surface of the roots takes place at that metal is mainly localized in rhizodermis, then the pollutant penetrates into the tissues of the stem according to its translocation factor with the formation of the complexes rich in cysteine.

An approach for identifying of the binding forms of the element the methodology based on HPLC separation of the plant extract with UV and ICP-OES detection to investigate peptide compounds formed in plants during bioaccumulation process was developed. The obtained results allow assuming that in water-soluble fraction isolated from hyacinth cadmium is bound mainly with the peptides similar to phytochelatins. It has been assumed that flavonoids perform an intermediate role in the accumulation process, taking part in the transport and combat an oxidative stress.

In contrast to the traditional black-box approach, investigations of contaminant speciation in plant tissues have given sound understanding of the phytoremediation phenomenon. Such advancements could provide a basis for future improving the efficacy of the biological remediation processes.





## POSSIBILITIES OF AIR POLLUTANTS REMOVAL: HEAVY METALS, PARTICULATE MATTER AND PAH

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### ABSTRACT

The air pollutants became in recent years on the top of harmful factors to man wellbeing and health. The ease of air movement together with pollutants makes difficult to clean it up. For purifying air the use of plants can be employed, what to some extent is possible to control by man via phytoremediation. Plants due to their large leaf area index – LAI (from 2 for grasses up to 14 in some trees), morphological characteristics and some metabolic properties are very efficient in air pollutants accumulation but they vary in this regard depending on species. Additionally an important role in cleaning up air plays rain which washes down pollutants suspended in air and these accumulated/deposited on surfaces including plants leaves. However, part of contaminants retained in the plants wax. Also wind participates in blowing up pollutants from surfaces but this part might be and often is re-suspended in air.

For years in our Lab we estimated accumulation of PM on leaves of over 60 trees, shrubs and climbers species.

In this work we attempt to evaluate the role of rain in washing off PM from surface *Tilia cordata* trees, For evaluation were chosen only slow rainfalls (not exceeding 20 mm). After every episode of the rainfall, amount of rain collected in plastic containers placed under tree was measured, the liquid was filtrated and after drying PM were determined gravimetrically. Results showed that for evaluation were chosen three, slow rainfalls (not exceeding 20 mm). After every episode of the rainfall, collected in plastic containers placed under tree, the amount of rain was measured and the liquid was filtrated and after drying PM were determined gravimetrically. Results showed that he amount of rainwater measured under the trees was the smallest near the trunk and it was increasing up to the last 60 cm to the edge of the crown when achieve the same amount as the rainfall outside the trees. However, in the water collected under the trees 7.9% higher content of PM it was noted.

We also determined amount of Pb in soil samples collected from underneath *Tilia cordata* trees planted in 1936 using XRF spectrometer Alpha 4000. In this experiment confirmed the role of rain in washing Pb off leaves to the soil under the crown. Lead is washed from the leaves but in very little quantities is transported to leaves therefore it become a good marker for the distribution of Pb under the tree. The results of this experiment on many years growing trees shows lower amount of Pb near the trunk increasing along with distance from trunk and again lower at the edge of the crown where number of the leaves is much lower.

The aim of other experiments were to assess the role of the plants in removing PAHs from the air, their flow from leaves to the soil, PAH degradation in soil and role of plants in the intensification of this process.

Plants of *Canna x generalis* were grown in containers with soil spiked with phenanthrene, chrysene, benzo(k)fluoranthene, benzo(g,h,i)perylene and in non spiked soil (control). The amount of PAHs was by 21.6% higher in combination with *Canna X generalis* plants compared to the combination without plants. Higher PAHs content in the soil with *Canna X generalis* plants is due to uptake of these pollutants from air, their accumulation on leaves and washing off the leaf surface to the soil.

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## MITIGATION MEASURES FOR GEOGENIC CHROMIUM RICH SOILS

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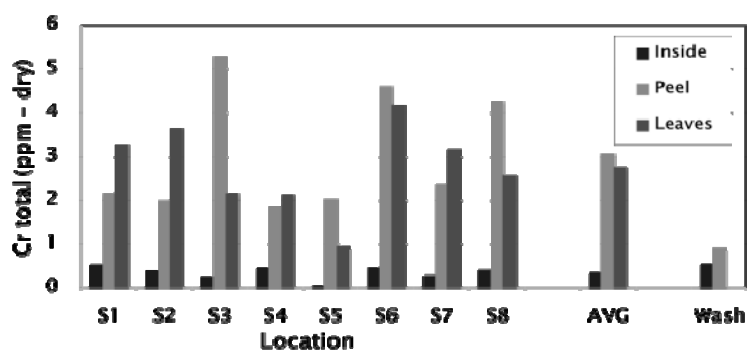
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### ABSTRACT

Heavy metals at high concentrations in soils cause toxicity to biota, toxic effects to plants, lead to losses in agricultural productivity as well as potential human health effects. Therefore, it is of great importance to reduce the mobility and bioavailability of these metals from the soil. Within the framework of the LIFE+ project CHARM, the Technical University of Crete has used mitigation technologies that can reduce the mobility of chromium (by converting Cr(VI) to Cr(III) and/or by sequestration), so that the overall amount of chromium uptaken by the cultivated crops or leached to the groundwater is significantly reduced. The objective of this study was to evaluate the efficacy of zero valent iron products and compost to stabilize chromium in the soil and reduce its bioavailability.

The overall experimental design was conducted in two phases. In the first phase, a sampling campaign was conducted in order to collect soil and plant (carrots) samples from cultivated sites in the Schimatari area of Asopos river basin. Asopos river basin has agricultural soils containing geogenic origin chromium and other heavy metals that have the potential to pose a threat to human health and environment. Groundwater samples from wells irrigating the carrots were also collected. Samples were analyzed for base cations and heavy metals and specifically chromium, according to EPA 3051A method and ICP-MS (Agilent- CX). Regarding carrots, the leaves, the peel and the inside of carrot were analyzed separately. In the second phase, a field scale experiment demonstration was conducted where carrots were cultivated in treatment plots with amendments of Zero Valent Iron, DARAMEND® and compost as soil stabilization agents. It is hypothesized that ZVI and DARAMEND® (which is a ZVI based product) will reduce Cr(VI) into Cr(III) in situ which in turn will precipitate, whereas the amendment of compost in the soil will stimulate the formation of water stable aggregates which will incorporate the silt-clay fractions into micro and macro aggregate, reducing in this way the mobility and bioavailability of Cr(VI) in soil solution.

The results of the initial sampling (before the application of the mitigation measures) showed that the average dry weight chromium concentration in carrots was 0.35 ppm for the inside of carrot, 3.07 ppm for the peel and 2.76 ppm for the leaves (Figure 1). After wash, the average dry weight chromium concentration was 0.54 ppm for the inside of carrot and 0.91 ppm for the peel. The results suggest that chromium is found on the peel of the carrot and washing can reduce its concentration by 70%. Amendments of Zero Valent Iron, DARAMEND® and compost all reduced the bioavailability and stabilize chromium in the soil.



The results suggest that chromium is found on the peel of the carrot and washing can reduce its concentration by 70%. Amendments of Zero Valent Iron, DARAMEND® and compost all reduced the bioavailability and stabilize chromium in the soil.

**Acknowledgements:** This research was co-funded by the LIFE+ CHARM project (LIFE10 ENV/GR/000601).



## CITRIC ACID-ASSISTED PHYTOEXTRACTION OF LEAD: A FIELD EXPERIMENT

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### ABSTRACT

Lead is one of the most common inorganic pollutants in soils. This metal has a high persistence in the soil and is potentially toxic, even at low concentrations. In the United States, the Environmental Protection Agency (EPA) considers levels of Pb in soil at between 400 and 500 mg kg<sup>-1</sup> as high risk to human health. Conventional techniques, such as excavation, backfilling, fixation and leaching are remediation strategies available for soils contaminated by heavy metals. Alternatively, emerging technologies, such as phytoremediation, which is especially suitable for moderately polluted areas, can also be used. Phytoextraction which removes metals from the soil through absorption and subsequent translocation from the plant's roots to its aerial parts is often proposed for the remediation of soils contaminated with metals due to lower cost, public acceptance and reduced environmental impact. This study was carried out to assess the performance of *Zea mays* and *Chrysopogon zizanioides* in the phytoextraction of Pb from a soil contaminated by battery recycling activities. The experiment was conducted in an area of slag deposition in the vicinity of a company operating in the recycling of automotive electric batteries, located in Rio Tinto, PB, Brazil (06°48'11" S latitude, 35°04'50" W longitude). The species were planted with different spacings between rows (0.80, 0.65 and 0.50 m). Citric acid (40 mmol kg<sup>-1</sup>) was applied on each experimental plot on the 61st d of cultivation in order to solubilize the Pb and assist the phytoextraction. The results showed that the chelating agent promoted a 14-fold increase in the Pb concentration in maize shoots as compared to the control, which accumulated only 111 mg kg<sup>-1</sup> of the metal. The citric acid induced a Pb concentration in vetiver shoots that was 7.2-6.7 fold higher than the control at both the 0.65 and 0.50 m plant spacing, respectively. The use of citric acid increased substantially the uptake and translocation of Pb to the shoots, regardless of plant spacing. The use of citric acid at 40 mmol kg<sup>-1</sup> was effective in the Pb solubilization of the soil and in the induction of Pb absorption by the studied species. Commercial citric acid is suitable for use in the area, due to low cost and high biodegradability. Due to the natural low solubility of Pb and to the long time required (more than 150 yr), phytoextraction without chelating agents is not feasible to remedy the studied area. Conventional spacings used for both species (0.80 m) and also 0.65 m for maize coupled with the application of the chelating agent is the recommended phytoextraction strategy for the remediation of areas with moderate Pb contamination. For higher concentrations of this metal, other remediation approaches should be adopted to make the process practical and economically viable.

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## CHELANT-ENHANCED HEAVY METAL UPTAKE BY *EUCALYPTUS* SP. UNDER CONTROLLED DEFICIT IRRIGATION (CDI)

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### ABSTRACT

Enhancement of phytoremediation of heavy metal polluted soils employs organic ligands, aimed to solubilize, phytoextract and translocate metals into the canopy. The use of EDTA, an efficient yet environmentally persistent chelants is phasing out. We tested the hypothesis that CDI of fast growing, salinity resistant *Eucalyptuses* coupled with timely EDTA application/cessation enhances sediment phytoremediation while minimizing leaching of metal complexes below the root-zone by allowing for their biodegradation prior to uncontrolled winter leaching.

Two experimental setups were examined: (i) 24 lysimeters (220-L) packed with a mixture of metal polluted sludge and quartz sand (planting in 2006), and (ii) two 60 m<sup>3</sup>, drainage-controlled basins filled with dredged, polluted, saline river sediment. Both were planted (in 2006 and in 2010, respectively) with *Eucalyptus* saplings (1 and 5 species, respectively). The trees were matured for a year before applying the CDI and chelants. In the next 2 summers the lysimeters were irrigated with tap or RO water amended with Na<sub>3</sub>EDTA, Na<sub>3</sub>EDDS and citric acid (each at 0.05-2 mM) or not amended (control). In late 2009 the trees were cut (for analyses) and then allowed to recover for 2 years before EDTA application restarted. This time EDTA was as either 4Na or 4NH<sub>4</sub> salt (by H<sub>4</sub>EDTA titration with NH<sub>4</sub>OH). The basins were irrigated with secondary wastewater effluent with intermittent additions of 10 mM EDTA during the summers of 2011 & 2012. In 2011 it was applied once a week from May through mid-September and in 2012 it was applied in every irrigation in May and then in July. Leaching was prescribed yet frequent (overall LF ≈2%) for soil solution monitoring. Tree leaves were analysed 2-3 times a year.

**Lysimeters:** EDTA was the only effective chelant in solubilizing and translocating metals into the canopy, and the growth of *E. camaldulensis* seemed unaffected by the salination of the soil solution (up to 35 dS m<sup>-1</sup>) and the build-up of EDTA concentrations in the soil solution (up to 100 mM). EDTA slowly degraded, with a half-life of ≥27-d at 10 mM solution concentration. Comparably, the EDDS half-life was 5-11 days. The peak average (6-18 replicates) metal concentrations in the lysimeter soil solution and in the *Eucalyptus* leaves, in the EDTA vs. control treatments were (all respective values): Cd: 200 mg L<sup>-1</sup> vs. 1.0, and 67 vs. 21 mg kg<sup>-1</sup>; Cu: 90 vs. 1.5 mg L<sup>-1</sup>, and 17 vs. 3.0 mg kg<sup>-1</sup>; Cr: 4.0 vs. 1.4 mg L<sup>-1</sup>, and 3.0 vs. 1.0 mg kg<sup>-1</sup>; Ni: 60 mg L<sup>-1</sup> vs. 14, and 20 vs. 6.0 mg kg<sup>-1</sup>; Pb: >44 vs. 0.1 mg L<sup>-1</sup>, and 9.0 vs. 1.0 mg kg<sup>-1</sup>; and Zn: 650 vs. 4.0 mg L<sup>-1</sup> and 200 vs. 70 mg kg<sup>-1</sup>. In 2013, ≈7 years after planting, metal's concentrations in the upper (15-30 cm) soil layer of the EDTA and control treatments decreased as follows: Cd (53 mg kg<sup>-1</sup>): -67% and -20% (*p*<0.05); Zn (206 mg kg<sup>-1</sup>): -58 and -6% (*p*<0.01); Pb (23 mg kg<sup>-1</sup>) (0-15 cm): -60% and -28%. Decreases in the concentrations of other metals (e.g., Cu (37 mg kg<sup>-1</sup>): -55 & -28%; Ni (14 mg kg<sup>-1</sup>): -29%; Mn (50 mg kg<sup>-1</sup>): -75%; Fe (4580 mg kg<sup>-1</sup>): -27%) were not significantly difference between the treatments.

**Sediment basins:** The concept worked similarly well, with respect to high growth rate of the selected species under high salinity and ensuing salination at the CDI (EC >20 ds m<sup>-1</sup> in the summer time). Average weights of 3.5 years old *E. camaldulensis* and *E. occidentalis* were 55 and 45 kg/tree. EDTA degraded fast in the sediment with peak soil solution concentrations not exceeding 17 mM immediately after addition. The concentration quickly decrease to 0-2 mM, and in the winter leachate water they were 0-0.1 mM. EDTA addition increased metal concentration by >2 orders of



magnitude. In 2011 they returned to near background concentration 2-3 months after the secession of EDTA application and in 2012 this return to background occurred within a month. Thus the uncontrolled winter leaching was not affected by the summer applied EDTA. Leaves metal concentrations followed EDTA addition in 2011 but not in 2012. Cd concentration in the above two species and in *E. Spatulate* were usually  $>15 \text{ mg kg}^{-1}$ .

To conclude, the study suggests that sustainable phytostabilization and phytoextraction of heavy metals are achievable under CDI with EDTA fertigation at low dose, continuous or intermittent. The biodegradable EDDS and citric acid were completely ineffective. RO water Irrigation with  $(\text{NH}_4)_{(3-4)}\text{EDTA}$  removes all salinity components, and further widened the scope of this CDI-based remediation technique.

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## CITRIC ACID- AND TWEEN® 80- ASSISTED PHYTOREMEDIATION OF MULTI-CONTAMINATED SOILS VEGETATED WITH ALFALFA

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### ABSTRACT

The combination of phytoextraction and rhizodegradation phytotechnologies can be employed together with the aim to remediate soils multi-contaminated by inorganic and organic pollutants [1]. However, low bioavailability of contaminants represents a significant limitation in phytoremediation [2]. To overcome this constraint, biodegradable amendments such as low molecular weight organic acids with metal chelating ability and surfactants, which enhance the solubility of organics, can be added to soils [3].

The present study assessed the phytoremediation potential of alfalfa (*Medicago sativa* L.) in a multi-contaminated soil as well as the effects of citric acid and Tween® 80 (polyethylene glycol sorbitan monooleate), applied individually and in combination, on the phytoremediation process.

The experimental design included a pot experiment in the growth chamber. One month old alfalfa seedlings were transplanted to a soil contaminated by heavy metals (Cu, Pb, Ni and Zn) and petroleum hydrocarbons. Pots were fortnightly treated with citric acid (15 mmol kg<sup>-1</sup> dry soil), Tween® 80 (0.036 mmol kg<sup>-1</sup> dry soil), or the combination of both compounds. Vegetated and non-vegetated controls received distilled water instead of amendments. Each condition was repeated in triplicates. Plants were harvested after 30, 60 and 90 days, every time three days after amendment application. Soil samples (from the rhizosphere in the case of vegetated pots) were taken at the same times. Plant parts were dried, weighed, mineralized and analyzed for their content in Cu, Ni, Pb and Zn by ICP-OES. The number of aliphatic hydrocarbon degraders and the activity of lipase enzyme were determined in soil samples by the most-probable-number method and a colorimetric assay, respectively [4,5].

The results showed that alfalfa plants could tolerate and grow in the multi-contaminated soils. Over the 90-day experimental time, shoot and root biomass increased and negligible plant mortality arose. Heavy metals were uptaken by alfalfa to a limited extent, and mostly by plant roots. Heavy metal concentration in plant tissues were in the following order: Zn > Cu > Pb > Ni. Alfalfa rhizosphere effect was manifest, enhancing both microbial population (alkane degraders) and activity (lipase enzyme), with rhizosphere effects of 28.11 and 2.04, respectively, after 90 days. Soil amendments did not significantly enhance plant metal concentration or total uptake. By contrast, the combination of citric acid and Tween® 80 significantly improved alkane degraders (5.3-fold increase) and lipase activity (1.0-fold increase) in the rhizosphere of amended plants, after 30 days of experiment.

This evidence supports the phytoremediation potential of alfalfa species to facilitate the remediation of multi-contaminated soils and the possibility to enhance the phytoremediation process through the joint application of citric acid and Tween® 80.

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## LEAD TOLERANCE AND ACCUMULATION IN METALLICOLOUS AND NON-METALLICOLOUS POPULATIONS OF *HIRSCHFELDIA INCANA*

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### ABSTRACT

Morocco is known for its mining activities that are sources of pollution by heavy metals. In the eastern region, the soil index pollution is considered very high because of the simultaneous presence of different metallic trace elements with a predominance of Pb. This makes the region toxic to flora, fauna and local populations.

*Hirschfeldia incana* is a member of the Brassicaceae family, that growth well in these contaminated sites. This plant, with a significant biomass, tolerates and accumulates high concentrations of Pb [1]. *H. incana* is a pseudometallophyte that grows also in unpolluted sites in the north of the country. In the context of phytoremediation program, we compared the growth, Pb accumulation, some physiological characteristics and genes expression in a metallicolous (MP) and a non-metallicolous population (NMP) of *H. incana*.

In soil culture, MP appears more tolerant than NMP, while both populations accumulate Pb at the same level. In hydroponic culture, Pb accumulations in the aboveground parts of the NMP were 2.5 times higher than the MP when plants were supplied with 100 µM of Pb. However, MP was more tolerant to Pb than the NMP. The MP had significantly lower concentrations of anthocyanins and higher concentrations of chlorophyll a than NMP.

An heterologous hybridization of *H. incana* transcripts on *A. thaliana* microarrays showed that several genes are regulated by lead. QPCR analysis for *HMA4*, *GCN2* and *MRP14* genes revealed that all of them are regulated by Pb in leaves of MP and NMP. This can suggest a functional role of the 3 genes in Pb accumulation. Interestingly, *MRP14* and *GCN2* genes were expressed constitutively higher in NMP. These results can explain the difference in accumulation between the two populations and suggest a possible adaptation of the MP by a regulation of Pb accumulation. Our results demonstrate that the MP has adapted to tolerate and accumulate Pb and thus has a good potential for phytoremediation of Pb contaminated soil.

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## CADMIUM UPTAKE AND REPARTITION OVER THE VEGETATIVE STAGE IN *HELIANTHUS ANNUUS* GROWN AT Cd 2 AND 20 nM

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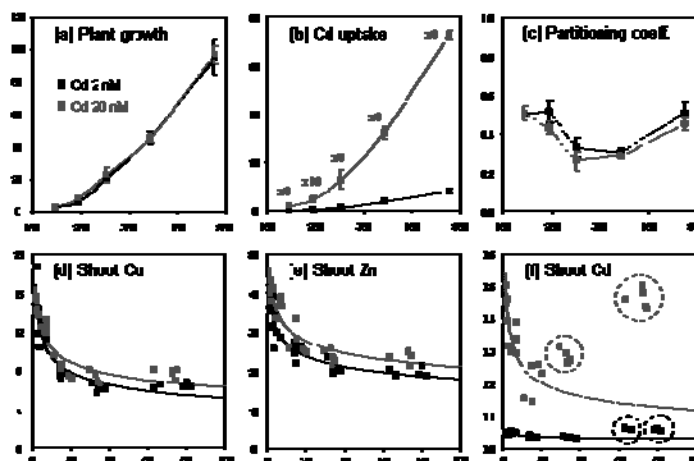
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### ABSTRACT

Although processes governing the fate of Cd in plant tissues are well described at high level of Cd exposure (vacuolar sequestration, xylem loading, xylem-to-phloem transfer), the literature is lacking of a formalization of this knowledge in a simulation model. There are some indications showing that the concentration of metals in a plant organ can depend on the growth of this organ due to a dilution effect (Chen et al., 2008; Ekvall and Greger, 2003). These observations can be summarized by the following model:  $C = a \cdot Biom^{-b}$  where  $C$  is the concentration of trace elements in the plant compartment (leaves, stems, seeds),  $Biom$  is the biomass of the plant compartment,  $a$  and  $b$  are parameters. The parameter  $b$  describes the dilution intensity when the parameter  $a$  synthesizes the effect of the bioavailability and that of the plant species. The main objective of this work is to test this model on *Helianthus annuus*, for variations in biomass resulting from the phenology. We worked at a level of Cd exposure that commonly occur in agricultural soils (nM range) and for which metals repartition between plant organs has rarely been described.

One cultivar of sunflower (ES BIBA) commonly cultivated in France was grown in greenhouse in a ¼ Hoagland nutrient solution with 2 or 20 nM Cd(NO<sub>3</sub>)<sub>2</sub>. Plant sampling was performed at five phenological stages (6, 9, 14, 19 and 31 expanded leaves). Plants were divided into roots, stems, leaves (excluding the petiole) and flower bud (when present). All plant tissues were freeze-dried, weighed and milled. Their total N content was measured by the combustion method of Dumas and their content in Cd, Cu and Zn by ICP-MS after a microwave digestion in a mix HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub>. Xylem sap was collected on every plant by the “root pressure” method and analysed for its concentration in metals (ICP-MS), organic acids and amino acids (LC-DAD).

The figure shows the evolution of [a] plant dry weight (g), [b] plant uptake of Cd (µg) and [c] partitioning coefficient of Cd to shoots (unitless) over thermal time (° days) and the relationships between [d] Cu content, [e] Zn content, [f] Cd content (µg g<sup>-1</sup>) and biomass (g) in plant shoots, at Cd 2 and 20 nM.



Plant growth was not affected by the level of Cd exposure. The 10-fold rise in the concentration of Cd in nutrient solution induced an almost 10-fold increase in the plant uptake of Cd. The partitioning coefficient of Cd to shoots varied over time, contrary to that of Zn, and was fairly similar at Cd 2 and 20 nM. All these results suggest that at low level of Cd exposure, which characterize agricultural soils, the uptake and the root-to-shoot translocation of Cd in sunflower are not much regulated. Investigations are in progress to check whether the molecular processes involved in Cd homeostasis (e.g. PCs synthesis) are induced at this low concentration.

A decrease in Cu and Zn content was observed with plant growth in stems, leaves (so shoots) and roots. This result confirms that the dilution of Cu and Zn in plant biomass has a physiological origin. The “dilution model” presented above described nicely the experimental data for Cu and Zn. A comparison of the  $b$  value with the one obtained for N is necessary to know whether the dilution of Cu and Zn can be explained in the same way as the one of N. For Cd, the re-concentration of the metal in aerial tissues observed at the two last stages of vegetative growth still needs to be figured out. Interestingly, a similar trend was observed in the concentrations of Cd and of some amino acids and organic acids measured in xylem sap.

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## SELECTION OF PHYTO- AND/OR PHYCO-REMEDICATION BASED SYSTEMS TO APPLY ON NUCLEAR CONTAMINATION

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### ABSTRACT

Although there are various studies dealing with the application of terrestrial plants, aquatic plants and eukaryotic or prokaryotic algae for the decontamination of certain radionuclides from aqueous solutions, no review exists that compares these different approaches to identify the most promising decontamination scheme for scenarios related to nuclear installations. In addition, these studies are conducted under specific environmental conditions with little information onto what extent the extraction capacity depends on the experimental conditions (e.g. pH, exposure time, amount of biomass used, etc.). In order to evaluate the applicability and feasibility of plant/algae based remediation systems for scenarios related to nuclear installations, a more in depth evaluation and testing of existing rhizofiltration/biosorption-driven remediation systems is much needed.

The goal of this research is to assess the potential of using algae (pro- or eukaryotic) and higher plants for the remediation of radioactively contaminated water within different scenarios related to nuclear installations.

A review is made of the existing literature on plant and algae based water decontamination systems for radionuclides relevant for contamination scenarios related to nuclear installations (e.g. <sup>131</sup>I, <sup>137</sup>Cs, <sup>90</sup>Sr, <sup>58</sup>Co, <sup>60</sup>Co, <sup>110m</sup>Ag, <sup>54</sup>Mn, <sup>59</sup>Fe, <sup>51</sup>Cr, <sup>56</sup>Zn, etc.). An important aspect of this literature review relates to the conditions in which the rhizofiltration/biosorption tests were performed as this determines the removal efficiency of the system (e.g. radionuclide loading, contact time, volume brought in contact with the contaminated water mass, the batch/flow-through setup, environmental conditions, etc.). Growth and nutrition of biomass and age can also affect rhizofiltration or biosorption due to changes in plant size/cell size, cell wall composition and extracellular product formation. For all organisms studied for bioaccumulation/biosorption, the minimum and optimal conditions of growth are identified. Environmental parameters such as pH, salt content, water hardness, temperature, etc. are considered. It is for example known that the biosorption of Cs and Sr by the *Laminaria* sp. (eukaryotic algae) is highly dependent on the pH of the solution causing up to 50 % variation in removal efficiency [1]. In addition the influence of competing ions on the uptake of radionuclides by plants is investigated. Sr<sup>2+</sup> uptake is for example reduced in the presence of Ca<sup>2+</sup> while Cs<sup>+</sup> uptake is restricted in the presence of K<sup>+</sup> [2-4].

The following step within this research is to perform a SWOT analysis (strengths, weaknesses, opportunities and threats) in a qualitative and descriptive way. Criteria that are considered are efficiency of radionuclide removal, robustness of the system to changing environmental conditions, efficiency of the system to remove a mixture of contaminants vs. specificity of decontamination, ease to grow or harvest biomass, ease to process the contaminated biomass, ease to apply the system in an industrial context, etc. Based on the results of the literature review and the preliminary SWOT analysis, the species and/or strains that are identified as most promising for the removal of radioactive pollutants from water systems will be selected as prime candidates for further research.

Although this study is still on-going, preliminary results based on 81 research studies indicate that phyto- and phyco-remediation techniques have a high potential for radionuclide removal in scenarios related to nuclear installations. Available data also point to rhizofiltration, using aquatic plants, and biosorption, using algae, as the most promising lines of techniques to decontaminate radionuclides from aqueous solutions. Because the accidental release of radionuclides will be mostly in a mixed form, it will be necessary to find out which combination of bioremediation schemes would be best for specific scenarios.

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## CHROMIUM BEHAVIOUR IN *SILENE VULGARIS*

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### ABSTRACT

Chromium is a transition metal which represents the seventh most abundant element on earth. Due to its corrosion-resistant properties it is used in many industrial activities. Its world production is in the order of 10,000,000 tons per year. The wide and increasing use of Cr in industry has led to the disposal and dispersal of large amounts of chromium into soils and waters making it a metal of important environmental concern. Phytomanagement could be a viable alternative in areas polluted with wastes from chromium-using industries. More important than the total chromium concentration is its speciation which determines its solubility, bioavailability and toxicity. Cr(III) and Cr(VI) are the two most common oxidation states of Cr but Cr(VI) is much more available and toxic to living organisms than Cr(III). *Silene vulgaris* (Moench) is a perennial dicotyledonous facultative methallophytewidely distributed throughout Europe, North America, Asia and North Africa. The tolerance of this species to a diversity of metals make it very useful in the initial stages of revegetation and soil remediation. This study investigated the ability of *S. vulgaris* to take up Cr(III) and Cr(VI).

Three experiments were performed. The first experiment was made with a population of *S. vulgaris* grown semi-hydroponically with different concentrations of either Cr(III) or Cr(VI). A combination of synchrotron X-ray spectroscopic techniques, scanning electron and light microscopy and infrared spectroscopy were used to determine the distribution and speciation of Cr and in *S. vulgaris*. A second experiment was made also in semi-hydroponic with different concentrations of either Cr(III) or Cr(VI) to determinate the influence of metal speciation in root exudation of two genotypes of *S. vulgaris*. The exudation rate, the elemental analysis and the concentration of organic acids were determined in the root exudates. And finally, a third experiment was made in soil conditions, artificially polluted with Cr(VI), to assess of rhizosphere bacterial communities in two genotypes of *S. vulgaris* grown on chromium polluted soil. The diversity of the microbial community was analyzed though the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and profiles of 16S rRNA gene fragments of culturable bacteria from bulk soil and rhizosphere.

The results showed that *S. vulgaris* accumulated more Cr when grown with Cr(VI), resulting in an overall reduction in biomass. Starch accumulation in leaves of *S. vulgaris* at higher Cr(VI) doses may be attributed to an impairment between carbon utilization and assimilation resulting in stunted plant growth but not the complete inhibition of photosynthesis indicating that *S. vulgaris* possess tolerance mechanisms that allows it to survive in Cr(VI) rich environments. One of these potential mechanisms is related to root exudates. Hexavalent Chromium expositions increased the root exudate release in *S. vulgaris*. Chromium accumulation and levels of lipid peroxidation in *S. vulgaris* genotypes were positive correlated to root exudates releases rates. The concentration of C, N and H, and specially S, clearly changes as consequence of hexavalent Chromium. The general banding profile corresponding to soil showed the largest number of bands in rhizospheric soil; similar pattern of bands among repetitions; different pattern of bands between the two studied genotypes; loss of bands and increased band intensity after chromium treatment.

The primary mechanisms implicated in Cr(VI) tolerance are a) the total reduction of Cr(VI) to Cr(III) in the rhizosphere or just after uptake in the fine lateral root tips and b) chelation of Cr(III) to the cell wall both of which reduce metal interference with critical cell functions and c) the rate of root exudates and the diversity of the microbial community analyzed. These mechanisms make *S. vulgaris* suitable for in situ remediation of Cr polluted soils.

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## ROLE OF PHRAGMITES AUSTRALIS (COMMON REED) FOR HEAVY METALS PHYTOREMEDIATION OF ESTUARINE SEDIMENTS

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### ABSTRACT

According to a recent study conducted by the European Commission, out of  $1.17 \times 10^6$  identified contaminated sites reported in 33 countries in Europe, only 58 have been identified remediated. The main contaminant category is heavy metals contributing 30-35% to soil and groundwater contamination respectively. There is a clear need for sustainable and appropriate sediment remediation technologies, which will depend on the site characteristics, concentration, types of pollutants to be removed, and the end use of the contaminated medium.

The focus of this study is on the investigation of phytoremediation of estuarine sediments contaminated with heavy metals. Therefore, for the purpose of this study, the ability of *Phragmites australis* to take up heavy metals (*i.e.*, Co, Ni, As, Mo, Cd, Pb, Se, Ba, Cr, Cu, Fe, Mn, Zn and Hg) from estuarine sediments on the northern Atlantic Spanish coast was investigated using a pilot plant experimental approach. The aim of this study was to elucidate the role of *Phragmites australis* in the restoration of sediments. Translocation factors were calculated for two ecotypes of *P. australis*, one adapted to contaminated estuarine sediments (M) and one adapted to non-contaminated estuarine sediments (G), both growing in estuarine contaminated sediment (R) saturated with freshwater during the length of the experimental research. Plants were harvested in October and December 2012, coinciding with the maximum annual vegetative growth and senescence periods respectively.

Results indicated that both ecotypes successfully grew in the contaminated sediment. For a period of four months ecotypes of *P. australis* adapted to sediment M increased their rhizosphere surface in a 1254%, while ecotypes of *P. australis* adapted to sediment G registered an increase of 219%, which suggests acclimatization in terms of vegetative development for ecotypes of *P. australis* adapted to contaminated sediment.

Results of heavy metals analyses indicated that a relative higher concentration of Fe, Mn and Zn, as compared to the rest of elements under investigation, was present in the sediments used as substrate for the experiment (R), as well as in the sediments where both ecotypes of *P. australis* were taken from in the field. All sediments presented a different profile in concentration levels. All elements analysed were present in all sediments. Hg was not detected in plant tissue samples. Translocation factors indicated that ecotypes of *P. australis* adapted to sediment M were able to upward translocate Ni, Mo, Se, Ba, Cr, Mn and Zn, and ecotypes of *P. australis* adapted to sediment G were able to upward translocate Ni, Mo, Se, Ba, Cr, Zn. Results also indicated that for both ecotypes most of the absorbed Co, As, Cd, Cu and Fe were retained belowground, phytostabilizing the metals and reducing their concentration in the sediments. These results suggested that *P. australis* can be used to immobilize some metals (Co, As, Cd, Pb, Cu, Fe) and store them belowground. The accumulation of some other metals in the aboveground biomass (Ni, Mo, Se, Ba, Cr, Mn, Zn) by *P. australis* showed a temporal variation with the highest values in the pre-senescence and senescence for ecotypes from G and M, respectively, which suggested the best time of the year to harvest within the first annual cycle.

A comparative analysis of results between ecotypes indicated that in pre-senescence period seven metals (Fe, Co, Se, Mo, Ni, Cr and Cu) were accumulated belowground in a major concentration by the ecotype M, while six (Cd, As, Pb, Zn, Mn and Ba) were accumulated in a major concentration by G. For the same period all the metals, with the exception of Cu, were accumulated aboveground in a major concentration by G. In senescence period all the metals were accumulated belowground in a major concentration in G, while aboveground five metals (Co, Mo, Ni, Cr and Ba) were in a higher concentration in G, and seven (Fe, Se, Pb, As, Zn, Mn and Cu) were highly accumulated by M, and Cd was found in the same concentration in both ecotypes. There is not a clear influence on the phytoremediation potential of *P. australis* associated to ecotypes adaptation to contaminated sediments.

**Acknowledgements:** The financial support by Ayuntamiento de Camargo, Cantabria, Spain.



## ZINC/CADMIUM ACCUMULATION AND CELLULAR LOCALIZATION IN A NEW HYPERACCUMULATOR *SEDUM PLUMBIZINCICOLA* GROWN IN MINE AREA

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### ABSTRACT

During the last decade there has been a growing interest in metal-hyperaccumulating plants, due to their unique physiology of metal tolerance and accumulation as well as their potentials for phytoremediation applications. Quantitative studies on the distribution of metals within plant tissues are important for understanding plant metabolisms, and the mechanisms of metal tolerance and hyperaccumulation. *Sedum plumbizincicola*, a new Cd/Zn hyperaccumulator has the potential phytoextraction for Cd/Zn polluted soils. But the accumulation ability and mechanisms of plant grown in mine area are still unknown. In this study, *Sedum plumbizincicola* plants and the corresponding rhizosphere soils were collected from four different plant growing places of mine area, and the localization of Zn/Cd within root, stem and leaf frozen-hydrated cross-sections of *Sedum plumbizincicola* was quantitatively investigated using Cryo-micro-Proton-Induced X-ray Emission (cryo- $\mu$ -PIXE). The results showed that *Sedum plumbizincicola* showed Cd/Zn hyperaccumulating ability with high Cd/Zn (1.47/14.6 g kg<sup>-1</sup>) in mine area, but the variation of Zn/Cd accumulation in plant was much more largely depended on the available metals than total metals in the soils. In root, 96.6% of Zn in frozen-hydrated root tissue was distributed in the cortex with an average concentration of 3111 mg kg<sup>-1</sup>, but Zn was low localized in central cylinder with an average concentration of 562 mg kg<sup>-1</sup>. In stem, Zn and Cd were preferentially accumulated in the epidermis with the average concentration of about 21375 and 1211 mg kg<sup>-1</sup>, respectively. Zn was also enriched in vascular bundle and its surrounding parenchyma. More interestingly, some of the vascular bundles were absent from Zn distribution. In leaf, the average concentration of Zn in the whole cross section of frozen-hydrated leaf was 1571 mg kg<sup>-1</sup>. Zn highly concentrated in the upper and lower epidermis with the concentration 5155 and 5660 mg kg<sup>-1</sup> respectively, while Zn concentrations in palisade and spongy mesophyll were 1201 and 1173 mg kg<sup>-1</sup>, respectively. While Cd extend relative evenly distributed in leaf with the highest concentration of 280 mg kg<sup>-1</sup> in vascular and the lowest concentration of 128 mg kg<sup>-1</sup> in spongy mesophyll, and 85.5 % of total leaf Cd was distributed in mesophyll when taking the tissue area into account. In conclusion, preferential sequestration of heavy metals especially Zn to epidermis in stem and leaf is one of the important mechanisms, and different mechanisms might operate Zn and Cd storage and detoxification in hyperaccumulator *Sedum plumbizincicola*.

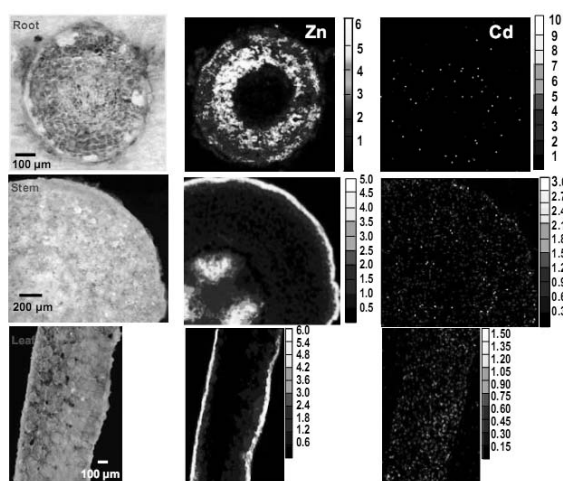


Figure 1 Optical image and elements mapping of *S. plumbizincicola* growth in mine soil (g kg<sup>-1</sup> FHW).

While Cd extend relative evenly distributed in leaf with the highest concentration of 280 mg kg<sup>-1</sup> in vascular and the lowest concentration of 128 mg kg<sup>-1</sup> in spongy mesophyll, and 85.5 % of total leaf Cd was distributed in mesophyll when taking the tissue area into account. In conclusion, preferential sequestration of heavy metals especially Zn to epidermis in stem and leaf is one of the important mechanisms, and different mechanisms might operate Zn and Cd storage and detoxification in hyperaccumulator *Sedum plumbizincicola*.

**Acknowledgements:** The financial support by the National Natural Science Foundation of China, project No. 41325003 is greatly appreciated.



## NATURALLY SELECTED BIO-ACCUMULATORS OF HEAVY METALS IN A BATTERY WASTE DUMPSITE IN IBADAN, SOUTH-WESTERN NIGERIA

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### ABSTRACT

A post impact assessment of a battery waste dumpsite was made in a local community acquired as a housing estate. Despite the devastation caused by the battery waste, certain group of plants grew luxuriantly. Soil and plant samples were therefore taken to evaluate the content of heavy metals in the soil and plants. Analysis was carried out in the Analytical Services Laboratory of International Institutes of Tropical Agriculture, (IITA), in Ibadan, Nigeria. Arrays of naturally selected bioaccumulation of heavy metals were identified by the level of Lead (Pb) in the roots and shoot/leaves of plants. The crops include: *Sporobolus pyramidalis* with (6,700 and 24,200 mgkg<sup>-1</sup>) lead in the leaf/stem and root respectively. In the same environment, *Panicum maximum* has 3,480 mgkg<sup>-1</sup> Pb in the leaf/stem and 21,000 mgkg<sup>-1</sup> in the root; *Imperata cylindrical* had 3,080 and 6,010 mgkg<sup>-1</sup> Pb in the /stem and root respectively. *Agaratum conizoides* contains 4,950 mgkg<sup>-1</sup> Pb in leaf/stem and 19,800 mgkg<sup>-1</sup> in the root. *Gomphrena celosoides* has 20,200 mgkg<sup>-1</sup> Pb in the leaf/stem and 23,610 mgkg<sup>-1</sup> Pb in the root. It was observed that there is generally more accumulation of Lead in the roots than the shoot. The above plants should be screened as phyto-mop of Pb and further investigation is being carried out to determine the efficiency of each plant for further use. Judging from allowable concentration of Pb in plants by WHO (0.02 – 0.05 mgkg<sup>-1</sup>), these plants are hyper accumulators and should be investigated further as phyto remediators.



## CADMIUM BIOAVAILABILITY IN AN INTEGRATED BIOSYSTEM, AND THE USE OF *AGARICUS SUBRUFESCENS* AS A REMEDIATION AGENT

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### ABSTRACT

**Introduction:** Cadmium in food stuffs receives considerable public and scientific attention due to this heavy metals toxicity and tendency to be taken up in crops. The bioavailability of Cd varies strongly in different materials, such as natural soils, organic wastes and inorganic fertilizers.

Integrated biosystems are connected food production systems where wastes are recycled into new crops in a sustainable way, often comprising industrial rather than traditional agricultural processes. We present here a case including anaerobic digestion of food waste connected to a closed greenhouse system in which mushroom and plant crops were grown using the digester residue (digestate) as well as some paper, straw and green waste. Cd enters with the raw waste; its mobility in the internal processes such as digestion, digestate dewatering, composting and crops growing was investigated. Some cultivated *Agaricus* mushroom species are known to bioconcentrate Cd. We were interested in testing if edible mushroom culture could be used to control Cd fate in the system.

**Methods:** Digestate was separated by filtration into a solid and liquid fraction. The solid fraction was composted 10 days with paper and straw for making mushroom compost [1]. *Agaricus subrufescens*, *A. arvensis* and *A. bitorquis* were grown in the mushroom compost. Some of the solid fraction was also vermicomposted using *Eisenia foetida*. Green wastes were composted thermophilically into a stable product. Lettuce, various herbs, tomatoes, beans and *Physalis* were grown in substrate mixes of digestate solids, vermicompost, spent mushroom compost and green waste compost. The liquid fraction of digestate was used for fertigation. Cd was measured by GF-AAS, ICP-AES and ICP-OES. Organic materials were also analyzed by a sequential extraction procedure for assessment of Cd bound to individual fractions: exchangeable, reducible, oxidizable and residual.

**Results:** Preliminary results from the overall system show that materials had total Cd concentrations between 0.1 and 0.9 mg kg<sup>-1</sup> dry matter (DM), thus within a “normal” range.

*A. subrufescens* had a strong effect on Cd mobility: Mushrooms from the first crop contained 2 to 30 mg kg<sup>-1</sup> DM, some far above the EU limit of ~8 mg kg<sup>-1</sup> DM (corresponding to the limit of 1 mg/kg fresh weight). However, concentration more than halved in the second crop and continued decreasing in consecutive crops. In digestate rich composts, the mushrooms extracted more than 80% of the cadmium present at start, mostly during the first crop, while mushrooms from the second and later crops in fact complied with the EU legislation for consumption.

Mushroom cadmium content was negatively related to mushroom yield and positively related to substrate total cadmium concentration. Within composts of similar cadmium content, but with varying digestate content, a higher digestate content lead to higher mushroom cadmium content. This will be more closely analysed using the sequential Cd extraction results when ready.

We are currently analysing the complete set of crops and materials for Cd and other heavy metals. The mass flow of the integrated biosystem will be presented.

**Conclusion:** When mushroom culture is appropriate in integrated biosystems or other waste-based cropping systems, *A. subrufescens* can be an efficient Cd remediation agent. The species has a strong ability to take up and translocate Cd to the fruitbodies. This should be of particular interest when working with more polluted wastes.

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1. Stoknes, K., Beyer, D. and Norgaard, E. (2013) Anaerobically digested food waste in compost for *Agaricus bisporus* and *Agaricus subrufescens* and its effect on mushroom productivity. *J Sci Food Agric* Jul 93(9), 2188-200

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## PLANT UPTAKE AND TRANSLOCATION OF $^{223}\text{Ra}$ AS A POTENTIAL RADIOPHARMACEUTICAL NUCLIDE

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### ABSTRACT

The radiopharmaceutical therapy and diagnosis play important role in the treatment and imaging of cancer diseases and often make possible healing or life prolongation at malignant prognosis. Together with the use of classical radiopharmaceutical nuclides such as  $^{99\text{m}}\text{Tc}$ ,  $^{51}\text{Cr}$ , or  $^{67}\text{Ga}$ , the new radioisotopes with better characteristics of emitted particles are continuously investigated. One of new radionuclide for bone therapy is the  $^{223}\text{Ra}$ . Radium-223 is an alpha emitter with a half-life of 11.4 days and after emission of 4 alpha and 2 beta particles decays finally to a stable  $^{207}\text{Pb}$ . The general problem at radiopharmaceutical therapy and/or diagnosis is the impossibility of total recovery of a radioactive material into the radioactive waste. Thus part of the activity is excreted, preferably by urine into municipal or hospital wastewaters and can cause problems in the contamination of the ecosystem. For these reasons it is needed to investigate the possibilities of the removal of these radionuclides. The effective technology should be phytoextraction as additional step to conventional sewage treatment plants. Simultaneously the uptake and translocation of these radionuclides in agriculturally interesting plants can be investigated.

The phytoextraction of  $^{223}\text{Ra}$  was investigated using in vitro cultures of common cultivated plants (*Zea mays*, *Helianthus annuus*, *Brassica napus* and corn plants).  $^{223}\text{Ra}$  was eluted from  $^{227}\text{Ac}/^{223}\text{Ra}$  generator as nitrate and added to cultivation solution. Murashige-Skoog or tap water was used as cultivation medium. To the 2-4 weeks old cultures (depending of plant species) the  $^{223}\text{Ra}$  contaminated medium was added and cultures were cultivated at common conditions. After one or two weeks the plants were removed, root system intensively washed and the radioactivity distribution in plants was evaluated using electronic autoradiography.

The results show that  $^{223}\text{Ra}$  is uptaken and absorbed by the root system, the uptake is markedly plant dependent. Moreover the uptake is strongly influenced by cultivation medium. The significant differences were found at cultivation in medium and tap water. Although the total uptake was below 10 % of inserted radioactivity, it was clearly documented, that the studied radionuclide is not only adsorbed on root surface, but it is effectively absorbed into plant tissue and translocated to shoot parts of plants.

It can be concluded, that the hypothesis about the possibility of food chain contamination by radiopharmaceutical therapy is true and the investigation of the methods for hospital sewage water is needed. The use of contaminant chelators or plant water transport accelerators will be used in further investigations to obtain more effective extraction of studied contaminant.

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## RECOVERING METALS FROM SEWAGE SLUDGE, WASTE INCINERATION RESIDUES AND SIMILAR SUBSTANCES WITH HYPERACCUMULATIVE PLANTS

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### ABSTRACT

Sewage sludges as well as ashes and slags from waste incineration plants are known accumulation sinks of many elements that are either important nutrients for biological organisms (phosphorus, potassium, magnesium, etc.) or valuable metals when considered on their own in pure form (nickel, chrome, zinc, etc.); they are also serious pollutants when they occur at localized anthropogenic end-of-stream points. Technologies to recover these metals in a reasonable and economically relevant manner from very diffuse sources are practically non-existent or require large amounts of energy and chemicals, which pose environmental risks. Often more than 90% of these same metals have to be imported from abroad for technological use. These primary resources are becoming more expensive as they become more scarce and remaining deposits more difficult to mine, which is a serious concern for industrialized nations. Basic economic and strategic reasoning demands an increase in recycling activities and waste minimization.

On the other hand agriculture uses large volumes of mineral fertilizers, which are often sourced from mines as well, and thus are also subject to the same principle of finiteness and potential shortage in supply. These converted biological nutrients are taken up by crops and through the food chain and human consumption end up in sewage systems and in wastewater treatment plants in great quantities. The metabolized nutrients mostly do not return to agriculture, but due to contamination with heavy metals are diverted to be used as construction aggregates or are thermally treated and end up rather uselessly in landfills.

The project BIO-ORE aims to explore new pathways to concentrate metals from diluted sources such as sewage sludge and wastewater by using highly efficient biological absorption and transport mechanisms. These enzymatic systems from plants work with very little energy input. The process is called bioaccumulation and can be most effectively observed in hyperaccumulating metalophytes, which are studied for its suitability to be incorporated in metal recovery processes.

In a systematic series of tests under laboratory conditions the accumulation behaviour for antimony, chrome, cobalt, manganese, molybdenum nickel, zinc and rare earth metals of a selection of candidate plants growing on sewage sludge is assessed (quantitatively and qualitatively). Growth performance of these plants as well as the most suitable substrate properties were evaluated. Furthermore, an overview of the locations in Austria will be compiled where wastewater streams with comparatively high concentrations of the selected metals occur in interesting quantities. Lastly, stakeholders from all the process steps, from the sewage sludge treatment until final metal refinement, were involved and consulted early on in order to develop the best metal recovery strategies.

The results of this project provide the groundwork for further research and development steps that may bring to practical implementation a technological option with potentially huge benefits:

- The recovery of valuable metal resources from sewage sludge, incineration ashes and metal rich waste waters by environmentally friendly and low energy means
- Simultaneous decontamination of the input substrates from heavy metals, opening the possibility for these nutrient streams to be redirected to biological regeneration processes (for example use as fertilizers in agriculture) without fear of polluting soils with heavy metal loads





- Simultaneous generation of biomass on contaminated substrates, which can yield usable energy surplus through incineration during processing

Metal accumulation occurred in all used plants under certain conditions. Nitrogen seems to play an important role for the plants and their decision, which metals to (hyper)accumulate. Unfortunately *Pteris cretica* showed the worst metal accumulation in comparison to the other plants. *Alyssum murale* showed interesting accumulations for molybdenum in nutrient rich conditions of sewage sludge and good accumulation for nickel, rubidium and manganese under poor nutrient conditions. The accumulator plant *Dryopteris filix-mas* could be interesting for further investigation about vanadium and molybdenum. Two varieties of sunflower were tested, where rubidium and manganese showed promising results. *Eichhornia crassipes* was grown on watered industrial sludge and showed high accumulation for cadmium, nickel, rubidium and zinc. *Phytolacca americana* will also be tested in further trials, as they are said to be one of the rare earth accumulators, which could be partly proven by this first trials.

All in all, the results look very promising to keep up the work and stretch it to other waste streams as well. Therefore a national follow-up research project started this March 2014, where two years of field trials with hyperaccumulative plants and further phytomining strategies (recovering of metals) will be developed.

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## POTENTIAL OF INDIAN MUSTARD FOR RHENIUM PHYTOMINING

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### ABSTRACT

Rhenium (Re) is one of the rarest elements found on Earth, with an estimate concentration of 0.4 ng g<sup>-1</sup> in the upper crust, 2.2 pg mL<sup>-1</sup> in freshwater, and 7-8 pg mL<sup>-1</sup> in seawater. Due to its scarcity, Re is also one of the most expensive metals in the world market, presenting a high demand in the aerospace industry where it is used as a high-temperature superalloy. Bioavailable ReO<sub>4</sub><sup>-</sup> ions are widely dispersed in areas surrounding copper-molybdenum mines, and their accumulation in plants of these regions has been reported. This opens a window of opportunity for phytomining, a cost effective and environment friendly plant based technique to extract precious metals from low grade surface ores or mineralized soils. The objective of this study was to evaluate the potential of Indian mustard (*Brassica juncea* (L.) Czern), a species well known for its aptitude in the field of phytoremediation, to phytomine Re.

Commercial organic substrate was amended with KReO<sub>4</sub> (potassium perrhenate) to attain 5 different treatments according to the substrate Re concentration: 5, 10, 20, 40, and 80 mg kg<sup>-1</sup>. Healthy 14-day-old Indian mustard seedlings were then transferred to plastic pots containing the spiked substrate, at the rate of 5 replicates per treatment/harvest. The plants were allowed to grow under greenhouse conditions, and soil moisture was kept at 35 ± 5% with deionized water, according to field capacity. The plants were harvested for analysis 50 and 80 days after sowing. Fresh and dry biomass weights of the roots and shoots were determined, and the Re content of both plant parts was assessed through inductively coupled plasma optical emission spectrometry (ICP-OES). Analysis of variance (ANOVA) and homogeneity of variance tests were performed (p ≤ 0.05). Tukey and Dunnett's T3 tests were carried out for post-hoc comparisons between groups.

At the end of the experiment, the Indian mustard plants developed in the 5 mg kg<sup>-1</sup> treatment presented significantly higher shoot dry weight values. The aboveground dry biomass reductions of the plants grown in the 10, 20, 40 and 80 mg kg<sup>-1</sup> treatments in relation to those of the 5 mg kg<sup>-1</sup> treatment were of 29.46%, 37.87%, 43.26%, and 58.72%, respectively. The concentration of Re in shoots at the final harvesting period was strikingly higher in the plants subjected to the 80 mg kg<sup>-1</sup> treatment, registering a mean of 23396.2 mg kg<sup>-1</sup> (Figure 1). In comparison to the results of the 5, 10, 20 and 40 mg kg<sup>-1</sup> treatments, this implied an increment of 1635.88%, 769.88%, 253.43%, and 200.38%, respectively. Nevertheless, the content of Re in the shoots of all treatments were well above the threshold levels of hyperaccumulation for this metal.

Accordingly, the bioconcentration factor for the plants of all treatments was two orders of magnitude higher than 1.

The findings of this study confirm the suitability of Indian mustard for Re phytomining. Although further research is required to assess the profitability of the process, the results suggest that potential earnings from Re phytomining with Indian mustard could be used to at least finance the phytoremediation of copper-molybdenum mining areas.

**Acknowledgements:** The authors gratefully acknowledge financial support from the National Council for Scientific and Technological Development of Brazil (CNPq) under process No. 150084/2014-5.

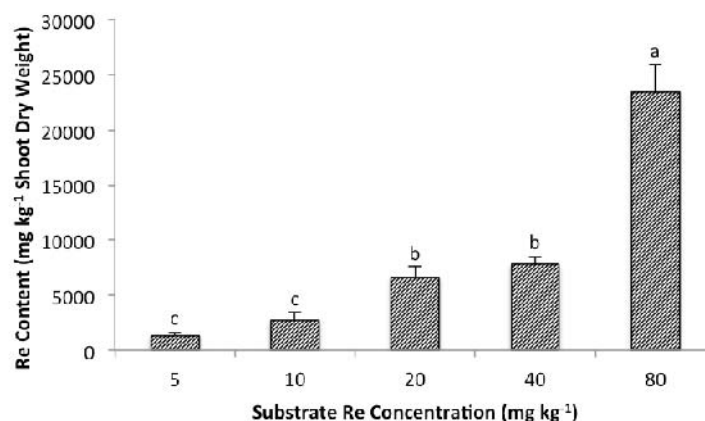


Figure 1. Concentration of Re in the shoots of Indian mustard 80 days after sowing. Data are means ± SD. Values followed by different letters differ significantly at p ≤ 0.05, n = 5.



## LOCALISED TRACE METAL SOLUBILISATION AND OXYGEN DEPLETION IN THE RHIZOSPHERE OF *SALIX SMITHIANA* UPON ADDITION OF ELEMENTAL SULFUR

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### ABSTRACT

Phytoextraction by metal-accumulating willows such as *Salix smithiana* has shown promising results for Zn and Cd. However, even on moderately polluted soils, the applicability of phytoextraction is often hindered by presumably long remediation times. Enhanced contaminant solubility and, in consequence, increased contaminant accumulation in plants (*i.e.* induced phytoextraction), may contribute to higher phytoextraction efficiency, lower remediation times and thus enhance the practical applicability. However, induced phytoextraction may also increase the risk of contaminant leaching to groundwater. This risk might be low when elemental sulfur ( $S^0$ ) is used as soil amendment for a controlled and steady mobilization of trace metals.

Here we present data from a rhizobox experiment where *S. smithiana* was grown on a slightly acidic, moderately metal polluted soil with and without application of  $S^0$ . We collected soil pore water from rooted and bulk soil compartments using Rhizon samplers and measured pH,  $SO_4^{2-}$ ,  $NO_3^-$  (IC) and metal concentrations (ICP-MS). After 61 days, plants were harvested and analysed for biomass production and metal concentrations. Additionally, willows were grown in rhizotrons for monitoring potential localised changes in metal solubility and soil respiration in the willow rhizosphere in response to  $S^0$ . Chemical images of the distribution of metals and  $O_2$  in the root zone were obtained using diffusive gradients in thin film (DGT) gels and planar optode (PO) sensors. We analyzed the DGT gels for the metal distribution in the rhizosphere using laser ablation-ICP-MS, whereas  $O_2$  concentrations (PO) in the rhizosphere were assessed using a colour-ratiometric approach.

Our results from the rhizobox trial showed significantly increased Mn, Zn and Cd concentrations in the sulfur treatments compared to the control due to soil acidification caused by the oxidation of  $S^0$ . Enhanced metal mobilization was particularly observed in the  $S^0$  amended rhizosphere compartments. The corresponding large concentrations of Mn along with  $O_2$  depletion in the rhizosphere indicated a reduction of Mn (oxy)hydroxides, serving as electron acceptors during sulfur oxidation in  $O_2$ -depleted zones. Enhanced metal solubility in the  $S^0$  treatments was generally associated with substantially increased uptake of Cd and Zn in willow tissues. Chemical images of DGT-available Zn and Cd show clear, root associated depletion zones in both, control and  $S^0$  treatments. Higher bulk soil metal concentrations and root associated solubilisation hot spots were observed in the  $S^0$  treatment. Increases of dissolved Mn were associated to both, sulfur application and patches of higher soil compaction. PO mapping results showed patchy, root associated, oxygen depletion in the  $S^0$  treatment, while  $O_2$  depletion was almost absent in the control, supporting the hypothesis of reduction of Mn (oxy)hydroxides upon oxygen depletion due to the oxidation of elemental sulphur.

We conclude that the observed magnification of metal mobilization in the partially anaerobic willow rhizosphere, *i.e.* at the location of plant uptake, upon addition of  $S^0$  has a good potential for enhancing phytoextraction efficiency while keeping the risk of metal leaching from the bulk soil relatively low.

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## HOW DO LOW DOSES OF DFOB AND EDTA AFFECT THE PHYTOEXTRACTION OF Cd, Cu, Pb AND Zn BY *HELIANTHUS ANNUUS*?

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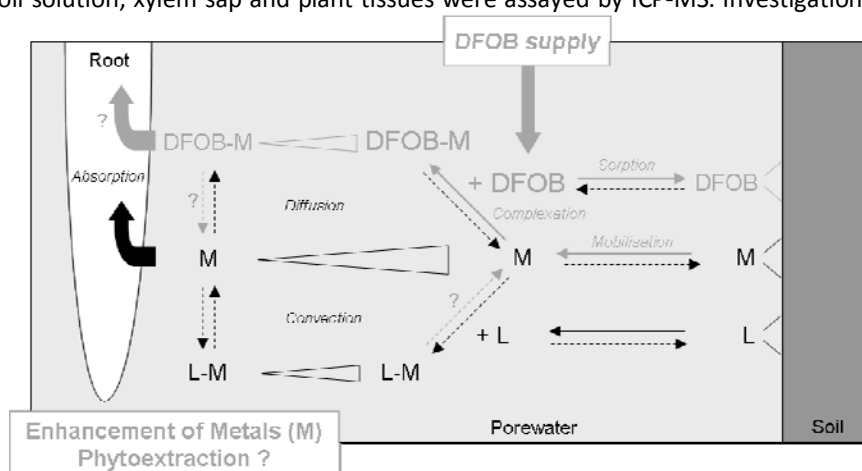
### ABSTRACT

Phytoextraction is the only way for the *in situ* cleaning-up of metal-contaminated soils. This technology is environmental friendly and cost-effective compared to physico-chemical clean-up process but its effectiveness must be improved to cope with the requirements of planners and users. The main weakness of phytoextraction is the long cleaning-up duration (Baker et al. 2000) primarily caused by the low phytoavailability of metals in soil. Inoculation of siderophore-producing bacteria (SPB) has been recently proposed as a strategy to optimize metal phytoextraction (Rajkumar et al. 2010). The localized and continuous production of siderophore in the close vicinity of plant roots, where most of the SPB are established, would help in promoting metal phytoextraction with minimizing the risk of metal leaching. However, there is a need to dissect how siderophores interact with metals at the soil-root interface to better assess the potential of coupling phytoextraction with the inoculation of SPB.

The present study focuses on desferrioxamine B (DFOB), the main siderophore produced by the actinobacteria *Streptomyces pilosus*. DFOB is characterized by a high selectivity for Fe(III) ( $10^{31} \text{ M}^{-1}$ ) but also by a good affinity for divalent metals including Cd(II) ( $10^{7.9} \text{ M}^{-1}$ ), Cu(II) ( $10^{14.1} \text{ M}^{-1}$ ) and Zn(II) ( $10^{11.1} \text{ M}^{-1}$ ). The purpose of this work was to compare the efficiency of a low dose of DFOB vs. EDTA on the phytoextraction of Cd, Cu, Pb and Zn by sunflower. The main goal was to dissect the impact both chelators have on (1) the metals mobilisation from the solid phase, (2) their speciation in soil solution, (3) their uptake by plant roots and (4) their translocation from roots to shoots, for two cultivars of sunflower grown on an agricultural poly-contaminated soil.

Two cultivars of sunflower (ES RICA, KAPPLAN) with contrasted pattern of metal repartition (Laporte et al., 2014) were grown for 28 days on a calcareous soil (pH 7.3) contaminated in Cu, Cd, Pb and Zn (334, 5.2, 1191 and 561  $\text{mg kg}^{-1}$  soil, respectively). Four days after transplanting, DFOB was supplied at the concentration of 200  $\mu\text{mol kg}^{-1}$  soil to mimic bacterial production. The same procedure was performed for EDTA. Soil solution was extracted every week (on days 9, 16 and 23) using Rhizon soil moisture samplers. At harvest, xylem sap was collected on every plant by the "root pressure" method. Then, plants were divided into roots and shoots, and all plant tissues were freeze-dried, weighed, milled and digested in a mix  $\text{HNO}_3\text{-H}_2\text{O}_2$ . The concentrations of metals (Fe, Cu, Cd, Pb, Zn) in soil solution, xylem sap and plant tissues were assayed by ICP-MS. Investigations are in progress to quantify in soil solution (i) the concentrations of DFOB and EDTA using the Fe-CAS complex, and (ii) the labile fraction of metals by anodic stripping voltammetry.

The results will provide new insights on the mechanisms by which DFOB alters the soil-plant transfer of metals and, thus, on the efficiency of coupling phytoextraction with the inoculation of siderophore-producing-bacteria.



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## NEW INFORMATION FOR PHYTOEXTRACTION AND PHYTOMINING

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### ABSTRACT

Ni-hyperaccumulators have the ability to store more than 10,000  $\mu\text{g Ni g}^{-1}$  of dry matter (DM) in their tissues without heavy metal toxicity symptoms [2]. Nickel content in the organs of 14 hyperaccumulators from three genera: *Alyssum*, *Leptoplax* and *Bornmuellera* collected in Greece and Albania were analyzed [4]. Moreover, two different growing systems aimed to improve the yields of nickel phytoextraction were investigated, and the better cropping system was chosen for the sustainable development of *A.murale*. Results showed *Alyssum murale* (*A.murale*) grown in the Balkans (Albania) had yields of at least 100 kg Ni per ha.

For the huge biomass treatment, an optimized process to obtain a high value of ammonium nickel sulfate hexahydrate (ANSH) was studied in detail [1,3]. Once harvested and dried, *A. murale* biomass is incinerated to obtain concentrated Ni ashes to ensure the success of phytomining. This process is based on acid leaching for Ni extraction, purification of other elements in the leachate and different crystallisation steps to prepare the product at a high purity ( $\geq 99\%$ ). The main features of this process are presented as well as the newest results obtained at the pilot scale. We have clearly demonstrated the feasibility of the synthesis of this nickel double salt after phytoextraction and a start-up project has been launched to develop this activity.

To complete this approach, other extraction methods and synthesis are currently investigated to obtain various Ni products and will be presented.

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## OPTIMISATION OF AMENDMENT EFFECTIVENESS IN STABILISATION OF METALS IN HEAVILY CONTAMINATED SOILS

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### ABSTRACT

Metals can be stabilized in soil by amendments increasing metal adsorption or altering their chemical form. Few experiments compare different amendments under similar environmental conditions, or consider whether all soil properties or functions (microbes, soil fauna, plant growth, retention, colloid stability, etc.) are similarly protected. Within the EU FP7 Greenland project (266124) we compared the impact of novel soil amendment combinations and traditional materials on metal solubility and response of plants, soil organisms and microbial activity.

One-year greenhouse pot experiments were established: soil A, less toxic agricultural soil contaminated through long-term Zn/Pb smelter emissions in Poland (pH 7.0); soil B, toxic soil contaminated through smelter dust spill in Poland (pH 6.8). Amendments were tested individually and in novel combinations in planted and unplanted soils: compost (GWDA), drinking water residue (DWR), iron grit (IG), Ca-phosphate (PO<sub>4</sub>), LD slag (LD), Thomas basic slag (TBS), gravel sludge and siderite (GS/SID) and cyclonic ash (CA). Soil B was planted with grasses, and soil A with lettuce. Plants were periodically harvested, yields recorded and metal content determined. Soil metal extractability and bioaccessibility, pH, EC and enzymatic activity were measured. Soil pore waters were analysed for trace element/nutrient concentrations.

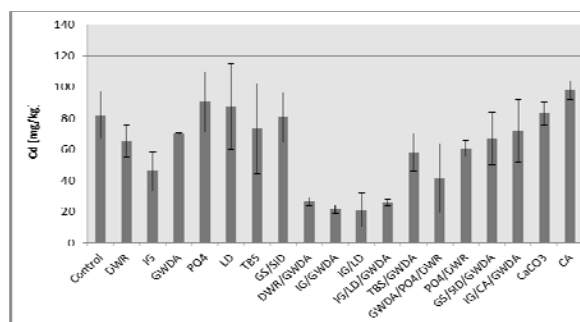
Parallel tests evaluated earthworm behaviour and metal accumulation. Earthworms *Eisenia veneta* were put into jars – five earthworms (previously weighed to record the initial weight) to each jar. The jars were stored at 20 °C and soil moisture was maintained at field water capacity. Earthworms were removed from the soil after 4 weeks, weighted and analysed for metals content (Figure). The soil was thoroughly mixed and samples were taken for the determination of metal solubility and pH.

The earthworms were removed from soil and kept on moist filter papers in glass vessels for 3 days for full depuration of worms. Then they were washed in deionized water, dried on paper towels, and killed by dry-freezing. The dry weight of the earthworms was recorded and the samples were dissolved in concentrated nitric acid (Baker Analyzed Instra). Metal concentrations in earthworm extracts were measured with inductively coupled plasma mass spectrometry (ICP-MS, AGILENT 7500CE).

Plant-associated bacteria (rhizospheric or endophytic) can improve plant establishment, growth and survival in contaminated sites. In the next study we tested whether effect of selected amendment combinations can be enhanced by bacterial inoculation of plants (*Festuca arundinacea*). Eight amendment combinations were tested at 2 rates and with 2 types of inoculants.

We measured plant yield and metal concentration and metal soil extractability as indicators of the inoculation effectiveness.

**Acknowledgements:** The financial support by FP-7 project GREENLAND contract No. 266124 is greatly appreciated.



## AIDED PHYTOSTABILISATION OF A Cr, Mo AND Ni-CONTAMINATED TECHNOSOL

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### ABSTRACT

The metal-contaminated technosol of a steel smelter heap at Rive-de-Gier, France, displays high potentially available Cr, Ni, and Mo pools with low organic matter and nutrient contents. At this site, aided phytostabilisation may restore a vegetation cover, reduce labile soil Cr, Ni and Mo concentrations, decrease exposure pathways and thus pollutant linkages, notably metal(loid) transfer through natural agencies in the environmental compartments and into the food chain. This study aimed at assessing in controlled conditions the efficiency of Ni/Cd-tolerant plants in mixed stand and soil conditioners to perform aided phytostabilisation on a metal (Cr, Ni, and Mo)-contaminated technosol, while avoiding to increase herbivore exposure.

Plots were implemented on the technosol of Rive de Gier. Soil treatments included: untreated soil (UNT), soil amended with either ramial chipped wood (RCW) or composted sewage sludge (CSS). Topsoils were collected in these plots and an uncontaminated kitchen garden (CTRL) and placed in pots (in triplicates). Potted Unt soil was also amended to singly test two other conditioners, i.e. vermiculite (5%) with compost (5%) (VOM), and iron grit (1%) with compost (5%) (OMZ). Seeds of Ni/Cd tolerant ecotypes of *Festuca pratensis* Huds., *Holcus lanatus* L., and *Plantago lanceolata* L were planted on all soils. Soil pore water was collected, pH and electrical conductivity were measured. After 15 weeks, shoots were harvested. Shoot ionome and soil pore water were analyzed by ICP-MS.

The pH of the soil pore water decreased from 10.2 (Unt soil) to 8.7 (CSS) and 8.8 (RCW) but did not change for the VOM and OMZ treatments. Soil amendment influenced metal concentrations (in mg L<sup>-1</sup>) in the soil pore water: Cr concentration decreased from 1.5 mg (UNT) to 0.01 - 0.3 for all amended soils; Mo concentration fell but to a lower extent from 3.5 mg (UNT) to 1.6 - 2.8 on amended soils; whereas Ni concentrations were similar for all soil treatment, i.e. 0.01 - 0.02. Nutrient concentrations and soluble organic matter (SOM) increased in the CSS soil pore water.

After 15 weeks, *F. pratensis* developed well on all soil treatments while *H. lanatus* and *P. lanceolata* only grew on the CTRL, UNT, CSS and CTRL soils and with a low growth rate. The shoot DW yield of *F. pratensis* for the CSS soil was 2 fold higher than that for the CTRL soil: it reached 375 ± 71 g m<sup>-2</sup> (CSS) compared to 162 ± 5 g m<sup>-2</sup> (CTRL). For the UNT, RCW, VOM and OMZ soils, it varied between 12 and 39 g m<sup>-2</sup>.

Shoot Cr, Ni and Mo concentrations of *F. pratensis* significantly decreased for the CSS treatment compared to the Unt one, i.e. from 31 to 0.8 mg Cr Kg<sup>-1</sup>, 16 to 1.1 mg Ni Kg<sup>-1</sup> and 308 to 108 mg Mo Kg<sup>-1</sup>. The RCW, VOM and OMZ treatments reduced between 3 and 7 times the shoot Cr concentration of *F. pratensis*. However, these treatments did not change significantly its shoot Mo and Ni concentrations, which remained between 186 and 376 mg Mo Kg<sup>-1</sup> and 8 and 16 mg Ni Kg<sup>-1</sup>.

The CSS treatment best performed to decrease Cr concentration in the soil pore water, increase shoot DW yield of *F. pratensis* and reduce its shoot Ni and Mo concentration. The increase of nutrient concentration in the CSS soil pore water may stimulate shoot production of this Ni/Cd-tolerant *F. pratensis* ecotype which allowed the dilution of the high Ni and Mo concentrations in *F. pratensis* shoot. Other soil treatments did not improve shoot yield of *F. pratensis* and such high shoot Ni, Cr and Mo concentrations may potentially affect herbivores.

Cultivation of a Ni/Cd-tolerant population of *F. pratensis* on this contaminated technosol amended with CSS is a successful combination to restore a dense grass cover. However the shoot Mo concentration of *F. pratensis* remains elevated. Direct evidences of an aided phytostabilisation of metals or only their in situ stabilization/phytoexclusion need further long-term assessment in plots.

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## TRACE METALS IN GROWTH MEDIUMS AND PLANT TISSUE MATERIAL FROM A REMEDIATED GOLD MINE TAILINGS DAM IN SOUTH AFRICA

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### ABSTRACT

Remediation and rehabilitation of gold tailings is not necessarily a new science but rather a collection of established technologies that have been adapted to suit a new situation. Environmental aspects fundamental to the remediation or rehabilitation of mine dumps and tailings dams are a) aesthetics – shape, form, colour; b) air quality – dust, radioactivity, fumes; c) water quality – surface and ground water; d) soil quality and functions – sterilisation, pollution, land use potential; e) sustainability; and f) post closure land use after mining. Based on the complex integration of different remediation attributes, the most difficult aspects to achieve are the sustainability of applied rehabilitation and remediation techniques, and the post closure land use of tailings dams and mine dumps.

Geochemical imbalances and ongoing chemical reactions, e.g. oxidation of pyrite, do not favour the physical stability of mine dumps and tailings dams. Restrictive factors include the germination potential and survival rate of established vegetation on a hostile growth medium as well as the uptake of potentially toxic trace metals which is an important factor in the consideration of grazing as a proposed post closure land use option.

The geochemical aspects of a remediated gold tailings dam, together with concentrations of specific trace metals in the plant tissue growing on the tailings dam are discussed in this paper. The concentrations of macronutrients and ten other potentially toxic trace metals from tailings and top soil as well as three grass species used for remediation, are compared with each other in order to identify the species most suitable for remediation purposes.

Different correlations exist between soil pH and the bioavailability of trace metals. At pH < 5.3, bioavailable concentrations are distinct for Cr, Co, Ni, Zn and Cd, moderate for Mn and As, and non-existent for Cu, Pb and U. The highest macronutrient concentrations are found in the grass *Digitaria eriantha*, and concentrations of Mn, Co, Zn and As are highest in *Cynodon dactylon*. *Hyparrhenia hirta* shows a great affinity for Cr, and *Digitaria eriantha* for Pb. Only Cu and U exceed toxic limits in *Hyparrhenia hirta* and *Digitaria eriantha* at pH < 5.5.

In conclusion, it is possible to determine the suitability of different species for grazing as a post closure land use option. Relative to this, pH control is an important factor in the limitation of trace metal bioavailability in growth mediums, i.e. gold tailings.

**Keywords:** remediation, rehabilitation, gold tailings, macronutrients, trace metals, absorption, bioavailability





## ASSESSMENT OF PHYTOSTABILIZATION OF Pb AND Zn IN A TAILING POND FROM SE SPAIN

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### ABSTRACT

Unreclaimed mine tailings are a worldwide problem due to tailings piles present a source of contamination for nearby communities. These mining areas are affected by the eolian dispersion and water erosion; moreover the wastes from tailing ponds are characterized by high contents of Fe-oxhydroxides, sulphides and heavy metals. Aided phytostabilization consists of using both plants and organic/inorganic amendments in order to reduce contaminants mobility and bioavailability and recover soil health. In this study, Santa Antonieta tailing pond, located in Cartagena-La Unión mining district (SE Spain), was selected for applying the aided phytostabilization. Different samplings (before application, 6, 12, 18 and 24 months after application of marble waste, raw pig slurry and the solid phase of pig slurry), were carried out. Soil physicochemical and chemical properties were determined, and the vegetation biomass and the Pb-Zn concentrations in different parts (roots, leaves and stems) of the vegetation were analyzed. Results showed that pH, total nitrogen, cation exchangeable capacity, total organic carbon, aggregate stability, available phosphorous and exchangeable potassium increased while salinity decreased after amendments application. In addition, the available concentrations of Pb were reduced from 2019 to  $< 1 \text{ mg kg}^{-1}$  and for Zn from 107 to  $< 5 \text{ mg kg}^{-1}$ . Fertility improvement and reduction of metals mobility promoted the development of plants and continuous increase of biomass during 18 months after amendments application. The roots of *Lygeum spartum* accumulated the highest Pb and Zn concentrations ( $\sim 146$  and  $\sim 290 \text{ mg kg}^{-1}$ , respectively), exceeding the Pb concentrations in the roots of this plant the toxicity values proposed by different authors; while the concentrations in shoots were lower than  $10 \text{ mg Pb kg}^{-1}$  and  $60 \text{ mg Zn kg}^{-1}$ . In addition, *Cynodon dactylon* accumulated high concentrations of Zn in roots and shoots ( $> 250 \text{ mg kg}^{-1}$ ).

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## (AIDED)-PHYTOSTABILISATION OF Cu-RICH MINE TAILINGS USING WOODY CROPS AND GRASS SPECIES

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### ABSTRACT

Mine-spoils and tailings present hostile environments for plant growth (low nutrients, low organic matter content, high acidity, and elevated trace metal content). Phytostabilisation techniques aim to establish a vegetation cover and progressively promote *in situ* inactivation of trace metals by combining the application of soil amendments with the cultivation of metal-tolerant populations of plant species.

This study was carried out in an abandoned copper mine in Touro, Galicia (NW Spain). The mine operated from 1974 until 1988 but is now confined to the extraction of materials for road construction. Mine tailings cover an area of approximately 550 ha. The objectives of the study were to reduce Cu mobility and availability in the mine-soils and establish either a short rotation coppicing system or a grass cover. The geological substrate is amphibolite, with significant quantities of metal sulphides (pyrite, pyrrhotite, and chalcopyrite). The mine-soils (Spolic Technosols (Episkeletic)) are characterised by their extreme acidity (pH 2.8-3.5), low C, N and P, and high concentrations of Cu (319-774 mg/kg). Field plots (10m x 45m) were established in Spring 2011 and treated with one of three amendments. Amendments consisted of composted municipal solid wastes (compost) and two technosols. Technosols were mixtures of organic (anaerobic and aerobic sewage sludge) and inorganic wastes (aluminium oxides, iron oxides, fly ash from wood bark combustion, and foundry sand). Application rates of all amendments were equivalent to 250 kg Mg<sup>-1</sup> dry tailings. Compost-amended plots were planted with different metal-tolerant clones of *Salix* (*S. caprea* and *S. viminalis*) and *Populus nigra*. The experimental plot was sub-divided into 5m x 5m sub-plots, and three replicate sub-plots were used for each plant species/clone. *Agrostis capillaris* cv. Highland was planted in three replicate sub-plots in both the compost- and technosol-amended plots. Soil samples were collected to determine the baseline trace metal content and general physico- and bio-chemical properties before amendment addition/planting. Plant growth/survival and soil properties (improvements in pH, nutrient availability and soil enzyme activities, and reductions in Cu bioavailability) were determined one and two years after planting. Shifts in the soil microbial community with time were evaluated using the denaturing gradient gel electrophoresis (DGGE) technique.

Soil Cu concentrations at time zero showed a high heterogeneity across the experimental field plots. Nonetheless, the addition of amendments led to an increase in soil pH, C/N content, cation exchange capacity and nutrient availability. At the same time amending soils led to a significant decrease in the NH<sub>4</sub>NO<sub>3</sub><sup>-</sup> and NaNO<sub>3</sub>-extractable concentrations of Cu (<0.2 mg kg<sup>-1</sup> extractable [Cu]) and shoot Cu concentrations remained consistently below 25 mg kg<sup>-1</sup>. Few differences were observed in soil physicochemical properties between the three amendments after 2 years of treatment. However, plant growth, soil enzyme activities and microbial community structure were significantly influenced by both the amendment type and plant species.

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## EDTA AIDED PHYTOREMEDIATION OF CADMIUM BY *BRASSICA NAPUS* L.

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### ABSTRACT

Heavy metals pollution of soil is a prevalent global problem and oilseed rape (*Brassica napus* L.) are considered useful for the restoration of metal contaminated soils. Phytoextraction is an in-situ environment friendly technique for the clean-up of contaminated soils. Response to cadmium (Cd) toxicity in combination with a chelator, Ethylenediaminetetraacetic acid (EDTA) was studied in oilseed rape grown hydroponically in greenhouse conditions under three levels of Cd (0, 10, and 50  $\mu\text{M}$ ) and two levels of EDTA (0 and 2.5 mM). Cd decreased plant growth, biomass and chlorophyll concentrations while the application of EDTA enhanced plant growth by reducing Cd induced effects in Cd-stressed plants. Significant decrease in photosynthetic parameters was found by the Cd alone. Addition of EDTA improved the net photosynthetic and gas exchange capacity of plants under Cd stress. Cd at 10 and 50  $\mu\text{M}$  significantly increased electrolyte leakage, the production of hydrogen peroxidase ( $\text{H}_2\text{O}_2$ ) and malondialdehyde (MDA) and a significant reduction was observed in the activities of catalase (CAT), guaiacol peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase under Cd stress plants. Application of EDTA at the rate of 2.5 mM alone and with combination of Cd increased the antioxidant enzymes activities and reduced the electrolyte leakage and production of  $\text{H}_2\text{O}_2$  and MDA. Oilseed rape (*Brassica napus* L.) actively accumulated Cd in roots, stems and leaves and the addition of EDTA boosted the uptake and accumulation of Cd in oil seed rape by dissociating Cd in culture media. The present results suggest that under 8 weeks Cd-induced stress, application of EDTA significantly improved the plant growth, chlorophyll content, photosynthetic, gas exchange capacity, improving enzymes activities and increased the metal uptake in roots, stems and leaves of oilseed rape (*Brassica napus* L.) respectively which shows that this technique is very useful for heavy metal pollution management.

**Keywords:** antioxidant enzymes, cadmium, chelator, EDTA, growth, oilseed rape



## PHYSIOLOGICAL STRESS FACTORS ASSOCIATED WITH DIFFERENT MINE TAILINGS ON THE CHLOROPHYLL FLUORESCENCE OF PLANTS

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### ABSTRACT

Phytoremediation is an emerging technology for the remediation of mine tailings. Phytostabilization is a technique within this field which involves the establishment of vegetation in mine tailings in order to immobilize potentially toxic trace elements, and which can also refer to the physical stabilization of the surface of tailings materials. Physical stabilisation is required to avoid or minimise erosion and reduce surface runoff, thereby decreasing the distribution of trace elements, e.g. arsenic, copper, nickel, cobalt, cadmium, into the surrounding environment and/or groundwater system.

The aim of this investigation was to evaluate the ability of selected plant species to adapt to the physiological stress factors associated with mine tailings, thereby facilitating a successful phytostabilization methodology. The vitality of the plants subjected to various soil stress conditions was measured as a function of photosynthetic ability. It is expressed as  $PI_{ABS}$ , and takes into consideration the ability of the plant to absorb and convert sunlight into chemical energy.

Chlorophyll fluorescence (JIP-test) was used to measure  $PI_{ABS}$  in six plant species subjected to various stress factors, i.e. water stress, acidity, salination and low nutrient status. The stress factors associated with the different mine tailings negatively affected plant vitality, thereby limiting the ability to convert sunlight into chemical energy. Comparisons between the reactions of the different plant species to the stress factors revealed significantly different behaviours. Very low water retention levels are characteristic of coarse tailings materials, i.e. kimberlites and coal discard, and this was reflected in the chlorophyll fluorescence results. Gold and coal tailings negatively impacted the plants by causing a decrease in the vitality index. Plants that were grown in more favourable conditions and plants adapted to survive in mine tailings showed a higher vitality index.

To conclude: Chlorophyll fluorescence assessments proved to be a very useful indicator in the identification of physiological stress in plants established on mine tailings.

**Keywords:** Chlorophyll fluorescence, Physiological stress factors, Phytostabilization, Plant vitality ( $PI_{ABS}$ ), Potentially toxic trace elements, Remediation, Mine tailings

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## HEAVY METAL TOLERANCE AND ACCUMULATION OF SWEET SORGHUM- POT OUTDOORS EXPERIMENT

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### ABSTRACT

High biomass producing species are considered as tools for remediation of contaminated soils by heavy metals, radionuclides or organic pollutants. Sweet sorghum (*Sorghum bicolor*), with high biomass, is a typical model plant for bioenergy crops. In this study, the characteristics of Zn, Cu, Cd, As, Hg or Cs tolerance and accumulation of sweet sorghum grown for three months were studied in pot outdoors, Hainan Province, P.R. China. Each treatment had three replications. No visual toxic symptoms on shoots of *S. bicolor* were observed at Cd level  $\leq 3$  mg/kg or Cs level  $\leq 100$  mg/kg or Hg level  $\leq 10$  mg/kg, with the height and dry biomass of the plants were similar to that of the control. Obvious toxic effects of *S. bicolor* growth occurred at Cu level of 200 or 400 mg/kg, at As level of 25 or 50 mg/kg, at Zn level of 500 mg/kg, at Cd level of 15 mg/kg, or at Cs level of 400 mg/kg, with height and dry biomass of the plant decreased much. The shoot Zn concentration and root of the treatment with 250 mg Zn/kg was 1657.6 mg/kg and 1370.0 mg/kg, respectively when the dry biomass of the plant decreased by 45%. The shoot and root Cs concentration at Cs level of 100-400 mg Cs/kg reached 687.5-2716.3 mg/kg and 336.9-1147.5 mg/kg, respectively. The TF value and BCF value of the plant under Cs pressure (100 or 400 mg/kg) reached 2.0 and over 6.0, respectively. It' estimated that 10.2 kg Cs/ha at Cs level of 100 mg/kg as well as 6.9 kg Cs/ha at Cs level of 400 mg/kg could be removed from the soil with one time of harvest (every three-month growth). Hg concentration in the plant was lower than detection limit. The above results showed that sweet sorghum was promising for phytoremediation on low-level Cs or Zn polluted soil.

*Acknowledgements:* The financial support by the National Natural Science Foundation of China (Grant No. 51378284;50830302; 50808111).



## NATURAL PLANTS ON BORON (B) RESERVE AREAS IN TURKEY AS PHYTOREMEDIATION AGENTS OF B CONTAMINATED ENVIRONMENTS

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### ABSTRACT

Boron (B) is one of the necessary micro nutrients for plant metabolism. However, the window between the amount which is necessary for growth and the amount which causes toxicity is very narrow and this spectrum varies among plant species. 4 (5) mg kg<sup>-1</sup> available B in soil for plants is generally accepted as toxicity border for most plants.

B excess is a common problem in the world and Turkey differs from all other countries with securing almost 70% of total B in the world. This situation is resulted in forming a special habitat in terms of soil-plant relationships due to high B concentrations at or near environments of the reserve areas.

In this research, therefore, we aimed to determine both (1) the most tolerant natural plant species to plant available B in soil and (2) their B accumulation potentials. To reach these aims, we studied in and around seven natural B reserve areas (tertiary deposits) located in four different provinces in Turkey. Soil and its corresponding plant samples were taken every 200 m from the centres of all of the B reserve areas to their environments (up to 2 km) along the transects (Belt Transect Method) on the main directions. "Minimum area method" was used in determining the size of sampling area. Plants species were identified and soil and plant samples were analysed chemically.

At the end of the study, it was determined that 11 natural plants distributed on the areas with high plant-available B concentrations over 10 mg kg<sup>-1</sup>. Two of them were aquatic (*Typha latifolia* L. (Typhaceae), *Phragmites australis* (Cav.) Trin. ex Steudel (Poaceae)) and the others were terrestrial species (*Isatis glauca* Aucher ex Boiss. subsp. *glauca* (Brassicaceae), *Elymus elongatus* (Host) Runemark subsp. *turcicus* (McGuire) Melderis (Poaceae), *Glaucium corniculatum* (L.) Rud. subsp. *corniculatum* (Papaveraceae), *Alyssum sibiricum* Willd. (Brassicaceae), *Polygonum equisetiforme* Sibth. & Sm. (Polygonaceae), *Chenopodium album* L. subsp. *album* var. *album* (Chenopodiaceae), *Tamarix tetrandra* Pallas ex Bieb. emend. Willd. (Tamaricaceae), *Gypsophila perfoliata* L. var. *perfoliata*, *Puccinella distans* (Jacq.) Parl. subsp. *distans*). Moreover, it was also revealed that these natural species also accumulated B in quite high amounts. The highest B accumulation amount was determined in *P. distans* subsp. *distans* (270.24 ppm in leaf, 85.74 ppm in stem and 124.30 ppm in root).

As a result, most of the mentioned plant species are reported for the first time as B tolerant and accumulator with this research. It is thought that these species could serve as phytoremediation agents for both aquatic and terrestrial environments affected with B. So that, this phyto-technological application could help sustainable management of plant-available B concentration in natural environments.

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## STUDY OF ORGANIC AMENDMENTS USED IN PHYTOREMEDIATION AND ITS INTERACTIONS WITH METALS BY $^{13}\text{C}$ CPMAS NMR SPECTROSCOPY

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### ABSTRACT

The application of organic amendments in soils of urban areas (gardens, parks, etc.) and in other sectors such as agriculture is a common practice since they provide organic matter and nutrients and therefore help to improve fertility. However, it is important to analyze its chemical composition in order to determine effect produced on the mobility and bioavailability of contaminants in soils, because depending on the amendment can have contradictory effects. The objective of this work is to study the components of different organic amendments (commercial humic acids and sheep and horse manure compost) by NMR  $^{13}\text{C}$  CPMAS, since in both cases, it has been found that when are used in combination with different plant species such as *Vetiveria zizanioides* and *Atriplex halimux* for phytoremediation of soils contaminated with heavy metals, immobilization of the same occurs. Furthermore it has been determined by spectroscopic analysis of mixtures of amendments with different concentrations of metals that functional groups of these amendments are involved in the retention of metals.

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## BORON TOLERANCE IN *PUCCINELLIA FRIGIDA*: AN EXTREMELY BORON TOLERANT SPECIES

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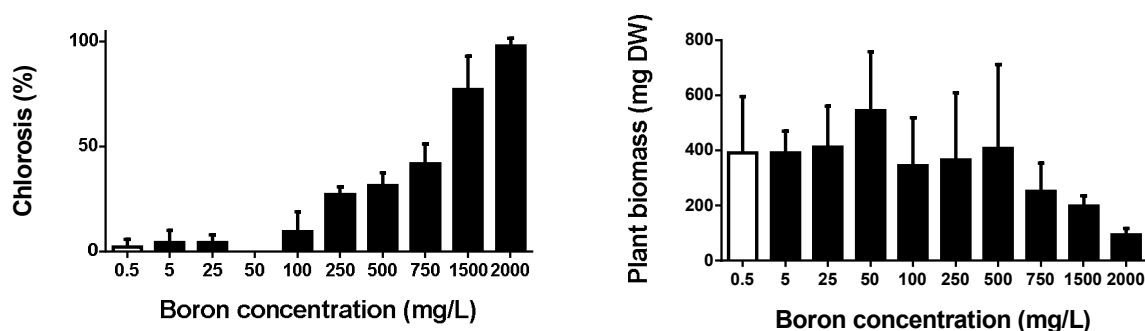
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### ABSTRACT

Boron (B) is an essential element for plant growth, but is required only at trace concentrations due to its high toxicity. Many arid and semi arid regions suffer from B toxicity, limiting their agricultural activities. Current B removal technologies are either impractical or too expensive for arid regions; hence, much attention has been paid to using B tolerant plants for phytotechnologies during the last decades. Different kinds of phytotechnologies for B-polluted soils and water have been studied: evapotranspiration, phytoextraction, phytoremediation, and constructed wetlands. For any of these, new B-tolerant plant species must be studied in order to find suitable candidates for the different applications and sites.

We recently discovered a new B-tolerant species - *Puccinellia frigida* - that spontaneously colonizes and acts a B hyperaccumulator in an extremely B-contaminated site in Northern Chile (Rámila and Pizarro, 2013). The aim of this work was to start evaluating the potential of *P. frigida* for B phytoremediation purposes.

To investigate this, we studied the tolerance of *P. frigida* to B in hydroponic culture in a controlled greenhouse environment. Seeds were collected from the Colpitas Basin, Northern Chile, and germinated in filter papers. Seedlings were transferred to soil and, after one month, plants were transplanted to hydroponic culture and acclimated during several weeks. Plants were then treated with 10 different B concentrations for 5 weeks. Each treatment was done in triplicate, with four plants per pot per replicate. Finally, plants were harvested. Shoot and root biomass were determined, and chlorosis (visual symptom of B-toxicity) was visually estimated.



We found that *P. frigida* tolerated more than 750 mg B/L without showing major symptoms of toxicity (32 % chlorosis). This concentration is extremely high, taking into account that concentrations over 3 mg B/L may be considered toxic for plants. Moreover, shoot growth was inhibited by 50% only at ~1500 mg/L, which exceeds the concentrations reported for other species with extreme B tolerance (Stiles et al., 2010). Plant biomass production was estimated in the range of 2 - 5 ton ha<sup>-1</sup> year<sup>-1</sup>. As a conclusion, *P. frigida* is the (or at least one of the) most B-tolerant species reported so far. Hence, this plant could be used to phytorestore extremely B-polluted sites or evapotranspire high B-waters to reduce B-leaching to adjacent areas. Analysis of the plant's tissue B concentration will reveal its applicability for phytoextraction purposes.

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## MYCORRHIZATION OF *BETULA CELTIBERICA* INCREASES THE TOLERANCE TO GROW IN A HEAVY-METAL POLLUTED SOIL

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### ABSTRACT

In natural conditions, roots of many plant species, especially in the case of trees are associated with mycorrhizal fungi. The presence of mycorrhizae, increases root absorption area and water and important nutrients bioavailability. That symbiotic association seems to gain importance when plants grow in poor or metal-polluted soils, where mycorrhizal fungi can improve the survival and development rates of trees under stress conditions, although the effect of the mycorrhization on metal uptake seems to be fungus and plant specific.

The aim of our research was to evaluate the effect of mycorrhization of *Betula celtiberica* in order to consider its use in phytoremediation programs, studying its physiological responses when growing in a heavy-metal polluted soil and verifying whether or not plant-fungus association persists along culture time. Plants of a selected clone of *B. celtiberica*, whose Cd accumulation capacity exceeded the hyperaccumulation threshold in a previous *in vitro* screening (Fernández et al., 2008), were cultured in greenhouse in a peat-perlite mixture (3:1, v/v) (control plants) or in a polluted soil coming from a deserted nitrate factory located in Asturias, Spain, with a wide range of heavy metals including Zn, Hg, Cd, Pb, As and Fe. In addition, some of these plants were inoculated with an isolation of the ectomycorrhizal fungus *Paxillus ammoniavirescens* collected also in a metal-polluted soil. After 60 days, plant growth, photosynthetic efficiency, pigment content and metal accumulation were measured.

In a controlled *in vitro* mycorrhization, the fungus present is known. However, when it pass to field or greenhouse, it is possible that the fungus inoculated disappears or is displaced by other mycorrhizal fungi. In order to assess whether plant-fungus interaction persisted along culture time and to verify that the observed ectomycorrhizae were formed by *P. ammoniavirescens*, the species of fungus was determined analysing the sequence of Internal Transcribed Spacer (ITS) region of the ribosomal DNA amplified with the primers ITS1 and ITS4. The obtained sequence was compared with the one previously obtained from an isolation of *P. ammoniavirescens* confirming that plant-fungus interaction persisted along culture time.

Results showed that mycorrhized plants had a higher fresh weight and shoot length than non-mycorrhized ones. No differences in root length or number of leaves were found among treatments. Regarding photosynthetic pigments content, chlorophyll, carotenoids and anthocyanin levels were significantly higher in mycorrhized plants than in the non-mycorrhized ones when plants were cultured in polluted soil. However, no differences in photosynthetic efficiency were found among treatments. Mycorrhized plants accumulated fewer metals than non-mycorrhized both in roots and in shoots. This result may indicate that *P. ammoniavirescens* presence in rhizosphere could reduce metal bioavailability in soil or immobilize the metals in its mycelium preventing therefore its transport to host plants. This behavior leads to an increasing metal tolerance of *B. celtiberica* plants as it could be observed in the results obtained from the analysis of biomass and photosynthetic pigments content. In any case, in both treatments, *B. celtiberica* plants were able to bioaccumulate several times the amount of Zn, Cd and Mn available in soil.

**References:** Fernández et al. (2008). *Env. Poll.*, 152 (3), 522-529.

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## SEED GERMINATION OF PLANT SPECIES USEFUL TO RECLAMATION OF WASTES AFTER Zn-Pb ORES FLOTATION USING *IN VITRO* AND *IN VIVO* ASSAYS

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### ABSTRACT

The reconstruction of habitats strongly contaminated with heavy metals require taking up significant measures. Waste material obtained after zinc and lead ore flotation are ranked among particularly hard to introduce biological life. Application of native local floristic biodiversity is the fastest way to create compact vegetation cover, what sometimes offer the possibility of restraining the mobility of toxic ions in relatively short time.

The Olkusz district Zn-Pb ore-mining region located in southern Poland is known to be a habitat of plant species adapted to grow in such waste areas. Among others *Alyssum montanum* L. (Brassicaceae), *Biscutella laevigata* L. (Brassicaceae), *Dianthus carthusianorum* L. (Caryophyllaceae), *Gypsophilla fastigiata* L. (Caryophyllaceae), *Scabiosa ochroleuca* L. (Dipsacaceae) spontaneously appear in areas contaminated with heavy metals. For above mentioned reasons fruit samples were collected from specimens representing calamine flora of the Olkusz Ore District in the autumn 2012. Seed samples were preserved at room temperature (22° C) and they were not subjected to any additional treatment. Representative samples of respective species were used to test the energy and ability of germination in the experiment which were set up in 2013. First of all, the energy and ability of germination were evaluated in the field condition on experimental plot established on the post-flotation waste heap (50°17'N, 19°30'E). Seeds were sown during the autumn on three types of substrates: (1) post-flotation material, (2) post-flotation material enriched with NPK fertilizers (3) post-flotation material mixed with municipal sewage sludge. Photoblastic seeds of *G. fastigiata* and *D. carthusianorum* were not covered by layer of substratum on the contrary to the others species seeds. At the field conditions the number of seedlings was started to be summed up after 28 days period. As additional control treatments, the seed germination *in vitro* using paper test (4) at room temperature (22° C), and (5) the outdoor temperature (28 - 0,3° C day/night) was carried out. In those two control treatments seedlings were counted 3, 7, 18 and 28 days after sowing.

At room conditions germination began in three days (except *S. ochroleuca*), and 18 days after sowing the germination level of every tested species was proved to be similar (above 80% of seeds germinated). The highest germination ability was ascertained for both *D. carthusianorum* (94.6 %) and *A. montanum* (98.66 %). Whereas in the outside conditions seeds started to germinate seven days after sowing. *G. fastigiata*, *S. ochroleuca* germinated as last two species. Consequently the number of *G. fastigiata* and *S. ochroleuca* seedlings were the lowest (36 % and 49.3%, respectively). On the contrary *Alyssum* and *Dianthus* quickly began the process of germination, so in a week period over 90 % of seeds germinated, and 18 days after sowing this result increased up to 97.3 % for *Alyssum*, 98.6 % for *Dianthus*. Due to the high germination ability of, *Alyssum* and *Dianthus* calamine ecotypes, the most promising in vegetation cover creating are proposed exactly these two species. Whereas during this experiment the germination of *B. laevigata* seeds were poor, except *in vitro* test at room temperature. The results of seed germination ability tested on the field plot with poor post-flotation material were similar to germination ability tested *in vitro* in case of *G. fastigiata* (41.3%) or significantly lower for *D. carthusianorum* (32 %). However, those results may be disturbed by intense flurry of wind. Uncovered seeds could be blown by the wind on neighboring plots or outside the experimental plots. Therefore, it should be underlined that for calculations only the seedlings growing in the respective plot were taken into account. In every treatment, germination of *A. montanum* was proved to be very high, and comparable to the paper test (88 %). Generally on "NPK plots" it was observed for most examined species significantly lower seed germination ability in comparison to all control treatments. It may be the effect of higher salinity of the substrate (additionally, some seedlings have been yellowish). The greater ability of seed germination was noticed on the plots with sewage sludge, probably due to better properties of the ground. Undoubtedly, the lowest germination ability was ascertained in the case of *Scabiosa ochroleuca*, and it amounted to 2.6% and 5.3% on the plots with NPK and sewage sludge, respectively.

Recapitulating, in terms of seed germination ability the most difficult plant material was *B. laevigata* while the best – *A. montanum*. Additionally easily germinated *D. carthusianorum* seeds can be helpful in the reconstruction of habitats degraded by centuries-old industrial activity.



## REMOVAL EFFICIENCY OF COPPER AND CHROMIUM BY AQUATIC MACROPHYTES *EICHHORNIA CRASSIPES* AND *PISTIA STRATIOTES*

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### ABSTRACT

Heavy metal contamination in water has become a serious issue in Pakistan and this problem requires environmentally safe technology like phytoremediation. Present study focussed on comparative phytoremediation potential of two aquatic plants *Eichhornia crassipes* and *Pistia stratiotes* for the removal of chromium (Cr) and copper (Cu) at their different concentrations (mg/l) i.e., 2 (T<sub>1</sub>), 4 (T<sub>2</sub>), 6 (T<sub>3</sub>) and 8 (T<sub>4</sub>) and Control (T<sub>0</sub>) for a period of 30 days. It was observed that during 30 days experiment, percentage removal of Cr by *Eichhornia crassipes* and *Pistia stratiotes* was 95 and 86% while the percentage removal of Cu was 99 and 97% respectively. The percentage removal of Cr by different parts (roots, shoots and leaves) of *Eichhornia crassipes* was 57, 25, 18% and 56, 28 and 16%. Whereas percentage removal of Cr by different parts (roots, shoots and leaves) of *Pistia stratiotes* was 51, 32, 17% and for Cu was 52, 32 and 16% respectively. Results showed significant removal ( $p < 0.05$ ) of chromium and copper by *Eichhornia crassipes* and *Pistia stratiotes*. Both the species are efficient for the removal of Cu as compared to Cr but roots are significantly ( $p < 0.05$ ) more efficient for removal of Cu and Cr as compared to shoots and leaves for both the species. Significant increase ( $p < 0.05$ ) in the plant biomass was observed for all the treatments after 30 days of experimentation but increase in biomass decreased with increase in both the metal concentrations for both the plants.

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## EFFECT OF Pb AND Cu TOXICITY ON ANTIOXIDANT ACTIVITY AND PHOTOSYNTHESIS IN SEEDLINGS OF *CITRUS AURANTIUM* L.

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### ABSTRACT

Heavy metal stress is a major environmental factor which adversely affects plant growth and development. However, low concentrations may ameliorate the toxic effects of metals and help to sustain development under stress. Rootstocks of fruit trees, commonly used to regulate growth, precocity, fertility, and yield, can also be used to solve problems associated with soils pollution such as copper and lead excess. Despite their potential use for phytoremediation, information on the physiological and biochemical aspects of heavy metal toxicity stress on fruit tree rootstocks remains limited. This study was conducted to determine the effects of varying concentrations of PbNO<sub>3</sub> and CuSO<sub>4</sub> on physiological operation, antioxidant activity and flavonoids content of *Citrus aurantium* L. subjected to heavy metal stress at different levels. The results showed that PbNO<sub>3</sub> and CuSO<sub>4</sub> addition significantly affected growth in a concentration-dependent manner; generally, low concentrations enhanced while high concentrations limited growth. The results also showed that, generally, low PbNO<sub>3</sub> and CuSO<sub>4</sub> concentrations significantly increased photosynthetic rate and water potential while it decreased flavonoids content and antioxidant activity. These results suggest that PbNO<sub>3</sub> and CuSO<sub>4</sub> influence many physiological parameters and especially for CuSO<sub>4</sub> the function of the photosynthetic apparatus and this is depends on the metals concentration.

**Keywords:** phytoremediation, *Citrus aurantium* L., heavy metal toxicity, stress

**Introduction:** Copper (Cu) is considered as a micronutrient for plants and plays important role in CO<sub>2</sub> assimilation and ATP synthesis. Copper is also an essential component of various proteins like plastocyanin of photosynthetic system. Lead (Pb) is one of the most abundant toxic elements in the soil. High level of Pb also causes inhibition of enzyme activities, water imbalance and oxidative stress by increasing the production of ROS in plants [3]. It is evident that heavy metals as Cu and Pb modify flavonoid content in plants. Accumulated heavy metals in plants may interact with the photosynthetic machinery and resulted in a wide variety of toxic effects, including photooxidative damage. In particular, they alter the chloroplast membrane functions and components of the photosynthetic electron transport chain (ETC), thus impairing the light phase of photosynthesis [4]. It is known that heavy metals decrease photosynthetic rate, stomatal conductance, transpiration rate, plant growth and agricultural productivity [6]. The aim of this work was to determine the effects of the abiotic stress caused by lead and copper on photosynthetic apparatus and on the flavonoid levels in young citrus plants.

**Materials and Methods:** *Plant Material:* One year old *C. aurantium* plants were grown with different concentrations of Cu and Pb in a greenhouse in Thessaloniki, Greece, (40°34' 35' N 22°57'19'E), 2013.

*Determination of total antioxidant:* The ability of the leaves to act as hydrogen or electron donors in the transformation of DPPH into its reduced form DPPH-H was investigated. Total antioxidant activity was determined following the method of Su *et al.* [2]. *Photosynthesis:* Photosynthetic parameters were monitored with a portable photosynthesis system (Li-Cor 6400, USA). The measurements were made on the third mature leaf in control and heavy metals treatments. Photosynthetic assimilation rate (A), stomatal conductance (gs),

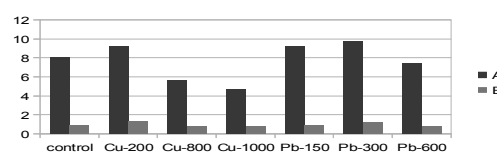


Figure 1. Assimilation rate (A) and Transpiration rate (E) photosynthetic parameters as affected by heavy metals concentrations [ $\mu$ M] in the nutrient solution.



intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) and transpiration rate (E) of leaves were measured using the youngest fully expanded leaves on a fine morning. *Determination of flavonoids*: The total flavonoid content was measured using a modified colorimetric method [7]. The flavonoid content was expressed as g rutin equivalents (CE) per 100 g of dry weight (dw).

*Results and Discussion: Effect of copper stress on photosynthesis*: In our study, at 800µM and 1000µM copper treatment, the photosynthetic assimilation rate (A), stomatal conductance (g<sub>s</sub>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) and transpiration rate (E) of leaves were significantly decreased compared to control. While at low concentration (200µM), copper promoted the increase of photosynthesis (A). Changes in CO<sub>2</sub> assimilation may be attributed to either stomatal or non-stomatal factors, or both. Photosynthetic assimilation rate (A) was significantly decreased at both 600µM and lead, accompanied by the decrease of C<sub>i</sub> and g<sub>s</sub> suggested the stomatal factor was the main factor influencing CO<sub>2</sub> assimilation. However, when A and stomatal closure (E and g<sub>s</sub> decreased) significantly declined in 600 µM Pb compared to that at 150 µM and 300 µM Pb, C<sub>i</sub> even showed increased, which implied that non-stomatal limitations to photosynthesis also occurred [1].

In conclusion, excess copper and lead inhibit photosynthesis and flavonoid content the inhibition of copper on the photosynthesis is not only by stomatal but also by non-stomatal factors. At low concentrations copper promoted photosynthesis. Pb treatment had also a stimulating effect on photosynthetic machinery, flavonoid content, antioxidant activity at low concentration and an inhibitory effect at higher concentration.

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## PRELIMINARY STUDY FOR THE PHYTOREMEDIATION OF HIGHLY ACIDIC MINE TAILINGS: SELECTION OF AMENDMENTS

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### ABSTRACT

Tailings formed from mine spoils accumulation and storage are considered one of the principal sources of contamination associated to mining activities. They generally contain high amounts of trace elements (TEs) whose availability and dispersion (by wind and water erosion and lixiviation) suppose a serious threat to surrounding populations and ecosystems. This is the case of the mining district of La Unión-Cartagena (Murcia, SE Spain), where 89 abandoned tailings have been identified and which remediation is needed [1]. Phytostabilisation using native halophyte shrubs such as *Atriplex halimus* seems to be an efficient remediation technique [2], because of their tolerance to TEs, high canopy and deep root system, and their adaptation to the Mediterranean semi-arid climate. However, given the extreme conditions of mine tailings, the application of an adequate combination of amendments is required to reduce soil toxicity and improve soil fertility [2]. The aim of this work was to assess the viability of two inorganic materials (calcium carbonate and ViroBind<sup>TM</sup> reagent), alone or in combination with organic amendments, for their use in the phytoremediation of highly acidic and TEs-contaminated mine tailings from La Unión-Cartagena.

Two pot experiments were carried out using two spoil soils (D1 and D2) from mine tailings situated in an area known as "El Descargador", within the mining district of La Unión-Cartagena. Both soils are extremely acid (pH 3.3), have high concentrations of TEs ( $\approx 1100$ ; 4700-6900; 5000-20000 mg kg<sup>-1</sup> of As, Pb and Zn) and elevated EC (2.9 and 9.0 dS m<sup>-1</sup>). As amendments, four different formulations of a commercial reagent (ViroBind<sup>TM</sup>, mainly composed by Fe and Al oxides and Ca and Mg minerals) and its combination with a mature compost were applied in soil D1, while calcium carbonate and its mixtures with a digestate from an anaerobic digestion plant or with the previous compost were selected for soil D2. Four weeks after amendments addition, 0.3 g of *A. halimus* seeds were sown in each soil and pots were kept in a climate chamber during 5 and 3 months for soil D1 and D2, respectively. The evolution of the treatment effect on soil physicochemical properties and TEs availability in soil and soil solution (periodically extracted *in situ*), as well as plant growth and foliar TEs accumulation were studied.

The application of the amendments led to a significant pH increase in both soils, reaching values of 6-7.8 at the end of the experiments. Soluble and extractable concentrations of TEs were very high, especially in soil D2, but treatments successfully decreased their solubility as a consequence of the pH rise (>99% decrease of TEs dissolved in pore water). *A. halimus* was not able to grow in untreated soils, but all the treatments allowed its growth, reflecting a clear improvement of soil conditions. The supply of essential nutrients by the organic amendments (principally compost) was crucial for the increase observed in biomass production and nutritional status of the plants, and for reducing foliar TEs accumulation (Figure 1). Therefore, the use of *A. halimus* with the combined application of calcium carbonate or ViroBind<sup>TM</sup> and compost can be a good phytostabilisation strategy to remediate highly acidic TEs-contaminated mine tailings.

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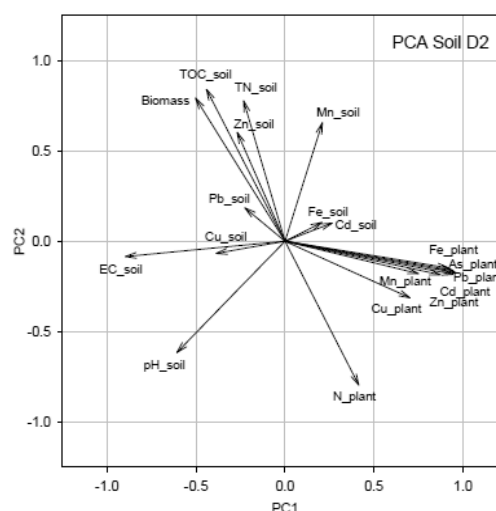


Figure 1. Principal components analysis resulting from the relation of soil and plant properties (soil D2).



## THE EFFECT OF ORGANIC AND MINERAL SUBSTANCES ON THE MOBILITY OF CADMIUM IN THE SOIL – PLANT SYSTEM

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### ABSTRACT

Current findings highlight the need for a comprehensive approach to address the contamination of soil and crop growth. The position of the Slovak Republic within Europe stipulates that this land is contaminated by long-term global transmission of large industrial and energy complexes. These impacts have a negative effect on soil with an increased to extremely high content of risk elements.

Ecological risks of accumulation of hazardous substances in the soil are reflected in the ability of soils to provide hygienically harmless food. The danger lies mainly in the change of their mobility influenced by various factors. This is particularly true for heavy metals with highest biotoxicity.

South Slovakia is the most intense producer of vegetables where Cd is shown to be the most dangerous heavy metal. Its accumulation in vegetables is affected mainly by different soil conditions. Soil remediation is very expensive, but its impact to some extent can be reduced by applying sorbents based on organic and natural resources. The aim of our study was to analyze the influence of selected sorbents on the accumulation of cadmium in carrots.

Two sorbents were used in the experiment: the Humavit (organic humus fertilizer) and the Zeolite (sorbent of mineral origin). The study showed that increased Cd concentration in soil resulted in a non-linear Cd accumulation in carrot roots. The study found that application of the Humavit (5 %) into soil contaminated by Cd (2 mg and 5 mg Cd.kg<sup>-1</sup> soil, respectively) significantly decreased Cd content in carrot roots (approx. from 25 % to 35 %). In comparison, application of the Zeolite (5 %) into soil contaminated with 5 mg Cd.kg<sup>-1</sup> soil significantly decreased Cd content in carrot roots (about 28 %). Application of lower rates of the Humavit (5 %) and the Zeolite (5 %) into soil contaminated by Cd did not significantly affect Cd content in carrot roots. The study confirmed that application of both sorbents into soil contaminated with 2 mg Cd.kg<sup>-1</sup> soil significantly decreased Cd accumulation in edible parts of carrot (the values below hygienic limits – 0,1 mg.kg<sup>-1</sup>). Under conditions of higher concentration of Cd in soil (5 mg Cd.kg<sup>-1</sup> soil), only application of the Zeolite (5 %) into soil significantly reduced Cd content in carrot (below the hygienic limits). The study showed that carrot cultivation in highly Cd-contaminated soils is not recommended. However, application of the Humavit (5 %) into soil lowly polluted with Cd establishes hygienic and safe conditions for cultivation of carrot.

Intensive and systematic research focused on specific ion-exchange properties of the Zeolite sources opens wide possibilities of the sorbents use in land protection.

*Acknowledgements:* The financial support by VEGA project No. 1/0456/12 is greatly appreciated.



## TISSUE KINETICS OF METAL ACCUMULATION IN THE RECOVERY OF INDUSTRIAL WATER SLUDGE BY PLANTS

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### ABSTRACT

The overall pollution in the planet is reaching unbearable limits for humanity. Never before mankind had come to adopt measures agreed between countries in the magnitude of the current ones. Among the measures taken to alleviate the problem are increasing green alternatives. In this paper, it is shown how GMOs operate which have proven over the last decade to be by far, the best overall tool for combating pollution. A kinetic study of the accumulation of heavy metals (Ni, Cu, Zn, Cd, B, Pb, and Hg) in sludge from a treatment plant of industrial water is performed. The results confirm again with another specific case, the capacity and usefulness of this tool as a solution to the problems of global pollution.

Some of the plants used in this work are hyperaccumulators as demonstrated in mining soil containing concentrations of heavy metals close to 20000 ppm [1]. Since the capacity of a hyperaccumulator plant, could be non-existent, in amounts of industrial sludge containing only a few hundred ppm, this study aims to determine whether these plants are able to maintain their usefulness in industrial sludge. Sludge from a treatment plant for industrial water were used to demonstrate the ability to decontamination of species used. The initial data (t0) were taken at 3 weeks of planting, and end at 3 months (tf) From a practical point of view the most important goal was to determine whether GMOs are the best tools available in regard to wild genotype, and above all to know exactly, the ability of the tested genotypes, to decontaminate sludge. In this regard it is noteworthy that GMOs, consistent with previous trials, produced a greater accumulation in virtually all cases. Besides, both wt and GMOs multiplied the overall accumulation in plant at the end of the assay. In the other hand, assuming that a good way to determine the ability to remediate a contaminated environment, is to calculate the bioconcentration factor (BCF). This BCF, is greater than 1 in most cases, (Table 1). Finally, regardless of the initial concentration and specific metal, a greater total accumulation for GMOs is observed, compared to wt. Therefore, it can be concluded that there is no threshold in both. There is no threshold concentration, in the limits of concentrations tested in this assay, for the plant to activate the mechanisms of accumulation. There is also no threshold to activate the differences, between GMOs and wt.

Table1 Metal	BFC t0		tf	
	wt	GMO	wt	GMO
Ni	0,09	0,09	0,53	0,65
Cu	1,03	0,77	2,57	3,22
Zn	2,97	2,52	12,90	14,99
Cd	0,26	0,34	0,56	0,74
Pb	0,03	0,05	0,60	1,13
Hg	0,61	2,22	0,31	1,37

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## GERMINATION OF COATED SEED FROM THREE GRASS SPECIES IN DIFFERENT MINE WASTES FOR PHYTOSTABILISATION

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### ABSTRACT

The adverse characteristics of mine waste materials, together with the loss of ecosystem functionality and stability caused by mining activities, create conditions undesirable for seed germination which can delay the success of phytoremediation and -stabilisation. Attempts to improve germination rates in the harsh environmental conditions associated with mine tailings include the enhancement of seeds. Advance Seed Company enhances seeds by the chemical application of water-soluble, lime-based coatings which are hypothesised to assist in the germination process, especially with regard to the facilitation of water movement and nutrient uptake.

The main objectives of this study were to compare the germination and root development of coated and uncoated seed types in different growth media (including mine tailings), and also to determine the water absorption of seeds relative to the coating. Three grass species native to South Africa (*Eragrostis curvula*, *Digitaria eriantha*, *Cynodon dactylon*) were selected for use in this study. Both seed types (coated and uncoated) were sown and replicated in eight different mine tailings and two control media. Seedlings were removed after six days and fixed for Scanning Electron Microscopy analysis to investigate root development. The hydration percentage of coated and uncoated seeds was also tested during germination by allowing for imbibition to occur; the water content was measured over a time-period of 50 hours.

The preliminary results of the germination experiments showed that the coated seed types had poor germination percentages compared to the uncoated equivalents for all selected species in the majority of the growth media, such as the coal, gold and gypsum mine wastes. The hydration results revealed higher water contents in the uncoated seeds of *Eragrostis curvula* and *Cynodon dactylon* compared to the coated seed types. Seedlings from the uncoated seeds of *Eragrostis curvula* displayed profound lateral root development in several of the growth media compared to seedlings from the coated seeds, while seedlings of *Digitaria eriantha* and *Cynodon dactylon* from both coated and uncoated seeds displayed lateral root development in several of the growth media.

Depending on the components used in the applied seed coating, a negative charge may exist on the coating which could retain water from the enclosed seed and consequently inhibit imbibition. This possibility is reflected in the higher water contents and improved germination results of the uncoated seed types when compared to the coated seed types of the three species; the coating may prevent water from reaching the embryo during germination. The ability of mine tailings to retain water was found to be a determining factor in water availability and consequently, the germination of seeds.

**Keywords:** phytoremediation, phytostabilisation, seed coating, germination, imbibition, mine tailings/waste

**Acknowledgements:** Financial support from Advance Seed (South Africa) is greatly appreciated.



## HEAVY METALS ACCUMULATION IN *CHLAMYDOMONAS REINHARDTII* AND *THALASSIOSIRA WEISSFLOGII* CELLS

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### ABSTRACT

Photosynthetic algae play a crucial role on environmental sustainability, being in the basis of the nutrition chain [1]. Exposure of algae to environments polluted with heavy metals results the accumulation of these toxic elements in them. Accumulation occurs via both adsorption of the metals on the cell walls and insertion into the cells. Examination of the accumulation of the heavy metals in the algae is important both for the information it gives about the condition of the ecosystems, and for the potential of exploitation of these organisms for the remediation of natural waterbodies, or as biofilters with a wide range of applications [2,3,4].

In this study, Cd, Cr, and Pb accumulation in two unicellular algae was investigated. One freshwater microalga (*Chlamydomonas reinhardtii*) and one marine microalga (*Thalassiosira weissflogii*) were cultivated in media polluted with several concentrations of the abovementioned heavy metals, up to lethality levels. Tolerance of these organisms to the heavy metals under examination was examined and the growth curves for the different pollution levels were constructed. The percentage of the adsorbed vs. the total accumulated metal for each experimental condition was estimated by atomic absorption spectrometry, and the biochemical impact of the inserted metals in the cells was examined.

Cells, grown in both unpolluted and polluted media were lysed and differential centrifugation was applied on lysates in order to isolate the different cellular fractions and organelles. Heavy metal-induced proteins were searched by SDS-PAGE Electrophoresis.

**Keywords:** *Chlamydomonas reinhardtii*, *Thalassiosira weissflogii*, heavy metals, cadmium, chromium, lead, heavy metal-induced proteins, bioindicators

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## STRATEGIES FOR ENHANCING THE PHYTOREMEDIATION OF HEAVY METALS-CONTAMINATED INDUSTRIAL SOILS BY NATIVE SPECIES OF PRINCIPADO DE ASTURIAS (SPAIN)

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### ABSTRACT

Phytoremediation of heavy-metal polluted soils resulting from industrial and mining activities is a useful technology to solve this important environmental problem. The selection of plants that will be used in phytoremediation is one of the most important factors to consider in order to achieve successful results. The use of species adapted to these soils can improve decontamination programs and in the case of trees increase the effectiveness of metal uptake by plants due to the deeper root system and greater biomass. Furthermore, the use of symbiotic mycorrhizal fungus increases the survival and development rates of trees, although the effect on metal uptake seems to be fungus and plant specific. On the other way, interactions plant-bacteria have strong impact in metal phytoextraction. Bacteria produce substances (siderophores, plant growth hormones, ACC deaminase) that improve the growth and increase the plant biomass. Besides, bacteria capable of tolerating high concentration of metals can affect plant metal uptake by altering the mobility and bioavailability through soil pH, release of chelators and/or oxidation/reduction reactions.

The objective of this study was to investigate the phytoremediation potential of different plant species (*Dittrichia viscosa*, *Melilotus alba*, *Eupatorium cannabinum*, *S. atrocinerea*, *Salix caprea* and *Betula celtiberica*) using different strategies for enhancing phytoremediation and to identify the effectiveness of these strategies for its application in decontamination of heavy metal-polluted soils "in situ" on a field scale. The plants used were clones which showed high metal accumulation capacity, exceeding the hyperaccumulation threshold in a previous *in vitro* screening. Plantlets were micropropagated by *in vitro* culture, acclimated in a greenhouse and transferred to soil. *Salix atrocinerea*, *S. caprea* and *B. celtiberica* plants were also mycorrhized by an isolated ectomycorrhizal fungus *Paxillus ammoniavirescens* collected in a metal-polluted site. The experimental site was a 600 m<sup>2</sup> field located within the industrial zone of Asturias (Spain) which presented high concentration of As, Cd, Cu, Fe, Hg, Pb and Zn. The field soil was ploughed to homogeneity prior to experimentation and was divided in plots, each of 2 m<sup>2</sup> size with at least 2 m between plots. Three different strategies were assayed: planting density, application of fertilizer and mycorrhization effect.

Results showed that planting density and fertilization had a significant influence on plant growth and therefore on total biomass obtained. The mycorrhization of *B. celtiberica* increased the length and diameter of shoots and Zn accumulation in leaves reached hyperaccumulation levels in both mycorrhized and non-mycorrhized plants. The highest levels of Pb and As accumulation were obtained in mycorrhized *B. celtiberica*. The extraction efficiency of *B. celtiberica* is even higher than that of other accumulator species as *D. viscosa*. To analyze interactions plant-bacteria, we isolated arsenic and other heavy metals resistant bacteria from inside the roots (endophytic bacteria) and the rhizosphere of *Betula* and *Salix*. The effects of these bacteria in *B. celtiberica* plantlets were assayed "in vitro" individually and in the form of a three-members consortia, in the presence of 300 µM sodium arsenate. The results showed a positive effect on the metal accumulation in the above ground parts of the plants, although the shoot and root length and number of leaves decreased. To check the penetration of added bacteria in the plant, sections of roots (10 µm) were stained with vital dyes (LIVE/DEAD® Viability Assays) and their presence confirmed by "in situ" hybridization (FISH) in a Confocal Laser Scanning Microscope.

In conclusion, the results from this field study show that *D. viscosa* and mycorrhized *B. celtiberica* have an interesting potential application for phytoextraction of heavy metals from contaminated soils.

**Acknowledgements:** The financial support by UE-12-IDARTS-LIFE-11-000547 project is greatly appreciated.



## PRELIMINARY ANALYSIS FOR A MINING WASTE PHYTOSTABILIZATION PROJECT WITH NATIVE PLANTS IN SW-SARDINIA (ITALY)

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### ABSTRACT

SW-Sardinia (Italy) had been a very important metalliferous mining region through the centuries since the Phoenician-Punic age. The main extracted metals were, among others, zinc, lead, silver and copper. Exploitation closed down at the end of the XX century, leaving hundreds of waste heaps spread across the whole region. The investigated mine area, called San Giorgio, is located in the municipality of Iglesias and it is characterized by several dumps, mainly composed of fine-grained materials derived from metallurgical treatments, leaved at the end of the last century. Because of the high levels of toxic elements, the colonization by plants is slow and the main surface of the waste is still bare. The risk assessment, carried out on the basis of the Italian regulation, indicated that the restoration actions are compulsory.

With the aim to planning the site reclamation using the phytostabilization technology, pioneer plants and soils were analysed in 6 waste deposits in part colonised by various plant communities. The research was focused on 7 garrigue communities, respectively dominated by: *Euphorbia pithyusa* ssp. *cupanii*, *Cistus salviifolius*, *Cistus monspeliensis*, *Limonium merxmulleri*, *Helichrysum microphyllum* ssp. *tyrrhenicum*, *Dittrichia viscosa* and *Genista corsica*. 15 uniform vegetation patches were examined in the studied dumps, furthermore the soil of the bare areas and the soil of the non-contaminated areas was sampled. In every patch, the soil and the dominant plant species were sampled in three different point. The plant communities have been analysed by the phytosociological method. In order to investigate the soil properties, in every recorded site, the soils taken in correspondence of the plant rhizospheres were analysed to determine: skeleton, texture, pH, conductivity, CEC, exchange complex, C, N, Fe, organic matter (SOM), total content of Cd, Cr, Hg, Pb and Zn and bioavailable fraction of Cd, Pb and Zn.

Analytical results show that most of the bare areas have pH soil of about 7 and SOM <10 g/kg. The rhizosphere soils have, in most of the sampled points, basic pH and SOM from 10 up to 90 g/kg.

Among the investigate species, *E. cupanii* forms the most pioneer garrigues, on very poor soils, comparable to those of the bare areas. On the contrary, the soils with the highest content of SOM are colonized by *G. corsica*, *C. monspeliensis*, *C. salviifolius* or *H. tyrrhenicum* communities. Among these, *Cistus* species seem to prefer clay soils, *G. corsica* grow in soils from clay to loam and *H. tyrrhenicum* can develop also on sandy-loam soils. Between the two *Cistus* species, *C. monspeliensis* is able to grow on soils very skeleton-rich, if compared with *C. salviifolius*. If considered for their tolerance to the heavy metals, the most of the studied species are wide spread in soils with more than 6.000 mg Pb/kg and 1.000 mg Zn/kg as total content. However, *L. merxmulleri* and *H. tyrrhenicum* show a higher tolerance to Cd (>1000 mg/kg) and Zn (up to 60.000 mg/kg). On the other hand, *L. merxmulleri* is the only specie not found on soils with more than 40 mg Cr/kg.

The analysis of plant communities allow to observe that the development of the garrigues dominated by deciduous shrubs cause the formation of a leaf litter and promote the growth of annual herbs. However, therophytes are absent on the soils not yet colonised by chamaephytic or nanophanerophytic species. The development of the garrigues or low maquis is not strictly related with the decreasing of toxic metals in soil, however it is possible observe the increasing of the contents of N and SOM at the same time as the plant cover evolves. The pioneer dwarf shrub contribute to create a continuous herbaceous layer that probably help to reduce the transport of the metals in the environment, reducing the risk for the human health. More investigations will be necessary to improve the present state of knowledge and verify how it would be possible carry out a restoration plan based on phytostabilization using native plants. Among the most metal tolerant species, those having low translocation factor could be selected (e.g. *C. salviifolius*).

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## PHYTOEXTRACTION POTENTIAL OF FOUR BLACK LOCUST HALF-SIB FAMILIES IN THE PRESENCE OF HEAVY METALS IN NUTRIENT SOLUTION

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### ABSTRACT

Phytoremediation of toxic metals has been widely studied during past decades. The possibility for uptaking and accumulating these non-essential elements was examined with different plant species, both perennial and woody tree species. Black locust is characterized as high biomass productive species, adaptive to different types of soils and climates. In relation with that, it represents a good model plant for phytoextraction tests with heavy metals. Present work examines phytoextraction potential of four black locust half-sibs (families 54, 56, 115 and 135), grown hydroponically under semi-controlled conditions in greenhouse. Plants were treated separately with 6 ppm of cadmium (Cd), 100 ppm of nickel (Ni) and 40 ppm of lead (Pb) added in Hoagland nutrient solution, accompanying with mixed treatment of all three elements. Presence of Cd, Ni and Pb was observed in all investigated treatments in tissues of the root, stem and leaves of four black locust half-sib families. High accumulation rates of nickel and cadmium were determined in all four half-sib families. Higher amounts of lead were accumulated at individual treatment, compared with mixed treatment, where lead accumulation was potentially inhibited with presence of nickel and cadmium in nutrient solution. A significant decrease in morphological and physiological parameters was present at all treatments in relation to control. Decrease in photosynthetic activity, transpiration rates and water use efficiency at nickel, cadmium and mixed treatments could be the consequence of higher accumulation rates of these two elements in plant tissue. Chlorosis, necrosis and stunted growth of the youngest leaves were visible at both nickel and mixed treatment, accompanying with decrease in chlorophyll pigment contents. Differences in terms of visible symptoms like chlorosis and necrosis were not noticeable among four analysed half-sib families.

*Keywords:* black locust, heavy metals, accumulation, photosynthesis, transpiration

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## PHYTOTOXICITY OF SODIUM FLUORIDE AND UPTAKE OF FLUORIDE IN WILLOW TREES

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### ABSTRACT

The willow tree (*Salix viminalis*) toxicity test and a cress seed germination test (*Lepidium sativum*) were used to determine uptake and phytotoxicity of NaF. Concentrations in hydroponic solutions were 0-1000 mg F/L and 0-400 mg F/L in the preliminary and definitive test. A third test was done with soils collected from a fluoride-contaminated site at Fredericia, Denmark. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub>-values for inhibition of transpiration were determined to 38.0, 59.6 and 128.7 mg F/L, respectively. The toxicity test with soil showed strong inhibition for the sample with the highest fluoride concentration (405 mg free F per kg soil, 75 mg F per L soil solution). The seed germination and root elongation test with cress gave EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub>-values of 61.4, 105.0 and 262.8 mg F/L, respectively. At low external concentrations, fluoride was taken up more slowly than water and at high external concentrations at the same velocity. This indicates that an efflux pump becomes overloaded at concentrations above 210 mg F/L. Uptake kinetics were simulated with a non-linear mathematical model, and the *Michaelis-Menten* parameters were determined to half-saturation constant K<sub>M</sub> near 2 g F/L and maximum enzymatic removal rate v<sub>max</sub> at 9 g/(kg d).



## BORON, A HIDDEN TOXIN IN FLUE GAS DESULFURIZATION WATER

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### ABSTRACT

Coal fired power plants commonly produce a complex mix called flue gas desulfurization (FGD) water containing precipitated sulphur from the flue gases. Usually ground limestone slurry is reacted with the hot gases under oxidizing conditions to precipitate calcium sulphate. The precipitate may have utility in production of gypsum board or for soil amendment, but the remainder is usually treated as wastewater. Such FGD water is poorly defined because it depends on the source of coal, conditions of combustion, and limestone source used for the precipitation. After removal of the gypsum, FGD water is typically high in calcium, magnesium, chloride, sodium, and sulphate. Often it is supersaturated with calcium sulphate. Some elements, including selenium, arsenic, mercury, fluoride and boron are present in much lower concentrations, but at levels exceeding standards for surface water to be used for drinking, or irrigation, or for aquatic life in streams, lakes or rivers. Reported boron levels vary widely (up to >80 mg/L). This exceeds general irrigation limits (~<5 mg/L as B) many-fold. FAO data indicate damage occurs at levels of 5 mg/L in soil water for most crops. High B levels are also inhibitory of growth of aquatic plants, including algae, with reported effects at concentrations below 5 mg/L for some species.

Methods for removing mercury to very low levels are in use. Selenium and arsenic can be precipitated under reducing conditions as selenide and arsenide. Simple anions (e.g. F<sup>-</sup>) and cations are removable by ion exchange techniques if necessary. Boron, present as borate, has very low affinity for ion exchangers. It can be sorbed by vicinal hydroxyls of some sugars, but that is a very expensive process.

Tests were conducted with a local source of FGD water to determine its toxicity to hydroponically grown sunflowers, a moderately tolerant crop. Hoagland's solution and simulated FGD water (Hoagland's solution supplemented with sodium chloride and magnesium sulphate) were compared to FGD water. Water collected at the outflow of a constructed wetland used for Se removal, and lake water from the same facility, were also examined. Essential nutrients were added to all solutions to approximate an optimal hydroponic culture solution. Two week old sunflower seedlings of matched weights were used in three replications, with their water use followed for 14 days. While the added salt, approximating that of FGD water, definitely was inhibitory of sunflower water use (>30 %) compared to Hoagland's solution, there was little decrease (~10 %) in net dry matter accumulation. In contrast, inhibition by FGD water was ~40 % for dry matter production, with > 50 % decrease of water uptake. Increasing levels of boron caused further growth inhibition, whether supplied in FGD water, wetland outlet water or lake water. A 20 mg/L level of B gave ~40 % inhibition of growth in the lake and wetland outlet waters. Inhibitory effects of added B were less obvious in the FGD water where growth was ~40 % less than Hoagland's solution, even without added boron.

These results have major implications for disposal strategies used for FGD water. Boron levels in receiving water bodies must be kept below about 5 mg/L of B to avoid significant inhibition of plant growth when that water is used for irrigation, or discharged to zones where aquatic plants grow.

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## METAL CONTENT IN SOIL AS A FUNCTION OF DISTANCE FROM SOURCE OF EMISSION, WIND DIRECTION AND METAL SPECIFIC GRAVITY

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### ABSTRACT

Transportation is, apart from industry, one of major sources of environmental pollution. Elevated levels of various contaminants including metals, are recorded in soil in the vicinity of roads with intensive traffic. Metals, which origin mainly from car exhaust, lubricants, tire abrasion and broke pads, accumulate in soils neighboring the roads edge in considerable amounts. Polluted road dust can be easily blown out by wind for further distances causing metal deposition on soils and this would depends on wind direction. Accumulation of heavy metals in urban soils becomes a source of serious hazard to environment and human health. Although literature on the accumulation of metals as a consequence of increasing car traffic is reach data on effect of distance from source of emission is quite limited and often controversial.

The aim of this study was to assess the impact of: (i) the distance from the roadway, a (ii) the most frequent wind direction on the accumulation and (iii) specific gravity of selected metals content in the soil nearby road of intensive traffic.

Study area was the strip along the Przychyłkowa Street (Warsaw, Poland) characterized by intensive car traffic. Samples of soil were collected at the depth of 15 cm from windward and leeward sides of the road from ten distances starting from the road edge (the source of emission) up to 128 m. Collected soil samples were dried, triturated in ceramic mortar and sieved at 0.75 mm sieve. Then sub-samples were placed in certified XRF cups (10 cm<sup>3</sup>) and were placed in the measuring window of the XRF spectrometer Alpha 4000 for content of 28 selected metals determination. The analysis of each sample was set at measurement lasting 420 second. Active acidity (pH) and electrical conductivity (EC) of every soil samples were also measured. Analysis was performed in three replications for every point of collected samples.

Content of metals in most cases was significantly higher in samples collected from windward than from the leeward sites of the road. At the close distances from road edge out of the 28 measured metals content of four (Pb, Cr, Ba and Ti) were exceeded the background soil values for central Poland. Moreover, content of Pb and Ba exceeded permissible levels for agriculturally used land but were far below the level permitted for industrial use. The distance at which the metals at higher levels were recorded depends on specific gravity being greater for lighter metals i.e. Ti and Ba were recorded in higher amount at further distances (up to 128m) than Pb and Cr (16 m).

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## MATHEMATICAL MODELLING OF RHENIUM PHYTOEXTRACTION WITH INDIAN MUSTARD

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### ABSTRACT

Rhenium (Re) is one of the scarcest elements on Earth's crust ( $7 \times 10^{-8}\%$ ). Due to its rarity, Re is also one of the most expensive metals and an interesting target for phytomining. This work introduces a new mathematical model for Rhenium (Re) phytoextraction with Indian mustard [*Brassica juncea* (L.) Czern], a species recurrently used in phytomining studies involving other valuable metals such as gold and silver.

Firstly, a theoretical model is proposed to explain the metal accumulation process in the plant. The theoretical model formulation is based on a simplified mathematical model for complex dynamic systems, by assuming several parameters to describe the uptake mechanism of Re and its effect on plant growth. The parameters were set into simpler groups in order to facilitate validation. In addition, the model relates the concentration of Re in the soil and the concentrations of Re in the root and shoot of the plant. The resulting theoretical model is calibrated and tested using two sets of data (two different harvesting periods, 50 and 80 days), considering five concentrations (5, 10, 20, 40 and 80 mg kg<sup>-1</sup>) of Re in the substrate. The model accurately describes the experimental results, demonstrating a non-linear relationship between metal concentrations in the soil, root and shoot and the plant biomass yield.

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## THE EFFECTIVENESS OF USING RADISH (*RAPHANUS SATIVUS* L.) FOR PHYTOREMEDIATION OF INCREASED LEVELS OF LEAD-CONTAMINATION IN SOIL

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### ABSTRACT

Phytoremediation is an emerging technology that employs the use of higher plants for the clean up contaminated environment. Phytoextraction, the use of plants to extract toxic metals from contaminated soils, has emerged as a cost-effective, environment-friendly clean up alternative. Pot culture experiments using radish (*Raphanus sativus* L.) was performed to investigate lead (Pb) phytotoxic effects on antioxidant enzymes and other early warning biomarkers of soil Pb exposure. The study included an assessment of heavy metal accumulation in root, shoot and leaf, effect of lead stress on growth parameter (root length, root and shoot dry weight), photosynthetic pigment content, bioaccumulation coefficient (BAC) and the activity of anti-oxidant enzymes. Results demonstrated that efficient Pb uptake was observed by the roots in contaminated plants. Root growth was higher in control plants, as compared to the contaminated. Lead exposure also influenced biochemical and physiological parameters.

Administration of excess of lead was followed by an increase of Pb accumulation in leaves, and associated symptoms of toxicity. Typical symptoms of Pb toxicity developed 30 days after the beginning of treatment. Chlorophyll concentration was decreased in response to heavy metal toxicity. Activity of anti-oxidative enzymes e.g. peroxidase and catalase were increased in response to oxidative stress. Atomic absorption spectrophotometer (AAS) was used for analysis of heavy metal in soil and plant samples. The results of this research showed that radish are hyperaccumulator plants that can concentrate heavy metals in their different parts, thus they can be used for remediation of polluted area. Study also showed that potential of metal accumulator plants for extraction of metal from soil occur up to a certain level of concentration, after that when the concentration of metal increased the phytoextraction rate of metal or bioaccumulation coefficient (BAC) were decreased.

*Keywords:* oxidative stress, peroxidase, phytoremediation, radish



## ELECTROPHORETIC PROTEIN PROFILING OF MACROPHYTES EXPOSED TO CHROMATE STRESS

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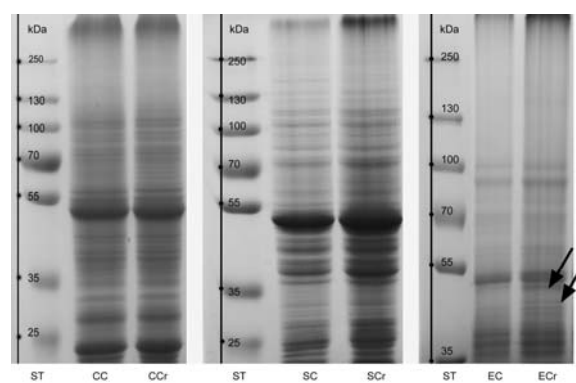
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### ABSTRACT

Macrophytes can serve as good models for the study of heavy metal interactions with assimilative plant organs. They are also preferable candidates for efficient phytoremediation of heavy metal contamination in aqueous environments. Anthropogenic pollution with chromium has become a worldwide problem due to the wide use of this metal in a number of industrial applications. Cr may appear at various oxidation states forming chemical agents of different toxicity, solubility and stability, and among these the Cr(VI) compounds are the most hazardous to living organisms. Chromate can easily enter cells *via* anion transporters and then it interacts with cell constituents as a strong oxidant. It undergoes reduction to the final Cr(III) form due to both biotic (enzymatic, non-enzymatic) and abiotic reactions. Cr(VI) and many of the intermediates that appear upon chromate metabolism are thus agents causing severe physiological stress.

The aim of the work was to reveal whether the incubation with chromate caused any changes in protein profiles of three common macrophytes which might be used as Cr efficient phytoremediators. Among the studied plants were: water-submersed macrophytes *Callitriche cophocarpa* and *Elodea canadensis* as well as the free-floating *Spirodela polyrhiza*. The source of the Cr(VI) anions was potassium chromate ( $K_2CrO_4$ ). The plants were incubated for 24 h with chromate levels evaluated experimentally to be sublethal (1, 1, and 0.5 mM, respectively), that is at conditions causing initial symptoms of growth inhibition and chlorosis, and thereafter enabling full recovery within several days after washing out the Cr(VI) solution and further cultivating in an MS medium (*i.e.* at conditions same as for the untreated control plants). Protein extracts were obtained after mechanically homogenizing the plant material in a mortar with liquid nitrogen. Then, an optimized technique of protein precipitation with acetone and phenol was elaborated to eliminate all the non-protein impurities that severely interfered with the electrophoretic analyses.

The Figure shows exemplary results obtained with the SDS-PAGE electrophoresis. As it can be seen chromate supplementation did not result in changes of proteomic profiles for *C. cophocarpa* (the path marked "CCr") and *S. polyrhiza* ("SCr"). *E. canadensis* ("ECr"), in turn, revealed only two protein bands induced by Cr(VI) treatment ( $M_w$  of approx. 43 and 48 kDa). Paths "ST" represent molecular weight standards and "C" – electrophoregrams of the respective control experiments.



Abiotic stress has been shown to alter proteomic patterns in many organisms. Our reference studies involving bacterial and yeast proteomes further proved that the Cr-induced stress resulted in protein profile changes where both suppressed and induced proteins as well as down- and up-regulated ones were revealed. So far, little or none has been done with regard to macrophytes with the aim to study their response to chromate at the level of detectable proteomes. Such research is of high interest since these plants can accumulate or interact with chromium compounds directly *via* their photosynthetically active organs.

In this context, the observed lack of changes or only subtle alterations in electrophoretic proteinograms upon Cr(VI) treatment are intriguing phenomena and will launch a detailed study to reveal biochemical and physiological background of the macrophyte adaptation to Cr(VI).

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## EFFECTS OF Cd, Pb AND Ni ON THE ROOT HISTOLOGICAL CHARACTERISTICS OF *SALIX ALBA* L. AND *SALIX NIGRA* MARSH.

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### ABSTRACT

Species of the genus *Salix* are nowadays increasingly used in phytoremediation projects. Therefore, in this research we focused our attention on the changes of anatomical characteristics of willow root in the presence of elevated concentrations of Cd, Ni and Pb.

Plant material consisted of two willow clones, clone of species *Salix alba* L. and clone of species *Salix nigra* Marsh. Plants were grown in semi-controlled conditions, using a water culture method. After 35 days of the beginning of the experiment, the treatment of plants with two concentrations ( $10^{-4}$  and  $10^{-5}$  M) of all three heavy metals was initiated, for a period of 45 days. Anatomical analysis was performed on cross sections of the roots, that are made in three zones: 1 cm from the apical region, in the middle part and in the base (neck) of root, using the Image Analyzing System. Detailed anatomical analysis included root cortex (number of cell layers, exodermis area and area of parenchymatic cells in the fourth to fifth parenchyma layer) and root central cylinder (percentage of the central cylinder, number and area of the vessels).

Treatments with heavy metals did not cause necrotic changes of the root, regardless of the applied concentration of heavy metals and clone. However, they caused significant changes in the values of analyzed root parameters in comparison with control samples. The largest number of negative effects on the parameters of root anatomical structure exhibited Cd. Significant reduction in the root cross section area of *Salix alba* clone under the influence of Cd is caused by significant reduction of the number of parenchymatic cells layers and area of exodermal cells. The total area of the vessels is significantly higher in the presence of both concentrations of Cd, compared with the control sample. The minimal number of negative effects were caused by Pb. Deposits of heavy metals were observed in the root tissues, mainly in the walls of cortical parenchymatic cells, rarely in the lumen of parenchymatic cells and in the walls of the xylem elements. Nickel and lead caused even positive effects on the *Salix nigra* clone, in terms of increased values of measured parameters, compared with the control, which could mean that applied concentrations had a stimulatory effect on this clone. Generally observing, clone of species *Salix nigra* shows greater tolerance of heavy metals.

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## INTERANNUAL AND INTRA-ANNUAL MERCURY VARIATION IN A TREE SPECIES (*QUERCUS ILEX* L.)

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### ABSTRACT

Plants which are exposed to environmental pollutants are able to accumulate them in their organs depending on specie of plant, type of organ or its age among other factors. The evergreen plants can hold in the same branch stems and leaves which are one, two or more years old. Thus, the amount of contaminants of these former organs could vary with the exposure time to contaminating source.

*Dehesa de Castilseras* is a forestry area located in Almadén mining district (Ciudad Real, Spain). In this district mercury mining and metallurgical activities have been carried out for centuries. Within *Dehesa de Castilseras*, close to Almadenejos village, the leaves and the stems of the main evergreen plant species (*Quercus ilex* L.) have been analyzed with the aim of studying how the mercury concentration varies between consecutive years and during the same year. For that reason, three different trees were selected and two branches were taken from each of them, one facing north, and the other facing south.

Considering each branch and each year, the results show that leaves have higher mercury concentration than stems on average. Furthermore, the lowest mercury concentration was measured in fruit (acorn). The obtained values are similar to mercury concentrations measured in acorns from oak crops that live under free mercury conditions.

With regard to ageing effect on mercury concentration and taking into account each branch separately, the results show the older leaves have higher mercury concentration than younger ones. Nevertheless the older stems not always have higher mercury concentration than younger ones. Moreover, the mercury concentration of the leaves and stems which have recently sprouted is very low and very similar while if these fractions are analyzed six months after its sprout, mercury concentration values are much higher and heterogeneous. This fact leads us to suggest that these kind of young organs are sampled between 6 to 9 months after their sprouting.



## RESISTANCE OF PLANTS TO SOIL CONTAMINATION BY CHROMIUM (VI)

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### ABSTRACT

Soil contamination by heavy metals, including chromium, leads to the gradual degradation of soils in many parts of the world; in some cases the damage can be permanent, so that the affected soil becomes infertile and its plant cover disappears. Heavy metals may also have a negative effect on the growth and development of plants, their chemical composition and the biological properties of soil, such as the enzymatic activity and counts of microorganisms. These considerations have encouraged us to undertake the current study, in which our aim was to determine the resistance of plants, including their chemical composition, to soil contamination by hexavalent chromium. In addition to this, activity of some enzymes engaged in the transformations of carbon, nitrogen and phosphorus has been studied as well as the physicochemical properties of soil.

An experiment, conducted in an unheated greenhouse at the University of Warmia and Mazury in Olsztyn (north-eastern Poland), was set up on soil material collected from the arable humus horizon of proper brown soils (Eutric Cambisol). According to the soil classification based on grain-size distribution, proposed by the United States Department of Agriculture, this soil represented loamy sand. It had the following properties: pH in 1 mol KCl dm<sup>-3</sup> - 6.2; C<sub>organic</sub> - 6.50 g kg<sup>-1</sup>; Cr - 11.35 mg kg<sup>-1</sup>; N<sub>total</sub> - 0.76 g kg<sup>-1</sup>; hydrolytic acidity - 14.10 mmol(+) kg<sup>-1</sup>; sum of exchangeable bases Ca<sup>++</sup>, Mg<sup>++</sup>, K<sup>+</sup>, and Na<sup>+</sup> - 80.00 mmol(+) kg<sup>-1</sup>; cation exchange capacity - 94.00 mmol(+) kg<sup>-1</sup>; base saturation - 85.11%. The experiment included 5 replicates. Two variables were examined: the degree of contamination by chromium (VI) in mg Cr<sup>6+</sup> kg<sup>-1</sup> of soil (0, 15, 30, 45 and 60 and several species of crops (oat, spring barley, spring oilseed rape, white mustard, yellow lupine and horse bean). All the treatments received the same fertilization composed of macro- and micronutrients. During the whole period of growing the plants, the soil moisture was maintained on the level of 60% water capillary capacity. After harvest (on day 50), the volume of yields and their chemical composition were determined (content of nitrogen, phosphorus, potassium, calcium, magnesium, sodium and chromium). Also, the activity of the following soil enzymes was determined: dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Based on the analytical results, values of the resistance index for all the plants to chromium (VI) in soil as well as the activity of individual enzymes in response to this soil contaminant and the biochemical index of soil potential fertility were calculated. The results were submitted to the Tukey's test at the level of significance p=0.05. The principal component analysis (PCA) method was applied to assess the effect of chromium on plant yields and activity of soil enzymes.

Hexavalent chromium had a very toxic effect on the plants, which was confirmed by a highly significant negative correlation between the dose of Cr(VI) and yield of aerial parts of horse bean ( $r = -0.993$ ), spring barley ( $r = -0.981$ ), spring oilseed rape ( $r = -0.968$ ), yellow lupine ( $r = -0.961$ ), white mustard ( $r = -0.960$ ) and oat ( $r = -0.929$ ). The values of the correlation coefficients themselves suggest that the tested crop species were variously tolerant to the pollution. Toxic symptoms, such as water balance disorders (wilting), chlorosis of new leaves or damage to the growth apex and roots, appeared in yellow lupine in all treatments, including the ones with the smallest chromium dose (15 mg · kg<sup>-1</sup> of soil) and becoming more intensive under higher doses. The smallest dose of Cr<sup>6+</sup> also caused a significant decrease in the yields of spring oilseed rape and white mustard. Such a change in yields was manifested by the resistance indices (RS) of individual crops in response to the heavy metals. For all the crops, the RS was the lowest in soil polluted by 60 mg Cr<sup>6+</sup> kg<sup>-1</sup> of soil. The most chromium-resistant plant proved to be horse bean (RS = 0.500). The resistance of the other crops was much lower. The RS of white mustard was 0.337, oat - 0.247, spring barley - 0.168, yellow lupine - 0.097 and spring oilseed rape - 0.062. Assessment of the effect of chromium cannot be limited to plant yields but should also consider the chemical composition of plants. The results of our analyses demonstrated that the soil contamination by chromium induced larger changes in the concentrations of nitrogen, phosphorus or potassium than magnesium, calcium and sodium in aerial organs of the plants. Another aspect that must not be neglected is the accumulation of chromium by plants. Its elevated content in a plant is one of the consequence of the presence of chromium in the environment due to anthropogenic activity. Chromium was also found to have inhibited the activity of dehydrogenases, urease, acid phosphatase and alkaline phosphatase. Moreover, it reduced the value of the potential biochemical index of soil fertility. Dehydrogenases proved to be the most sensitive to the excess of chromium in soil, in contrast to alkaline phosphatase, which was the most tolerant of all the analyzed enzymes.



## LABORATORY AND PILOT-SCALE PHYTOFILTRATION OF URANIUM-CONTAMINATED WATER

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### ABSTRACT

The objective of the present study is: 1) developing a phytofiltration prototype system; 2) to test the potential of the species *Callitriche stagnalis*, *Potamogeton natans* and *Potamogeton pectinatus* for the decontamination of U-contaminated waters.

The installed prototype consists of a closed circuit of channels. The system was initially contaminated with 500 µg/L of U as uranyl.

The aquatic plants tested in the U phytofiltration experiments showed a high ability for U accumulation and, therefore, they can be useful choices for future *in situ* applications. The results described for the phytofiltration prototype show a reduction of U concentration, in the water, from 500 to 72.3 µg/L of U, thus representing an efficiency of 85.5%. At the end of 15 days of the test the U concentration in *C. stagnalis* increased from 0.98 to 1567 mg/kg, in *P. natans* increased from 3.46 to 271 mg/kg and in *P. pectinatus* increased from 2.63 to 1588 mg/kg. All these species showed a rapid U uptake and the results highlight the effectiveness of the selected plants to remove uranium from the water.

The advantage of the phyto-system proposed in this study is related to the high tolerance of the species tested to U contamination and with their abundance and natural occurrence in the Portuguese rivers which prevent the invader factor for an *in situ* application. In fact, the native character of these species represents a very important factor for the establishment of this technology taking into account the sociability relationships with other aquatic species and their ecosystems adaptation. The combination of several species, resembling the natural environment, can prove more effective than the usual mono-specific culture option.

The selected plant species are a promising choice for the remediation of low to medium U contaminated waters. However, to assess the real phytotechnological potential of the proposed system, and before an *in situ* application, laboratory tests are needed to determine the toxicity level, the biomass productivity and what enhancement strategies of U uptake can be used. The issue of the final disposal of the generated residues also has to be addressed.

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## OPTIMIZATION OF HARVEST TERM OF PHYTOEXTRACTION SHORT ROTATION COPPICE

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### ABSTRACT

Soil contamination by risk elements belongs to significant contemporary ecological problems. Following this reason, the interest for economically reasonable methods of soil decontamination, as phytoextraction is increasing. Fast growing trees from *Salix* spp. and *Populus* spp. are suitable plants for phytoextraction of risk elements, especially on medium and weakly contaminated soils [1]. The majority of risk elements is usually accumulated in leaves than in twigs [2] therefore a biomass harvest can be preferable realise within vegetation period.

The aim of our study was to optimize rotation period and term of harvest of fast growing trees in field conditions and to improve the phytoextraction potential. The experimental short rotation coppice plantation near Příbram town (49°42'24"N, 13°58'32"E), was planted in April 2008 and 2009 with clones of *Salix* and *Populus* species: (*Salix schwerinii* x *S. viminalis*) x *S. viminalis* (S1), *S. smithiana* (S2), *Populus nigra* x *P. maximowiczii* (P1) and *P. nigra* (P2). Both plantings were completely harvested in February 2012. Other samples were taken after next 8 months of growth in September 2012. The yield parameters and the content of risk elements were determined at all samples. We investigated the differences between three years (planting in 2008) and four years harvest rotation (planting in 2009) as well as differences between winter (February) and summer (September) harvest of trees.

The biomass yield at three and four years rotation was similar. The differences between biomass yield from winter and summer harvest weren't significant, clones reached the same yields 8 months after winter harvest as after 3 and 4 years of growth. In September, the trees were harvested with leaves. The ratio of leaves to twigs weight was different at individual clones: S1=0.28, S2=0.36, P1=0.61, P2=0.50. The dry biomass yield decreased in sequence: P1>>S2>P2>S1 (433-97g plant<sup>-1</sup>).

The contents of observed risk elements weren't different in trees harvested after 3 or 4 years. The significant differences were found between winter and summer harvest. The highest concentration of nutrients K, Ca and Mg were determined at all clones in leaves (K 10.09-13.85 g kg<sup>-1</sup>, Ca 13.76-22.43 g kg<sup>-1</sup>, Mg 3.49-5.91 g kg<sup>-1</sup>) and consequently in twigs in September, while the lowest concentration of these elements were detected in twigs harvested in February (K 0.63-2.29 g kg<sup>-1</sup>, Ca 1.29-2.79 g kg<sup>-1</sup>, Mg 0.10-0.32 g kg<sup>-1</sup>). Also the other elements (P, Cd, Fe, Mn, Pb and Zn) showed the highest concentrations in leaves (e.i.: Cd 12.67-51.43 mg kg<sup>-1</sup>), and very low concentrations in twigs harvested in September (e.i.: Cd 4.04-9.78 mg kg<sup>-1</sup>) and high concentrations in twigs harvested in February. Copper content in twigs harvested in February was higher than content in leaves harvested in September. This is related with changes of element concentration in xylem sap [3]. The element uptake was significantly influenced by biomass production. The highest uptake was determined at the most productive clones P1 and S2. The concentration of elements was also highly affected their uptake, as Fig. 1 and 2 show. Higher amount of Ca, K and Mg was removed at summer harvest, their high concentrations were determined in leaves and twigs as well; conversely Cd, Cu, Fe, and Pb were predominantly removed by winter harvest.

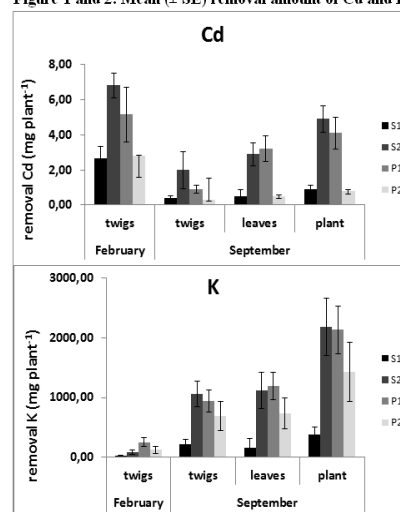
Our study showed the shortening of rotation period by one year does not influence both the biomass yield and element uptake of older trees. Summer harvest is less suitable for phytoextraction of contaminants: the risk elements removal was low and nutrient removal high at September compare to February harvests.

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Figure 1 and 2: Mean (± SE) removal amount of Cd and K





## ACCUMULATION OF RISKY METALS IN SEEDS AND ABOVEGROUND BIOMASS OF AMARANTH

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### ABSTRACT

Amaranth is an 8000 year old cultivated crop originating on American continent. It is not demanding on soil and climatic conditions, is resistant to stress and high temperatures and produces a lot of biomass. A few years ago amaranth was rediscovered as a multipurpose crop applying high nutritional quality seeds and leafy vegetables for food and animal feed. In some parts of the world amaranth seed is considered as a staple food rich in lipids, proteins, carbohydrates, dietary fiber and also another components such as squalene, tocopherols, polyphenols, phytates and vitamins. In West Africa amaranth is cultivated also for its edible leaves rich in vitamins and dietary minerals. The objective of the study is to compare the degree of accumulation of risk metals by amaranth seeds and aboveground biomass and to evaluate the safety of consumption parts of five different amaranth cultivars from the aspect of heavy metal content. Soil as a starting place for input of risk substances into a human food chain was analysed, too.

In soil the agrochemical characteristics (pH/KCl, content of K, Mg, P, Ca and humus) were determined. Pseudototal content of risk metals (Zn, Cu, Ni, Cr, Pb and Cd) including all the form besides residual metal fraction was assessed in soil extract by *aqua regia* and content of mobile forms of selected heavy metals in soil extract by  $\text{NH}_4\text{NO}_3$  ( $c=1\text{mol/dm}^3$ ). Gained results were evaluated according Law No. 220/2004 (valid in the Slovak Republic) as well as threshold values proposed JRC EC (2006). Used analytical method was flame AAS (AAs Varian AA Spectr DUO 240 FS/240Z/UltrAA). Seeds and aboveground biomass of five amaranth cultivars (Golden Giant, Rawa, Annapurna, Oscar Blanco, Koniz) were manually separated, dried at 105°C to constant weight and powdered. After previous microwave decomposition of plant samples the contents of risky metals were determined by AAS method (AAS Varian AA Spectr DUO 240FS/240Z/UltrAA) and expressed as mg/100 DM (dry matter). Gained results were evaluated according to FC SR (Food Codex of the Slovak Republic valid in the Slovakia) as well as according Commission Regulation (EC) 1881/2006.

The soil of amaranth cultivation was uncontaminated. Only determined Cd content was by 40% and Pb content by 10% higher than limit values given by Law No. 220/2004 for the soil extract by *aqua regia* and for the soil extract by  $\text{NH}_4\text{NO}_3$ , respectively. But on the other hand the determined Cd content did not exceed the threshold value given by EC (2006). In aboveground amaranth biomass the followed values of heavy metal content were determined (in mg/kg DM): Zn: 26.20-30.38; Cu: 6.20-16.84; Ni: 0.63-1.75; Cr: 3.90-8.00; Pb: 0.70-2.00; Cd: 0.69-1.87. In amaranth seeds these values were measured (in mg/kg DM): Zn: 10.33-20.20; Cu: 2.37-4.43; Ni: 0.45-0.68; Cr: 0.40-1.70; Pb: 0.25-0.60; Cd: 0.15-0.20. In seeds of all investigated amaranth cultivars the maximal allowed amounts (given by FC SR as well as EC 1881/2006) for Cd and Pb were by 60-100% and 25-200% (respectively) exceeded. In aboveground amaranth biomass 0.17 – 12.25 fold higher amounts of heavy metals were determined in comparison to amaranth seeds. The statistically significant differences in values of heavy metal content measured in seeds as well as in aboveground biomass between investigated amaranth cultivars were confirmed. Our results confirm, that amaranth seeds and leaves as food raw materials could represent a risk to the health of the consumer from the aspect of high Cd and Pb amounts.

Because of ability to produce abundance of biomass and at the same time to accumulate high amounts of risk heavy metals amaranth could be used as a potential plant for a soil phytoremediation. The results confirmed the ability of amaranth to accumulate risk metals even from relatively „clean“ soil.

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## EFFECT OF URANIUM ON PHOTOSYNTHETIC PARAMETERS IN *PISUM SETIVAM* IN CONJUGATION WITH ANTIOXIDANT DEFENSE

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### ABSTRACT

Large uranium accumulations in vegetable foodstuffs may present risks of human health if they are consumed. The toxic effects of U in living organisms depend on uranyl ion speciation and their solubility. In our present study we studied the impact of U on photosynthetic pigments (chlorophyll/carotenoid contents) and some antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and the cysteine and non-protein thiol (NP-SH), as well as lipid peroxidation as oxidative-damage markers. Disturbances in nutrient status induced by U are another feature previously noted in other plants. It has been also observed that ion transporters could be the target of ROS hence, U and macro/micronutrients uptake was also studied by analyzing the content of those elements in roots and leaves of *Pisum setivum* plants.

In tested plants photosynthetic pigments significantly altered depending on the concentration of uranium uptake in comparison to control, the decrease in net photosynthesis as a consequence of reduced absorption of essential mineral nutrients is an indirect reason for plant chlorosis. CAT and GR activity was lower in roots and shoots in compare to control at higher concentration but it was interesting to note that both cysteine and NP-SH was higher in root as well as in shoots in higher concentration U treated plants after 5 days. The decline in CAT activity might be due to the inhibition of the enzyme by post-transcriptional alterations or down-regulation of gene expression. The increase in the level of thiols and cysteine may be due to stimulation of enzymes of sulfate reduction pathway such as APS reductase and Serine acetyltransferase. The enhancement in NP-SH concentration possibly also reflects a defense reaction to enhanced production of ROS. Increment in lipid peroxidation indicates the prevalence of oxidative stress and perhaps this may be one of the possible mechanisms by which toxicity due to U could be manifested in the plant tissues. However, the level of lipid peroxidation was lower in roots as compared to that of shoots. This was attributed to low U load in shoots that probably resulted in low oxidative stress and membrane damage. Among all tested macro and micro elements (Ca, Fe, K, P, S, Mn, Zn and Cu) only Ca uptake is higher in shoots rather than roots. U accumulation is also higher in roots rather in shoots after 5 days of treatment. Our observation suggests that U severely disturbed the nutritional status of the experimental plant, a situation that could induce alterations in cell metabolism and thereby stunt growth. It has been hypothesized that the metal detoxification mechanism was more pronounced at the site of its uptake, which is restricting the mobility of metals to shoot from root. The detail of this work is going to be discussed in the meeting.



## RELATIONSHIPS OF FREE AMINO ACIDS AND FATTY ACIDS WITH CADMIUM IN *NOCCAEA CAERULESCENS* ECOTYPES

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### ABSTRACT

Metal hyperaccumulation is a physiological process that enables the plant to store large quantities of metal ions in above-ground tissues without suffering toxic effects. Cadmium (Cd) is one of the most toxic metals for living organisms and *Noccaea* (previously named *Thlaspi caerulescens*, J. & C. Presl) is one of the four identified Cd hyperaccumulators identified up to now. The uptake of Cd from contaminated soil affects the plant metabolism of amino acids and lipids. This effect is indicated by change in the content of free amino acids and fatty acids in tissues of hyperaccumulators. The aim of the study was to determine the changes in free amino acids and fatty acids content in selected ecotypes of *N. caerulescens*.

A pot experiment was conducted in a vegetation hall under controlled conditions (temperature day/night 24 °C/18 °C, relative humidity 60 %, light intensity: day/night 16 h/8 h) using three ecotypes of *Noccaea caerulescens* (F.K. Mey): "Ganges" (France), "Mežica" (Slovenia) and "Redlschlag" (Austria) and four Cd level 0, 30, 60 and 90 mg.kg<sup>-1</sup> applied as Cd(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O. Plants in growing period of four leaves were placed in 3 L plastic pots filled soil (chernozem modal: habitat Prague-Suchdol; pH<sub>KCl</sub> = 7.1 ± 0.3; C<sub>ox</sub> = 3.1 ± 0.7 %; KVK = 225 ± 19.5 mmol<sub>(+)</sub>.kg<sup>-1</sup>; contents of Cd = 0.42 ± 0.05 mg.kg<sup>-1</sup>) with nutrients and contaminations. The dose of nutrients was: 0.3 g N, 0.10 g P, and 0.24 g K applied in the form of NH<sub>4</sub>NO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>. The first harvest was performed 90 days and the second harvest 120 days after planting of *N. caerulescens* into soil. The Cd concentrations in plant biomass were determined by ICP-OES with axial plasma configuration (VarianVistaPro, Varian, Mulgrave, Australia). Total free amino acids (AA) compounds were determined using an EZ-faast amino acid analysis procedure (Phenomenex, Santa Clara, USA). Samples were analyzed for AA contents by the GC-MS (Agilent Technologies, Torrance, USA). The contents of methyl esters of fatty acids after transesterification were measured by GC-MS (Thermo Fisher Scientific, USA). The statistical analyses were performed using hierarchic analyses of variance (ANOVA) with interactions at 95% (P < 0.05) significance level with subsequent Tukey's HSD test and linear correlation (R<sup>2</sup>) by using Statistica 12.0 software (StatSoft, Tulsa, USA).

Uptake of Cd was the highest in the Cd-accumulating ecotype, Mežica, than in the low Cd-accumulating ecotypes, Ganges and Redlschlag. The free AA content in the biomass of ecotype Mežica declined progressively with increasing Cd concentrations during both harvests (R<sup>2</sup>=0.99-1 and R<sup>2</sup>=0.90-1). The same trend, decrease of free AA content was observed in biomass of ecotype Redlschlag. Results confirmed the significant relationship between contents of free AA and Cd content in biomass (R<sup>2</sup>=0.93-1 for both harvests). Opposite trend, increase of free AA content was observed in biomass of ecotype Ganges. The significant relationships between free AA content and Cd content in biomass was confirmed using linear regression (R<sup>2</sup>=0.84 and R<sup>2</sup>=0.62). The major free AA determined in all ecotypes of *N. caerulescens* were glutamic and aspartic acid, glutamine, asparagine and proline. The Cd soil contamination did not significantly modify the total contents of saturated and unsaturated fatty acids in biomass of ecotypes Ganges and Redlschlag. Different results were showed in biomass of ecotype Mežica. Linear correlations confirmed closed relation of saturated and unsaturated fatty acid contents and Cd content in biomass. Content of saturated fatty acids decreased with concentration of Cd (R<sup>2</sup>=0.92-1 and R<sup>2</sup>=0.99-1). On the other hand content of unsaturated fatty acids increased with concentration of Cd (R<sup>2</sup>=0.92 and R<sup>2</sup>=0.99).

Results of the pot experiment showed Cd effect on total content free AA and fatty acids playing an important role in plant adaptation to stress. Analyses of free AA and fatty acids confirmed the higher cadmium tolerance of ecotype Mežica in contrast to the ecotypes Ganges and Redlschlag. These results indicate that the mechanisms of Cd hyperaccumulation and metabolism of free AA and lipids are not identical in this species.

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## INFLUENCE OF ESSENTIAL METALS ON CADMIUM UPTAKE BY SORGHUM PLANTS

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### ABSTRACT

Increased concentrations of toxic metals in agricultural soils can cause death of plants or reduce their production. Contaminants accumulated in food crop may also cause danger to human health. On the other hand, plants grown on contaminated soil can reduce the pollution through a transfer of the contaminant from soil to harvestable plant parts. Phytoextraction can be a solution how to prevent the spread of pollution while ecologically enhanced the site with economical aspect. The method is suitable for low contaminated sites and it is used for its low cost and wide public acceptance. Appropriate plant species should be tolerant to pollutants toxicity and it should have also high biomass production. This condition meets energy plants such as woody plants, and grasses.

*Sorghum bicolor* is an important crop due to its wide use. Due to a high biomass production and known agronomic techniques, sorghum can be used as an energy crop and at the same time it can clean up the environment. The aim of this study was to expand knowledge about protection mechanisms of different species of sorghum (*S. bicolor* var. *eusorghum*, *S. bicolor* x *S. sudanese*) under cadmium stress. The effects of combination of cadmium with essential metals such as copper, iron, and zinc were studied.

Cd (mM)	Cu, Fe, or Zn (mM)	Cd (mM)	Cu, Fe, or Zn (mM)	Cd (mM)	Cu, Fe, or Zn (mM)	Cd (mM)	Cu, Fe, or Zn (mM)
0.01	0	0	0.01	0.01	0.2	0.2	0.01
0.05	0	0	0.05	0.05	0.2	0.2	0.05
0.1	0	0	0.1	0.1	0.2	0.2	0.1
0.5	0	0	0.5	0.5	0.2	0.2	0.5
1	0	0	1	1	0.2	0.2	1
5	0	0	5	5	0.2	0.2	5

Results showed that cadmium toxicity depended on chosen sorghum variety. EC<sub>50</sub> value range was from 1.2 to 2.0 mM for cadmium, 1.2 - 2.2 mM for copper, 3.5 – 8.2 mM for iron, and 5.4 – 12.6 mM for zinc. Essential elements had an influence on toxicity of cadmium to sorghum plants and also an effect of cadmium on copper, iron, and zinc toxicities was evident.

*Acknowledgement:* This work was supported by projects LD14106 and LD14107.



## HYPERACCUMULATOR *THLASPI CAERULESCENS* (GANGES ECOTYPE) RESPONSE TO INCREASING LEVELS OF DISSOLVED CADMIUM AND ZINC

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### ABSTRACT

Phytoextraction or phytoremediation of heavy metals has been thought to be an environmentally friendly, cost-effective strategy to clean-up soils or waters contaminated with these toxic pollutants. Efficient metal extraction relies basically on the use of hyperaccumulator plants with exceptional metal-accumulating capacity, known as natural phytoextraction. Hyperaccumulator plants possess several exceptional characteristics, such as the ability to take up and translocate exceedingly large amounts of metals and hypertolerate their phytotoxic effects. In this regard, *Thlaspi caerulescens* (Alpine pennycress) is considered as one of the best known examples of hyperaccumulator plants, being able to primarily hyperaccumulate Zn and Cd as well as Ni in some accessions.

In the current study, *Thlaspi caerulescens* (Ganges ecotype) was separately exposed to Cd and Zn at 0, 50, 100 and 200  $\mu\text{M}$  for 7 d to monitor plant physiological response in hydroponics. Significant dose dependent accumulation was observed for both metals, mainly in roots (up to 3.2 and 9.2  $\text{mg g}^{-1}$  for Cd and Zn, respectively). However, Cd was more phytotoxic in terms of plant growth and photosynthesis. This higher toxicity was also evidenced by the MetPLATE bioassay applied to exposure solutions. Root exudation was significantly correlated to Cd and Zn translocation ( $r > 0.85$ ) proving its involvement in facilitating metal uptake.

As for antioxidative responses, plants reacted to Cd and Zn by broadly exhibiting an elevation of glutathione reductase (GR) activity before declining at 200  $\mu\text{M}$  due to higher phytotoxicity. In contrast, superoxide dismutase (SOD) activity was unlikely to be affected by both metals. Root-to-shoot apoplastic flow was traced using a fluorescent dye (PTS), whose concentration in leaves increased to a certain extent with Cd and Zn accumulation indicating that heavy metals have a comparable effect to drought or salinity in promoting the passive diffusion of water and solutes. Nevertheless, Cd at 200  $\mu\text{M}$  hindered the diffusion of PTS and consequently affected the apoplastic transport in plants by damaging root cells.

**Keywords:** *Thlaspi caerulescens*, cadmium, zinc, exudation, antioxidation, apoplastic flow



## ASSESSMENT OF EFFICACY OF EDTA AND CITRIC ACID IN RHIZOFILTRATION ENHANCEMENT OF CADMIUM USING *LEMNA MINOR*

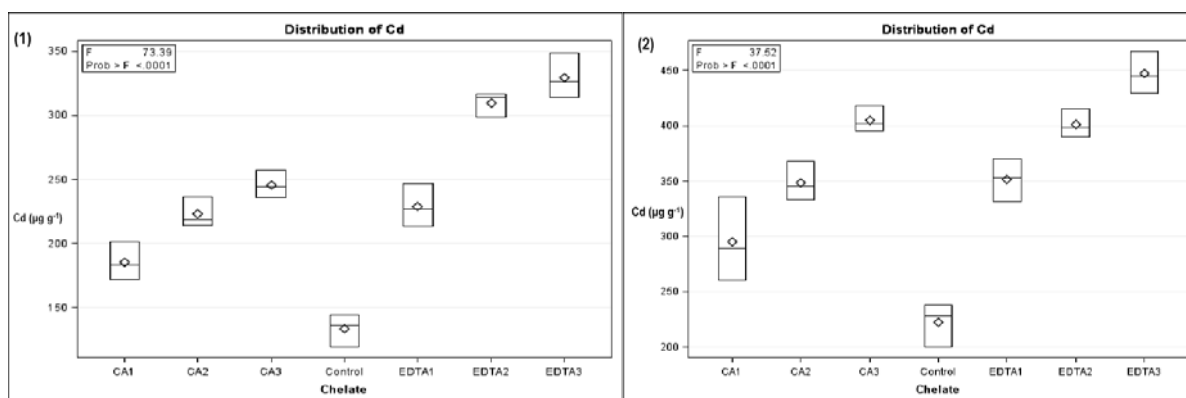
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### ABSTRACT

Free floating aquatic macrophyte namely *Lemna minor* (Linnaeus, 1753) was exposed to different concentration of cadmium ( $1, 5$  and  $10 \text{ mg L}^{-1}$ ) for a period of 30 days to evaluate its cadmium (Cd) accumulation capability in the presence of chelating agents such as EDTA and citric acid. The chelating agents were added at the rate of 1, 2 and 3  $\text{mg L}^{-1}$  separately and the experiment was conducted in triplicate. The water and plant samples were collected at 15 days interval for the analysis of cadmium. There was a significant difference in the Cd uptake ( $P < 0.05$ ) by the plant in the presence of chelating agents when compared to the control. Bioconcentration factor (BCF) of cadmium by the plants showed an increasing trend in the presence of chelating agents. The percentage uptake of cadmium by *L. minor* in the presence of EDTA was significantly higher than that of citric acid ( $P < 0.05$ ). The overall results suggest that EDTA can be effectively used to enhance phytoremediation efficiency of cadmium by *L. minor* in the contaminated water.



**Fig. 1, 2 - Cadmium accumulation ( $\mu\text{g g}^{-1}$ ) by *L. minor* on 15 and 30<sup>th</sup> day from  $1 \text{ mg Cd L}^{-1}$  respectively**

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## THE CURRENT STATUS OF *BISCUTELLA LAEVIGATA* L. (BRASSICACEAE) SPECIMENS TESTED FOR THE SUITABILITY TO PHYTOREMEDIATION TECHNOLOGY

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### ABSTRACT

The well known facts are that phytoremediation has become an advantageous way to recover degraded lands, and that numerous *Brassicaceae* members are easy accommodating into almost every kind of habitats. Many species are even known to perform well on waste areas as they are well adapted to recently formed, non-structural substrates. *Biscutella laevigata* L. is perennial herbaceous species, which has developed different ecotypes, and in Europe is recorded on rocky, often alpine stands, metal enriched soils, and anthropogenic substrates like waste heaps polluted with heavy metals. Simple leaves are gathered in a basal rosette with a woody root-stock. The leaved inflorescence has branched stalk with numerous bright yellow flowers. The fruit dehiscent is short-ovate with false-septum. In Poland *B. laevigata* has been described from two different stands: first is located in the Tatry Mountains, and second in the Zn-Pb ore-mining region in district of Olkusz located in southern part of the country, where it occur as an endemic metallophyte typical of calamine flora. There is a strong possibility that this calamine ecotype will be useful to reclamation of waste material polluted with Zn, Pb and Cd. Therefore, experiments were undertaken to investigate the fitness of *B. laevigata* specimens representing the calamine ecotype from the Olkusz ore-bearing district, during culture under vegetation hall conditions on wastes after zinc and lead ores flotation.

Pot experiment was set up in April 2011, so until now it was conducted for 3 years. Pots with a diameter of 22 cm were filled with substrate in an amount of 5.5 kg per pot. The substrate was wastes after flotation of zinc and lead ores collected from the settling of tailings materials, belonging to metallurgical and mining plant situated in Bukowno near Olkusz (the southern part of Poland). The experiment was conducted in six replications, and included three treatments: control, without fertilization; mineral fertilization at a dose of 1.2 g N; 0.4 g P<sub>2</sub>O<sub>5</sub>, 1g K<sub>2</sub>O per pot, using a mixture of aqueous solutions: NH<sub>4</sub>NO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and KCl; fertilization with municipal sewage sludge of 135g per pot, and enriched with potassium dose 0.8 g K<sub>2</sub>O/pot. The dose used was the maximum permissible dose of sewage sludge, which can be used for reclamation purposes, in accordance with applicable Polish Regulation. Substrate moisture was maintained at 35% of capillary water capacity. Observations of *B. laevigata* growth and development started a month after planting, and detailed biometrical measurements were conducted every four weeks. After each growing season one replication of each fertilizing objects was taken for analyses of the plant material and the substrate.

Flotation material used as a substrate for growing *Biscutella* was characterized by alkaline reaction, trace contents of organic carbon and total nitrogen, and very low contents of available phosphorus and potassium. The content of total forms of zinc, lead and cadmium (respectively: 10690 mg/kg, 8058 mg/kg and 78,8 mg/kg) several times exceeded the permissible contents of these metals as defined in the Regulation of the Minister of Environment for industrial and post-mining lands. Cultivation of plants with fertilization contributed to the slight increase in the content of organic carbon and total nitrogen in each object with respect to the contents of these elements in the initial flotation material. The increase of the carbon and nitrogen contents was set in the following range: control object < object fertilized with mineral fertilizers < object fertilized with sewage sludge. This



arrangement was maintained throughout the whole period of the experiment, and the observed changes intensified with time. During the experiment changes in zinc lead and cadmium contents in the substrate were also observed. After each growing period contents of Zn, Pb and Cd in the substrate gradually lowered. The decreases in contents for Pb and Zn were similar in all experimental treatments, and amounted to 35,9-40,4% and 35,9- 36,5% respectively. However in the case of Cd the phytoremediation activity of *Biscutella* specimens, it was strongly related to particular treatment, and it was the lowest in the object fertilized with sewage sludge in spite of the fact that plants developed the biggest biomass in this substrate. The contents of Zn and Pb were the lowest in the substrate of the object fertilized with sewage sludge, and the largest in the substrate of the object without fertilization.

Thus the investigated ecotype was proved to accumulate relatively high doses of Zn, Pb and Cd without any visible symptoms of phytotoxicity. Detailed morphological features will be presented during poster session. The next planed step is the investigation of the mechanisms responsible for accumulation of metallic elements, using *Biscutella laevigata* as model plant species.





## POTATOES – A CROP RESISTANT AGAINST INPUT OF HEAVY METALS FROM THE METALLICALLY CONTAMINATED SOIL

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### ABSTRACT

Cultivation of potatoes in Slovakia has a long tradition, determined by suitable climatic conditions and the development of agriculture. On the other hand, its development together with the development of industrial activity is related to the contamination of the environment. Enhanced levels of heavy metals in soil present a risk not only for cultivated crops, but especially for consumers. The ability of plants to accumulate heavy metals varies depending on the species: pea < cereals < potato < sugarbeet < corn < clover and their penetration influences soil-ecological conditions such as soil types, soil pH, concentrations and bonds of heavy metals, humus content in soil, oxidative-reductive conditions around root system connecting with microbial decomposition of inorganic and organic substances, soil moisture, temperature, utilized fertilizers and preparations for the plant protection.

The objective of our study was to assess the extent of accumulation of cadmium, lead and zinc in potato tubers depending on the concentration of these heavy metals in soil and to compare the resistance of 12 cultivars of potato (Laura, Red Anna, Marabel, Manitou, Cicero, Silvana, Rosara, Mozart, Cicero, Desiree, Volumisa, Adora), cultivated in 5 localities of the Slovakia (Odorin, Ivanka pri Nitre, Spissky Stvrtok, Belusa, Imel) against input of these heavy metals into the consumption part of potato.

Soils on which potatoes were grown, can be characterized as acidic to strongly alkaline (pH / KCl = 5.15 - 7.86), with low to moderate supply of humus (% hum. = 1.62 - 2.85), poor to good phosphorus content (P = 35.85 - 82.76 mg/kg) and moderate to very high potassium and magnesium contents (K = 189 to 456 mg/kg, Mg = 135-368 mg/kg). In soil extracts by ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub> c = 1 mol/dm<sup>3</sup>) and *aqua regia* contents of Pb, Cd and Zn using method of flame atomic absorption spectrometry (AAS Varian Spectr AA DUO 240FS/240Z/UltrAA) were determined (mean values of determined Pb, Cd and Zn content in all sampling sites were as follows: 0.146 - 0.640; 0.030 to 0.188 and 0.035 to 0.238 mg/kg in soil extract by NH<sub>4</sub>NO<sub>3</sub>, and 14.0 - 24.90; 0.92 - 2.72 and 35.0 - 72.60 mg/kg in soil extract by *aqua regia*).

The contents of Cd, Pb and Zn were determined in potatoes in extracts of freeze-dried samples. Mineralization of the samples was performed by microwave digestion (MARS X –press, CEM USA). Contents of Pb, Cd and Zn were determined at a wavelength of 217.0, 228.8 and 213.9 nm (respectively) using AAS method and expressed in mg/kg of fresh matter (FM). Determined contents of heavy metals were in the range of 0.020 – 0.630 mg Pb/kg FM, 0 – 0.058 mg Cd/kg FM, resp. 2.265 - 3.457 mg Zn/kg FM. Enhanced levels of heavy metals in soil partially corresponded to their accumulation in potatoes. Moderate statistically significant correlations between the content of heavy metal in soil and that in potato tubers were confirmed as follows: Zn: soil – potato tubers (cv. Marbel – loc. Odorin, Belusa, cv. Red Anna – loc. Odorin, cv. Volumia – loc. Imel), Pb: soil – potato tubers (cv. Red Anna – loc. Odorin, cv. Cicero and cv. Volumia – loc. Imel), resp. Cd: soil – potato tubers (cv. Laura – loc. Spissky Stvrtok). According to EC No 1881/2006 the maximal level 0.01 mg/kg FM for Cd as well as for Pb (for potatoes the maximum level applies to peeled potatoes) are given. The maximum limit for Zn is not given by this regulation. According Food Codex valid in the Slovak Republic (in which the maximum level for Zn in potatoes 10.0 mg Zn/kg FM is given) as well as according EC No 1881/2006 the allowed maximum values for Zn and Cd in potatoes were not in any cultivar exceeded. The maximum allowed amount of Pb was exceeded in cultivars Marabel (loc. Odorin, Belusa), Cicero (loc. Ivanka pri Nitre), Laura (loc. Spissky Stvrtok) and in all potato cultivars from locality Imel.

Varietal dependence as the most significant factor participating on the production and quality of potatoes (content of starch and other chemical compounds such as carbohydrates, organic acids, minerals, vitamins, amino acids, glycoalkaloids), was also reflected in a different extent of accumulation of heavy metals in the tubers, when in 80% of investigated cultivars statistically significant differences were confirmed (Multiple Range Tests for heavy metal by a variety Method: 95.0 percent LSD).

*Acknowledgements:* The financial support by project VEGA1/0456/12 is greatly appreciated.



## EVALUATION ABILITY TO ACCUMULATE Cd AND Zn OF *ARABIDOPSIS HALLERI* SSP. *GEMMIFERA* IN FIELD AND HYDROPONIC STUDY

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### ABSTRACT

Cadmium (Cd) is one of the most toxic heavy metal and concerned for human health in the world. Cd contamination of agricultural field and water causes serious problem in human health(ex. Itaiitai disease) through the food chain. Therefore, it is required that to clean them up from an environment at minimal cost and environmental load. *Arabidopsis halleri* ssp. *gemmifera*, known as Cd and Zinc (Zn) hyperaccumulator, has been researched for using phytoextraction in recent years, however detail of Cd and Zn accumulation pathway and characteristics remains obscure. In the present study, we conducted that field experiment in Cd and Zn contaminated site using *A. halleri* to evaluate Cd and Zn accumulation from the field in Miyagi Prefecture, Japan. In addition, characteristics of Cd and Zn accumulation were investigated under hydroponic condition.

Field trial of Cd and Zn phytoextraction was conducted from September, 2012 to May, 2013. *A. halleri* ssp. *gemmifera* was planted in the field by seed sowing and seedling transplantation, and then harvested after 9 months. During the experiment, plant samples (n=5) and soil core samples (n=4) were collected from each plot in December 2012, March, April and May, 2013. Harvested plant was washed with tap and milli Q water, then dried for 2 days in 60°C. Dried sample was digested with HNO<sub>3</sub> using wet ashing method. Soil sample was screened 2 mm sieves then digested using inverse aqua regia. Cd and Zn concentration of each sample was determined by ICP-MS. In the hydroponic study, *Arabidopsis halleri* ssp. *gemmifera* was conducted pre-cultivation using 1/5 Hoagland solution for 2 weeks. The plant was transplanted to 5 mM MES buffer which contained 5 μM of Cd and 5, 50, 500 μM of Zn and cultivated for 24 hours then harvested. During cultivation, part of a solution was collected in 1, 2, 4, 6, 8, 12, 24 hours from the start. Cd and Zn concentration of plant and solution was determined same way of the field trial.

In the field study, the biomass of seedling transplantation was 2.1 t/ha and it was three times higher than seed sowing. Cd and Zn concentration of the plant was maintained constant during the experiment. Final Cd concentration of seedling and sowing was about 1,400 mg/kg DW and 850 mg/kg DW(Fig.1) and Zn concentration was both of 12,500 mg/kg DW(Fig.2). Cd and Zn removal by plant was 1.8 kg/ha (23% of soil content) and 27.4 kg/ha (3% of soil content). Bioaccumulation factor (BF) of Cd showed almost 200, it was 10 times higher than that of Zn. These results suggested that the plant has independent pathway of Cd and Zn, additionally plant more accumulates Cd actively than Zn. In the 24 hours hydroponic experiment, Cd concentration of the solution was declined until 12 hours. However, decline ration was depended on Zn concentration (Fig.3). It was suggested that Cd and Zn adsorption to surface of the root was competitive. Interestingly, Cd concentration of shoot was raised with Zn concentration of solution. This result represented *A. halleri* ssp. *gemmifera* accumulated much Cd even Zn existed 100 fold higher in solution.

In conclusion, in the field experiment, *A. halleri* ssp. *gemmifera* showed 10 times higher BF values of Cd than its Zn. In hydroponics, it was suggested Cd and Zn accumulation pathway was independent.

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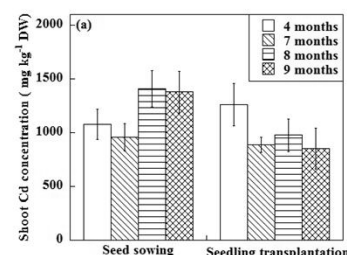


Fig.1 Changes of Cd concentration in shoots of *Arabidopsis halleri* ssp. *gemmifera*

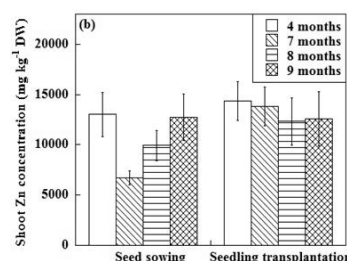


Fig.2 Changes of Zn concentration in shoots of *Arabidopsis halleri* ssp. *gemmifera*

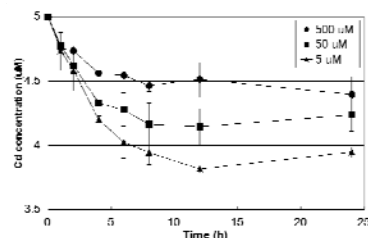


Fig.3 Changes of Cd concentration in solution in 24h.



## LOCALIZATION OF SOYBEAN HMA8 TRANSPORTER AND ITS IMPLICATION IN COPPER HOMEOSTASIS

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### ABSTRACT

Copper (Cu) is an essential micronutrient in living organisms. In plants, the Cu physiological content is generally in the range of 5–20  $\mu\text{g g}^{-1}$  in the vegetative organs [1]. However, the Cu concentration in soil has a wide variation. Concentrations less than 5  $\mu\text{g g}^{-1}$  were observed in different countries, such as Belgium, Croatia or Greece, whereas values higher than 100  $\mu\text{g g}^{-1}$  were also found regularly in several countries as Italy, Norway or Spain [2]. An appropriate Cu concentration in the plant is essential for its correct development, therefore, the study of Cu status in crops has an important agronomical interest. Furthermore, we have seen in previous works that Cu limiting conditions also induced a decrease in iron (Fe), another essential element [3]. To overcome this disadvantage, plants have developed a complex homeostatic network to maintain the Cu concentration in the tissues under physiological values. In that network, there are several proteins involved in the uptake and distribution of Cu, such as the heavy metal ATPase 8 (HMA8), responsible for Cu (I) transport across the thylakoid membranes [4].

In the model plant *Arabidopsis* (*A. thaliana*), there is only one gene encoding for HMA8, whereas in soybean (*Glycine max* (L.) Merr), there are two different genes subjected to alternative splicing, *HMA8-1* and *HMA8-2*. *HMA8-1* encodes a complete membrane protein and *HMA8-2* encodes a putative small truncated protein. The expression of both genes has been studied by quantitative real time PCR in soybean plants grown hydroponically under different Cu regimes. In mature leaves, the *HMA8-1* expression is downregulated by Cu, whereas the *HMA8-2* expression is upregulated. These results suggest that *HMA8-1* and *HMA8-2* could perform complementary functions related to Cu regulation. To further investigate the function of the HMA8-2 protein, its cellular localization was analysed using a C-terminus YFP tagged construct in combination with transient expression experiments in *A. thaliana* protoplasts. Preliminary results indicated that the HMA8-2 protein was localized in the chloroplast. It is thought that the chloroplast is the organelle that contains most of the cellular Cu pool in plants [5], suggesting that the localization of HMA8-2 may be in agreement with a role of this protein in Cu homeostasis. The study of the Cu homeostasis in soybean, an important crop plant, may be of agronomical importance. Therefore, the study of the regulation and localization of the Cu-related genes and proteins HMA8-1 and HMA8-2 will improve our understanding of plant Cu homeostasis, facilitating the selection of plants that may be able to compete in limiting nutrient soils as well as heavy metal tolerant plants used in phytoremediation.

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## HEAVY METAL EFFECTS ON SUNFLOWERS: BIOMASS PRODUCTION AND ACCUMULATION CAPACITY

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### ABSTRACT

Phytoremediation is an emerging technology using selected and engineered metal-accumulating plants for environmental clean-up. Sunflower (*Helianthus annuus* L.) has been known as one of the metal-accumulating plants. In this study, biomass productions and heavy metal accumulation capacities of sunflowers were monitored in the heavy metal contaminated soils and the correlation was analyzed.

Sunflower seeds were obtained from a commercial market, and the specie and cultivar is frequently and widely cultivated in the Republic of Korea. Soil specimen was collected from a farmland in the Gyeongsangnam-do, the Republic of Korea was used after taking a series of treatments. It was air-dried, mixed thoroughly and then screened through a 2 mm (10 mesh) sieve to remove large particles. The soil was then amended with the heavy metals: Pb as PbCl<sub>2</sub>; Cd as CdCl<sub>2</sub>·2.5H<sub>2</sub>O and Cu as CuCl<sub>2</sub>·2H<sub>2</sub>O. Each heavy metal concentrations of soil used in this study were 250, 1,000, and 2,000 mg-Pb/kg; 50, 100, and 600 mg-Cd/kg and 100, 300, and 450 mg-Cu/kg. The sunflowers were grown for 98 days in a greenhouse. After the 30 days, 65 days and 98 days, the sunflowers were harvested. The biomasses of sunflowers were measured and Pb, Cd and Cu in sunflower was analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES).

All values are presented as average values ± standard deviation (SD) obtained with at least three replicates. Student *t*-tests were used to assess differences between the sunflower biomass of 65 days and that of 98 days. Differences of heavy metal mass in sunflower among heavy metal treatments were determined using ANOVA, according to Duncan's multiple range test. A significance level of *P* < 0.05 was used throughout the study. The IBM SPSS statistics (Version 21.0) analysis program was used for the statistical analyses.

Table 1. Heavy metal accumulation by sunflower after 98 days growth

Heavy metal	Conc. (mg/kg)	Mass (mg)	Heavy metal	Conc. (mg/kg)	Mass (mg)	Heavy metal	Conc. (mg/kg)	Mass (mg)
	250	90.40 ± 10.16a		50	225.29 ± 112.69b		100	91.28 ± 31.49a
Pb	1,000	362.06 ± 23.69a	Cd	100	305.81 ± 40.65b	Cu	300	192.06 ± 41.46b
	2,000	719.71 ± 264.72b		600	0.00 ± 0.00a		450	40.69 ± 15.09a

Values followed by the same letters are not significantly different at (*P* > 0.05), Means ± SD, *n*=3

The biomass productions of sunflower in 65 days and 98 days were not significantly changed. The accumulated Pb in sunflower was increased as Pb concentrations increased in soil. There was no harvest in 600 mg-Cd/kg contaminated soil because of high toxicity. Accumulated Cd in 50 and 100 mg-Cd/kg were not significantly different from each other. Sunflower has the larger accumulation capacity in 300 mg-Cu/kg than those in 100 and 450 mg-Cu/kg.

According to the results, the sunflower has a relatively stronger tolerance to Pb contaminated soil than that to Cd and Cu contaminated soil. That means sunflower is a suitable remediation tool in high Pb contaminated soil.

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## ELEUSINE INDICA - A NEWLY DISCOVERED ANTIMONIC HYPERACCUMULATOR PLANT

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### ABSTRACT

China is the main reserves area of antimony. According to the review about the world's mineral resources issued by U.S. Geological Survey in 2011, there are 52.8 percent of the world's proven reserves of antimony in China; it ranks first in the world. The antimony reserves area of china is mainly distributed in Xi Kuang Shan in Leng Shui Jiang city of Hu Nan province. Xi Kuang Shan is called as the stibium capital of the world. To know the antimonitic pollution status in the mine of Xi Kuang Shan, the antimonitic concentration in the plants and the soils in the mine of Xi Kuang Shan were investigated. Different varieties of plants in the antimony mine area of Xi Kuang Shan including the factory area and the residential area are collected random. The topsoils of the plants grown are also collected. The plant samples were washed and divided into overground parts and underground parts and then cutted into pieces ( $<1\text{ cm}^2$ ) then air dried. The soil samples were air dried and grinded and sieved with 100 mesh. The air dried plant and soil samples were digested with  $\text{HNO}_3$  and  $\text{HClO}_4$ , antimonitic concentration in the digestive solution was measured with inductively coupled plasma atomic emission spectrometry (ICP-AES). Unexpected, it was found that *Eleusine indica* is an antimonitic hyperaccumulator plant! The investigation results show that the average antimonitic mass fraction in the overground parts of *Eleusine indica* reaches  $1864.7\text{ kg mg}^{-1}$ , the range is about  $933.8\sim 2638.9\text{ kg mg}^{-1}$ ; the average antimonitic mass fraction in the underground parts of *Eleusine indica* is only  $321.7\text{ kg mg}^{-1}$ , the range is about  $163.8\sim 502.6\text{ mg kg}^{-1}$ ; the average antimonitic concentration in the soil is  $102.3\text{ mg kg}^{-1}$ , the range is about  $81.2\sim 110.0\text{ mg kg}^{-1}$ . It is the first time that *Eleusine indica* is discovered as an antimonitic Hyperaccumulator plant and it is the first time that the antimonitic hyperaccumulator plant is discovered in China, this discovery fills in gaps on antimonitic hyperaccumulator plant in china, it supply a new plant resource for phytoremediation of antimonitic contaminated soil. However *Eleusine indica* is only proved as an antimonitic hyperaccumulator plant by field investigation. Artificial cultivation experiments have not been executed yet. Antimonitic hyperaccumulation mechanism and characteristics of *Eleusine indica* have not been researched yet, especially that whether antimony is hyper-accumulated through underground parts of *Eleusine indica* from the soil or it is hyper-accumulated by overground parts of *Eleusine indica* from the air. These are the further research contents.



Fig.1 The collected place of Eleusine Indica in Xi Kuang Shan in Leng Shui Jiang city of Hu Nan province in China



## EFFECTS OF CADMIUM STRESS IN STRAWBERRY PLANTS

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### ABSTRACT

The effects of water-applied cadmium (Cd) treatments on antioxidative enzyme activities were investigated in strawberry (*Fragaria x ananassa* cv. Camarosa) plants. Cold stored seedlings were grown in perlite:torf:soil (1:1:1) mixture in a greenhouse. Four weeks after planting, plants were treated by Actagro (7-7-7) nutrient solution containing 0, 500 and 1000 mg/l Cd (CdSO<sub>4</sub>) during 8 weeks. Fully expanded leaf and roots were collected from the plants at the end of the treatments for the analyses of cell membrane injury, lipid peroxidation (Malondialdehyde, MDA content) and chlorophyll content. In general, Cd treatments caused a linear increase in cell membrane injury and MDA content in both leaf and root tissues with increasing Cd concentrations. Root tissues were more injured than leaf tissues by Cd treatments. The accumulation of Cd in root of the plants was determined higher than leaf, which were parallel to cell membrane injury and MDA content. In addition Cd treatments significantly increased the chlorophyll contents of the leaf tissues in comparison to control plants.



## IMPORTANT ROLE OF ENHANCED LEVEL OF GLUTATHIONE IN *CRAMBE ABYSSINICA* TO DETOXYFY Ag NPs AND Ag<sup>+</sup> IONS

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### ABSTRACT

Widespread use of silver nanoparticles (AgNPs) in the environment has raised some serious concerns about their adverse effects on the environmental and human health. However, the biochemical mechanisms that plants use to detoxify the nanotoxicity are not understood well. *Crambe abyssinica* (a member of Brassicaceae), which is capable of tolerating abiotic stresses, such as cold, salt, and heavy metal, is an ideal industrial oil seed crop for phytoremediation of toxic metals, biofuels, and bioproducts. The importance of glutathione (GSH) in heavy metals and xenobiotic detoxification is well established. In this study, we engineered crambe plants overexpressing *E. coli*  $\gamma$ -glutamylcysteine synthase ( $\gamma$ -ECS), which is critical in GSH biosynthesis pathway. Several-folds higher levels of GSH were shown in all transgenic plant tissues compared to wild type control plants. In order to demonstrate that enhanced levels of GSH can protect plants from Ag nanotoxicity, two independent transgenic lines of crambe were exposed to various concentrations of Ag NPs and AgNO<sub>3</sub> (Ag<sup>+</sup> ions). Compared to WT plants,  $\gamma$ -ECS crambe showed higher tolerance and attain significantly higher fresh biomass along with the increase in total chlorophyll contents and transpiration rate. Moreover, transgenic lines can take up more Ag and translocate in shoots. Analysis of the levels of cysteine,  $\gamma$ -EC, GSH and its derivatives phytochelatin (PCs), showed significantly higher levels of these peptides. Additionally, exposure of transgenic lines to Ag NPs and Ag<sup>+</sup> ions further increased the levels of  $\gamma$ -EC, GSH, and PCs in transgenic lines compared to WT plants. These results indicate that metabolic pathway for GSH biosynthesis was strongly induced to detoxify Ag nanotoxicity. On the other hand, analysis of the important nutrient elements (such as Ca, K, Fe, Zn, Mg, P, and Mn) in plants showed that the levels of both exchangeable P and Zn in transgenic lines treated with Ag NPs and AgNO<sub>3</sub> were significantly higher than control groups. All other elements were not affected. These results clearly show that GSH is involved in the detoxification of Ag NPs and AgNO<sub>3</sub> (Ag<sup>+</sup> ions). Last, but not the least, polyamines (PAs) biosynthetic pathway can protect plants from abiotic stresses. Analysis of the regulations of putrescine (Put), spermidine (Spd) and spermine (Spm), showed that Put level was strongly induced in transgenic lines treated with Ag NPs and Ag<sup>+</sup> ions compared to transgenic control. There were no significant changes of the level of Put in WT plants. Also, Spd/Put ratio in transgenic lines treated with Ag NPs and Ag<sup>+</sup> ions was specifically higher than WT plants. However, no significant changes of the levels of Spm were found in both WT and transgenic lines. These results suggest that PAs biosynthetic pathway may also respond to detoxification of Ag NPs. This study is highly helpful to understand the fate, transport and toxicity of NPs in plants and could provide a useful way for remediation NPs in the environment.

**Keywords:** *Crambe abyssinica*,  $\gamma$ -glutamylcysteine synthase, silver nanoparticles, glutathione, putrescine



## THE RELATIONSHIPS BETWEEN CONTENT OF HEAVY METALS IN SOILS AND IN STRAWBERRIES

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### ABSTRACT

Strawberry can grow in a wide range of climatic conditions - from the subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere. Strawberries are one of the most popular edible fruits in Slovak Republic and their consumption has increased with the development of new varieties available at almost all seasons. Strawberry is a good source of bioactive compounds due to its high level of vitamin C and phenolics. These compounds present antioxidant effects, and therefore a consequent beneficial effect on the maintenance of consumer health [Da Silva Pinto et al., 2008; Giampieri et al., 2012].

The environmental study, carried out in Slovak Republic, was aimed at assessment of quality of strawberry based on the contents of heavy metals (Zn, Cu, Ni, Pb, Cd) as well as the possible correlations between selected heavy metals in soil and strawberries (*Fragaria x ananassa* Duch.). Seven different varieties were analysed (Clery, Sonata, Alba, Korona, Azia, Antea, Joly). Seven sampling sites were selected and the analyses of pH/KCl, P, K, Mg and chosen heavy metals in 2 different extracts were carried out (*aqua regia*, 1 M NH<sub>4</sub>NO<sub>3</sub>). The soils on which the strawberries were grown, can be characterized as strongly to weakly acidic (pH/KCl = 4.73 - 6.56), with low to moderate supply of humus (% Hum. = 1.27 to 2.24), with low to high phosphorus content (P = 41.89 - 120.41 mg/kg), with good to very high content of potassium (K = 295.7 - 431.70 mg/kg) and good to high content of magnesium (Mg = 192.6 - 300 mg/kg).

The results of our study revealed that from all observed metals in soil determined in *aqua regia* only in the case of cadmium the maximum permissible limit in comparison with the limit resulting from the law no. 220/2004 (valid in the Slovak Republic) as well as threshold values proposed by European Commission (EC) (2006) has been exceeded. In our paper the values of cadmium in the soil ranged in the interval 1.30 – 1.690 mg/kg representing 1.86 to 2.41 times higher values than limit valid in the Slovak Republic (0.7 mg/kg) and 2.6 to 3.38 times higher in comparison to European Commission (EC) (0.5 mg/kg). In our study in 1 M NH<sub>4</sub>NO<sub>3</sub> the values of lead ranged from 0.125 to 0.205 mg/kg representing values exceeded the limit valid in Slovak Republic (0.1 mg/kg) about 0.037-0.105 mg/kg. Despite exceeded values of heavy metals in soil, no values above the limit directly in strawberries when compared to Food Codex of Slovak Republic (FC SR) as well as to Commission Regulation 1881/2006 (CR) were recorded. Among the varieties statistically significant differences (P<0.05) in intake of heavy metals were found (Multiple Range Tests for heavy metal by cultivar, Method: 95,0 percent LSD).

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## STUDY ON As UPTAKE AND REMOVAL BY As HYPER-ACCUMULATOR *PTERIS VITTATA* AND IT'S RHIZOSPHERE BACTERIA

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### ABSTRACT

Arsenic hyper-accumulators such as *Pteris vittata* have been known applicable for phytoremediation of arsenic-contaminated soil. Previous studies show that *P. vittata* mainly absorbs arsenic as arsenate. Therefore, arsenic uptake by *P. vittata* is largely determined by arsenic speciation, which is strongly influenced by microbial activities. However, little is known about interactions between *P. vittata* and its rhizosphere bacteria. The objective of present study was to characterize the details of arsenic transformation prior to arsenic uptake by *P. vittata* and the participation of the rhizosphere bacteria on arsenic uptake.

Pot experiments of *P. vittata* cultivation and arsenic oxidation in the soil were applied to investigate the transformation and transition of arsenic from the soil to *P. vittata*. The concentration of total arsenic and arsenite in the soil and *P. vittata* was monitored by atomic absorption spectrophotometry. The terminal restriction fragment length polymorphism (TRFLP) targeted to microbial arsenite oxidase gene, *aroA*, was performed to analyze the microbial community in the rhizosphere of *P. vittata*. The results of arsenic analysis showed that the arsenite in the soil was promptly oxidized to arsenate and subsequently absorbed by *P. vittata*. The arsenite oxidation was inhibited by antibiotics, which indicated that bacteria played an important role. The results of TRFLP suggested that four *aroA* containing bacterial species were increased during early phase of cultivation when arsenic oxidation was observed and were subsequently decreased during late phase. The rhizosphere samples of *P. vittata* in the pot experiments were applied to isolate culturable bacteria. Thirty-seven bacterial strains with different morphologies and arsenic tolerance were isolated, and *aroA* gene was detected from four of these isolates. The selected isolate was further investigated about the arsenite oxidative activity, and the results showed that 89.2% of arsenite in the medium was oxidized to arsenate by the isolate after 48 hours incubation. Besides, the rhizosphere re-colonization of this isolate was also confirmed two days after inoculating to *P. vittata*.

According to these results, we concluded that the rhizosphere bacteria play a role in arsenite oxidation prior to arsenic uptake by *P. vittata* and thus may have potential to improve phytoremediation of arsenic-contaminated soil by *P. vittata*.

**Keywords:** arsenic, *Pteris vittata*, phytoremediation, rhizosphere bacteria, arsenite oxidation, *aroA*

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## ROOT UPTAKE OF Cs 134 EARLY AFTER RADIOACTIVE FALLOUT

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### ABSTRACT

Our investigation is focused on the dynamism of Cs 134 increase in the plant tissues after soil contamination by radioactive fallout. We assume that the harvesting early after fallout could reduce the quantity of the contaminated biomass. Our experiments were carried out with 3 weeks old sunflowers as a model plants in a phytotrone. The 200 ml of the Cs 134 solution with the activity 3 kBq/L was spilt on the top-soil. The first group was harvested after 1 day. The plants were washed. Roots, stems and leaves were separated, dried and finally, their activity was determined by gamma spectrometry.

The main finding is that the harvesting in the first two days could be effective in reduction of the quantity of contaminated biomass. However, the Cs 134 activity in the green biomass was low even at the end of experiment. The leaves of the plants from the first group contained the 5 Bq/kg (dw) or less. From fifth to tenth day the Cs 134 activity concentration in plant tissues still slightly increases but after ten days it remains almost constant. Twenty days after contamination the Cs 134 activity concentration in leaves was 26 Bq/kg (dw). Contrary to leaves, the activity in the roots permanently increased until the end of the experiment when it reached 430 Bq/kg (dw). It corresponds well with the ability of sunflower roots to adsorb a high quantity of cesium from contaminated water. The lowest activity was caught in the stems.

This approach is applicable in the case when the negligible quantity of radionuclides is retained on plants during rain.



## VARIOUS SPECIES FOR PHYTOREMEDIATION OF Ag, Cu AND Zn IN DIFFERENT CONCENTRATIONS FROM A SOIL MATRIX

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### ABSTRACT

Transition metals are often present in post-industrial soils in concentrations that require specialized attention because of their potential health risk for nearby biota. Two different soils (S1 and S2) contaminated with high level of Ag (34.6 and 113.6 mg kg<sup>-1</sup> respectively), as well as with Cu (47.5 and 20.0 mg kg<sup>-1</sup> respectively) and Zn (162.2 and 177 mg kg<sup>-1</sup> respectively), were collected from the same brownfield in eastern Montréal (Canada). In a pilot experiment, four plant species (*Brassica juncea*, *F. arundinacea*, *Medicago sativa* and *Salix miyabeana*) were grown in different pots containing the soils to investigate their potential use in phytoremediation of the contaminated site. Analysis showed that the only influence of soil type on plant growth was to reduce aerial biomass production of *F. arundinacea* grown in S2 soil compared to S1. Higher Ag concentrations were found in underground parts of all species grown in S2 substrate compared to S1. The three herbaceous species performed equally well, and all were more effective than *S. miyabeana* in uptake of Ag in both substrates. For Cu and Zn, no difference in root concentrations was recorded between substrates. *F. arundinacea* showed the highest root concentration of Cu, while all species showed similar results for Zn, regardless of substrate. The highest aboveground concentration of Ag was observed in *B. juncea* grown in S2 soil (12.2 mg kg<sup>-1</sup>); *S. miyabeana* was the only specie translocating Zn aboveground, performing equally in both substrates (mean=118.25 mg kg<sup>-1</sup>). To our knowledge, this is the first study to demonstrate bioaccumulation and translocation of Ag by higher plants from a soil matrix. These findings suggest that *B. juncea* could be used as a phytoextractor of Ag from a soil matrix, and that the four species have potential for use in complementarity to remediate an Ag/Cu/Zn contaminated brownfields.



## THE EFFECT OF SUPER ABSORBENT POLYMER ON PHYTOEXTRACTION OF TRACE ELEMENTS

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### ABSTRACT

In general the mechanism of plant root uptake is classified to root interception, mass flow and diffusion. Plant root distribution is an essential factor to determine the capacity of phytoextraction. However the direct uptake by root interception is not sufficient because most of ions in soils are taken up through mass flow or diffusion. Therefore soil water movement forward to rhizosphere can be more critical. In this study, super-absorbent polymer (SAP) was used to make a matric potential gradient of soil water between rhizosphere and its ambient soil. Sunflower was planted with SAP and without SAP. The concentration of SAP treatment was 0.1% in the soil volume basis. During test period water content was monitored. Sunflower biomass yield and the TE concentration in plants were also analyzed. Water content of soil with SAP remained 2~5% higher than one without SAP. Biomass yield on soil with SAP was estimated higher: in the SAP treated case, the yield of root, stalk, leaf, flower, and seed was 2,125, 6,909, 2,710, 2,435, 2,756 kg ha<sup>-1</sup> respectively, in the SAP non treated case, 1,338, 4,449, 1,906, 1,892, 2,004 kg ha<sup>-1</sup>. The result of the analysis of Cd, Cu, Zn, and Pb in each plant part showed some tendency that these ions were accumulated more in the harvestable parts, especially stalks, when SAP was treated. From this test it is expected that SAP treatment can be used as an alternative soil conditioner to enhance the phytoextraction of TE.

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## SACCHARIFICATION OF SUNFLOWER STALKS WITH HIGH METAL CONTENT USING LIGNOCELLULASES FROM A FUNGAL CONSORTIUM COMPRISING *PHOLIOTA ADIPOSA* AND *ARMILLARIA GEMINA*

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### ABSTRACT

Lignocellulases from *Armillaria gemina* and *Pholiota adiposa* are efficient in hydrolyzing aspen and poplar biomass, respectively. In the present study, lignocellulosic enzymes obtained from a fungal consortium comprising *P. adiposa* and *A. gemina* were used for the saccharification of sunflower stalks. Sunflower stalks were thermo-chemically pretreated using 2% NaOH at 50°C for 24 h. The saccharification process parameters including enzyme loading, substrate concentration, pH, and temperature were optimized using response surface methodology to improve the saccharification yield. The highest enzymatic hydrolysis (84.3%) was obtained using the following conditions: enzyme loading 10 FPU/g-substrate, substrate 5.5%, temperature 50°C, and pH 4.5. The hydrolysis yield obtained using the enzymes from the fungal consortium was equivalent to that obtained using a mixture of commercial enzymes Celluclast and Novozyme  $\beta$ -glucosidase. Addition of up to 500 ppm of heavy metal ions (As, Cu, Fe, Mn, Ni, Pb, and Zn) during saccharification did not significantly affect the saccharification yield. Thus, the biomass grown for phytoremediation of heavy metals can be used for the production of reducing sugars.

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## **PHRAGMITES AUSTRALIS' DEFENSIVE RESPONSE TO METAL CONTAMINATION**

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### **ABSTRACT**

Saltmarshes are complex coastal ecosystems of great productivity and environmental relevance. Nevertheless, these ecosystems are constantly subject to anthropogenic pollutants. Saltmarsh plants have been proven to influence the distribution and bioavailability of metals in rhizosediment being also able to uptake and bioaccumulate considerable amounts of this kind of pollutants [1,2]. For these reasons, saltmarsh plants have been recognised as potential phytoremediators/phytostabilisers. Halophytes seem to present advantages over non-halophytic plants since they are able to survive and reproduce in salt-rich environments [3], feature that may ensure their tolerance to other environmental stresses as metal contamination. This ease to cope with harsh environmental conditions is thought to be associated to efficient internal biophysicochemical mechanisms.

As knowledge on this matter is still limited, a battery of studies was performed with *Phragmites australis*, a perennial macrophyte, commonly found in European temperate saltmarshes. The purpose of this work was to attain a better understanding how metal contamination could condition the liberation of aliphatic low molecular weight organic acids (ALMWOAs) and the production of antioxidant thiolic compounds (as cysteine (Cys), oxidised glutathione (GSSG) and reduced glutathione (GSH)) [4]. Experiments were performed in three complementary media: (I) freshwater from the river (a natural medium simpler than estuarine water); (II) rhizosediments soaked in the respective elutriates; and (III) rhizosediment cores containing *P. australis* stands (a microcosm scale up study).

Results demonstrated that contamination by metals, particularly Cd and Cu, could trigger several specific responses in the marsh plant. For instance, experiment I showed that the liberation of oxalic and citric acids by *P. australis* roots was influenced by the exposure to Cd or Cu contaminated freshwater, being the plant response dependent upon the metal. Likewise, in experiment II, both metals could induce a specific influence on the production of thiolic compounds in roots and leaves cells. A short-term exposure of *P. australis* to metal (Cd or Cu) contaminated rhizosediments soaked in the respective elutriates caused a disequilibrium in cell's homeostasis, which was confirmed in experiment III. In fact, in experiment III, there was a significant decrease of GSSG levels in roots cells and of Cys, GSH and GSSG levels in leaves cells of *P. australis* after short-term exposure to Cd-contaminated sediment. However, after long-term exposure (two months) to this Cd-contaminated sediment, *P. australis* re-established the basal levels (levels in plants not exposed to contaminated sediment) indicating that defensive mechanisms involving thiolic compounds were activated in the organs of the plant. Furthermore, no external toxicity symptoms were observed in the specimens in any of the experiments. Considering the remarkable ability to cope with contaminated environments and harsh conditions allied to its ability to bioaccumulate metals, *P. australis* is therefore a suitable choice for phytoremediation procedures.

Estuarine ecosystems are very sensitive areas, their management and phytoremediation strategies facing severe challenges. For this reason, pursuing research on the dynamics behind salt marsh plants' success to cope with the harsh conditions of marsh areas and to remove pollutants from sediments is utterly important and will contribute for the planning of more efficient remediation projects of impacted marsh areas.

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## INPUT OF RISK METALS INTO FABA BEAN CULTIVATED IN TARGETED CONTAMINATED SOIL IN MODEL CONDITIONS

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### ABSTRACT

Agricultural production is the main source of foodstuffs, it is important to evaluate negative effects of risky elements on quality of agricultural products. Metals, such as iron, zinc and manganese are essential metals, since they play an important role in biological systems. Cu and Zn are essential micronutrients, they can be toxic when taken in excess. Lead and cadmium are nonessential metals as they are toxic, even in trace – these elements are present in the soil above the hygienic limit on most territory of the Slovak Republic. We investigated the effect of accumulation of heavy metals in faba bean grown in model conditions in the targeted contaminated soil with increasing rates of the selected heavy metals (cadmium, lead, zinc and copper, separately).

For cvs. Zobor and Saturn, four variants in each of the experiments were realised: A: control (without Zn or Cu addition), B: 40 mg Zn.kg<sup>-1</sup> or 20 mg Cu.kg<sup>-1</sup> of soil (hygienic limit), C: 250 mg Zn.kg<sup>-1</sup> or 50 mg Cu.kg<sup>-1</sup> of soil (half dose of analytically significant contamination, D: 500 mg Zn.kg<sup>-1</sup> of soil or 100 mg Cu.kg<sup>-1</sup> (analytically significant contamination). Faba beans were harvested in full ripeness. For cv. Merlin were made 4 variants: A-control (with basic fertilization) and cadmium and lead was applied in gradual specific doses with 5 (variant B), 10 (variant C), 15 (variant D) multiple as the limit value by the Law no. 220/2004 Z.z. to assess the state of soil contamination.

Trace elements (Zn, Cu, Co, Ni, Cr, Pb, Cd) – in soil and matter - were determined using a Varian AA240FS atomic absorption spectrometer equipped with a D2 lamp background correction system, using an air-acetylene flame. Pseudototal content of risk metals was assessed in soil extract by *aqua regia* and content of mobile forms of selected heavy metals in soil extract by NH<sub>4</sub>NO<sub>3</sub> (c = 1 mol.dm<sup>-3</sup>) and in the solution of 2 M HNO<sub>3</sub> was determined. The results were evaluated according to the legislative valid in the Slovak Republic. The soil from two localities Cakajovce (cvs Saturn and Zobor) and Vycapy-Opatovce (cv. Merlin) used in the pot trial was uncontaminated.

Faba beans matter was harvested in full ripeness. The samples of faba bean were analysed after the decomposition by HNO<sub>3</sub> on the microwave digestion. Gained results was evaluated by the content of risky elements in seeds were evaluated according to the Food Codex of the Slovak Republic.

With increased Zn (Cu) doses applied into the soil the strong statistical relationship between soil Zn (Cu) content and Zn (Cu) amount in seeds of both of investigated faba bean cultivars was confirmed. Despite of high Zn doses applied into the soil in model conditions, in all variants the determined Zn amount in seeds of faba bean cv. Saturn was just below the maximal allowed content in foodstuffs given by the legislative. In cv. Zobor the determined Zn content was in C as well as in D variant higher than maximal allowed amount (by 2% and 12%, respectively). In variants with Cu application the determined Cu contents were far below the limit value. Faba bean seeds cvs. Saturn and Zobor in full ripeness accumulated (in all variants higher than hygienic limits) high amounts of Pb and Cd (Zn application into soil) and amounts of Pb and Ni (Cu application). The contents of all other heavy metals were lower than hygienic limits. In variants with Zn application the determined Zn and Cu contents in seeds were increased, Cd content was decreased and contents of Ni, Cr and Pb were changed only slightly. In variants with Cu application the determined elements were changed only slightly.

With increased Cd doses applied into the soil the relationship between soil Cd content and Cd amount (R=0,92) as well as Zn and Cd values in seeds of investigated faba bean cultivar Merlin was confirmed. In all variants the determined Cd and Ni amount in seeds was exceeded the maximal allowed content in foodstuffs given by the legislative.

With increased Pb doses applied into the soil the relationship between soil Pb content and Pb amount as well as Zn, Pb and Cd values in seeds of investigated faba bean cultivar Merlin was not confirmed. The accumulation of lead into seed was lower in comparison to cadmium. The determined Pb content was in D variant higher than maximal allowed amount (by 45%). In all variants the determined Cd amount in seeds was exceeded the maximal allowed content in foodstuffs given by the legislative.

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## OPTIMIZING VALORIZATION OF METAL-CONTAMINATED SOILS USING SRC OF WILLOW, PLANT-ASSOCIATED BACTERIA AND FERTILIZERS

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### ABSTRACT

The production of non-food crops on metal-contaminated soils is an economically beneficial and environmentally sustainable land use. In case metal accumulating crops, like short rotation coppice of willow are used, produced biomass does not only serves bioenergy purposes, but also gradually remediates metal contamination by the extraction of metals out of the soil. To improve biomass production (farmer's income) and metal extraction (shortening clean up time) of short rotation willow, we investigated the use of plant-associated bacteria which can have a beneficial effect on plant growth, metal uptake and translocation of metals to harvestable plant parts. Besides, also fertilization of willow was tested as a metal-phytoextraction improving strategy.

Bacteria isolated from a selected *Salix alba x alba* clone were screened for their plant growth-promoting characteristics (e.g. production of indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase), for characteristics enhancing the uptake of toxic metals (e.g. production of siderophores and organic acids) and for Cd and Zn tolerance. Six strains were selected for a pot inoculation experiment with cuttings of *S. alba x alba* planted in a Cd-Zn-Pb contaminated soil. In a parallel experiment, two fertilization strategies were tested: (i) a commercial slow release fertilizer mixed with the soil and (ii) an adapted solution of N, K and Mg weekly provided to the plants. This work summarizes biomass production of the inoculated or fertilized cuttings and metal concentrations in the produced biomass after 2 months of growth in pots.

Compared to non-inoculated and non-fertilized control cuttings, the production of biomass (roots, shoots and leaves) increased after an inoculation with the bacteria *Bacillus sp.*. Both fertilizers did not increase dry weight biomass production. The concentrations of Cd and Zn inside the plant were, compared to control plants, higher for cuttings inoculated with *Sphingobacterium sp.* and after treatment with the slow release fertilizer. Calculating the extracted amount of Cd and Zn after only 2 months of growth in metal-contaminated soil revealed already a slightly increased uptake of both metals after inoculation with *Bacillus sp.* and 2 different *Sphingobacterium sp.* as well as the slow release fertilizer treatment. These results indicate that the valorization of metal-contaminated soil can be optimized by the bioaugmentation of willow with selected plant-associated bacteria as well as the application of appropriate fertilizers.





## TRANSGENIC LINSEED (*LINUM USITATISSIMUM* L.) WITH $\alpha$ HMT1 $\alpha$ : THE EFFECT ON THE ACCUMULATION OF SEVERAL METAL ELEMENTS

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### ABSTRACT

The results presented here reassume our long-term research on transgenic linseed (*Linum usitatissimum* L.) that we generated by transformation with  $\alpha$ HMT1 transgene for overproduction of heavy metal-binding recombinant peptide (mammalian metallothionein) and cadmium (Cd)-binding CP peptide. The evidence of improved Cd accumulation was recorded as related to parental, non-GM line [1]. Because this material showed high genetic stability in subsequent generations, based on official EU field trials we focused on testing its tolerance and accumulation potential in relation to further metallic compounds. Transgenic lines of linseed were grown, selected and investigated in controlled field conditions and compared with conventional linseed varieties. Further *in vitro* screening of several GM-lines (homogenous T5 generation) for accumulation potential in relation to selected metallic micro- and macroelements (Se, Mg, Fe) was carried out.

The hypocotyl segments of linseed line AGT917 with partially removed epidermis were infected with *A.tumefaciens* strain EHA105 harboring pBI121-derivatives in the presence of acetosyringone and cellulase. After regenerating transformants *in vitro*, further generations were grown in greenhouse and later in field conditions, respectively. The compositional analysis of transgenic plants from natural field background was conducted. In T<sub>5</sub> plants germinated *in vitro*, the presence of  $\alpha$ HMT1 $\alpha$ ::gus and CP::gus fusions and their expression driven by 35S CaMV promoter were confirmed by  $\beta$ -glucuronidase histochemical assay and PCR. In T<sub>5</sub> plants, the influence of metallic compounds on growth parameters was evaluated using three *in vitro* systems on MS-based media: a) callus cultures in liquid media, b) cultures of multiple shoots and c) germinating seedlings. The determination of metallic compounds content in the biomass by atomic absorption spectroscopy (AAS) was done.

First results of compositional analysis in GM plants (both  $\alpha$ HMT and CP) from field (T3 generation) showed evident increase of Cd and Pb content in above-ground biomass in comparison with parental, non-GM cultivar. The contrasting results were obtained for Se content and no difference was detected in case of Mg content.

*In vitro* experiments with GM-lines (T5 generation) exposed to increasing concentrations of Mg showed differences in growth parameters between GM- and non-GM lines and positive effect of transformation on accumulation of Fe and Cu within the seedlings.

In conclusion: This type of transformation in linseed may be considered as stable. The transgenes used show the metabolic activity in transgenic plants even after the several years of selection in field conditions. The preliminary results can lead us to the idea, that this transgenic linseed can have high phytoremediation potential. Nevertheless, many questions concerning this interesting and unique plant material still remain unanswered and have to be an area for further basic and applied research.

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## THE EFFECT OF HUMIC ACIDS ON CADMIUM ACCUMULATION BY *AGROPYRON REPENS* L. AND BIOCHEMICAL PARAMETERS

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### ABSTRACT

The effect of humic acids (HA) on the uptake of lead by quack grass plants was studied. Cadmium levels in the variant (Cd (250 mg/kg + 2,5 g/m<sup>2</sup> HA) increased in the roots in 1,6 times and in the shoots – decreased by 12% compared to control with cadmium without HA. With increasing content HA (5 g/m<sup>2</sup>) in the soil Cd content in the shoots and in the roots increased by 2.1 times compared to variant with cadmium without HA. Biochemical parameters were studied with plants grown hydroponically in 5 variants 1- 0 (control); 2 – 0.4 mM Cd (Cd1); 3 - 0.8 mM Cd (Cd2); 4 - Cd1 + HA (0,5 mM); 5 - Cd2 + HA (0, 5 mM). Chlorophyll (Chl) *a* content decreased in all treatments, except [Cd1 + HA (0,5 mM)] -variant. Content of carotenoids remained unchanged or increased in variant with HA. Proline content increased significantly in the shoots in all variants, especially in [Cd2 + HA (0, 5 mM)]-variant in 13 times, and in the roots - about in 5-9 times. Proline content in the shoots in variants without HA was lower than in the presence of HA, and in the roots, vice versa. In variant [Cd2 + HA (0, 5 mM)] was observed the most increasing of ABA content: in the shoots in 3 times and in the roots - in 2 times. Thus, HA stimulated Cd uptake by roots and shoots. Cadmium caused decrease of Chl *a* content, but increased Chl *b* and carotenides, it might be adaptive reaction. Cadmium significantly increased proline content, this increasing roles as osmotic compatible and osmoprotector under heavy metals stress. ABA content increased in all variants, but the most increasing was observed in variant with high concentration of humic acids.

*Keywords:* cadmium, humic acids, chlorophyll, carotenoids, proline.



## WILLOWS UNDER ARSENIC EXPOSURE: PHYSIOLOGICAL RESPONSES, ACCUMULATION AND DIFFERENTIAL GENE EXPRESSION IN ROOTS AND ABOVE GROUND PARTS

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### ABSTRACT

The toxicity of (arsenic) As in plants is mainly mediated by the competition between arsenate (AsV) and phosphate in metabolic processes. In addition, As could disrupt enzymatic activities by the binding of arsenite (AsIII) to thiol groups present in proteins. Regardless of the negative effect of As in plants metabolism, several species have shown the capacity to survive or avoid the stress associated to As. The objective of this study is to understand how some of them are even able to extract, degrade, or immobilize As contaminants.

Results from a four weeks hydroponic study with *Salix purpurea* 'Fish Creek' saplings showed that these shrubs are able to support up to 5 ppm As without showing any significant symptoms while taking up and accumulating As in its tissues. Physiological measurements, including photosynthesis, transpiration, and biomass production, were measured in plants exposed to 0, 5, 30 and 100 ppm of As. Preliminary differential gene expression analyses (RNA-seq) led to the identification of 164 differentially expressed genes in stem, 864 in leaves and 1348 genes in roots. These results will help us to develop a better understanding of the mechanism implied in As metabolism in willows and in plants in general.



## EFFECTS OF HEAVY METALS ON PLANT RECOMBINANT GLUCOSE-6P-DEHYDROGENASE ISOFORMS

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### ABSTRACT

Glucose-6-phosphate dehydrogenase (G6PDH - EC 1.1.1.49) is the key enzyme of the oxidative pentose phosphate pathway (OPPP); its reaction provides the reduction of NADP<sup>+</sup> to NADPH, a co-factor essential in many metabolic processes in plant cells, and in response to biotic and abiotic stress, such as salinity, pathogen attack, ABA response, drought, nutrient starvation.

Previous studies suggested a possible correlation between metals and changes in total G6PDH activity. The plastidial P2-G6PDH isoform shows a redox regulation, localised at the level of two conserved cysteines; at least other 3 cys residues have been identified within its protein sequence, with structural and/or unknown functions.

We obtained mutagenized recombinant P2-G6PDH from *Populus trichocarpa*, changing the cysteine in serine, in order to investigate a possible direct effect of heavy metals in the regulation of G6PDH activity involving cys residues. The recombinant proteins were purified to apparent homogeneity, and the activities of the purified enzymes were measured in the presence of different elements (Ni, Cd, Pb, Cu, Zn).

Similar measurements were made on recombinant barley cytosolic G6PDH; this isoform presents several cysteines as well, but doesn't show any redox regulation.

Changes in the activity and kinetic properties of Cyt and P2-G6PDH were observed, suggesting peculiar role(s) in this regulation played by cys residues.



## PHYTOEXTRACTION POTENTIAL OF *VETIVERIA ZIZANIOIDES* FOR REMOVAL OF COPPER FROM CONTAMINATED SOIL IN PRESENCE OF VARIOUS ORGANIC AMENDMENTS

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### ABSTRACT

Copper (Cu) toxicity is a problem of both agricultural and environmental significance. Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. The present study was undertaken to evaluate the growth response and phytoextraction capacity of *Vetiveria zizanioides* growing at different concentrations of Cu ranging from 500 to 2000 mg kg<sup>-1</sup> without and with organic amendments viz. farm yard manure, mycorrhizae, *Azotobacter chroococcum*. The results indicate that the *Vetiveria zizanioides* was not survived in as such Cu contaminated soil (without amendment) at various concentrations. The test results also indicate that the *Vetiveria zizanioides* exhibited high tolerance to Cu in the soils and their normal growth attained even at Cu concentration of 2000 mg kg<sup>-1</sup> after addition of organic amendments. Significant improvement in physico-chemical and microbiological properties in Cu contaminated soils was observed in presence of various organic amendment. The accumulation of Cu in roots was higher than the shoot of *Vetiveria zizanioides* and varied in the range of 50-70% depending upon the concentration of Cu in soils. The inoculation of plant with mycorrhizae and *Azotobacter chroococcum* increased the root length by 5-7 times and biomass by 8-10 times with respect to control (as such contaminated soil). Thus, *Vetiveria zizanioides* can be used as best potential accumulator of Cu from contaminated soil in presence of farmyard manure, *mycorrhizae* and *Azotobacter chroococcum*.

**Keywords:** phytoextraction, copper, farmyard manure, mycorrhizae, *Azotobacter chroococcum*, *Vetiveria zizanioides*



## PHYTOREMEDIATION POTENTIAL OF NATIVE FLORA OF CONTAMINATED SOIL OF VALE DAS GATAS MINE IN PORTUGAL

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### ABSTRACT

The objective of this study is to evaluate the phytoremediation potential of native flora of soils enriched with arsenic and heavy metals in the area of the old Vale das Gatas mine (Northern Portugal).

Very high maximum values for Pb (6,299 mg/kg), As (5,770 mg/kg) and W (636 mg/kg) were observed at soil and tailings of the Vale das Gatas mine area. The Cu-Mn-W-As-Pb-Zn association, which reflects the presence of mineralised veins in the area, is inversely correlated with pH.

In general, the content variations in plant materials were strongly related to the content variations in soils. It has also been verified that in contaminated locations or tailings, the concentration of metals in plant tissues is high due to the high metal concentrations in the soil. The leaves of *Agrostis castellana* and *Holcus lanatus* reflect the Cu, Pb and Ni pedogeochemical anomalies. The aerial parts of *Pteridium aquilinum* and *Juncus effusus* seem to be indicative of Zn anomalies in the soil. *Holcus lanatus* and *A. castellana* were the main accumulators of As, Cu, Fe and Pb and good accumulators of Zn. *Pteridium aquilinum* was a good accumulator of As, Pb and Zn. *Juncus effusus* appeared to be a Zn accumulator. The *P. pinaster* trees growing on the tailings and contaminated soils accumulated the studied elements in quantities greater than observed in plants of the areas representative of the local geochemical background. These values were also higher than those typically observed in this species. In the *P. pinaster* samples from tailings and contaminated soil locations, the older needles (2- and 3-years-old) show a tendency to accumulate higher concentrations of As, Fe, Zn, Pb and W while Ni and Cu were preferentially accumulated in young needles and stems (1-year-old).

The metal/metalloid concentrations higher than toxic level in some species indicate that internal detoxification metal tolerance mechanisms might also exist; therefore, their utility for phytoremediation is possible.

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## PERFORMANCE OF *ATRIPLEX HALIMUS* L. IN ABSORPTION AND TRANSLOCATION OF Cr(III) AND Cr(VI), AND EVALUATION OF HIS TOLERANCE TO METALS

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### ABSTRACT

Metal pollution has become, due to industrial and technological advances in recent decades, a major threat to human health and environment. It is thus important to have effective tools to assess the bioavailability of metallic element and their environmental effects in contaminated soils. The purpose of the present work was to evaluate the tolerance and capacity to accumulate chromium in its two valence states, Cr(III) and Cr(VI) of *Atriplex halimus* L., grazing species, one of the most abundant perennial halophytes which is found as hedges in agricultural soils near a discharge of a bolts, cutlery and fittings factory (BCR, located in Oued Rhiou, Algeria).

The obtained results show a variation of the accumulation of chromium according to the plant organs, the metal form and the rate of chromium.

Chemical tests reveal an evolution of the chromium content in the aerial parts (leaves and stems) and the ground organs (roots) of *Atriplex halimus* L. This variation is related to the increase in dose and type of the used chromium.

It was also found that chromium absorption by plants treated with Cr(III) is more important than those treated with Cr(VI).

The obtained results also show that *Atriplex halimus* L. is more tolerant to trivalent chromium than to hexavalent chromium.

We finally found that this species present a large absorption capacity in roots and a low translocation to the above ground organs, which suggests the existence and evolution of the absorption, transport and detoxification mechanisms by storage or biochemical reactions in this species.

**Keywords:** *Atriplex halimus* L., heavy metals, chromium, accumulation, translocation, tolerance.



## EFFICIENCY OF CADMIUM ACCUMULATION IN EUROPEAN AND HYBRID LARCH FOR USE IN PHYTOREMEDIATION

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### ABSTRACT

The aim of this study was to compare the efficiency of cadmium (Cd) phytoextraction by two species of gymnosperms widely used in Limousin (France) for reforestation: a hybrid larch (*Larix x eurolepis* Henry) and one of its parents, European larch (*Larix decidua* Mill.). Larches were chosen because of their fast growth rate. The study focused on Cd, one of the most toxic metallic trace element (MTE) for plants and was conducted on two-years-old trees in greenhouse.

Larches were planted in November 2012 in pots containing a mixture of horticultural soil and sand (75/25, v/v). The temperature was maintained frost-free without artificial illumination and trees were watered with distilled water. After an acclimation of 3 months, larches were divided into 3 batches of 10 trees: a control group and two batches watered once with an aqueous solution of cadmium chloride, resulting in final concentrations of 50 and 100  $\mu\text{M}$  Cd in soil, corresponding respectively to 12.5 and 25  $\text{mg}\cdot\text{kg}^{-1}$  (the threshold for considering a polluted soil is 1-3  $\text{mg}\cdot\text{kg}^{-1}$  according to the European Directive [1]). Plants were subsequently watered with distilled water and were harvested in November 2013 when needles just began to turn yellow. When trees were harvested, root system was briefly washed with water and then incubated for 2 hours in 20 mM EDTA in order to remove Cd adsorbed on root surface. Stem height and diameter (at the root collar) of each tree were measured just before potting in greenhouse and the day of harvest. Each tree was divided into 9 samples: taproot, roots, needles, buds, branches, bark and wood (for these organs, the last year was separated from the first two years). The obtained samples were weighed to determine their fresh weight and were then dried in a ventilated oven at 40°C to obtain their dry weight (DW).

Each dry sample was reduced in powder, mixed with 68% nitric acid and 30%  $\text{H}_2\text{O}_2$  and mineralized by microwaves (Multiwave 3000, Anton Paar, Perkin Elmer). Cd concentration was then measured with a flame atomic absorbance spectrometer (HGA 900, Perkin Elmer) and the results were expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  DW.

Results showed that the treatments with 50 or 100  $\mu\text{M}$  Cd had no significant effect on European and hybrid larch growth. Indeed, stem height and collar root diameter, as well as fresh and dry biomass were not reduced. This result thus indicates that both larch species can tolerate a moderately polluted soil, what is required for further study. The measurements of Cd concentrations are in progress but the first results indicate that Cd would be mainly stored in root system, what is observed in non hyperaccumulating plant species and in most Cd hyperaccumulators [2]. Moreover, buds accumulated less Cd than other tested organs, what could be interpreted as a strategy of protection for the following year (in order to maintain development of young organs). Cd quantification in all organs will allow to determine which larch species is more suitable for Cd phytoextraction.

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## ACCUMULATION PATTERNS OF Cr(VI)/Cr(III) – QUALITATIVE AND QUANTITATIVE ANALYSIS OF XRF MAPS

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### ABSTRACT

The aims of this study were both the qualitative and quantitative analysis of Cr accumulation patterns in shoots of *Callitriche cophocarpa*. This globally-distributed, submersed macrophyte exhibits outstanding Cr phytoremediation capacity in an aquatic environment. Plants were immersed in 100  $\mu$ M (5.2 mg/L) Cr solution for seven days, in environmentally-relevant conditions. Cr was applied separately at two stable forms as Cr(VI) and Cr(III), known from their diverse physicochemical properties as well as different influence on biota. The maps of Cr distribution in young leaves, mature leaves and stems, were obtained according to micro X-ray fluorescence spectroscopy ( $\mu$ XRF). This method is a non-destructive technique that allows studies of elements with a very narrow beam of X-rays, at concentration levels in  $\mu$ g/g range. The detailed analysis of XRF maps were done according to Image-Pro PLUS (Media Cybernetics) software.

Distinct patterns of Cr arrangements were found in respect to Cr speciation as well as the plant organ. Cr was found solely in spot-like structures when the shoots were exposed to Cr(III). However, Cr was visualized both in vascular bundles and spot-like structures after plant incubation in Cr(VI). The element was found exclusively in vascular bundles in the case of mature leaves exposed to Cr(VI). Preferentially, Cr deposition were observed in vascular bundles of Cr(VI)-treated stems, too. On the other hand the element was detected in high amount in spot-like structures in young leaves treated by hexavalent Cr. In this work we also demonstrated the statistically significant differences in the Cr concentrations dependent on the Cr valency and the type of plant organ. The concentration of Cr increased in the following order: Cr(VI) mature leaves < Cr(VI) young leaves < Cr(VI) stems < Cr(III) young leaves < Cr(III) mature leaves < Cr(III) stems. The relation between the tissue surface (%) that bounds Cr and Cr concentration (expressed in counts per second; cps) was described by the function:  $y=9.1\ln x+8.4$ ; where y – surface (%) occupied by Cr, and x – Cr content. According to this function we might conclude that the tissue structures of *Callitriche* responsible for Cr binding are already saturated in the conditions of our experiment; though, the maximal sorption capacity of the plant tissue toward Cr ions was not achieved.

This work has contribution into the knowledge concerning mechanisms of aquatic phytoremediators response to elevated concentration of Cr ions. It also provides information concerning application of particular organs of *Callitriche cophocarpa* in efficient Cr remediation.

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## HEAVY METALS AND METALLOIDS ACCUMULATION BY NATIVE FLORA OF MINING AREAS IN PORTUGAL: PHYTOREMEDIATION POTENTIAL

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### ABSTRACT

The aim of this study is to evaluate the phytoremediation potential of native flora of soils enriched with metals and metalloids, in distinct abandoned mining areas of Portugal.

Analytical methods included colorimetry for W, atomic absorption spectrophotometry (AAS, Perkin-Elmer, 2380) for Ag, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn, hydride generation system (HGS) for As and Sb and fluorometry (Fluorat-02-2M analyzer, Lumex) for U.

Significant accumulation of heavy metals and metalloids in both soils and native wild flora suggests that metal contamination is a matter of great concern in the studied mining areas. The native flora displayed its ability to withstand high concentrations of heavy metals/metalloids in the soil. However, accumulation patterns of metals/metalloids in the plants tested differed. As metal concentrations in above ground parts were maintained at low levels, metal tolerance in most cases may mainly depend on their metal excluding ability.

However, metal/metalloid concentrations higher than toxic level in some species like *Agrostis castellana* (As, Fe), *Cistus ladanifer* (Cr, W), *Cistus salvifolius* (Ni, Pb), *Digitalis purpurea* (Sb, W, Zn), *Helichrysum stoechas* and *Hypochaeris radicata* (U), *Holcus lanatus* (As, Cu, Fe), *Lonicera periclymenum*, *Mentha suaveolens* and *Phytolacca americana* (Pb, Zn), *Pinus pinaster* (As, W, Zn), *Polystichum setiferum* and *Solanum nigrum* (Zn), *Pteridium aquilinum* (As), as well as the serpentine plant species *Alyssum serpyllifolium* subsp. *lusitanicum*, *Lavandula stoechas* subsp. *sampaiana*, *Linaria spartea* subsp. *virgatula* and *Ulmus procera* (Cr, Ni) and *Bromus hordeaceus* and *Plantago radicata* subsp. *radicata* (Ni) indicate that internal detoxification metal tolerance mechanisms might also exist; therefore, their utility for phytoremediation is possible.

Furthermore, the plants could grow and propagate in substrata with low nutrient conditions which would be a great advantage in the revegetation of mine tailings. It was also observed that despite lower accumulation, trees of the studied regions can be very effective due to their higher biomass.

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## HEAVY METAL ACCUMULATION BY WILD PLANTS IN INDUSTRIALLY DISTURBED HABITATS OF THE MIDDLE URALS

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### ABSTRACT

Heavy metals (HM), providing a cytotoxic, mutagenic and carcinogenic action are among the most dangerous pollutants. In plants HM inhibit photosynthesis and growth, change mineral nutrition and water balance. HM pollution demands the elaboration of the ways of their remediation and safe use. In connection with this the present study deals with the plant HM accumulation capacity both in roots and leaves to identify plant hyperaccumulators or excluders suitable respectively for phytoremediation, or cultivation in contaminated areas for economic purposes.

Accumulation capacity of *Tussilago farfara* L., *Plantago major* L., *Plantago media* L., *Taraxacum officinale* Wigg., *Melilotus albus* M., *Trifolium medium* L. were studied in 5 habitats near Nizhny Tagil (NT) and Kachkanar Mining and Processing Plant (KMPP). Soils, roots and shoots of plants were collected for HM determination – at least 10 soil samples and/or sludge, 10 flowering plants of each species separately underground and aboveground parts. The heavy metal content was determined by atomic absorption spectral analysis (PerkinElmer AAS 300). According to the level of pollution the habitats were defined as: background, buffer, impact.

The content of HM mobile ion forms in soil/sludge varied: 7 - 951 mg/g for Cu; 6-390 for Zn; 377-5700 for Fe; 0,2-124 for Co; 0,5-37 for Cr; 0.01-0.2 for Cd; 0.2-5 for Pb; 4-23 for Ni. The underground parts of plants accumulate 5-100 ug/g Cu; 8-150 ug/g Zn; 0,4-17 ug/g Co; 1-30 ug/g Cr and 110-3300 ug/g Fe. *P. major*, *P. media*, *T. officinale*, *T. farfara* revealed a high ability to accumulate copper in the roots, *P. major* – zinc, *M. albus*, *P. major*, *P. media*, *T. farfara*, and *T. officinale* - iron, *M. albus*, *P. major* - cobalt; *T. farfara*, *P. major*, *M. albus* - chromium. In most species the positive correlation between the content of HM in soils and in the roots was shown.

Accumulation coefficient (AC) less than 1 is usually observed at elevated HM levels in the soil due to the active work of the root barrier.  $AC \geq 1$  indicates unlimited diffusion or absorption of the metal ions into the plant. For plants from N.-Tagil sites (table) KH in most cases does not exceed 1, and with the growth of the toxic load decreased.

Table. AC for HM in wild plants from sites with different relative toxic load

Species	Relative toxic load (Nizhniy Tagil)					
	1,00	2,55	3,33	6,19	8,36	22,78
$AC_{Cu}/AC_{Zn}$						
<i>P. major</i>	0,7/0,9	0,8/0,4	0,4/0,4	0,3/0,4	0,3/0,2	0,1/0,2
<i>T. farfara</i>	2,0/0,4	1,3/0,1	0,3/0,2	0,2/0,1	0,20,1/	0,1/0,04
<i>T. officinale</i>	1,6/0,6	1,6/0,3	0,6/0,4	0,3/0,1	0,3/0,1	0,1/0,1
<i>M. albus</i>	0,5/0,9	0,4/0,2	0,1/0,2	0,8/0,6	0,1/0,1	0,02/0,1
<i>T. medium</i>	1,0/0,9	0,7/0,3	0,2/0,2	-/-	0,2/0,1	-/-
Relative toxic load ( KMPP)						
	1,0 – 2,9	1,1- 4,3	2,6 -3,7	4,6		
$AC_{Cu}/AC_{Zn}$						
<i>T. officinale</i>	23,6/8,32	14,2/4,1	30,8/14,4	21,8/3,5		
<i>P. media</i>	84,4/9,83	33,4/6,37	-/-	52,7/10,4		

Heavy metals concentration in soils, roots and shoots, accumulation and translocation coefficients allowed revealing different strategies in the accumulation of such essential elements as copper and zinc in plants. *P. major*, *P. media*, *T. officinale*, *T. farfara* have shown the high ability to accumulate Cu, *P. major*, *P. media* – Zn and *M. albus*, *T. officinale* – Fe. Species revealed difference in the effectiveness of soil/root and root/shoot barriers.



## PHYTOREMEDIATION OF URANIUM TAILINGS: ROLE OF CHELATORS IN TRIGGERING URANIUM ACCUMULATION IN WHEAT

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### **ABSTRACT**

Radionuclides including toxic heavy metals mobilization through extraction from ores and processing for diversified uses has laid to the discharge of these harmful metals into the ecosystem. These pollutants pose a great risk to environment and human health. Conventional methods of remediation applied for contaminated environments have many limitations; alternatively, phytoremediation can serve as a prospective method for decontamination and rehabilitation of contaminated sites. Several approaches may be applied to further enhance the efficiency of metal phytoremediation. Chelator-assisted phytoextraction has been proposed as a potential tool for phytoremediation of U contaminated tailings. In the present study, a greenhouse experiment was set up to scrutinize the ability of citric acid (CA), oxalic acid (OA), nitrilotriacetic acid (NTA) and EDTA for phytoremediation of U tailings by wheat (*Triticum aestivum* L.). Uranium tailings were mixed with garden soil to yield 25:75 mixtures. Prepared pots were divided into four sets and treated with following different concentrations- 0.1, 0.5, 2.5 and 12.5 mmol kg<sup>-1</sup> soil amendments for each of the chelator whereas, control pots were remain untreated. Experiment was conducted in completely randomized block design with triplicates. The observations were recorded during the vegetative stage and effects of different concentrations of chelators were noted on growth parameters, which includes, shoot-root length and shoot-root fresh as well as dry mass. An optimum concentration for each of the chelator was recorded on the basis of biomass production, tolerance and accumulation potential. Uranium accumulation in different plant parts was recorded through pellet fluorometric method. Severe toxicity in the form of reduced growth and plant death was recorded at 12.5 mmol of each chelator. Lowest growth inhibition produced by chelators occurred in NTA which was followed by OA, moderate in CA and highest was recorded in EDTA treatments at their respective levels. Uranium uptake and accumulation were concentration dependent for each amendment applied. Maximum U uptake (3.4- fold) in the roots occurred at 2.5 mmol of CA while NTA proved to be the weakest for the same purpose. Chelator induced U uptake in the present study follows the order: CA> EDTA> OA> NTA.

*Keywords:* phytoremediation, uranium tailings, chelators, EDTA, NTA



## IN *IN VITRO* CULTURE OF *DITTRICHIA VISCOSA*, MICROPROPAGATION AND ITS POTENTIAL USE IN PHYTOREMEDIATION

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### ABSTRACT

*Dittrichia viscosa* (L.) Greuter is a ruderal plant species, widely distributed in the Mediterranean basin; it has a considerable biomass and is able to grow on heavy metal (HM) polluted soils, as reported by several authors [2,3,4,5]. However, only few reports indicate *D. viscosa* as a phyto-remediator plant, useful for reclamation of HM polluted soils. The principle aim of this work was to develop and set up a protocol for *D. viscosa* seed sterilization and for *in vitro* seedling culture. Furthermore, *D. viscosa* plants, derived from seed propagation, collected from three different natural populations located in central and southern Italy (Roma, Potenza, Crotona), were assessed for their genetic dissimilarity by RAPD analysis. On the basis of morphological characteristics, some clones were selected and tested to evaluate their capacity to grow on axenic medium in the presence of high Zn concentrations (up to 10, 20, 40, 60 and 100 fold of Zn present in the culture medium). Different tests carried out on *Dittrichia* seeds allowed us to define a simple and effective sterilization protocol, providing the combination of ethanol and sodium hypochlorite solutions, at 70 % and 1.0 % respectively. Seeds were sown on three different media: Murashige and Skoog (MS), Gamborg and Mohr (modified by Basile et al. [1]). Seedlings grew similarly on MS and Gamborg medium, while they showed an improved growth rate, in particular at root level, on modified Mohr medium. Plants were vegetatively reproduced transplanting nodal-internodal portions as microcuttings to fresh medium. Clonal propagation was extremely effective and allowed us to multiply vegetatively several seedlings of *D. viscosa*. When seedlings, grown in *in vitro* culture, reached 10-15 cm in length, were transferred to peat-based potting soil provided with a transparent plastic bag, necessary to limit the excess of seedling evapo-transpiration. Plants were regularly irrigated every 2-3 days with tap water and, after 15 days of greenhouse adaptation (23 °C; 14 h light and 10 h dark), plastic bags were removed from plants. The percentage of survival was larger than 80%. Some plants were vegetatively micropropagated on medium at increasing concentrations of Zn, as previously described. However microcuttings were able to produce new roots and to grow only at the lower Zn concentration (10 fold). The performed RAPD analysis on the three *D. viscosa* populations grown in *in vitro* culture revealed a close genetic relationship between Roma and Potenza populations, whilst the Crotona population showed a clear genetic differentiation from the other two.

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## EVALUATION OF ARSENIC REMOVAL POTENTIAL OF FIVE AQUATIC PLANTS GROWN SINGLY OR IN COMBINATIONS

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### ABSTRACT

Arsenic (As) is a ubiquitously present highly toxic metalloid, which poses a serious threat to the environment and human health. Mitigating environmental As contamination is desirable. Exploiting the As accumulation potential of plants has been considered a potential solution; the technology is referred to as phytoremediation. In this backdrop, we opted for a combinatorial approach and concentrated on evaluating the potential of five aquatic plants, namely *Hydrilla verticillata*, *Ceratophyllum demersum*, *Lemna minor*, *Wolffia globosa* and *Eichhornia crassipes*, for growth and As

accumulation for one month in single and combined (in two plant combinations) culture. Plants ( $1 \text{ g L}^{-1}$ ; total 4 g) were subjected to 4 L of  $2500 \mu\text{g L}^{-1}$  As (as sodium arsenate;  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ) in 10% Hoagland solution for a period of 30 d. In two plant combination treatments, 2 g of each plant was used. Total As in the plant material was estimated after digestion of oven-dried plants by using an

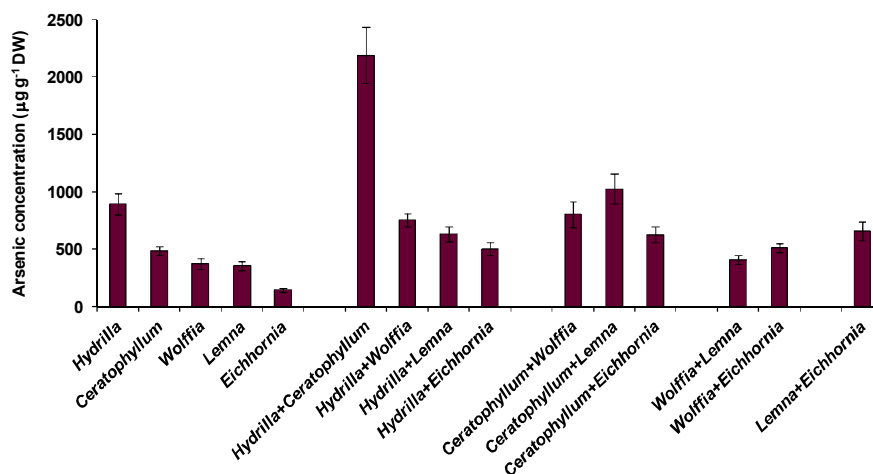


Figure 1. Arsenic concentration ( $\mu\text{g g}^{-1} \text{ DW}$ ) of plants grown singly or in combinations in control and arsenic treatment for 30 days. All values are means of triplicates  $\pm$  SD.

atomic absorption spectrophotometer. *Eichhornia* exhibited the maximum growth rate under control conditions. Under As stress, *Hydrilla* showed an increase in growth rate (19%) in comparison to control, while other plants demonstrated a decline. Among different combinations, *Ceratophyllum+Lemna*, *Hydrilla+Ceratophyllum* and *Hydrilla+Wolffia* showed higher growth in As (92%, 43%, and 33%, respectively) than under control conditions. As concentration ( $\mu\text{g g}^{-1} \text{ dw}$ ) was found to be maximum for *Hydrilla+Ceratophyllum* (2188) followed by *Ceratophyllum+Lemna* (1026) and *Ceratophyllum+Wolffia* (804) (Figure 1). Total As removal ( $\mu\text{g}$ ) in 30 days was found to be the maximum for *Ceratophyllum+Lemna* (4365) followed by *Hydrilla+Ceratophyllum* (3326) and *Hydrilla+Wolffia* (1896). In all combinations of plants, *Hydrilla* always contributed more than 50% (52% to 78%), while *Eichhornia* less than 30% (17% to 28%). The study advocates the use of *Hydrilla* or *Ceratophyllum* as one plant of choice to be used in combination. It also proposes to screen for different plant combinations for better application of phytoremediation technique in field.





## **PERSISTENT ORGANIC CONTAMINANTS**





## PROPOSED METHODOLOGY FOR RESTORATION OF UNCONTROLLED LANDFILLS IN INSULAR COMMUNITIES

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### ABSTRACT

Despite the promotion of waste management being held throughout the world, unfortunately the disposal of untreated waste into final landfill sites is the most widely solution these days. Obviously, this cannot be part of an integrated waste management system where waste are recovered, reprocessed and reused as new or existing resources, since nothing is controlled.

Uncontrolled waste disposal on landfill sites causes pollution that can be hazardous on the environment and introduce several problems on socio-economic welfare of the planet, thus moving away from them is a major issue that requires immediate actions. Waste disposal pollution is caused by either normal operations, such as the emissions of landfill gas into the atmosphere and the leakage of leachate in subsoil (and then to the aquifer) or the accident from subsidence and fires; either way they constitute serious threads to public health and the environment.

In this research we try to develop a methodology of restoration of landfill sites on large islands. If landfill sites are problematic in mainland scenarios, in large islands the situation is even worse. The limited surface of islands, the volume of waste which is steadily increased (due to fishing, tourism or other sources) and the waste washed up from open seas, magnify the problem and makes them a priority.

For the proposed methodology we used Cyprus as a case study. We evaluated different management plans for landfill restoration through multi-criteria analysis on the conditions found currently on the island. We examined the life cycle assessment (LCA) plan of uncontrolled landfill sites, their waste forms and the risk assessment of Cyprus landfills. Finally, we evaluated various recovery methods of soil and water contaminated with heavy metals (since these are very common in waste disposal sites) such as Permeable treatment walls, phytoremediation, biochemical process, and their suitability to Cyprus. Different scenarios were evaluated based on the effects of the pollution, the restoration and aftercare costs, the gas emissions, the simplicity of implementation, the perspective of land development, and, finally, for each open dump site the best option was chosen.



## HYDROPONIC PHOTOBIOREACTORS FOR THE REMOVAL OF TOXIC ORGANIC POLLUTANTS

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### ABSTRACT

Hydroponic bioreactors were recently developed for the removal of the toxic herbicide, atrazine, by Sorghum plants, and for the removal of bisphenol-A by *Cyperus corymbosus* and *Juncus acutus*.

Sorghum plants, were germinated and cultivated in a thin layer of solid nursery medium, developed their roots into a deeper solution containing high concentration of atrazine, up to 30mg/L.

The plants tolerated atrazine and gradually removed the atrazine from the medium, leading to its complete removal within 4 weeks. Aeration facilitated root activity. Root associated bacteria involved in atrazine removal were defined. <sup>14</sup>C-atrazine breakdown products were identified in Sorghum shoots, including GSH-atrazine and atrazine metabolized products.

Bisphenol-A, recognized as an endocrine disruptor in mammals, fish and invertebrates, was tested for its remediation by the wetland plants, *Cyperus corymbosus* and *Juncus acutus*, set in a model hydroponic system of glass containers. Bisphenol-A, at concentrations between 50-200ppb, was remediated within 1-4 days. The mechanism of bisphenol-A remediation is currently investigated, aiming to identify mode of metabolism of the pollutant by the plants, involvement of root associated bacteria. We aim to determine the effectiveness of hydroponic bioreactors outdoors.



## FULL-SCALE REMEDIATION OF CONTAMINATED SLUDGE USING PLANTS - INVESTIGATIONS, RESULTS AND EXPERIENCES

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### ABSTRACT

At a central waste water treatment plant of a city with about 530,000 inhabitants in Germany municipal as well as industrial waste water were treated. The sewage sludge was pumped to a site (56 ha) outside the city. On this site the water content of the sewage sludge was decreased and the sludge was used in agriculture afterwards. In 1990 the operation of the dewatering site was stopped and about 800,000 m<sup>3</sup> of sewage sludge remained there in several lagoons.

An environmental impact assessment (EIA) was developed for the site. The EIA identified the need of remediation measures to prevent hazard risk. Different remediation techniques were compared using economical, social and environmental aspects. The biological *in situ* remediation using plants was chosen for remediation of the sludge lagoons. It is one of the biggest and best monitored phytoremediation projects of its kind in Europe.

On the site common reed (*P. australis*) is successfully used for the remediation of sewage sludge contaminated with metals (Cd, Cr, Cu, Ni, Pb, Zn). At the beginning of remediation (1996) the water content of the sludge was approximately 65%. These conditions were favourable for establishment of reed population as biological capping of the sludge lagoon. A special technology for the out planting, on coconut mats pre-cultivated reed plants, was developed and successfully used. A biomass yield of about 19 t dry matter × ha<sup>-1</sup> by mowing the reed shoots in winter and a metal removal of 6.7 kg × ha × yr<sup>-1</sup> could be achieved.

Due to humification and mineralization of the sludge, linked to the remediation process, a decrease in plant density and biomass yield and a lower efficiency of metal removal were determined between 2005 and 2007. That's why BioPlanta has carried out extensively investigations based on crop management and agronomic practice to continue the phytoextraction in an optimal way and to improve the phytoremediation technology.

The selection of phytoremediating species has been shown to be the most important factor affecting the extent of metal removal regarding the metal content and yield. Using sunflower (*H. annuus*) an increase of total metal extraction by factor 8 was reached in comparison to reed. Sedge (*C. acutiformis*) shows a more efficient extraction for five out of six investigated metals (by factor 2 to 12) compared to reed. In field studies, BioPlanta proved that after application of N-P-K fertiliser reed showed a significant higher yield (2 to 3 fold), resulting in improved phytoextraction of metals by factor 3 to 14. Increase in yield was also achieved by mowing reed in winter, sunflower in autumn and rape in summer. Highest metal extraction (9.7 kg × ha<sup>-1</sup> × yr<sup>-1</sup>) was reached combining agronomic practices with selected plant species, e.g. fertilization and mowing of reed in autumn. The application of plant supplements and herbicide revealed a plant and element specific increase of the phytoextraction efficiency.

As result of these investigations sunflower and also maize were used (instead of reed) for full-scale remediation on one of the four sludge lagoons. About 10 t dry matter × ha<sup>-1</sup> of sunflower as well as 7 t dry matter × ha<sup>-1</sup> of maize could be yielded. Due to the harvest of sunflower and maize about 2.3 kg × ha<sup>-1</sup> × yr<sup>-1</sup> and 3.5 kg × ha<sup>-1</sup> × yr<sup>-1</sup> of metals could be removed, respectively. Within 17 years remediation (1996 – 2013) in total 321 t dry matter were mowed and removed as well as 99.7 kg metals (92.6 kg Zn; 0.7 kg Ni; 4.4 kg Cu; 0.4 kg Cr; 0.5 kg Cd; and 1.1 kg Pb) were extracted using plants.



## ENVIRONMENTAL FATE OF PPCPs AT A 2,000 Ha MUNICIPAL WASTEWATER LAND-APPLICATION SITE

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### ABSTRACT

The occurrence and fate of pharmaceutical and personal care products (PPCPs) in the environment are of increasing public importance due to their ubiquitous nature and documented effects on wildlife, ecosystems, and potentially humans. One potential, yet undefined, source of entry into the environment of PPCPs is via the land application of municipal wastewater onto permitted lands. The objective of this study is to determine the extent to which PPCPs are mitigated by or exported from managed tree plantations irrigated with municipal wastewater. A specific focus of the study is PPCP presence or removal in groundwater before surface water discharge at the catchment scale. The study site is a municipality that land-applies primary treated wastewater onto 2,000 ha of managed hardwood and pine plantations. Initial sampling and analysis includes grab sampling of groundwater throughout the study site and targeted analysis of a suite of PPCPs utilizing GC-MS and LC-MS/MS. Further study scope, methods, and preliminary results of targeted PPCPs in groundwater and surface waters will be presented.

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## MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF MAIZE (*ZEA MAYS*) EXPOSED TO FORMERLY MULTICONTAMINATED SOILS

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### ABSTRACT

Phytoremediation is promising, but depends on understanding contaminants' impact on plant functioning. We therefore focused on the impact of formerly contaminated soils on cultivated plants (*Zea mays*) especially on (i) the impact of polycyclic aromatic hydrocarbons (PAH) on plant functioning and (ii) the PAH transfer to plant. Cultivation was conducted under controlled conditions on former contaminated soils (coking plant, steel industry). Maize growth and organ development were assessed, by measuring both its morphological and nutrition parameters. PAH transfer was assessed by measuring soil total and available concentration using passive sampler method (polyoxymethylene membrane, POM) or resin extraction (Tenax®) and PAH concentration in roots and shoots. First results showed that, after five weeks, plants exposed to contaminated soils presented decreased biomasses and modified root architecture with fewer thin roots. PAH availability, using POM method, of all contaminated soils was low and was not well correlated to PAH concentration in roots. Other analyses are on going: nutrients up-taking, PAH concentration in shoots and available PAH using Tenax® extraction.

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## IN SITU PHYTORECLAMATION OF SEWAGE SLUDGE AFTER ITS AMENDMENT WITH SOIL AND GRAVEL

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### ABSTRACT

Sewage sludge is often contaminated by heavy metals, organic pollutants, and pathogens. It is also characterised by a high water holding capacity and anaerobic conditions, and malodours associated with the presence of some volatile organic compounds (VOCs), even after digestion and alkaline stabilisation. These aspects present the main obstacles to applying sewage sludge to the land and explain why treated sewage sludge, if not landfilled or incinerated, often remains within the territory of Wastewater Treatment Plants (WWTPs) without further utilisation. Economic considerations and traditions may also be of relevance. In Greece, for example, owing to a lack of acceptance by farmers, most sludge is landfilled. According to Matthews (1999) [1] and IEEP (2009) [2] the country uses a very small quantity of sludge in agriculture (~10 %), and this mostly in the frame of research projects and pilot studies. In addition to agricultural use, trials have been performed in the timber industry [3], mine spoils reclamation [4], vermiculture [1] and cement industry [5]. However, this is still very much at the experimental stage in Greece. Meanwhile, the volume of sewage sludge is continuously growing, not only in landfills, but also within the area of Greek WWTPs, and this has an adverse effect on the environment and human health. In order to protect the environment, areas which already have an accumulation of sewage sludge should be reclaimed.

This paper reports on the possibility of using woody species, namely *Populus alba* L., *Robinia pseudoacacia* L., *Quercus pubescens* Willd. subsp. *pubescens*, and *Pinus pinea* L., for the reclamation of sewage sludge from the Thessaloniki WWTP (Greece) after its amending in different rations with soil of the neighbouring area and gravel. The establishment of the experimental site initiated in early January 2012. Seven ditches were dug using a tractor. Each ditch (13x1.5x0.8m) was filled with a mixture as follows: sludge/soil/gravel 33.33/33.33/33.33% applied as layers (T1), sludge/soil 50/50% (T2), sludge/soil 30/70% (T3), sludge non-amended (C4). In early March 2012 the treatments T1, T2, and T3 were planted with early mentioned plants. Four plants of each species separated by a distance of 0.5 m were used for each treatment. These plants were purchased from the Greek Forestry Service. The control units C1, C2, and C3 had corresponding mixture to T1-T3 respectively, but they were not planted. An automatic dripping irrigation system was installed in all experimental units and used for three hours early in the morning three times per week during the dry season. After six months since the plantation of the treatments, leaves of plants and samples of sludge mixture were collected for nutrients analyses. Macronutrients (Ca, Mg, K, P) trace elements (Mn, Cu, Zn, Fe, Cr, Ni, Pb) and Na were measured. The results showed that plants took up macronutrients at relatively high rates, in some cases reducing their excessive levels of concentration in the sludge by as much as 95 %. Trace elements were removed at a lower rate compared to hyperaccumulator plants, this was possibly affected by their reduced bioavailability in the substrate, but the bioconcentration factor, which was greater than one for most of the species and for most of the trace elements, indicated that the plants were capable of phytoextraction. It was concluded that the best effect on the rates of plants survival was produced by amending the sludge with greater amounts of soil (e.g., to 70% as for T3 and its own control C3). The fact that no native vegetation was established themselves in the unit C4, which was composed of 100 % sludge, means that it is



impossible to introduce plants directly to fresh sludge with no amendments. The nutrients uptake did not differ significantly between the treatments, but did between the plant species. Thus, *P. alba* and *R. pseudoacacia* had better ability to concentrate macronutrients, while *Q. pubescens* – trace elements, reaching in some cases the threshold of hyperaccumulators for Cr (1,000 mg kg<sup>-1</sup>).

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## PHYTOREMEDIATION OF PHARMACEUTICALS IN WASTE WATERS – FROM LABORATORY TO REAL SCALE

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### ABSTRACT

In the recent time, the global production of the human and veterinary pharmaceuticals strongly increases together with their affordability not only in developed countries, but due their availability and decreasing prices also in Third World countries. Nevertheless, among the most polluted areas belong developed and industrial countries with high standard of living and population density.

The one of the most significant problems is persistence of the pharmaceutical residues and their metabolites in the environment. Many of them do not exhibit acute toxicity for water ecosystems, but have a cumulative effect for nontarget organisms.

Groundwater analysis showed, that the non-steroidal antiflogistics (e.g. diclofenac, ibuprofen and ketoprofen) together with paracetamol and antibiotics were the most abundant pharmaceuticals in the samples collected in Czech test localities.

The laboratory experiments with *in vitro* cultures of *Arabidopsis*, *Melilotus officinalis* proved presence of the several common metabolites of oxidation in cultivation medium contained common NSAID. Achieved result suggests the ability of the plant cells to transform human and veterinary pharmaceuticals to the products of the firsts detoxification step.

These results were verified in reed (*Phragmites australis* L.) cells, tissues and whole plants and finally tested in real scale in constructed wetland with combined vertical and horizontal flow designed for agriculture waste waters cleaning. Elimination of phosphorus, nitrogen and ammonia in this arrangement will be discussed too.



Fig. 1. CW in construction 2012.

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## PHYTOREMEDIATION OF PCBs CONTAMINATED AGRICULTURAL SOIL BY LEGUMINOSAE-GRAMINEAE INTERCROPPING

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### ABSTRACT

Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by leguminosae (alfalfa) and gramineae (ryegrass and tall fescue) was studied in a field trial. All planted treatments had significantly higher PCBs removal efficiency compared with those of unplanted control after 270 days of in-situ phytoremediation. Alfalfa monoculture received the highest removal efficiency of soil PCBs by 59.6%. Analysis of PCBs congeners composition showed that the percentage of di-chlorinated biphenyl decreased in all planted treatments. Alfalfa produced the maximum biomass among the 3 plants. Total PCBs concentration in alfalfa roots reached 355.1 µg/kg, which was significantly higher than those in ryegrass and tall fescue. The phytoextraction efficiency of different treatment was in order of alfalfa > alfalfa-ryegrass-tall fescue > alfalfa-ryegrass > ryegrass > alfalfa-tall fescue > tall fescue. Furthermore, the comparison of the soil microbe taxonomic assignment at the level of phylum under different treatment showed that intercropping with alfalfa could increase the microbial richness and diversity, thus enhance the PCBs removal from the field soils. The results suggest that alfalfa may be an ideal candidate for the phytoremediation of PCBs contaminated soil.



## IMPACT OF EKKMI ADDITION TO SOIL ON WEATHERED *p,p'*-DDE ACCUMULATION IN XYLEM SAPS OF PLANTS

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### ABSTRACT

DDT (2,2-bis(chlorophenyl)-1,1,1-trichloroethane) was one of the most extensively used organochloride pesticides globally during the 1960s to 1970s and was widely applied in Turkey until being banned in 1985. In soil, *p,p'*-DDT can be biotically and abiotically converted to 2,2-bis(chlorophenyl)-1,1-dichloroethane (*p,p'*-DDD) and 2,2-bis(chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE) residues. Both *p,p'*-DDT and its metabolites are classified as persistent organic pollutants (POPs) and bind strongly to soil and sediment organic matter. Except phytoremediation, application of many in situ remediation technologies for POP contaminated soils are both ineffective and expensive. Previous studies have shown that *Cucurbita pepo* ssp. *pepo* (zucchini) has a significant and unique potential to accumulate weathered POPs such as *p,p'*-DDE, polychlorinated biphenyls (PCBs), and chlordane from contaminated soil, but that other plants, including closely related squash and melon species, do not have this ability. However, the mechanism of POP uptake by *Cucurbita pepo* ssp. *pepo* remains unknown.

EKKMI is a new material produced from activated sludge of domestic wastewater treatment plants. This research focuses on DDE accumulations in xylem saps of *Cucurbita pepo* ssp. *pepo* (Zucchini) plants grown in both DDT contaminated soil and DDT contaminated soil amended with EKKMI. Pot experiments were conducted to assess the effect of EKKMI amendment on the phytoextraction of weathered *p,p'*-DDE from soil by plant systems. Soil contaminated with 526-691 ng/g *p,p'*-DDE was amended with EKKMI at 0 % (Control) and 1.0 % (w/w) levels and *Cucurbita pepo* (cv Raven and Zephyr) was then planted. The plants were harvested after 35-days of growth and *p,p'*-DDE concentrations were measured in xylem saps of Raven and Zephyr plants by SPME methods coupled with GC/ $\mu$ -ECD.

Average plant biomass of Raven and Zephyr plants grown in EKKMI amended pots were 15 and 8 times higher than their control plants grown in unamended (0% of EKKMI) pots. For 1% EKKMI amended Raven plants, an average xylem *p,p'*-DDE concentration was measured as 0.59  $\mu$ g/L which is almost 4 times more than that of xylem *p,p'*-DDE concentration obtained from Raven control plants. While average xylem *p,p'*-DDE concentration was 0.07  $\mu$ g/L for control Zephyr plants, xylem contaminant concentration was increased approximately 8 times relative to control plants. Further investigation needs to be performed of the changes of DDE concentrations of plant compartments and metal contents in xylem sap of the cultivar and root to xylem interactions of the plants grown in unamended and amended pots.



## USING PLANTS TO REMEDIATE TNT AND RDX POLLUTION

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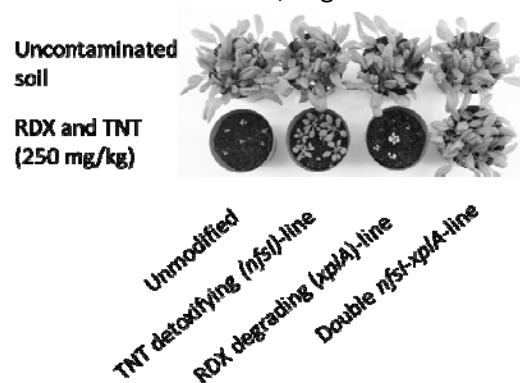
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### ABSTRACT

The containment and remediation of the explosive compounds 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) on military ranges are of high priority to the US Department of Defense. While RDX is highly mobile in the soil column, where containment and remediation strategies are urgently needed, highly phytotoxic TNT remains tightly bound to the organic material in the soil, hindering the use of plant-based approaches for remediation. RDX is now a significant threat to drinking sources such as those close to the US Massachusetts Military Reservation. Currently, there are no cost effective processes to contain RDX or remediate these vast areas of contaminated, vegetated land on military training ranges.



**Figure 1.** Six-week-old *Arabidopsis* plants growing in soil contaminated with TNT and RDX [2].

all three genes. These NR-XpIA-XpIB-expressing plants are both resistant to TNT and able to metabolise RDX, using the nitrogen obtained for growth (Figure 1) [4]. These genes have now been transferred into switchgrass (*Panicum virgatum*), a species native to temperate military training ranges in the US. We are now in the process of conducting field trials (Figure 2) to test the ability of transgenic switchgrass plants to remediate RDX.

While plants have an inherently low ability to degrade RDX, we have established that expression of a unique bacterial cytochrome P450, XpIA and its partnering reductase, XpIB [1,2] in the model plant species *Arabidopsis* (*Arabidopsis thaliana*) enables these plants to remove, and degrade, saturating concentrations of RDX from soil leachate. To enable plants to contain and metabolize RDX, they have to be able to withstand, and grow, in the presence of the co-contaminating, toxic TNT. To overcome this, we engineered *Arabidopsis* plants to express a bacterial nitroreductase, (NR) [3] and demonstrated that this conferred resistance to TNT. We then produced *Arabidopsis* plants expressing



**Figure 2.** Developing field trial plots in the US.

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## ARABIDOPSIS GLUTATHIONE TRANSFERASES DETOXYFY TNT

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### ABSTRACT

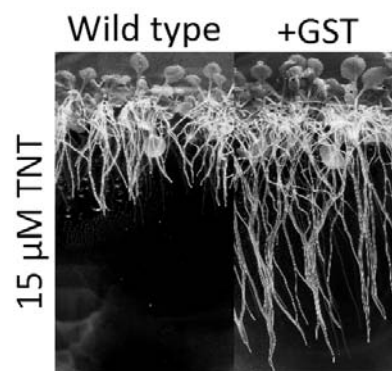
The explosive 2,4,6-trinitrotoluene (TNT) is a major worldwide military pollutant. The presence of this toxic and highly persistent pollutant presents various health and environmental concerns. Due to its chemically resistant structure, TNT has proven to be highly recalcitrant to microbial degradation. In the present study we investigate the role of two glutathione transferases (GSTs), GSTU-24 and GSTU-25, from *Arabidopsis thaliana* (Arabidopsis), which are specifically upregulated in response to TNT exposure.

Microarray analysis on TNT-treated Arabidopsis plants and subsequent quantitative PCR analysis revealed eight upregulated GSTs, all in the Tau class (GST-U1, U3, U4, U7, U12, U22, U24 and U25), with GST-U24 and GST-U25 being the most up-regulated (252-fold and 177-fold respectively). Following affinity chromatography purification and characterisation of recombinant forms of both enzymes, we identified three distinct TNT-glutathionyl products. HPLC-based assays with subsequent mass spectrometry and NMR spectroscopy proved that GSTU-25 was able to produce 2-glutathionyl-4,6-dinitrotoluene, with the concurrent release of nitrite. This conjugate is chemically weaker than trinitrotoluene structures and is likely to be more susceptible to biodegradation.

In order to further investigate the detoxification abilities of these enzymes *in planta* the CaMV-35S promoter was used to drive the over-expression of GST-U24 and GST-U25 in Arabidopsis. These GST overexpressing lines exhibited significantly increased ability to withstand and detoxify TNT, displaying higher shoot and root biomasses than untransformed plants when grown in the presence of TNT. In addition, the overexpressing lines were able to remove more TNT from soil, with a corresponding reduction in glutathione levels, when compared to wild type plants.

In conclusion, GSTs contribute to the TNT detoxification pathway in Arabidopsis, while over-expression of plant GSTs confers enhanced resistance to TNT, along with an increased ability to remove and detoxify this environmental pollutant. Furthermore, the 2-glutathionyl-4,6-dinitrotoluene conjugate produced presents the opportunity for subsequent degradation and mineralization of TNT, rather than the indefinite storage of TNT-transformation products in the environment. Ultimately the GST-mediated detoxification pathway demonstrated here could be exploited in more robust plant species for the phytoremediation of TNT.

**Acknowledgements:** This work was funded by the Strategic Environmental Research and Development Program of the US Department of Defence. HS and EJ acknowledge funding from the Biotechnology and Biological Sciences Research Council (BBSRC) and KT acknowledges funding from a Burgess studentship.



## CONTRIBUTION OF ARBUSCULAR MYCORRHIZAL INOCULATION IN THE PHYTOREMEDIATION OF PAH POLLUTED SOILS: MOLECULAR CHARACTERIZATION OF THE PROTECTIVE EFFECT

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### ABSTRACT

Soil contamination by polycyclic aromatic hydrocarbons (PAH) originating from intensive anthropic activities has now become a priority concern in industrialized countries. Indeed, some of these compounds exhibit mutagenicity and/or carcinogenicity leading to human toxicity and disrupting ecosystem functioning. Among remediation methods of polluted soils, phytoremediation has the advantage of being both ecological and economic. This phytotechnology uses the natural ability of plants to eliminate persistent organic pollutants directly and/or indirectly by stimulation of the rhizospheric degrading microflora activities *via* root exudation.

Nowadays, it is known that the arbuscular mycorrhization improves PAH dissipation [1,2,3] and allows plant protection against PAH toxicity [4]. Recent results have shown that PAH induced an increase of malondialdehyde (a biomarker of lipid alteration) but that mycorrhizal reduce this PAH-oxidative stress through inducing antioxidant enzyme system such as superoxide dismutase [4,5]. Moreover, the protective effect of mycorrhization against PAH is linked with some lipid modifications [6,7,8]. However, the molecular mechanisms involving in the tolerance against PAH toxicity in both symbiotic partners are still unknown. Thus, our work goes into this context and aims to study, both in the plant host and the mycorrhizal fungus, the expression of some genes involving in the resistance against reactive oxygen species (ROS) such as superoxide dismutase, peroxidase, glutaredoxin and pyridoxine; two DNA repair genes: transcription elongation factor II-S and Tyrosyl-DNA phosphodiesterase; one gene of metabolization of pollutants: glutathione S-transferase and two genes of lipid metabolism: sterol methyl oxidase and acetyl CoA but also verify the presence of oxidative stress by the dosage of H<sub>2</sub>O<sub>2</sub> (ROS) in presence of benzo[a]pyrene (a high molecular weight PAH).

Our experiments have been carried out using a monoxenic culture system containing *Medicago truncatula* cv. *Jemalong* (A17) roots transformed with *Agrobacterium rhizogenes* and mycorrhized or not by the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* in absence or presence of benzo[a]pyrene. Some plant antioxidant genes (superoxide dismutase and peroxidase) and the two DNA repair genes have been up-regulated in non mycorrhizal plants in presence of pollutant whereas no difference of these genes expressions were observed in mycorrhizal plants. Up-regulations of fungal genes involved in the protection against ROS (pyridoxine) and in the metabolization of pollutants (glutathione S-transferase) have also been observed. Taken together, these results suggested that arbuscular mycorrhization is able to give protection to *Medicago truncatula* against oxidative stress induced by benzo[a]pyrene.

**Keywords:** PAH, phytoremediation, arbuscular mycorrhizae, resistance gene expression, oxidative stress

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## PCB REMOVAL IN LAGOON SEDIMENTS USING POPLAR AND WILLOW PHYTO PROCESSES

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### ABSTRACT

This report details activities to reduce the mass of Polychlorinated Biphenyl (PCB) at the Altavista Virginia waste water Lagoon Site using phytoremediation (Phyto) techniques. PCB concentrations as high as 10,000 ppm have been measured in sediments deposited over 30 years ago. The Virginia Department of Environmental Quality has established a 50 ppm total PCB cleanup concentration goal.

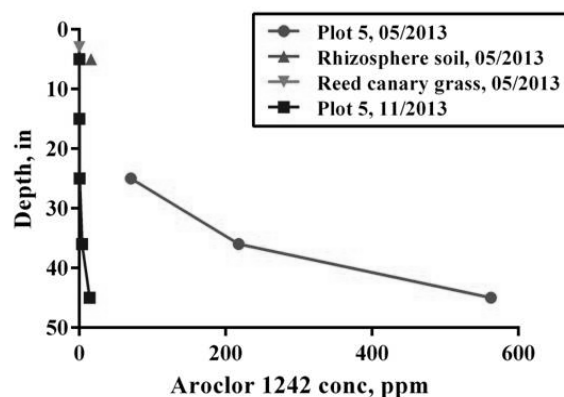
Research at The University of Iowa has proven that a grass rhizosphere accelerates the PCB mineralization rate in planted soils. The ECap<sup>®</sup> poplar root system performs the same processes though deeper at full-scale. This project demonstrates how poplar and willow can grow a healthy plant rhizosphere in three feet of sediment within an operating lagoon. Roots support a more diverse microbe population. During the growing season, flooding and drying within the lagoon sediment pulse the water content, oscillating the redox potential.

Six ECap<sup>®</sup> plots were installed in 2012 – 2013 by placing a layer of soil blended using local materials over sediment. Unrooted poplar whips were then pushed through the soil and sediment layers to the top of the clay liner.

The planted poplar and willow trees grow well in the cover soil to increase water transpiration from sediment. The ECap<sup>®</sup> tree cover will help contain PCBs in the subsurface by keeping the surface soil layer intact while sustaining microflora intimate with roots.

Sediment samples taken before and after installing ECap plot #5 show PCB removal patterns. Native microbes in the rhizosphere appear to be achieving PCB 'disappearance' in sediment. Research is being proposed by The University of Iowa to define the microbial PCB transformation mechanisms and transport processes in a healthy poplar rhizosphere.

*Keywords:* PCB, sediment, rhizosphere, phytoremediation, poplar



## PHYTOREMEDIATION OF CONTAMINATED SOIL WITH POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) BY *SALICORNIA PERSICA*

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### ABSTRACT

Oil and salinity pollutions have been a serious problem of the modern age, due to loss of plant cover and soil organism diversity. In order to investigate the possibility of enhancing biodegradation of halophytes, this study was conducted to evaluate the mechanisms of natural biodegradation of crude oil under salinity conditions. For that, *Salicornia Persica* were collected from Nayband of Forest Park, Bushehr, Iran, and cultured in pots with soil texture of Sandy loamy. Six levels of crude oil, including 0, 0.4, 0.8, 1.6, 2.4 and 5.3% (w/w) and four levels of Salinity including 0, 200, 400, 600 mM NaCl were considered as treatments with 3 replications. PAHs concentrations of shoot and root were determined by (GC-MS) after 21 days of start of oil treatments. Results showed that the concentrations of crude oil and NaCl were significant on PAHs concentrations in shoot and root. Furthermore, by increase of NaCl concentration, uptake and concentration of PAHs compounds decreased considerably. Benzo (b) fluoranthene and Benzo ((ghi) perylene in shoot with 1874.461 and 1860.27, and Phenanthrene, Benzo((ghi)perylene in root with 2531.9988 and 2149.0945 µg/kg dw respectively were the most compounds. Absence of PAHs in control suggest that root was the main pathway for uptake of PAHs in this halophyte and it also seemed that phytoextraction and rhizodegradation have occurred as a major mechanism to remove contaminants. By this capacity of uptake PAHs from contaminated soil with crude oil, *Salicornia Persica* as potential candidate of native plant can be used as Bioaccumulator of marine pollution.

**Keywords:** contaminated soil, petroleum pollution, halophytic plant, accumulation, biodegradation, phytoextraction



## DO CROSSTALKS WITH PRIMARY AND SECONDARY METABOLISM LIMIT OR STIMULATE THE DETOXIFICATION OF XENOBIOTICS?

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### ABSTRACT

Because plants are static and live in a competitive and sometimes hostile environment, they have evolved efficient mechanisms that protect them from abiotic and biotic stresses. These mechanisms include detoxification and sequestration of xenobiotic compounds and of toxic trace elements, exploited in any phytoremediation process. However there must be a limit on the amount of pollutants that can be accumulated and detoxified without disrupting the normal plant biochemistry and physiology. This limit seems to depend not only on plant species, but also on the ecotype or cultivar. The presentation aims to highlight some biochemical mechanisms, suggested or supposed to of importance for the successful phytoremediation of organic contaminants.

Enzymes involved in xenobiotics detoxification are often linked to the redox chemistry of the cell. The activities of cytochrome P450 monooxygenase, peroxidase and glutathione transferase have implications on the regulation of cellular redox status, closely related to mitochondrial respiratory chains, also involved in maintaining the cellular and plant energy balance and carbon flow. For example, overloading a plant with high concentrations of xenobiotics requiring oxidation by P450 may compete with the normal functions of these enzymes. An increase in their activity may also impose a major demand on both intracellular O<sub>2</sub> and NAD(P)H pools, disturbing plant redox or energy status, and thus affecting both primary and energy metabolism.

Plant mitochondrial respiratory chains differ from the mammalian one. In addition to Complex I, they contain at least four other dehydrogenases that enable the controlled oxidation of matrix and cytoplasmic NAD(P)H. Furthermore, plant mitochondria are characterized by the presence of an alternative respiratory pathway, through which reducing equivalents can be transferred to oxygen. This pathway branches at the level of the ubiquinone pool and comprises a single enzyme, the alternative oxidase, not coupled to the synthesis of ATP and insensitive to cyanide. Increase in the expression and/or activity of the alternative oxidase has been observed during temporal events such as seed conditioning, leaf development, elevation of salicylic acid levels, thermogenesis, fruit ripening, oxidative stress, physical wounding and plant pathogenic attack. The alternative pathway is inhibited by benzhydroxamate compounds, antioxidants such as propylgallate and copper chelators such a disulfiram. The alternative oxidase is able to oxidise several added polyphenolic substrates and could also oxidise intracellular polyphenolic compounds. It is not yet known if it could directly participate in the metabolism of some xenobiotics.

Depending on the structure of the organic pollutant to be detoxified, the secondary metabolic processes in the plant could also be affected. Many plant specific metabolites, often involved in plant interactions with its environment, have a structure similar to xenobiotics, and detoxification of the latter does probably use, at least partially, the metabolic pathways of the former. For example, most of the natural anthraquinones are glycosylated, whereas glycosyl-transferases are known to be involved in the conjugation of many xenobiotics, probably including sulphonated anthraquinones. It is not yet known if the detoxification mechanisms of sulphonated aromatic compounds are unique and specific to anthraquinone producing plants.

Molecules involved in the conjugation of xenobiotics, like glutathione, also play a major role in normal plant metabolism. The presence of xenobiotic compounds can induce the biosynthesis of glutathione transferases and thus an increased use of glutathione. Plant glutathione level and redox status are thus affected under such conditions. On the other hand, phytochelatins are derived from glutathione and involved in the detoxification of trace elements with their –SH groups. In either case, implications on sulphur supply and metabolism are expected.

Experimental conditions do also affect plant physiology and biochemistry, and it is not obvious if results obtained with young and small plantlets under well-defined laboratory conditions can be extended to mature plants in the field. However, the large-scale implementation of phytoremediation will be successful only if the “right plant is used at the right place”. Basic physiological and biochemical knowledge is thus required to select the most appropriate plant species, ecotype or cultivar, tolerant to the contaminants to be treated and able to accumulate and detoxify them without impacting its growth and survival.





## EXOGENEOUS OXYSTEROLS INCREASE THE NET PHOTOSYNTHETIC RATE, O<sub>2</sub> PRODUCTION AND CO<sub>2</sub> BINDING

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### ABSTRACT

An examination of the involvement of exogenously applied oxysterols, 24-epibrassinolide (24E) and the 20-hydroxyecdysone (20E) in the regulation of primary photosynthetic processes was performed together with the analysis of changes of the content of the above sterols in plant tissue. Phytoecdysteroids are steroid compounds present in many plant species (sometimes in rather large amounts), but their biological role is still far from being clear. We have found that the exogenous application of 20-hydroxyecdysone (20E) to leaves of New Zealand spinach causes stimulation of its net photosynthetic rate (PN) but does not positively affect the photosynthetic electron transport or the content of photosynthetic pigments. The increase in PN was observed shortly after 20E treatment and was statistically significant during the 4th and 6th hours after treatment but not later, which could be perhaps caused by a strictly short-term window of opportunity for ecdysteroids to significantly affect photosynthetic processes. To our knowledge, these results are the first to suggest a new potential biological function of phytoecdysteroids — regulation of photosynthesis. The effect of 20-hydroxyecdysone (20E) applied in low (10 nM) concentration on various photosynthetic parameters was made in spinach and maize. It also positively influenced the content of photosynthetic pigments in maize (not in spinach). The efficiency of the photosynthetic whole electron-transport chain responded negatively to the 24E or 20E treatment in both species, but there were interspecific differences regarding Photosystem (PS) II response: a positive effect on its oxygen-evolving complex and a slightly better energetical connectivity between PSII units were observed in maize whereas the opposite was true for spinach. The size of the pool of the PSI end electron acceptors was usually diminished due to 24E or 20E treatment. Plants treated with combination of both steroids mostly did not significantly differ from the control plants. We have demonstrated for the first time that 20E applied in low concentration can affect various parts of photosynthetic processes similarly to 24E and that brassinosteroids regulate not only PSII but also other parts of the photosynthetic electron transport chain – but not necessarily in the same way. The results confirm our previous results obtained with *in vitro* models, aimed at plant ecdysteroid-binding proteins identified using affinity chromatography (*e.g.* ribulose 1,5-bisphosphate carboxylase/oxygenase - RuBisCO, oxygen evolving complex OEC and others). Ecdysteroids as insect hormones have been investigated thoroughly but their function and the mechanism of action in plants and other organisms is still unknown. Our results show that ecdysteroids are able to increase the yield of RuBisCO-mediated reaction in which CO<sub>2</sub> is fixed into organic matter, influence the water cleavage reaction evolving oxygen, and the chromatography analyses with MS detection show that application of exogenous 24-epibrassinolide can influence the ecdysteroid content in plant tissues.

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## BEFORE THE BLAST: THE INTERACTION BETWEEN PLANTS AND EXPLOSIVES

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### ABSTRACT

Landmines are a global threat; at least 110 million landmines remain in place from conflicts in over 60 nations. Today, certain landmine detection methods place individuals in harm's way. Some risk can be mitigated using currently available remote sensing techniques when vegetation is present. As a landmine degrades and releases explosive compounds into the surrounding soil, nearby vegetation may absorb those compounds via bulk water movement into roots. Contaminant uptake causes plant stress which is detectable remotely using hyperspectral reflectance sensors. These sensors measure reflected light over a range of wavelengths which reveals information about the health status of a plant. Our study focused on using plants as bio-indicators to detect buried explosives. We exposed three species representing different functional types (*Ulmus alata* (tree), *Vitis rotundifolia* (vine), *Cyperus esculentus* (sedge)) to 500 mg kg<sup>-1</sup> of Composition B (Comp B; 60/40 mixture of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4,6-trinitrotoluene (TNT)), a commonly used explosive, and took leaf fluorescence and reflectance measurements over a nine week period. These measurements were analyzed within and across species using standard reflectance indices to discern stress caused by exposure to Comp B in soil. Reflectance indices use specific wavelengths to reveal information about plant functional traits (i.e., pigment content, specific leaf area (SLA), and biomass). Our objective was to compare standard reflectance indices to specific wavelengths that are altered when vegetation responds to the presence of explosives in soil.

Plants in soil contaminated with Comp B exhibited changes in pigment content, SLA, and biomass as well as plant reflectance for *U. alata* and *V. rotundifolia*. *Cyperus esculentus* was not a good indicator for the presence of explosive compounds, only showing significance in one index at week five and another at week nine. Woody species *U. alata* and *V. rotundifolia* both showed significance in more indices representing changes in chlorophyll content, fluorescence, and SLA including the Photochemical Reflectance Index (PRI),  $R_{750} - R_{710}$ ,  $R_{740} - R_{850}$ , and the Normalized Difference Vegetation Index (NDVI). By week five, leaves of the woody species exposed to Comp B were necrotic and senesced, which were then replaced by new growth. By week nine only two indices indicative of chlorophyll fluorescence revealed signs of stress, likely due to leaf drop and subsequent regrowth. Our next step is to use wavelengths that show differences due to the presence of Comp B to develop an explosives-specific index (ESI). Using plants from different parts of the globe and of various functional types will aid in the creation of an ESI for real-life application. Scaling up landmine detection efforts to the landscape level will decrease the inherent risk of current detection methods.



## **IN-SILICO STUDIES OF Cu-Zn SUPEROXIDE DISMUTASE ENZYME FROM *HELIANTHUS ANNUUS* WITH POLYCYCLIC AROMATIC HYDROCARBONS TO FIND OUT INSIGHT INTO ITS PHYTOREMEDIATION CAPABILITIES**

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### **ABSTRACT**

Cu-Zn superoxide dismutase is an enzyme that is reported to detoxify heavy metals and thus aid in phytoremediation. In this study an effort has been made to explore the wide potential of Cu-Zn superoxide dismutase enzyme found in *Helianthus annuus* for its capability to degrade pollutants of several industries which are mainly polycyclic aromatic hydrocarbons. It includes pollutants from iron, steel, rubber, stone and glass industries. Comparative genomics approach was employed in order to deduce the similarity between Aldehyde Keto Reductase [AKRs] related ESTs and gene corresponding to Cu-Zn superoxide dismutase [SOD1] in *Helianthus annuus*. This was followed by modeling of Cu-Zn superoxide dismutase protein. Motif and profile studies correlated the findings. Physico-chemical and functional characterization of the modeled protein was performed. The modeled protein is thermostable over wide range of temperatures and also has high aliphatic index. For molecular docking, a set of pollutants were selected from five different industries. The data were obtained from Environment Protection Agency [EPA] for iron, steel, rubber, stone and glass industries. Protein-ligand docking was carried out using Discovery Studio. Dibenzofuran, anthracene, Butyl benzyl phthalate, Tetrabromobisphenol, Mercaptobenzothiazole, Flumeturon and phenyl isocyanate dimer from different industries showed better binding capability with the Cu-Zn Superoxide Dismutase protein. These findings need to be further tested in the field condition for its validation. These studies may open new avenues for utilizing the medicinal plants and their detoxifying properties for remediation of hazardous pollutants.



## ASSESSING THE USE OF *SALIX ALAXENSIS* FOR THE RHIZOREMEDIATION OF DIESEL-CONTAMINATED SOIL

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### ABSTRACT

Throughout Alaska there are many contaminated sites that cannot be reached by roads that require remediation. In the case of the remote community of Kaltag, Alaska tank farms were established in the early 1960's and since that time there have been multiple diesel spills. Rhizoremediation using native plants is a potentially cost-effective method for remediating soils contaminated with organic contaminants, but has not yet been extensively tested in Alaska. Native plants have the added benefit of being adapted to local conditions. The willow *Salix alaxensis* is native to Alaska and in previous studies has been shown to promote diesel biodegradation in soil and alter the diversity of active naphthalene degraders. These effects could potentially be related to the release of salicylic acid as a secondary root compound. Due to the presence of weathered diesel, the remote location and the costs of ex-situ remediation, the use of *Salix alaxensis* for rhizoremediation of the site was studied. Microcosm studies were performed with crushed *Salix alaxensis* root, mimicking root turnover, as well as salicylic acid and fertilizer on the biodegradation of fresh and weathered diesel and 1-chlorooctadecane in Kaltag soils. With cold weather dominating much of the year, half of the microcosms were incubated at 4°C as well as 20°C to simulate summer conditions. Diesel and 1-COD losses were quantified using GC-MS, respiration was monitored using respirometry, and populations of diesel-degraders were quantified using most probable number (MPN) methods. Community analyses using 16S rRNA sequencing are also planned. Preliminary results indicate that the soils possess substantial diesel-biodegradation abilities, and analyses of the effects of willows and other treatments are in progress. If found to be effective for biostimulation, the use of *Salix alaxensis* would be advantageous as cuttings could be taken directly from the local region. Future field-scale tests are planned in Kaltag to determine the effectiveness and costs of phytoremediation using native willows and other plants in relation to land farming methods.

*Acknowledgements:* The financial support of Alaska Department of Environmental Conservation and National Institutes for Water Resources.



## A CONSISTENTLY ENHANCED PHOTOSYNTHETIC CAPACITY IMPROVES BRASSINOLIDE-MEDIATED PHENANTHRENE STRESS TOLERANCE

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### ABSTRACT

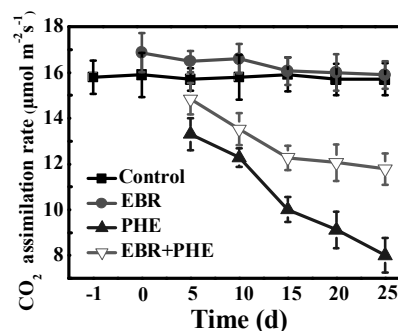
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants well-known for their mutagenic, carcinogenic and teratogenic activities. PAHs exist in the atmosphere in the form of solid and liquid aerosols. Because of lipophilic nature, PAHs are easily accumulated in the cuticle of leaf and thereby causing phytotoxicity. Plant growth regulators are used to improve plant tolerance in order to strengthen phytoremediation program as well. Brassinolide (a plant steroidal hormone) has been extensively documented for its role in abiotic stress tolerance. Previously, it has been reported that 24-epibrassinolide (EBR) alleviates root imposed phenanthrene (PHE) stress. However, role of EBR against PHE foliar spray-induced phytotoxicity is unknown. Here, we investigated the effects of EBR on photosynthetic apparatus at different time course challenged with PHE foliar spray in tomato.

Foliar parts of tomato plants were pretreated with 0.1  $\mu\text{M}$  EBR and at the next day treated with 100  $\mu\text{M}$  PHE inside specially constructed open-top chamber in green house. The foliar spray was repeated every 5 days for EBR and every 3 days for PHE and the experiment was terminated 25 days after first PHE application. At the end of treatment period, it was observed that PHE foliar spray significantly inhibited plant growth and biomass as indicated by reduced plant height, stem diameter, dry and fresh weight of shoot and root. Transmission electron microscopic observation showed obvious ultrastructural alterations especially in the chloroplast of leaf cell in PHE-treated plants. PHE-treated plants showed distorted or disorganized chloroplasts particularly in grana stacking and development of plastoglobuli. Elevated levels of lipid peroxidation and accumulation of reactive oxygen species confirmed that PHE foliar spray caused an oxidative stress in the leaf. However, application of EBR remarkably improved plant growth and biomass, and lowered the oxidative damage to cell membrane and photosynthetic apparatus.

PHE foliar spray significantly reduced  $\text{CO}_2$  assimilation rate ( $P_n$ ), stomatal conductance and also transpiration rate ( $T_r$ ) constantly throughout the experimental period. Supplementation of EBR not only improved  $G_s$ , but also  $T_r$  and consistently kept higher  $P_n$  in EBR+PHE-treated plant compared to PHE alone. Maximum photochemical efficiency and related chlorophyll fluorescence parameters studied at different time points during PHE treatment were all higher in EBR treated PHE-stressed plants. Activity of antioxidant and detoxification related enzymes such as superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase, glutathione S-transferases, phenylalanine ammonia-lyase and polyphenol oxidase etc. are all remarkably induced by EBR in PHE-stressed plants throughout the study period. Transcripts for different genes related to antioxidant enzymes are also in agreement with those observations.

It could be concluded that EBR-mediated PHE foliar spray-induced phytotoxicity alleviation was associated with a consistent enhancement of photosynthetic capacity, antioxidant enzymes activity and redox homeostasis for a prolonged period of time in tomato. In addition, results of current study suggest that EBR may have a protective role against atmospheric PHE-induced phytotoxicity and EBR could be considered as a potential phytohormone for assisted phytoremediation to design a feasible and efficient clean up technology.

**Acknowledgements:** The financial support by National Basic Research Program of China (2009CB119000) and National Natural Science Foundation of China (31071790) is greatly appreciated.



## EXPLOSIVES CONTAMINATED SOIL AS A PHYSIOLOGICAL FILTER AGAINST PLANT SUCCESSION AND ESTABLISHMENT

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### ABSTRACT

Soils contaminated with explosive compounds pose a threat to individual organisms and whole ecosystems. While many studies have focused on individual species responses to explosive compounds there are relatively few community or ecosystem scale explosive contaminant studies. In an attempt to bridge this gap our goal was to investigate impacts on community structure and diversity in an area contaminated with three explosive compounds/mixtures (RDX, TNT, Composition B). Community data were collected from an experimental minefield which was cleared 17 years ago, contaminated with explosives, and allowed to revegetate naturally. Plots within contaminated and reference sites were established and presence/absence for both woody and herbaceous species was recorded. Indicator species analyses were used to identify species related to environmental variations. Ordination and cluster analyses were used to determine if contaminant presence caused shifts in community composition.

Diversity metrics (i.e. species richness, diversity, Shannon-Weiner indices) identified trends present in the communities which differentiated reference and contaminant plots. Species area curves showed that there were large separations between the plot richness with explosives compound contamination and reference plots in both the woody and herbaceous assemblages. Each contaminant type had a different set of woody and herbaceous indicator species. Ordination and cluster analyses both showed separation of reference and treatment areas based on community composition. Differences between reference and contaminant sites in species composition, diversity, and richness suggests that presence of explosive compounds in the soil is acting as a physiological filter which influences species establishment and success in the contaminated areas. In addition the various contaminants may act in different ways resulting in slightly modified communities. Explosive contaminated soils can have noticeable large scale impacts long after an initial contamination event.



## APPLICATION OF PLANTS FOR BENZENE REMOVAL

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### ABSTRACT

An indoor gaseous benzene removal technology that has been widely researched is phytoremediation because plant can completely degrade benzene for use as a carbon source by P450 (mono-) or (di-) oxygenase. 8 ornamental plants that were screened for benzene removal showed that *D.sanderiana* can completely removal 20 ppm of benzene within 7 days, and the comparison between benzene removal by *D.sanderiana* under dark and light conditions found that benzene uptake under light conditions has higher benzene removal efficiency than under dark conditions. In addition, closing of stomata was found when *D.sanderiana* was grown under dark conditions. However, benzene removal by *D.sanderiana* still occurred under dark conditions. This result suggested that cuticle of plant could affect benzene uptake efficiency. To confirm the relation between benzene uptake and quantity of plant cuticle, 22 plant leaf grinding materials were screened for benzene adsorption and the quantity of crude wax in each plant species was studied. The results showed that *D. picta*, *A. aureum*, *F.religiosa*, *L. macrocarpa*, *A. scholaris* and *D.sanderiana* grinding leaf materials have high benzene removal efficiency, and the increase of crude wax quantity enhances benzene adsorption ( $r^2=0.6512$ ). Not only crude wax quantity but also wax composition was analyzed, and it was found that high quantity of  $\alpha$ -linoleic acid might enhance high benzene adsorption efficiency ( $r^2=0.4410$ ). For industrial application, continuous benzene adsorption system and plant leaf material immobilized on glass beads were also designed. The result showed that removing 55 ppm of benzene continuously at retention times of 3-5 mins, *D. picta*, *A. aureum*, *A. scholaris* grinding leaf materials could remove around 70-90% of benzene, and the saturation of benzene on *A. aureum* and *A. scholaris* grinding leaf materials, which had the highest benzene adsorption capacity, was about 132 hours. The benzene adsorption mechanism was also studied by benzene desorption using solvent and it was found that more than 99% of benzene could be desorbed easily by hexane. This result confirmed that physical sorption is involved in benzene adsorption mechanism of plant leaf materials. In addition, functional groups on surface materials before and after benzene adsorption were analyzed by FTIR to confirm the physical sorption appearance. Although there are several technologies that have been developed to treat benzene in industries, these technologies usually generate secondary waste and require high cost for maintenance and waste disposal. The application of plant and plant materials is an attractive method that should be studied and developed.

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## COMPARISON OF STIMULATED PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS IN AN AGED AND A FRESHLY SPIKED SOIL

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### ABSTRACT

Remediation of organic contaminants in soils is a challenging problem in environmental science and engineering. Phytoremediation is a promising technology that uses plants and associated microorganisms for the remediation of petroleum hydrocarbons (PHC). *Lolium multiflorum* and *Lotus corniculatus* were used in a pot experiment in the greenhouse to compare phytoremediation in an aged contaminated soil (collected from a historically contaminated site, Austria) and a freshly spiked soil. The effect of simple plant growth in comparison to additional treatments with nutrients and biochar as well as inoculation of plants with PHC-degrading microorganisms was investigated. Plant performance was compared between the two contaminated and a non-contaminated soil.

Spiked and non-contaminated soils were made by mixing sand and loess in a proportion of 9:1 to obtain the same grain size distribution as the aged contaminated soil. 50 g of crushed coal was added per kg soil to simulate the soil from the contaminated site. The spiking concentration for diesel was 22.65 ml/kg soil to obtain the same petroleum hydrocarbon concentration that was measured in the aged contaminated soil (16292mg/kg soil). To stimulate phytoremediation nutrients, biochar and PHC-degrading microbial strains were added. Nutrients NH<sub>4</sub>Cl (150 mg/100 g soil) and KH<sub>2</sub>PO<sub>4</sub> (20 µg /100 g soil) were added, biochar was mixed with soil (5% w/w) and the microbial consortium (mixture of strains: *Pantoea* sp. strains, ITS110 and *Pseudomonas* sp. strain, MixRI75) was added by seed inoculation before sowing.

Germination percentage (GP) was monitored weekly until three weeks after seed sowing. Biometric parameters (plant height, fresh and dry weight of shoots) and leaf chlorophyll content were recorded in periodic intervals. Soil samples were taken in regular intervals and PHC content was measured by GC-FID. The pot experiment started in spring 2014 and is planned until autumn 2015. The current results show that the germination rate of both plant species was reduced on aged contaminated soil, but not on spiked soil compared to the non-contaminated control. However 49 days after sowing biomass was significantly lower on spiked soil and on aged contaminated soil compared to the control soil. Amendment of nutrients and nutrients + microbial inoculation increased the biomass of *Lolium multiflorum*, but not in *Lotus corniculatus* in spiked soil. These amendments had no effect in aged contaminated soil. During the application of this abstract only one plant harvest was performed. In the presentation we will report about the development of plants and the degradation of PHCs in the first 6 months of the experiment.





## RECLAMATION OF OIL SAND MINING SITES IN CANADA: OPPORTUNITIES AND CHALLENGES FOR PHYTOTECNOLOGIES

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### ABSTRACT

A vast majority of Canada's oil reserves are found in the Athabasca oil sands of Alberta as bitumen. Large-scale mining operations used to extract bitumen create disturbance footprints that require innovative reclamation strategies. Developing new phytotechnologies for reclamation of these mining sites might assist industry to meet regulatory and societal expectations. Nevertheless, despite opportunities for phytotechnologies, there are significant challenges in moving these technologies from theory to practice. Our research is focused on developing plant-microbe associations, especially those involving bacterial and fungal endophytes, as potential reclamation tools.

Our research sites were established in 2013-2014 at three field locations in Alberta. One is the Bitumont provincial historical site, which is considered the location of two of the world's first oil sand extraction plants. Despite no reclamation efforts by man, vegetation has colonized areas within this site, some of which contain hardened bitumen. The second is Wapisiw Lookout oil sands reclamation area at Suncor Energy Inc., which is planted to native grasses, shrubs. The third is the CNRL RA1 site where peat or LFH cover material is planted with various vegetation and fertilizer treatments. Plant and soil samples were collected in summer and fall 2013 and summer 2014 at all field sites. Soil samples were analysed for nutrients and residual hydrocarbons. Culture dependent and independent techniques were used to characterize microbial communities in soil and to assess the diversity of microbial endophytes in selected plant roots. An objective was to elucidate beneficial endophytes that might be used to assist plant growth at these sites.

As expected, unique and diverse microbial communities were detected in soils at all field sites and linked to variations in plant communities. Similarly, different plants harboured unique bacterial root endophytes. Many of these endophytes were only detected by culture independent techniques, and some isolated endophytes had hydrocarbon degrading genes. The relationship or benefit of specific bacterial endophytes to the growth of plants on these reclamation sites is unknown. This is being assessed in on-going studies to determine how these endophytes might contribute to remediation of hydrocarbons.

Recent and current studies on reclamation of oil sands mining sites in Canada demonstrate that phytotechnologies can be effective tools to establish plant cover. Nevertheless, reclamation of large areas of land can pose serious logistical challenges when using plants that benefit from specific microbial associations. Discovery of new, unique endophytes might lead to development of specific biological inoculants to assist plant establishment and growth at these sites. Development of inoculation strategies or delivery systems is required, as well as an understanding of the ecology of such associations.

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## SUCCESSIONAL TRAJECTORIES OF STRUCTURAL AND CATABOLIC BACTERIAL COMMUNITIES IN OIL POLLUTED HYBRID *POPULUS* RHIZOSPHERES: A 2-YEAR FIELD STUDY

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### ABSTRACT

Poplars have widely been used for rhizoremediation of a broad range of organic contaminants for the past two decades. Still, there is a knowledge gap regarding the *Populus* rhizosphere associated bacterial communities and their dynamics during the remediation process. It is envisaged that a detailed understanding of rhizospheric microbial population will greatly contribute to a better design and implementation of rhizoremediation. In a recent study, we demonstrated the successional patterns of bacterial communities in oil polluted aspen rhizosphere in a 10-week greenhouse experiment [1].

In order to further investigate the long-term succession of structural and catabolic bacterial communities associated with aspen rhizospheres in oil contamination, we carried out a field study. Hybrid aspen (*Populus tremula x tremuloides*) seedlings were planted in polluted soil excavated from an accidental oil-spill site. Rhizosphere and bulk/un-vegetated soil samples were collected for microbial community analyses at 7 different time-points during the course of 2 years and sampling time-points were chosen to cover the seasonal variation in the boreal climate zone. Structural bacterial communities were accessed by means of 16S rRNA amplicon pyrosequencing whereas catabolic communities were monitored by pyrosequencing of alkane hydroxylase genes and extradiol dioxygenase genes. The functional/catabolic genes in this study were aptly chosen to target the population of aromatic and aliphatic degraders as PAHs and alkanes are the most important constituents of crude oil.

We observed a clear succession of bacterial communities on both structural and functional levels during the study period. Two distinct phases- early and late, were observed in terms of the differences in the bacterial community structure during the successional process. Analysing the co-occurrence patterns 3 different marker genes during the course of succession provided important clues about the early and late phase community members along with the rhizosphere prevalent groups. Sphingomonas type extradiol dioxygenases and alkane hydroxylase homologs of *Rhodococcus* clearly dominated the early phase communities. These high dominance-low diversity functional gene communities underwent a transition to low-dominance-high diversity communities in the late phase. These results pointed towards the increased catabolic capacities and a change from specialists to generalist strategy of bacterial communities during the course of secondary succession. Additionally, a significant seasonal effect was visible on the bacterial community structure during the winter months.

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## CLEANSED PROJECT: SUSTAINABLE MANAGEMENT OF POLLUTED DREDGED RIVER SEDIMENTS

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### ABSTRACT

The continuous stream of sediments, dredged from water bodies in order to maintain the navigation, is a common practice, but the fate of these sediments is an issue recognized worldwide.

The CLEANSED project (LIFE12 ENV/IT/000652) will demonstrate, evaluate and disseminate an innovative, integrated, multisector approach for the smart and sustainable management of polluted dredged river sediments.

The implementation of the project involved the dredging of sediments from Navicelli Canal in Pisa (Italy) and their characterization. These contaminated and nutrient poor sediments, after a phytoremediation process, developed in the framework of a previous EU project AGRIPORT (ECO/08/239065/S12.532262), were transformed into a valuable material with characteristics approaching those of an uncontaminated natural soil (techno-soil).

In the CLEANSED project, the AGRIPORT treated sediments, will be tested as growing substrate for the cultivation of ornamental plants with different saline stress tolerance, thus allowing to prevent the loss of soil due to plant nursing activities. On the other hand, fresh sediments will be tested, after a landfarming pre-treatment, as a filling material for the construction of a road under real working conditions. The whole cycle will take place within a real natural and productive district, i.e. the basin and alluvial plains of the Arno river, Italy.

*Acknowledgements:* Financial support by LIFE+ project CLEANSED ENV/IT/000652.



## PHYTO-DEHYDRATION OF POLLUTED SEDIMENTS IN AN ARTIFICIAL POND

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### ABSTRACT

In a former plant using for the production of biogas the manure collected in the rural district, an artificial pond of about 2.6 hectares and 4 meters of depth was filled by the fine solid fraction residual after the digestion process.

The sediment is characterised by a water fraction of about 80% and the remaining solid fraction is made by sand (15%), lime (60%) and clay (25%), without any significant vertical stratification. The pH is around 8 and the electrical conductivity around 2.5 ms cm<sup>-1</sup>. The concentrations, on a dry weight basis, of organic carbon and nitrogen are around 25% and 5%, respectively. The only heavy metals present in high concentrations are the copper (around 1200 mg kg<sup>-1</sup> d.w.) and the zinc (around 4000 mg kg<sup>-1</sup> d.w.).

The first target of the remediation is the reduction of the water fraction (dehydration), to avoid that during heavy rains the spillage from the lagoon reaches the adjacent river. Secondary targets are related to the transformation of the quality of the sediments, increasing progressively the aerobic conditions of the whole mass and decreasing the methane emissions. As final target a naturalization of the area is under evaluation, with the creation of a new habitat of particular interest for the protection of the birds presents in the region.

The phytodehydration will be obtained by means of different plant species adapted to the wetland environment (and so to the anaerobic conditions of the sediment), "planted" on the surface, by floating frames realised in organic material. The frames will be fixed to the lagoon borders to avoid the movement of the plants under the pressure of the wind. Six species (*Tamarix gallica*, *Tamarix Africana*, *Phragmites australis*, *Juncus effusus*, *Iris pseudacorus* and *Carex gracilis*) and three planting technique (cutting and rooted cutting for *Tamarix* species, pre-sowed coconut material for the other species) will be tested. Each *Tamarix* species will be represented by four different genotypes. The space arrangements of the different species will guarantee a high resilience to the system and a continuous evapotranspiration activity.

In this paper we present the results obtained in the pilot phase, conducted in mesocosms, where we tested and evaluated the dehydration capacity of different species and genotypes, as well as the plant effect on the CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emissions.

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## THE INFLUENCE OF ACTIVATED CARBON ON BIO/PHYTOREMEDIATION OF SOILS CONTAMINATED WITH CHLOROPHENOLS

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### ABSTRACT

Chlorinated phenols is one of the most important groups of priority pollutants due to their high toxicity, mutagenicity and carcinogenicity. They are widely used as wood preservatives and in other fields of national economy. Although bioremediation and especially phytoremediation are the most economically effective and environmental friendly technologies they cannot be applied for remediation of highly contaminated soils because of their high microbial and phytotoxicity. Previously we developed the approach of adsorptive bioremediation for soils highly contaminated with such compounds like chloroanilines, petroleum hydrocarbons and others. This method is based on the use of activated carbon (separately or in combination with microbial preparations) for reduction of soil toxicity and mobility of contaminants during a soil bioremediation. The main subject of this investigation was to study the influence of activated carbon on the bio/phytoremediation rate of soils highly contaminated with chlorophenols. Vegetation experiments were carried out in a 2.7 L pots with 1.5 kg of soil samples (dry weight). Three types of soils (alluvial-meadow, grey forest and chernozem) were spiked with 4-chlorophenole (4CP, 5700 mg kg<sup>-1</sup>) or mixture of 4-chloro phenol and 2,4,6-trichlorophenol (TCP) (totally 2400 mg kg<sup>-1</sup>). After 1 day, granular activated carbon (GAC, Electrostal, Russia) was mixed with the contaminated soils at doses 0, 1, 2 or 4% (w/w), separately or in combination with chlorophenol-degrading microorganisms. For that purpose, biomass of bacterial strain *Rhodococcus opacus* GM-14 was grown in a minimal liquid medium with 4-chlorophenol and 2,4-dichlorophenol as sole carbon and energy sources. The soil samples were incubated under outdoor conditions on a soil surface covered with agrotexile to protect from rains. All samples (except untreated controls) were mixed weakly (when unplanted) and watered regularly when needed. In the end of the 1st and 2nd seasons (after 3 and 15 months) the pots were seeded with white clover (*Trifolium repens* L.) and in 2 months the grown plants were taken out and weighed. In addition, pure controls with uncontaminated soils and similar doses of activated carbon were also created. The soil samples were taken every 2 or 4 weeks for chemical and biological analyses. The 4CP and TCP were extracted from the soil with dichloromethane and their concentrations were determined by GC-MS. Besides, phytotoxicity of those soil samples was determined regularly by express-method on white clover germination.

It has been demonstrated that the soil amendment with activated carbon substantially reduced soil toxicity for chlorophenol-degrading microorganisms and phytoremediators, that remarkably accelerated degradation of the chlorophenols in all the soils. Removal efficiency of chlorophenols in soils amended with GAC in combination with *Rh. opacus* GM-14 was above 87-97% compared to 5-10% in control samples without any adsorbent independent on microbial preparation. Thus it was concluded that adsorptive bioremediation and phytoremediation are perspective approach for cleaning up chlorophenol contaminated soils.



## BIODEGRADATION OF 2,4-DINITROTOLUENE BY SELECTED PLANT SPECIES

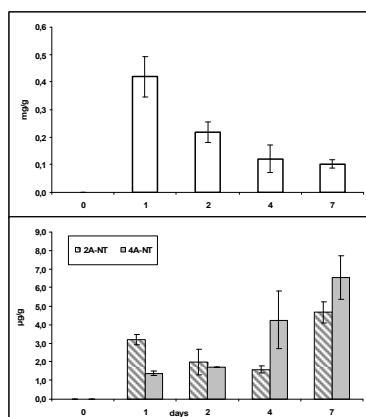
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### ABSTRACT

The contamination of the environment by nitrocompounds is a worldwide environmental problem. 2,4-DNT is a toxic and recalcitrant by-product of 2,4,6-trinitrotoluene manufacturing, and is also used in dye processes and in the manufacture of polyurethane foams. 2,4-DNT is considered to be carcinogen to the humans. The adverse impact of this explosive on living organisms, its environmental persistence and low susceptibility to degradation demonstrates that the remediation of contaminated soil is essential. In our study we focused on the possibilities to use phytoremediation as a progressive cleaning method of the waste water and soil in the areas of present or former ammunition plant. In this contribution, we study the potential of plants to remove 2,4-DNT from contaminated soils and waters *via* phytoremediation. In the first step, the toxicity tests were performed. The germination of range of plants (hemp, flax, sunflower, mustard and maize) were not effected by the 2,4-DNT up to concentration 200mg/l except the maize, which was sensitive to concentration 50mg/l. Next, 2,4-DNT degradation in plant cells was studied too. First degradation products 2-aminonitrotoluene and 4-aminonitrotoluene were found both in the medium and in the plant cells. The mustard ecotoxicity test showed that the toxicity of these metabolites were in low concentrations (< 50mg/l) higher than the toxicity of parent compound, but the 2,4-diaminotoluene, the product of next reduction step, was less toxic in all concentration (0-200mg/l).



Degradation of 2,4-DNT by plant cell culture of reed (*Phragmites australis*).

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## THE EFFECT OF STRESSFUL STIMULI (MECHANICAL AND CHEMICAL) ON THE EXPRESSION OF SECONDARY METABOLITES IN POPLAR BUDS

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### ABSTRACT

In response to biotic stress poplars synthesize a number of secondary metabolites. Some of them act as defense molecules against biotrophic and/or necrotrophic parasites. We tested the long-term effect of chemical and mechanical stresses on the expression of secondary metabolites in buds of two poplar clones from different poplar sections, *Populus nigra* clone Italica and *Populus trichocarpa* clone Fritzi Pauley. Both mechanical (perforation of leaves) and chemical (5mM salicylic acid) stimuli were applied two times in the end of the vegetation season. The buds were harvested after the trees had shed leaves.

A further aim of the study was to establish surface enhanced Raman spectroscopy (SERS) as a new technique for studying poplar secondary metabolites. This technique may complement the standard chromatographic techniques (HPLC, GC). For successful employment of SERS, the optimization of the extraction procedure with sequential application of carefully selected extractants and the proper choice of metal surface was crucial.

Preliminary SERS results confirm that the profile of secondary metabolites in the buds differed in the two examined clones. *P. trichocarpa* spectra were dominated by terpenoids while those of *P. nigra* were dominated by phenolic compounds. Further analyses should disclose whether stressful stimuli influenced the pattern of secondary metabolites in poplar buds.

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## CONTENT OF TRACE ELEMENTS IN MAIZE ON SOIL CONTAMINATED WITH HEATING OIL AFTER APPLICATION OF NEUTRALIZING SUBSTANCES

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### ABSTRACT

The purpose of this study has been to determine the effect of increasing rates of heating oil (0, 5, 10, 15 and 20 g kg<sup>-1</sup> of soil) and neutralizing substances (nitrogen, compost, bentonite, zeolite and calcium oxide) on the content of cadmium, lead, chromium and manganese in maize (*Zea mays* L.). The experiment with three replications was conducted in a plant house of the University of Warmia and Mazury in Olsztyn (north-eastern Poland). Soil in each pot was enriched with identical amounts of mineral fertilizers (100 mg N, 30 mg P, 100 mg K, 50 mg Mg, 0.33 B, 5 mg Mn and 5 mg Mo per kg of soil). In addition, each experimental treatment was enriched with mineral fertilizers in the following quantities: 100 mg N, 30 mg P, 100 mg K, 50 mg Mg, 0.33 B, 5 mg Mn and 5 mg Mo per kg of soil. Soil (9 kg) was first carefully mixed with heating oil and the aforementioned nutrients and substances and then placed in polyethylene pots. The detail specification of soil and substances used in experiment was given in our previous article [1]. A Polish cultivar of maize (*Zea mays* L.) called Reduta, grown at a density of 8 plants per pot, served as the experimental plant. Maize was harvested during the intensive stem elongation phase. At the same time, soil samples for analysis were collected. Having mineralized the plant material in 65% nitric acid, concentrations of the trace elements: cadmium, lead, chromium and manganese, were determined by the flame atomic absorption spectrophotometry (FAAS) on a SpectrAA240FS atomic absorption spectrophotometer. The results were processed statistically using two-factor analysis of variance ANOVA from a software package Statistica.

The increasing rates of heating oil and contamination alleviating substances produced different effects on the content of the analyzed elements in soil. In the non-amended series, the relationship between the increasing rates of heating oil and the cadmium content in plants followed a unidirectional trend, but the range of changes was relatively small. Under the higher rates of heating oil, the content of manganese increased by 64% up to the rate of 10 g kg<sup>-1</sup> of soil in maize compared to the control treatments (without heating oil). An increase in the concentration of lead in maize was observed under the influence of 15 g of oil per kg of soil. Higher rates of heating oil caused a decrease in the content of manganese and chromium in maize. In response to heating oil in soil, the content of chromium in maize rose by 89% (20 g oil kg<sup>-1</sup> of soil) relative to the treatment without this petroleum derivative.

Application of compost, bentonite, zeolite and CaO to soil contaminated with heating oil had a positive, limiting effect on the content of cadmium in maize. All the soil amending substances contributed to a considerable increase in the content of Pb<sup>+2</sup> in maize: compost by an average of 32%, nitrogen by 35%, bentonite by 63%, CaO by 105% and zeolite by 123% versus the series without any of these substances. As for chromium, all the substances except nitrogen slightly stimulated its higher concentrations in maize, and the highest quantities were determined in the series with zeolite (+18%) and CaO (+22%). The content of manganese in maize decreased significantly under the effect of neutralizing substances in comparison to the series without soil amending additives. The substance which most strongly inhibited the uptake of manganese by maize was bentonite, which reduced its content by 68%. Calcium oxide reduced the content of manganese in maize by 44%, compost by 22% and zeolite by 19%. Nitrogen was exceptional as it stimulated an increase in the content of this element in maize by an average 18% versus the series without neutralizing substances.

### References:

1. Wyszowski M., Sivitskaya V. (2013) Effect of heating oil and neutralizing substances on the content of some trace elements in soil. *Fresenius Environmental Bulletin*, 22(4), 973-978.

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## THE INDUCED PHYTOREMEDIATION OF ORGANOCHLORIDE PESTICIDES-POLLUTED SOILS USING DERIVATIVE AND COMPOSITION OF OKSAN

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### ABSTRACT

Phytoremediation is technology of restoration of polluted soil and water by using different plant species. Phytoremediation technology is applied directly in the area of contamination (*in situ*), helping to reduce costs and reduce exposure contaminated pollutant with the people and the environment. Most importantly, after phytoremediation soil does not lose its fertility. Consequently, this technology is ecologically safe and cost effective.

Exigency to develop phytoremediation technology of soils contaminated by pesticides arose from the fact that Kazakhstan as "environmental heritage" from the now defunct Soviet Union got many territories of the former storehouses of obsolete pesticides and surrounding land. In each region of the country, there are 5-20 the inactive former warehouses of pesticides. Soil around the warehouses has multicomponent pollution. Soil contains organochlorine pesticides and heavy metals.

Most important components of technology restoration of soil polluted with organochlorine pesticides with plants are phytoextraction and phytostabilization. Phytoextraction potential of the plant organism depends from the hydrophobicity of the pollutant. Degree of hydrophobicity (log Kow) largely determines the efficiency of absorption pollutants and their movements in the plants.

Low phytoextraction percentage promotes slow growth of plants and limited biomass production. The efficiency of phytoremediation of soil depends from the productivity of plants: t than larger biomass, consequently the higher accumulation of pesticides. In this regard, for increasing the phytomass of tolerable plants to pesticides and bioavailability of the pollutants from the contaminated soil to plant were used the new synthesized growth stimulator (derivatives of the oksan 2, 2-thiosemicarbazone dimethyltetragidropiran-4-one) and his compositions.

In the green and field was studied introducing derivatives of the oksane (SC in concentration 0,0001%) and their compositions (the wood charcoal 250 g + SC in concentration 0,0001%) in contaminated soil in period of germination on the physiological parameters of the plants (*Xanthium sturmarium*, *Amaranthu retroflexus* and *Cucurbita pepo* ).

In green was found that the introduction of SC and their compositions in artificially polluted by pesticide (4.4' DDE metabolites in concentration  $878 \pm 77 \mu\text{g kg}^{-1}$ ) soil increased biomass to 2-4 times, rise the content of chlorophylls and carotenoids in the leaves, changing the ratio of chlorophylls *a* and *b*, and the chlorophylls and carotenoids, the coefficient of biological absorption, translocation coefficient and promote reduction of pesticides in soil as compared with control by 18%.

In field the introduction of SC and their compositions in soil polluted with pesticides (4.4 'DDT and 4.4' DDE metabolites in concentration  $1426 \pm 241 \mu\text{g kg}^{-1}$ ) increased biomass plants and phytoextraction pesticides from soil to plant from 0.4% to 1.6% of pesticides from the square  $\text{m}^2$ .

This work can be required for the development of pesticide remediation technologies for organochloride pesticides-contaminated sites.



## IMPACT OF ORGANIC MATTER ADDITION TO SOIL ON WEATHERED p,p-DDE ACCUMULATION IN *CUCURBITA PEPO* CULTIVARS

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### ABSTRACT

*Cucurbita pepo* cultivars (cv:raven and zephyr) were grown in a 900 cm<sup>2</sup> mound at 2 m intervals containing soil with weathered DDE at 299-908 ng/g soil under field conditions. Soil removed from each mound (30 cm x 30cm x 25 cm) mixed with either 500 g or 1000 g organic matter as long as their unamended controls and each cultivar was planted into organic matter amended and unamended mounds. Cultivars were harvested at 42 days and DDE concentrations at plant compartments were analyzed by GC/ $\mu$ -ECD.

A method previously published was used for extraction of plant tissues such as roots, shoots, leaves, fruits. For xylem sap collection, soil was oversaturated with tap water. After 3h, stem of each cultivar was cleaned of the soil particles and severed 5 cm above the soil surface and the severed stem was placed into a 100 mL amber glass vial. The sap volume was measured at the end of 8 h collection period and xylem sap DDE content measured by SPME method coupled with GC/ $\mu$ -ECD.

The objective of this study was to assess the effect of organic matter amendments on the phytoextraction potential of *cucurbita pepo* cultivars under field conditions. Average shoot p,p'-DDE concentrations of raven grown in mounds with 500 g, 1000 g of organic matter amendments, and 0 g (unamended mound) were determined in as 2484 ng/g, 1621 ng/g, and 2068 ng/g (dry weight), respectively. For Zephyr, DDE contents for the same treatment types were 247 ng/g, 118 ng/g, 138 ng/g, respectively. Xylem sap p,p-DDE concentrations followed a similar pathway with shoot DDE concentrations of the cultivars grown in organic matter amended and unamended mounds under field condition.

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## PHYTOREMEDIATION OF ORGANIC WASTE LEACHATE USING IPOMOEA AQUATICA IN TROPICAL COUNTRY

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### **ABSTRACT**

This research was conducted to determine the performance of Ipomoea Aquatica as phytoremediator to treat leachate originated from vegetable waste from wet market in Malaysia which is considered as tropical country. Phytoremediation system was designed using Ipomoea Aquatica as phytoremediator which acted as a low cost and environmental friendly leachate treatment process. The performance of Ipomoea Aquatica to remove zinc, phosphorus and nitrate were tested and evaluated. It was also discovered that Ipomoea Aquatica able to reduce the concentration of Ammoniacal Nitrogen, Biochemical Oxygen Demand, Chemical Oxygen Demand and suspended solids by 1.88%, 1.71%, 2.49% and 4.44% respectively. The volume of vegetable leachate produced from a specified weight of solid vegetable waste was also determined. The optimum number of days for efficient removal performance capacity of Ipomoea Aquatica to treat leachate from vegetable waste was identified.

*Keywords:* phytoremediation, leachate treatment, vegetable waste, of Ipomoea Aquatica



## PRELIMINARY EVIDENCES FOR THE ACCUMULATION OF DIOXINS AND FURANS IN PLANT TISSUES ON A PHYTOREMEDIATION SITE CONTAMINATED WITH ACC AND PCP

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### ABSTRACT

Wood preservatives like ACC and PCP are widely used by utility companies worldwide. Unfortunately, the use of wood treatments such as ACC and PCP produces leachates that degrade to As, Chrome and Cupper, and dioxins and furans, respectively. These contaminants are highly toxic and new containment strategies are being explored. The objectives of this study was i) to determine which plant species can tolerate the mineralized, compacted and contaminated soil of a site used for storing wood-treated electric poles, and 2) evaluated the potential of selected plant species to extract or degrade contaminants originating from the leaching of the wood treatments.

A phytoremediation experiment was established in the summer of 2012 on the site of a facility used to handle electric poles treated with ACC and PCP. Four plant species (willow *Salix miyabeana*, tall fescue *Festuca arundinacea*, alfalfa *Medicago sativa*, indian mustard *Brassica juncea*) were established in a split-plot randomized block design, with fertilization as a treatment and repeated four times. After the second year of growth (i.e. 2013), the survival and the productivity of the plants were monitored and chlorophyll content evaluated. Leaf and root samples were also harvested and analysed for metal content, PCP, dioxins and furans.

The four species planted tolerated well the harsh conditions of the site and produced important quantities of biomass. Willows in particular had the highest biomass found. Fertilization had significant effects on the amount of above and belowground biomasses produced in all species. Similarly, stomatal conductance and chlorophyll content were significantly increased by fertilization. At the end of 2013, only willows were able to extract Cd, Cu and Zn from the soil. Surprisingly, preliminary analyses revealed that all the species planted accumulated dioxins and furans in their leaves and roots (willows > alfalfa > mustard > tall fescue).

The results obtained so far point toward the potential of willows to phytoextract specific metals. Moreover, the four plant species used in this study accumulated dioxins and furans in their roots and leaves. The next stages of the project is to evaluate to what extent the plant will accumulate large quantities of dioxins and furans and to investigate molecular processes potentially induced to degrade these compounds.

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## **PLANT-MICROBE INTERACTIONS**



## TRANSGENERATIONAL CHARACTERIZATION OF SEED ENDOPHYTIC BACTERIA OF A PLANT GROWING ON MINE RESIDUES

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### ABSTRACT

Plant endophytic bacteria can be defined as bacteria that live within plant tissues without causing substantive harm to the host plant. Endophytes have been isolated from different plant organs: roots, leaves, stems, flowers, fruits and seeds. Much of the research on endophytic bacteria focused on roots, stems or leaves. More and more reports mention isolation of endophytic bacteria from seeds. In seeds cultivable bacteria are not as abundant as in other plant organs but most likely only a limited fraction of the endophytes are cultivable. It is mentioned that some endophytes that might be important during germination, or even seedling development, are transferred to next generations through seeds. Endophytic bacteria promote plant growth by similar mechanisms like soil plant growth promoting bacteria, helping host plant to overcome environmental stress. Additionally, it has been demonstrated that endophytic bacteria increase metal tolerance and affect metal translocation to the aerial plant parts.

In the mining district of Zimapan, Hidalgo state, central part of Mexico, the annual plant species *Crotalaria pumila* was found growing spontaneously on multi-metal contaminated mine residues. The plant accumulates, without toxicity symptoms, Zn, Cd, Pb and Cu in shoots at concentrations higher than those phytotoxic for other species. *Crotalaria pumila* has been observed for three consecutive years completing its life cycle and producing viable seeds. The persistence of the mentioned species could be attributed to the presence of beneficial endophytic bacteria transferred from one generation to another through the seeds. Therefore, the aim of this work is to study the composition and functions of seed endophytic bacteria of three generations of *C. pumila* growing naturally on mine tailings in a Mexican semiarid region (Zimapan). Seeds from three different generations (2011, 2012 and 2013) were collected from *C. pumila* plants growing on metal mine residues, as well as seeds from a non-contaminated site. Twelve different surface sterilization protocols including variations on concentration and exposition time to NaClO and ethanol were tested. Then, the seeds were crushed and dilutions were plated on solid growth medium. Cultivable strains were characterized genotypically and phenotypically. Phenotypical characterization of cultivable bacteria included tolerance to metals (Zn, Cd, Pb and Cu), siderophore, acetoin and indol-3-acetic acid production, 1-aminocyclopropane-1 carboxylate deaminase activity, ability to fixate N<sub>2</sub> and capacity to solubilize phosphate. Non-contaminated site seeds contained more colony forming units (cfu) per gram seed than those from multi-metal contaminated mine residues. More cfu were observed in seeds collected in 2013; however, in the three different seed generations similar bacteria were observed. The surface sterilization protocol was a key factor to obtain as many cultivable seed bacterial endophytes as possible. Besides cultivable strains, also the non-cultivable population were characterized using culture independent techniques.



## AUTOCHTHONOUS BIOAUGMENTATION AS A STRATEGY TO ENHANCE Cu PHYTOREMEDIATION BY A SALT MARSH PLANT

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### ABSTRACT

This work aimed to evaluate the potential of the salt marsh plant *Phragmites australis* for phytoremediation of Cu contaminated sediments and to evaluate if this potential can be enhanced by bioaugmentation with autochthonous microorganisms resistant to Cu.

Plants were collected in an estuarine area, placed in vessels and kept in greenhouses under tidal simulation. Sediments were contaminated with Cu (ca. 270 µg g<sup>-1</sup>) and autochthonous microbial consortia resistant to Cu (prepared in the laboratory) were added to half of the vessels. After two months, metal concentrations in plant structures and sediments were determined, as well as metal speciation on sediments. Plants physiological parameters (chlorophylls, carotenoids and thiolic compounds) and plant biomass weight were also determined to assess possible stress signs.

Plant accumulated significant amounts of Cu in all tissues although in higher amounts in its belowground structures. Cu induced some stress signs in the plants belowground structures, with lower biomass weight being observed, although thiolic compounds levels have increased. Belowground structures biomass weight was even lower when the microbial consortium with Cu resistant microorganisms was added probably due to the increase of the Cu bioavailable fraction in sediment observed when bioaugmentation was applied. However, in this case Cu translocation increased with higher amounts of Cu being observed in the stems of the plants. This increase did not caused any visual toxicity signs, being accompanied, for instance, by an increase in the production of thiolic compounds in the plant leaves, indicating that the plant had mechanisms to respond to the uptake of the metal.

Therefore, this work demonstrated that *P. australis* is capable of phytoremediate Cu contaminated sediments and that autochthonous bioaugmentation can change metal bioavailability in the rhizosphere, increasing the phytoextraction potential of this plant. This technique will contribute for the removal of this contaminant from contaminated sediments reducing its toxicity risk and contributing for an efficient risk management strategy of estuarine and coastal areas.

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## METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES FROM A Hg-CONTAMINATED RHIZOSPHERIC SOIL: DIVERSITY, STRUCTURE AND CO-OCCURRENCE NETWORK

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### ABSTRACT

The goal of phyto (dendro) remediation is to reinstall a green cover on highly anthropogenic/derelict soils, and when possible to valorize the woody biomass produced. A detailed understanding of the effect plants may have on the activities of rhizosphere microorganisms could help enhancing the productivity. We hypothesized that the composition of and interactions between bacterial and fungal communities under trees that have naturally recolonized the anthropogenic area are profoundly altered as compared to those from the undisturbed area. To test this hypothesis, we used a high-throughput DNA sequencing technology that generates large environmental datasets. We further applied modelling of co-occurrence network to investigate potential interactions between microbial taxa and to decipher complex interactions between different components of the microbial communities. In this study, rhizospheric soils were collected from a strongly anthropogenic and a control areas. Bacterial and fungal 16S rRNA and ITS genes were amplified and sequenced using next-generation sequencing technology (Ion Torrent). Soils were also analyzed for chemical and physical characteristics. Sequence data were processed using Mothur. Metagenomic semi-quantitative data on microbial communities were used to model the microbial networks of ecological co-occurrence interaction in control and tailing pond areas.

Detailed analyses of the structure of microbial communities revealed that 26.4 and 8.6% of bacterial and fungal genus, respectively, were over-represented in the tailing pond soil, corresponding to 40.0 and 19.6%, of bacterial and fungal DNA sequences, respectively. Conversely, 9.2 and 12.6% of bacterial and fungal genus, respectively, representing 24.8 and 17.5% of bacterial and fungal DNA sequences, were over-represented in the undisturbed soil. Interestingly, the two soils shared 64.4% and 78.8% of bacterial and fungal genus, respectively. Within the emergent bacteria genus in the tailing pond soil, all are only minor genus, representing individually less than 2% of total sequences. A noticeable exception concerned *Pseudomonas* species, from which 17.0% of sequences isolated from tailing pond soil derived, whereas only 0.9% of the sequences were derived from *Pseudomonas* species in undisturbed soil. Conversely, the bacteria genus MC18 is a major component of the undisturbed soil (2.3% of DNA sequences), whereas it did not significantly emerge in the tailing pond soil (0.007% of total DNA sequences). The global co-occurrence networks including bacterial and fungi genus consisted in largely connected communities with some variations between the undisturbed and the tailing pond soils. These results are further discussed at the whole community level, and the interactions between the different microbial compartments undiscovered.

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## PROMISCUOUS DISPERSAL OF BIODEGRADATION GENES: A KEY TO IMPROVE ENDOPHYTE-ENHANCED PHYTOREMEDIATION?

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### ABSTRACT

Because many bacteria possess a natural capacity to cope with contaminants, they can be exploited to improve the efficiency of phytoremediation. In case of phytoremediation of organic contaminants, plants can benefit from endophytes possessing appropriate degradation pathways and metabolic capabilities, leading to more efficient contaminant degradation and reduction of both phytotoxicity and evapotranspiration of volatile contaminants. We were able to obtain a 90% reduction in TCE evapotranspiration after inoculation of a TCE degrading poplar root endophyte in field grown poplar trees. To move this concept towards wide-ranging, large-scale applications, it is important that the positive effect that could be achieved after inoculation is **stable at the long term** and that the concept of endophyte-enhanced phytoremediation can easily be extrapolated to a **broad variety of contaminants**.

In order to achieve a stable endophyte-enhanced phytoremediation, it is essential that the degradation genes from the inoculated endophyte are transferred to members of the indigenous population. When these degradation genes are located on a plasmid, they can be transferred to other bacteria by means of horizontal gene transfer. The host range of the plasmid strongly determines the success of such a horizontal gene transfer while the frequency of the transfer is regulated by its dispersal capacity. Therefore, we designed a **broad host-range plasmid** for cloning and **promiscuous dispersal of biodegradative genes**.

Two plasmids with a broad host-range origin of replication from the SEVA (Standard European Vector Architecture) plasmid collection [1] were selected as a backbone in which different units can be introduced: the SEVA 221 and 251, equipped with the origin of replication of the RK2 and the RSF1010 plasmid respectively. Next to the broad host range and promiscuous dispersal, the stability of the plasmids is of high importance, the more, because the final introduction of the degradation genes will result in rather large plasmids which involve high energy needs for their host cell. To ensure the stability of the plasmids, the HOK/SOK toxin-antitoxin (TA) system was introduced into the SEVA plasmids 221 and 251 resulting in pSEVA221 $\alpha$  and pSEVA251 $\alpha$ . Next, the obtained plasmids were introduced into *Pseudomonas putida* W619, a plant growth promoting poplar endophyte, and were compared in a stability experiment. For pSEVA251 $\alpha$ , 100% stability could be achieved, even after 100 generations.

In order to equip the SEVA251 $\alpha$  plasmid with TCE degradation capacity, TCE degradation genes were isolated from the pTOM plasmid originating from *Pseudomonas putida* G4. These genes, together with a constitutive promoter, are cloned into pSEVA251 $\alpha$ . In the final step, the complete plasmids (including the degradation genes) will be transferred to *Pseudomonas putida* W619, which can be used for inoculation experiments.

In future work, the pSEVA251 $\alpha$  can be equipped with other desired degradation genes after which the entire plasmid can be transferred to the endophyte that will be used for inoculation. The broad host range and promiscuous dispersal of pSEVA251 together with the HOK/SOK TA system that optimizes the stability will ensure a successful transfer of the degradation genes to the indigenous population resulting in an improved phytoremediation efficiency that is stable at the long-term. Moreover, this rather easy way to extrapolate the concept (of using degrading endophytes to reduce phytotoxicity and evapotranspiration) to a broad variety of contaminants could move endophyte-enhanced phytoremediation towards a wide-ranging application potential.

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## WATER HYACINTH AND ITS ENDOPHYTIC BACTERIA AS A POTENTIAL TOOL FOR PHYTOREMEDIATION OF Ba

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### ABSTRACT

Background: Energy is one of the biggest problems humanity is facing nowadays. Global energy demand is expected to double by the year 2050. The concern about the availability of energy has directed people towards shale-geologic formations containing imprisoned gas and/or oil within its pores. Technologies making shale gas extraction possible require huge amounts of water for drilling and fracking. In consequence, flowback waters contaminated by different pollutants, among many also heavy metals such as barium (Ba), are produced. Concentrations of Ba was found to reach on average  $661 \text{ mg} \cdot \text{dm}^{-3}$  and maximum even up to  $15500 \text{ mg} \cdot \text{dm}^{-3}$ . One of the successful hyperaccumulators, efficiently absorbing heavy metals (Pb, Sr, Hg, Zn, Cd, Cu and Ni) and other contaminates ( $\text{NO}_3$ ,  $\text{NH}_4$  and phenols) from wastewater is water hyacinth (*Eichhornia crassipes* (Mart.) Solms). Fast growing and producing enormous amounts of biomass water hyacinth may be also considered as potential biofuel crop. In this study, usefulness of water hyacinth plants in Ba phytoextraction from contaminated water was evaluated and community of cultivable endophytic bacteria from its roots was characterized.

Materials and Methods: Water hyacinth plants were cultivated, under strictly controlled growth conditions, in plastic containers filled with nutrient solution (1/4 of modified Hoagland solution, pH 6.2) enriched with increasing concentrations of Ba (between 0 and  $1500 \text{ mg} \cdot \text{dm}^{-3}$ ). Non-destructive measurements characterizing efficiency of photosynthetic apparatus (gas exchange, chlorophyll *a* fluorescence) were conducted in two week intervals. Biomass accumulation, reproductive capability and Ba content in particular plant organs were assayed at the end of the experiments. Cultivable endophytic bacteria strains were isolated from surface sterilized (active chloride and ethanol) roots of water hyacinth plants grown in  $400 \text{ mg} \cdot \text{dm}^{-3}$  Ba and characterized (i) morphologically (size, colour, shape of the colony), (ii) phenotypically in plant growth promoting features (production of siderophores, organic acids and IAA, nitrogen fixation, phosphorus solubilization, activity of ACC deaminase and tolerance to selected heavy metals: Zn, Cu, Cd and Ni) and (iii) genotypically with amplified rDNA restriction analysis of 16S rRNA gene using HpyCH4 IV enzyme, sequencing and identification based on naïve Bayesian rRNA Classifier and Sequence Match at the Ribosome Database Project II.

Results: Obtained results showed that water hyacinth was able to grow exuberantly in concentrations not exceeding  $500 \text{ mg} \cdot \text{dm}^{-3}$  Ba and accumulate extremely large quantities of this element, in concentration  $1000 \text{ mg} \cdot \text{dm}^{-3}$  Ba even up to  $35.4$  and  $83.50 \text{ g} \cdot \text{kg}^{-1}$  DW, for leaves and roots respectively. Lower Ba concentrations did not show any toxic effects on examined plants. However in higher concentrations (above  $500 \text{ mg} \cdot \text{dm}^{-3}$  Ba) Ba had negative impacts on plant's reproductive capability and biomass production. Gas exchange (i.e. intensity of photosynthesis, stomatal conductance and transpiration rate) and values of the selected chlorophyll *a* fluorescence parameters were also lowered. Bacterial community associated with water hyacinth grown in the presence of Ba was dominated by Firmicutes, most of all with *Bacillaceae* family (the nearest strains from RDPII database was similar to *B. pumilus*, *B. cereus*, *B. aquimaris*, *B. subtilis*, *B. licheniformis*, *B. bombysepticus* and *B. amyloliquefaciens*). Much less represented were Proteobacteria (*Enterobacter asburiae*) and Actinobacteria (*Micrococcus* sp.). Bacteria communities isolated from water hyacinth roots hold important plant growth promotion features. Among isolates derived from plants exposed to Ba pollution 38% produced siderophores, 24% organic acids and 30% IAA. ACC deaminase activity, nitrogen fixation and phosphorus solubilisation was noted in 14, 28 and 53% of isolates, respectively. Vast majority of those bacteria were resistant to Zn, additionally *E. asburiae* could grow also in the presence of Cu and Cd.

Conclusions: These results support the conclusion that water hyacinth can be used in phytoextraction of Ba from polluted water and endophytic bacteria are most probably making this process more efficient.

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## INTERACTIONS PLANT – SOIL MICROORGANISMS FOR REMEDIATION OF METAL MINE RESIDUES

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### ABSTRACT

Mining has been an important commercial activity in Mexico. As a result tons of mining waste is observed in many areas causing a potential risk not only for the environment, but also for the human population settled very near to mine tailings. In order to reduce the dispersion of potentially toxic elements (PTE) contained in tailings the establishment of a well-developed vegetation cover is required. However, mine tailings are drastically physico-chemical and biological altered environments and natural restoration may occur in many years. Microbe assisted phytoremediation and amendments are eco-friendly and cost-effective alternatives proposed to increase biological productivity; to improve fertility of mine tailings and induce sooner site remediation. The results obtained by using different plants such as *Jatropha curcas*, *Ricinus communis*, *Brassica napus*, *Medicago sativa*, *Acacia farnesiana*, *Senna multiglandulosa*, in combination with soil amendments such as perlite, vermicompost and biochar, as well as different beneficial soil microorganisms for instance arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria (PGPR) will be presented. The tested plants were selected because they are found as naturally established plants in mine tailings (except *J. curcas*, *M. sativa* and *B. napus*), their tolerance to PTE and role to increase soil fertility, or their use as raw material for potential biodiesel production. Soil amendments were able to decrease PTE availability; perlite reduced Cd availability, biochar (5%) decreased availability of Cd (97%), Cu (32%), Pb (56%) and Zn (34%). Vermicompost at 10% or 15% also diminished availability of several PTE. Several biochemical properties were analyzed for the PGPR (indol acetic acid, ACC-deaminase, phosphate solubilization, tolerance to different PTE, siderophore production) under study and their effect on plant growth into mine tailings substrates amended with organic compounds. All these factors (amendments, tolerant plants, and selected microorganisms) represent a very useful tool to deal with the harsh environment such as mine tailings and propitiate their phytoremediation, but their efficiency varies depending of the combination.



## THE COUPLING OF THE PLANT AND MICROBIAL METABOLISMS OF PAHs IN THE RHIZOSPHERE OF ALFALFA

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### ABSTRACT

Plant-microbial associations and symbioses, with a flexible metabolism and unique enzyme systems, have great advantages for survival in rugged environments, and their survival is due not only to an increase in tolerance to xenobiotics but also to an active removal of toxic substances from the habitat. Despite the increased use of plants and their associated microorganisms in the form of phytoremediation technology, biochemical interactions between partners in the rhizosphere that are aimed at the degradation of organic pollutants are not fully understood. We studied the catabolism of polycyclic aromatic hydrocarbon phenanthrene by several rhizosphere bacterial strains and the possibility of enzymatic oxidation of the PAH by the root exudates of alfalfa (*Medicago sativa* L.) in order to detect the possible coupling of the plant and microbial metabolisms under the rhizospheric degradation of the organic pollutant.

A comparative study of phenanthrene degradation pathways in the isolated PAH-degrading rhizobacteria *Pseudomonas stutzeri*, *P. viridiflava*, *Arthrobacter* sp., and *Ensifer meliloti* was conducted. Identification of the key metabolites formed during biotransformation of phenanthrene and its derivatives, including 9,10-phenanthrenequinone, 9-phenanthrol, 2-carboxybenzaldehyde, 1-hydroxy-2-naphthoic, salicylic and *o*-phthalic acids, was performed by using TLC, GC, HPLC, and mass spectrometry. It was found that the metabolic pathways of phenanthrene in these bacteria were different: *E. meliloti* catabolized phenanthrene through the salicylate pathway, while the remaining rhizobacteria converted this PAH through the *o*-phthalate pathway. 1-Hydroxy-2-naphthoic acid was the predominant metabolite in all strains.

Alfalfa plants were grown under sterile conditions in quartz sand in the presence and absence of phenanthrene for 3 weeks under controlled environmental conditions to obtain plant root exudates for study. The root exudates were concentrated by ultrafiltration, and the activity of peroxidases, tyrosinases and other oxidases was detected through an oxidation test with various substrates [2,20-azino-bis-(3-ethylbenzo-thiazoline-6-sulphonate) (ABTS), 2,7-diaminofluorene (2,7-DAF), *o*-dianisidine and 2,6-dimethoxyphenol], detectable by spectroscopy. It was found that the most marked activity was that of peroxidase, whereas the presence of oxidase and tyrosinase was detected on the verge of the test sensitivity. The optimal substrates for the detection of peroxidase activity of alfalfa root exudates were ABTS and DAF, and the optimum pH values were in the range 4.5-5.5. The presence of phenanthrene at a concentration of 0.03 g kg<sup>-1</sup> in the medium insignificantly reduced the peroxidase activity of the alfalfa root exudates.

Using alfalfa root exudates as a crude enzyme preparation, we found that the plant peroxidase in the presence of the synthetic mediator ABTS can oxidize phenanthrene (6-26 %) and its microbial metabolites 9,10-phenanthrenequinone (22-27 %), 1-hydroxy-2-naphthoic acid (28-100 %), and 1-naphthol (92 %). It was revealed that 9-phenanthrol significantly inhibited the peroxidase activity toward ABTS, which indicated a higher affinity of the enzyme to 9-phenanthrol and an indirect confirmation of PAH binding to the zymophore.

The results indicate the possibility of active participation of plants in the rhizospheric degradation of PAHs and their microbial metabolites, which makes it possible to speak about the coupling of the plant and microbial metabolisms of PAHs in the rhizosphere.

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## ENHANCED DEGRADATION OF POLYCHLORINATED BIPHENYLS USING MICROBE-ASSISTED PHYTOREMEDIATION

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### ABSTRACT

The presence of PCBs in the environment poses great risk to plants, animals and humans and their removal seems to be great ecological and economical problem due to their persistence in the environment. Thus, remediation of PCBs using microbes and plants represents one of the important mechanisms by which the concentration of this xenobiotic compound can be decreased and subsequently eliminated from the environment. However, it has been postulated that complete degradation of PCBs congeners can be achieved by use of microbial consortium along with plants i.e. microbe-assisted phytoremediation (MAP) rather than individual use of bacterial culture/consortium or plant. This study deals with MAP of PCBs (in Clophen oil) at microcosm level using bacterial consortium (T1) and combination of consortium and plant (T2) in the soil pots, contaminated with 2% Clophen oil along with a control (soil + 2% Clophen oil) over a period of 90 days. The plant used in this study was *Vetiveria zizanoides*. The samples were collected every 30<sup>th</sup> day and various physicochemical and microbial parameters were analysed to evaluate the degradation of PCBs in soil. The results showed decrease in total organic carbon (TOC), increase in chloride ion (Cl<sup>-</sup>), electrical conductivity (EC) in the treatment T2 with no significant changes in treatment T1 for the same as compared to control. The treatment showed increase in dehydrogenase activity in the following order; T2>T1>control. Even though, the results are yet to be confirmed by GC-MS analysis; the results of preliminary analysis performed in this study are encouraging. It can be inferred from the results that microbe-assisted phyto-remediation can be employed as an effective option for enhanced remediation of PCBs contaminated soil.

**Keywords:** PCBs, degradation, consortium, microbe-assisted phytoremediation, dehydrogenase activity



## ISOLATION, CULTIVATION, AND CHARACTERIZATION OF ENDOPHYTES IN FUNCTION OF PHYTOREMEDIATION OF DDE-CONTAMINATED SOILS

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### ABSTRACT

2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) is a pesticide that was used worldwide from 1943, but has been forbidden in most western countries since the 1970s. In soil, it quickly degrades to 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (DDE). Both molecules are considered as persistent organic pollutants (POPs), which are highly recalcitrant in soil, resulting in a worldwide spread of DDE contaminated soils.

*Cucurbita pepo* has been shown to accumulate high concentrations of DDE from surrounding soil [1]. This research focuses on optimizing the phytoremediation process with the help of plant-associated endophytic bacteria. To enhance the extraction of DDE from the soil and improve its degradation in the plant, endophytic bacteria have to be isolated and cultivated. Previously, it has been shown that only about 1-5% of the total bacterial population can be isolated and cultivated successfully [2]. To increase the cultivable fraction of the total bacterial population, a first part of this research explores different isolation and cultivation techniques. Results reveal that the medium composition as well as the addition of plant extract can affect the extent and the diversity of the cultivable of the bacterial population.

To investigate the role of endophytes during phytoremediation of DDE, in the second part, a comparison was made between the endophytic population isolated from plants that were grown with 100 µg L<sup>-1</sup> DDE and control plants. The isolated bacteria were identified genotypically using ARDRA and were characterized phenotypically using several tests concerning their plant growth promoting capacities such as the production of indol-3-acetic acid (IAA), siderophores, organic acids, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Bacteria that can be beneficial to plant growth were found in both control and exposed plants, however, a general trend was noticeable where endophytes isolated from DDE-exposed plants scored better in the tests for plant growth promoting capacities. Furthermore, a screening for possible DDE degradation by endophytic bacteria was performed. Endophytes isolated from DDE-exposed plants showed a DDE degrading capacity more often than endophytes isolated from plants grown without DDE.

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## PLANT-MICROBIAL-SOIL SYSTEM OF Ni-HYPERACCUMULATING PLANTS: METAL AVAILABILITY ASSESSED VIA CONVENTIONAL SOIL ANALYSES AND DGT

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### ABSTRACT

Hyperaccumulator plants have been described as good candidates for phytoextraction (or phytomining) techniques. The majority of these plant species have been identified as Ni hyperaccumulators, and their distribution is closely associated with serpentine soils. Phytomining of such Ni-rich soils provides an opportunity for the recovery of high-value metals with potential application in metallurgical processes. Success depends to a great extent on Ni availability to the roots and the ability of the plant to take up and accumulate this element in its shoots. Although controversial results can be found these plants have often been thought to access metal fractions not available to non-accumulating plants (thus increasing metal uptake), or that their root activity leads to a faster replenishment of soluble metal pools. Recent studies have also shown that hyperaccumulators harbor a higher number of metal-tolerant microorganisms compared to surrounding non-vegetated soil or non-accumulators, and that these plant-associated microorganisms can enhance metal availability for plant uptake. Here we characterize the plant-microbial-soil system of two Ni-hyperaccumulating subspecies of *Alyssum serpyllifolium*, both of which are endemic to the serpentine outcrops of the Iberian Peninsula. The objectives of this work were (1) to describe the culturable microbial community associated with these plants and to identify potential candidate microbial strains for application in phytomining; and (2) to further unravel the influence of this plant type on soil metal bioavailability and the potential implications in the efficiency of the phytomining process.

Plant and soil (non-vegetated and rhizosphere) samples were collected from *Alyssum serpyllifolium* subsp. *lusitanicum* growing in Samil and Morais (Tras-Os-Montes, N of Portugal) and Barazón (NW of Spain), and from *Alyssum serpyllifolium* ssp. *malacitanum* in Sierra Bermeja (S of Spain). For comparative purposes non-accumulating plants (*Dactylis glomerata*, *Holcus lanatus* and *Santolina semidentata*) growing at the same sites, and the non-hyperaccumulator *Alyssum serpyllifolium* ssp. *serpyllifolium* growing in a calcareous soil in S. Nevada (S of Spain), were also sampled. Plant Ni accumulation was determined, and soil samples were used for physicochemical analysis and the isolation and characterization of plant-associated culturable bacteria (total bacterial density, metal tolerance, plant growth promoting traits and diversity indices). Metal availability and fractionation was assessed using simple extraction ( $\text{Sr}(\text{NO}_3)_2^-$ ,  $\text{Ca}(\text{NO}_3)_2$ -extractable and sequential extractions), while metal kinetics and soil replenishment capacity was assessed using the DGT (Diffusive Gradient in Thin Films) technique employing Chelex resin gels. DGT is a passive sampling technique and for diffusion-limited uptake the DGT-labile solute fraction ( $C_{\text{DGT}}$ ) has been shown to predict plant uptake better than chemical extraction. Metal replenishment capacity in soils can be evaluated through the ratio  $C_{\text{DGT}}:[\text{metal}]_{\text{soil solution}}$ . DGT was also applied to plants grown in serpentine soil in rhizotrons for the chemical imaging of labile Ni distribution along the root axis and rhizosphere.

The *Alyssum serpyllifolium* subsp. *lusitanicum* population from Barazón showed the highest Ni accumulation in the shoot tissues. Total bacterial densities were higher in the calcareous soil, and the diversity of the culturable bacterial community associated with this non-hyperaccumulator was also





higher. However the densities of metal tolerant bacteria were higher in the serpentine soils and in the rhizosphere of the hyperaccumulator, and these were significantly correlated with soil Ni availability. Most bacterial strains belonged to the *Actinobacteria* phylum. Several strains were selected for PGP traits and Ni resistance, and promoted plant growth and Ni phytoextraction capacity in pot experiments. Simple extractants indicated a higher Ni availability in the rhizosphere of the hyperaccumulator, and this was corroborated by  $C_{DGT}$  values. Images showed Ni depletion in the rhizosphere of the hyperaccumulator and *H. lanatus*, but this was less pronounced in the grass species than the hyperaccumulator. On the other hand, a zone corresponding to an increase in Ni was seen in the rhizosphere of the non-hyperaccumulating *Alyssum* subspecies. Soil Ni availability and replenishment is discussed in relation to plant type and accumulation traits.

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## CHARACTERISATION OF ENDOPHYTIC BACTERIAL COMMUNITY IN THE HALOPHYTE *JUNCUS ACUTUS* L.

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### ABSTRACT

Phytoremediation is the use of plants and their associated microorganisms to degrade toxic contaminants in soil, air or water. The idea of exploiting endophytic bacteria in phytoremediation strategies is becoming increasingly popular because endophytes can effect beneficially the plant growth and contribute to the plant higher tolerance towards biotic and abiotic stress. Halophyte species are widespread in the Mediterranean basin and are of special interest since these plants are adapted to cope with environmental stresses. *Juncus acutus* L. has a wide ecological range and was found to be tolerant to organic and inorganic contaminants.

The culturable endophytic community of this halophytic species (*Juncus acutus*) growing on Bisphenol-A contaminated soil and groundwater was assessed. The genotypic characterisation of the isolated strains using BOX-PCR was performed and the 16s rRNA gene of the isolates with distinct BOX-PCR profiles were amplified and sequenced. The ability of the isolates to grow in the presence of BPA as the sole carbon source was also investigated and the most promising isolates were tested for their capacity to degrade Bisphenol A. The strains were further phenotypically characterised for plant growth promoting characteristics (ACC deaminase, IAA production P solubilisation, siderophore and organic acid production) and trace metal tolerance (Zn, Ni, Cd, Pb). Bioaugmentation experiments were performed with selected endophytic bacteria in order to study the potential enhancement of BPA degradation rates.

Eighty endophytic strains were isolated from the roots and upper and lower part of the leaves of the halophyte. The majority of the strains (84%) exhibited an increased ability to tolerate BPA. Concerning plant growth promoting traits, many bacteria had at least one PGP characteristic. The potential benefits of bioaugmentation were investigated and the results indicate that the indigenous endophytic microbial populations of the halophyte may be exploited in a phytoremediation strategy.

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## PLANT MICROBIOME POTENTIAL IN ENHANCING SOIL RHIZOREMEDIATION

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### ABSTRACT

Losses of contaminants during industrial and commercial operations, municipal and industrial waste treatment, oil extraction and production and inadequate storage often can result in soil contamination. More than 1,800,000 potentially contaminated sites have been identified in Europe. Among those, many are large scale sites affected by a mixed contamination *e.g.* by organic and inorganic pollutants, whose remediation implies technological and economic limitations on the choice of the remediation technique. The use of plants to extract and/or modify the pollutants (*phytoremediation*) together with root associated microbes to degrade organic pollutants or modify the heavy metals (*rhizoremediation*), has the advantages of being environmentally sustainable and based on a bioeconomy approach, applicable on a large scale, on extended sites and in long-term treatments, and suitable to mixed contamination. Microorganisms can be exploited for the magnification of the phyto- and rhizo- remediation efficiency by Plant Growth Promotion (PGP) activity. PGP bacteria are positively associated with the plant: they exert direct and indirect beneficial activities on the host plant by stimulating its growth and stress tolerance, receiving in turn a benefit from root exudates.

Aim of the work was to evaluate the potential of plant associated microbes for PGP enhanced rhizoremediation in a soil contaminated by heavy metals (Cu, Ni, As, Hg) and oil derived long chain (C>12) aliphatic hydrocarbons. A collection of 65 bacterial isolates was established from contaminated soil and from the rhizosphere of wild plants naturally selected in the most polluted area of the site, enriched on aliphatic hydrocarbons (C12, C13, C15, C16 and C17) as unique carbon source. The isolates, identified by 16S rRNA gene sequencing, belonged to different classes *i.e.* Actinomycetales,  $\alpha$ - and  $\beta$ -Proteobacteria, Bacilli. The collection has been then functionally screened by i) resistance to heavy metals (Ni, Cu, Hg, As species), ii) PGP traits (modulation of phyto-hormone omeostasis; increase of nutrient availability; production of hydrolytic enzymes, EPS and organic volatile compounds), iii) presence of alkane degrading genes (alkane hydroxylase, *Alk*). Eighty percent of the strains showed to tolerate one or more metals in the growing medium and to hold at the same time multiple potential PGP traits.

The most promising strains, having potential degrading activity on alkanes and resistance to heavy metals and multiple PGP activities, have been selected and tested *in vivo* for the positive association with plants. Rhizocompetence of the strains was demonstrated as the ability of the bacterial cells to colonise the plant root of *Lupinus albus*, species with phytoextraction potential, and the model plant *Arabidopsis thaliana*. Direct PGP activity was tested on *Solanum lycopersicum* and *A. thaliana*. Five strains demonstrated to induce a statistically significant increase in length and fresh weight of stem of the bacterized plants, as compared with not inoculated ones. In *A. thaliana* plants growing in synthetic medium containing Cu and Ni, bacterial inoculation induced a change in the root architecture and an increase in the number of lateral roots and the overall root biomass.

The results of the work demonstrated that the microbiota of contaminated soil and in particular associated to the rhizosphere of naturally selected wild plants has the potential of sustaining soil rhizoremediation, particularly magnified by PGP activity. The most promising isolates belong to the genera *Gordonia*, *Sporosarcina* and *Cupriavidus*, and could be exploited *on field* as bioaugmentation and PGP inocula for the bioremediation of soil polluted by hydrocarbons and heavy metals.

*Acknowledgements:* The financial support by Eni Syndial s.p.a. is greatly appreciated.



## ENDOPHYTIC BACTERIA CONTRIBUTE TO CARBAMAZEPINE REMOVAL IN *PHRAGMITES AUSTRALIS*

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### ABSTRACT

Carbamazepine is an anticonvulsant and mood-stabilizing drug used primarily in the treatment of epilepsy and bipolar disorder and prescribed widely in Europe and North America. Due to this fact, it is found ubiquitously as a persistent and recalcitrant contaminant, being one of the most prominent compounds in the list of hazardous PPCPs found in Waste Water Treatment Plants (WWTPs). Usual concentrations in WWTPs range between 1 µg/L in influents and 0.7 µg/L in effluents, indicating a remarkably low removal efficiency in activated sludge processes. Hence, the ultimate fate of this compound is the groundwater in many cases. Recent studies revealed concentrations up to 16 ng/L in confined and semi-confined groundwaters in France.

Up to now, current bioremediation approaches have focused on the role of plants in the uptake or absorption of carbamazepine from waters. Studies have been performed in edible plants (cucumber, soybean and *Brassica* sp.) and also in some grass and macrophyte species. Nevertheless, investigation about the degradation of carbamazepine by plants or their inhabiting microbes is scarce. However, since endophytes have been proposed to play an important role in phytoremediation, this study focuses on the identification and characterization of endophytic candidates for biodegradation of carbamazepine in wetland systems.

When *Phragmites australis* was exposed to carbamazepine (5 mg/L) for 9 days in semi-hydroponic conditions, different bacterial strains could be isolated from rhizomes and roots. Numerous of these strains were able to grow either in PDA or in R2A media supplemented with 10 µM carbamazepine. The species were identified by sequencing of the 16S rDNA. Furthermore, their growth in minimal medium with carbamazepine as sole carbon source was studied. Phylogenetic studies and identification of non-cultivable strains will shed more light on the behavior of the bacterial community in response to this important pharmaceutical.



## PHYTOREMEDIATION OF SOIL CONTAMINATED WITH AROCLOR BY *CHROMOLAENA ODORATA* (L) KING & ROBINSON INOCULATED WITH RHIZOSPHERE ORGANISMS

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### ABSTRACT

*Chromolaena odorata* was planted on soil contaminated with 10, 30 and 50% Aroclor and inoculated with rhizosphere organisms to study the phytoextraction of PCB congeners present in the soil. Fertilizer (NPK 5%) was applied to the soil and watered regularly for ten weeks. Growth enzyme activities and changes in PCB concentrations in the contaminated soil and plant tissues were measured. Rhizosphere microorganisms were characterized by 16S rRNA base sequencing. Plant growth was not significantly impeded by the presence of Aroclor in the concentrations used in the experiments. Increases in enzyme activities in the soil were observed in all the experiments. Reduction of between 67 and 87% in PCB concentrations were observed at the end of the ten-week period. The concentrations of PCBs in the roots of all experimental plants were more than in the shoots. Rhizosphere organisms did not significantly enhance the growth of plants and PCB reduction in soil or PCB concentration in plant tissues. *Chromolaena odorata* has shown the potential to grow in Aroclor contaminated soil and extract PCB from such soil. However, it is not clear whether degradation of PCB occurred in plant tissues or in the soil. The difference in amount of PCB recovered from the soil and plant tissues may indicate that biodegradation has taken place. However, volatilization and translocation, or a combination of both may have occurred along with some degradation.

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## CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM SELENIUM HYPERACCUMULATORS AND GM PLANTS

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### ABSTRACT

The goal of this work is to optimize the symbiotic synergism between plants and microbes resulting in more effective bio/phytoremediation processes. This approach is being performed for plants hyperaccumulating selenium (hyperaccumulators, HA) and genetically modified (GM) plants remediating organic or inorganic contaminants. We focus on the cultivable and also not cultivable (viable but not cultivable, VBNC) endophytic and rhizospheric bacterial communities responsible for contaminant accumulation and degradation.

An important aspect of this research is to compare the bacterial diversity of selenium hyperaccumulators and nonhyperaccumulators, to see how the bacterial community is shifted in HA and whether accumulation of selenium in plants can be facilitated by certain bacteria. Studied HA were *Stanleya pinnata* and *Astragalus bisulcatus* from Pine Ridge Natural Area, Fort Collins, Colorado, USA. Endophytic microorganisms were isolated from plant roots, stems and leaves. The most endophytes were isolated in the order roots>leaves>stems. Characterization of endophytes was performed by analysis of part of the 16S rRNA gene and MS MALDI-TOF Biotyper. Among others, the bacterial isolates were *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Staphylococcus*, *Variovorax*. Isolates were tested for the ability to produce siderophores and to metabolize selenium and nitrogen compounds. The isolates were able to reduce selenite into elemental selenium and some were able to reduce nitrite into nitrogen. Further, the metagenomic DNA of endophytes was isolated in order to determine the bacterial community (VBNC bacteria). We used T-RFLP analysis that will be continued by pyrosequencing.

A similar approach has been performed to study the changes of bacterial diversity in GM plants, as compared with wild type plants. Studied plants were *Nicotiana tabacum* containing bacterial *bphC* gene. The results so far indicate that the rhizospheric bacterial community of GM plants has not been changed. Cultivable endophytic bacteria have been isolated, and among these *Pseudomonas*, *Rhodococcus*, *Arthrobacter*, *Microbacterium* and *Leifsonia* have been identified. The metagenomic endophytic DNA from GM plants is under investigation.

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## HALOPHYTE-ASSOCIATED PLANT GROWTH PROMOTING BACTERIA: KEY TOOL FOR LAND RECLAMATION AND RESTORATION

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### ABSTRACT

Plant Growth Promoting (PGP) bacteria are known for their role in enhancing plant fitness both in conventional and extreme environments. PGP bacteria can directly and indirectly boost plant growth by providing nutrients and metabolites and by reducing the incidence of pathogenic attacks. Soil salinization and drought are among the most severe environmental stressors affecting crop production around the world. High salt content in the soil is a typical feature of arid and semiarid regions, hosting different halophyte species including those belonging to the genus *Salicornia*. Halophytes comprise several plant species of emerging interest as source of valuable products and services, including phyto-remediation and –reclamation technologies. One of the most promising and sustainable approach to improve plant growth and production in salt and drought affected lands is represented by the selection of PGP bacteria able to cope with the extreme geochemical conditions of saline soils.

In this perspective we investigated by cultivation dependent methods and molecular fingerprinting the microbiome composition associated to *Salicornia* rhizosphere and bulk soils collected from Sebket and Chott hypersaline ecosystems in Central Tunisia. Denaturing Gradient Gel Electrophoresis (DGGE) and Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting were applied to depict the overall bacterial community structure of the hypersaline soils, displaying the occurrence of a rich and highly diverse microbiome associated to the *Salicornia* plants roots. A large collection of bacteria was established from bulk soils and *Salicornia* rhizospheres. Plate counts showed that culturable halophilic/halotolerant bacteria were abundant in the bulk soil as well as in the rhizosphere of *Salicornia* specimens. The PGP traits of the halophilic/halotolerant bacteria collection were assessed in vitro in presence of 5% of sodium chloride, to simulate those environmental condition that likely hamper the efficacy of PGP inocula selected from conventional ecosystems. Tolerance to abiotic stresses typical of desertic and semi-desertic areas, such as extreme temperature, salt concentration and low water availability, was widespread within the bacteria collection and represented a common trait in strains belonging to different phylogenetic groups. A high number of isolates showed the ability to influence the nutrients and hormonal balance in vitro at high salinity value. In addition, some strains expressed protease activity, potentially correlated to biocontrol capacity against phytopathogens. In particular, the screening allowed the identification of several strains resistant to the tested abiotic stresses and performing different PGP activities, including ammonia and indol-3-acetic acid production and phosphate solubilization. One of the best promising isolates, *Halomonas elongata* BDV11S17A, was successfully *gfp*-labelled and used to demonstrate its ability to colonize *Salicornia* roots under laboratory conditions. Furthermore, *in vivo* test were performed on a subset of bacteria, identifying strains able to sustain the growth of *Salicornia* plantlets.

The results confirmed that halophytes are valuable source of PGP bacteria. The halophilic/halotolerant strains isolated in the present study could be exploited to setup specifically designed biofertilizer to sustain plant growth in soils impacted by salinity and drought stresses in a sustainable perspective. According to the climate change scenario and the future extension of salt affected lands this powerful approach should be carefully considered for a successful land restoration through phytoremediation and to reclaim land traditionally considered unsuitable for agriculture.

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## ISOLATION AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA AND THEIR EFFECTS ON PHYTOREMEDIATION OF PETROLEUM-CONTAMINATED SALINE-ALKALI SOIL

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### ABSTRACT

**Background:** Contamination of soil environment by petroleum hydrocarbons is becoming prevalent across the globe. Phytoremediation is a green technology that uses plants to remediate contaminated soils. Our previous study showed that growing tall fescue significantly increased microbial activity and the number of TPH-degraders of the soil. In addition, inoculating with Plant growth-promoting rhizobacteria (PGPR) may facilitate plant growth and development both directly and indirectly. Though earlier work has involved isolation of salt-tolerant rhizobacteria from halophytic environments, little is known about their tolerance to alkaline environments where the soils have high pH.

**Aims:** The objectives of the present study were: (1) to isolate and characterize efficient ACC deaminase producing PGPR from the rhizosphere of tall fescue grown in saline-alkali soils, (2) to evaluate other PGP activities of the most promising ACC deaminase producing isolates under various saline-alkaline stresses, and (3) to study the effect of the selected isolates on tall fescue growth and phytoremediation of a petroleum-contaminated saline-alkaline soil.

**Methods:** PGPR with ACC deaminase activity were isolated by a conventional method. The strain D5A isolated from rhizosphere soil of tall fescue was evaluated for their plant growth-promoting characters and effects of pH and salinity on the growth and IAA production of strain D5A. Different levels of Na<sub>2</sub>CO<sub>3</sub> and NaHCO<sub>3</sub> stress on the growth of strain D5A was tested. A pot experiment was then conducted to study the PGPR assisted phytoremediation of petroleum contaminated soil.

**Results:** A total of 115 PGPR strains were isolated from the rhizosphere of tall fescue grown in petroleum-contaminated saline-alkaline soils. Of these, 5 strains shown ACC deaminase activity >1.0 M α-KB mg<sup>-1</sup> h<sup>-1</sup> were selected for further study and the isolate D5A presented the highest plant-growth-promoting activity and was identified as *Klebsiella* sp. It grew well on the LB medium containing 9% NaCl and at a pH range of 4-10. The inoculation of D5A significantly improved seed germination, biomass production and chlorophyll contents in leaves of tall fescue grown in the petroleum-contaminated saline-alkaline soil. Shoot dry weight was almost 3 times greater in the inoculated than non-inoculated treatment by the end of the experiment. The inoculation also increased root dry weight, root activity and chlorophyll contents by 73%, 101% and 170%, respectively. The TPH contents in the soil after 120 d decreased by 42%, 50% and 66% in the non-plant control, tall fescue and tall fescue + D5A treatments, respectively.

**Conclusions:** The isolate D5A showed the highest PGP activities and growth-promoting ability. It also had a good adaptability to extreme pH and high salinity and alkalinity level. The D5A was identified as *Klebsiella* sp. The inoculation of this isolate enhanced the phytoremediation efficiency by further 16% as well as promoted the growth of host plants in the petroleum-contaminated saline-alkaline soil. Thus, the D5A can be used as a potential inoculum to improve phytoremediation of the organic contaminants in petroleum-contaminated saline-alkaline soils. Further studies are needed to validate the results in multiple field sites.

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## PHYTOREMEDIATION OF SALT-AFFECTED SOILS USING HALOPHILIC BACTERIA

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### ABSTRACT

Salinity is a most important ecological constraint for cereal crop including rice nowadays. Phytoremediation have been used to remediate salt affected soil but the use of halophilic bacteria is limited. In this study, the salt resistant *Thalassobacillus denorans* (NCCP-58) and *Oceanobacillus kapialis* (NCCP-76), halophilic bacteria isolated from salt mines near Karak, Pakistan were used to evaluate its effect on rice variety (Basmati-385) in soil contaminated with different concentrations (control, 50, 100 and 150 mM) of NaCl. It has been observed that significant increase in germination percentage and germination rate occurred in seeds primed with bacterial strains as compared to non primed seeds. Root and shoot length was more in plants raised from primed seeds than non treated seeds. Plants raised from inoculated seeds showed a significant increase in fresh and dry weight of seedling after 15 days. Significant increase in photosynthetic pigment; chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid contents occurred in 28 days old plants inoculated with bacterial strains under different saline condition. Similarly significant increase occurred in total nitrogen and protein contents in inoculated plants.  $Ca^{+2}$  and  $K^{+}$  ion concentration significantly increased while  $Na^{+}$  ion concentration decreased in plants inoculated with bacterial strains as compared to non inoculated plants under different saline condition. Bacterial strains *Oceanobacillus Kapialis* (NCCP-76) was more responsive in term of physiological and biochemical parameters than *Thalassobacillus denounce Sp.* (NCCP-58). The corresponding strains have a positive effect in alleviating the salt stress in plants growing in saline condition.



## EFFICIENCY OF *PSEUDOMONAS PUTIDA* MTCC 4391 TO ENHANCE ARSENIC REMEDIATION BY DIFFERENT PLANT SPECIES

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### ABSTRACT

A study was conducted to investigate the effect of arsenic tolerant bacteria *Pseudomonas putida*, having plant growth promoting characteristics, on growth and arsenic uptake by *P. vittata*, *A. capillus veneris*, *C. dentata* and *P. karka*. This arsenic tolerant bacterium mobilised arsenic from the treated soil and enhanced the arsenic uptake in the plant species. It was found, that soil inoculation of this bacterium significantly enhanced plant biomass and arsenic uptake from root to shoot. The present study also aimed to assess the role of arsenate reductase in detoxification of arsenic in the plant varieties. The results showed that due to increased accumulation of arsenic in plants by the inoculation of bacteria, arsenate reductase activity was also increased in the plant species. The results of the study denote that the arsenate tolerant bacteria have potential to enhance phytoremediation of arsenic-contaminated soils by plant species (*P. vittata*, *A. capillus veneris*, *C. dentata* and *P. karka*).

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## EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI SPECIE ON MAIZE GROWTH AND HEAVY METALS UPTAKE

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### ABSTRACT

Consumption of plants accumulating heavy metals is a health risk for humans and animals. Several studies reported that arbuscular mycorrhizal fungi (AMF) have a significant effect on plant growth and can reduce plant uptake of heavy metals. However, promotion of heavy metals uptake in soils inoculated with AMF has been shown. Two different species of AMF (*Glomus geosporum*; Gg and *Glomus intraradices*; Gi) were isolated from heavy metals contaminated soils. Species of AMF might have different effects on plant growth and heavy metals uptake.

This study was conducted to elucidate the potential of AM fungi Ga and Gi on maize growth and heavy metals uptake.

Soil was collected from the area of El-Madabegh, Assiut, soils treated with wastewater over 50 years. Soil (3 kg), was placed into pots. Fifty gram of AMF inoculum of Gg, or Gi was placed below seeds of maize. A control soil (no AFM) was included. The soil was treated with 120 and 30  $\mu\text{g N-KNO}_3$  and  $\text{P-KH}_2\text{PO}_4 \text{ g}^{-1}$  soil and irrigated to the field capacity throughout the experiment. After 45 days of growth, maize was harvested, and the shoot dry matter (DM) and content of Cd, Pb, Fe and Zn were determined.

Inoculation with AMF increased the dry weights of shoot and root with no significant difference between Gg and Gi treatments. Inoculation with Gi and Gg mycorrhizal fungi resulted in accumulation of higher concentrations of heavy metals in the maize roots than in the shoot.

With no AM inoculum, Pb, Cd, Fe and Zn concentrations in the shoot were approximately 67, 0.06, 370 and 197  $\mu\text{g}$  and in the root were approximately 57, 0.37, 3197 and 232.9  $\mu\text{g g}^{-1}$  DM, respectively. With Gg and Gi inoculation, Pb concentration in the shoot decreased by approximately 5 and 32 and that of Zn decreased by 9 and 28%, respectively. Inoculation with Gi resulted in lower Cd concentration in the shoot than in the root; the opposite is true for Gg inoculum. Shoot Fe concentration was optimal, but lower in the Gg compared to the Gi inoculated soil. In the root, compared to Gi, Gg inoculation resulted in higher Fe concentration.

AMF have a significant effect on maize growth in heavy metals contaminated soils, but maize uptake of heavy metals is dependent on AMF specie.

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## MICROBE ASSISTED PHYTOREMEDIATION TECHNOLOGY FOR ENHANCED REMEDIATION OF OIL SLUDGE CONTAMINATED SOILS

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### ABSTRACT

Oil sludge contamination due to increased use of petroleum products as an effect of increased urbanization and industrialization has emerged as a principal problem of concern. To improve the quality of contaminated soils/sites an eco-friendly option i.e. microbe assisted phytoremediation technology (MAPT) was adopted for remediation of the contaminated soil. An investigation was undertaken at CSIR-NEERI, Nagpur premises over a period of 180 days with an objective of remediating oil sludge contaminated soil using plant (*Vetiveria zizanioides*), bulking agent (wheat husk), microbial consortium and nutrients. Oil sludge collected from oil refinery, was diluted to 1:1(wt/wt) ratio with soil and laid as top layer in eight plots of size 1m<sup>2</sup> over 15m<sup>2</sup> area. The overall microbial population was assessed by monitoring dehydrogenase activity and TPH degradation was estimated on gravimetric basis. Polyaromatic hydrocarbons were quantified on GC-MS. Plant analysis included the measurement of root and shoot length after 180 days. The results showed 85.21% degradation of TPH component in bulked treatment with consortium, plant and nutrients (T3[b]). TPH degradation in bioaugmented treatments with and without nutrients was 70.2% and 74.62% respectively as compared to control (36.38%). Furthermore, the dehydrogenase activity was found to increase initially till 4 months after which it remained more or less constant and finally was found to decrease slightly at the end of the study in all the treatments with treatment T3[b] showing highest activity. Also, root length and shoot length of vetiver grass increased by 88.04% and 90.74% respectively in T3[b] as compared to 73% and 85% respectively in treatment with plant and oil sludge only. It can be concluded that vetiver can serve as a promising plant for remediation of oil sludge contaminated soils owing to its tolerance to toxic contaminant. Also, the combination of amendments such as plant, bulking agent, microbial consortium and nutrients together can be taken as a solution which is both economic and eco-friendly to decontaminate/remediate oil sludge contaminated soils.

**Keywords:** MAPT, contamination, oil sludge, TPH, remediation, amendments



## IMPROVEMENT OF GROWTH OF *HELIANTHUS TUBEROSUS* L. BY SOIL AND ROOT ENDOPHYTIC BACTERIA ON A Cd-Zn CONTAMINATED SOIL

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### ABSTRACT

The utilization of high-biomass crops in phytotechnologies can help to stabilize or remove toxic metals in soils as well as produce a renewable energy resource on marginal lands that can no longer be used for food and feed production [1]. Some energy crops are effective in removing metals from soil, but show reduced biomass in the presence of toxic concentrations of metals. The interaction between plants and beneficial bacteria can enhance biomass production and metal tolerance of the plants. Some plant growth-promoting bacteria (PGPB) can improve biomass by different mechanisms such as nitrogen fixation, nutrient solubilization or production of phytohormones and siderophores. These PGPB can also reduce the metal-induced stress in plants, by decreasing the plant ethylene levels through 1-aminocyclopropane -1-carboxylate (ACC) deaminase activity [2]. *Helianthus tuberosus* L. is an energy crop often used as bio-ethanol resource. Recent studies have shown the tolerance of this crop to toxic metals, particularly to Cd [3]. The aim of this work is evaluate the effects of bacteria on the growth and metal uptake of *H. tuberosus* in the presence of Cd and Zn.

Cultivable bacteria were isolated from soil, rhizosphere, root and stem of *Brassica napus* growing on a Cd-Zn polluted soil in Belgium. These bacteria were tested for their metal resistance and potential plant growth-promoting characteristics (nitrogen fixation, phosphate solubilization, ACC deaminase activity, production of siderophores, organic acids, acetoin and indole acetic acid). Three Zn-resistant strains and four Cd-resistant strains were selected to inoculate roots of *H. tuberosus*, according to the best characteristics showed in plant growth-promoting tests. Tubers of two cultivars of *H. tuberosus* (VR and D19) were collected from an experimental field (IMIDRA, Madrid, Spain), transferred to plastic pots filled with quartz sand and watered with ½ diluted Hoagland's solution spiked with 0.1mM of Cd (CdSO<sub>4</sub>.8H<sub>2</sub>O) or 1mM of Zn (ZnSO<sub>4</sub>.7H<sub>2</sub>O). Control plants were fertilized with nutrient solution. Bacterial strains were grown in 869 liquid medium at 30°C. The bacterial suspension (10<sup>8</sup> cfu mL<sup>-1</sup>) was sprayed into the pots after the appearance of the first roots. After three weeks of growing, plants were harvested. Fresh and dry weight, malondialdehyde (MDA) content and metal uptake were evaluated. In general, the effect of the bacterial strains on the growth of *H. tuberosus* was different depending on the metal and cultivar. In presence of Zn, the inoculation of *Pseudomonas sp.* 228 and 256 strains significantly increased the aerial biomass of both cultivars and decreased the MDA content in roots, with respect to non-inoculated plants. Regarding Cd, *Pseudomonas sp.* 262 and *Serratia sp.* 246 significantly increased the aerial biomass of VR cultivar and D19 cultivar, respectively. The inoculation of these bacterial strains could enhance biomass production of this crop in presence of Cd or Zn, improving the efficiency of the phytotechnology. Experiments on field soils are performed in order to evaluate the bacterial strains effects on the growth of *H. tuberosus* when metals and nutrients are less available and there is competence with endemic soil microorganisms.

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## POPLARS HOST DIFFERENT COMMUNITIES OF FUNGAL ENDOPHYTES IN HEAVY-METAL POLLUTED AND UNPOLLUTED SOILS

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### ABSTRACT

The potential of endophytic fungi to enhance poplar growth and phytoremediation efficiency is a yet untapped resource. We investigated communities of fungal endophytes in *Populus maximowiczii* × *P. nigra* clone Max-4 at heavy metal polluted and unpolluted soils with a culture-dependent approach. Root and leaf endophytes were isolated from surface-sterilized poplar tissues using nutrient rich (2% MEA) and nutrient poor (SNA) media. The fungal isolates were grouped into morphotypes based on their colony morphology and growth characteristics.

The heavy metal-polluted site (Nové Podlesí, Czech Republic) displayed lower diversity and abundance of poplar endophytes than the unpolluted site (Průhonice, Czech Republic). Only 56 fungal endophytes belonging to 16 morphotypes could be isolated from the poplars grown at the polluted site whereas we were able to isolate 120 fungal endophytes of 33 different morphotypes from poplars growing at the unpolluted site. Isolated endophytes exhibit a typical long tail abundance with a higher diversity of rare morphotypes while the majority of isolates belong to few dominant morphotypes. The communities from the polluted and the unpolluted site appear to overlap only to a limited extent which is yet to be confirmed by molecular means. The obtained isolates will be molecularly identified by their ITS sequences and tested for their potential to improve growth of poplars and to enhance phytoextraction of heavy metals.

*Acknowledgements:* The financial support by Technological Agency of the Czech Republic, project Improvement of phytoremediation capacity and production potential of energy crops grown in contaminated and poor soils by means of endophytic and mycorrhizal symbionts contract No. TA03011184, is greatly appreciated.



## REDUCTION OF Cr(VI) To Cr(III) BY THE ENDOPHYTIC BACTERIA OF THE HALOPHYTE *JUNCUS ACUTUS* L.

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### ABSTRACT

The idea of exploiting endophytic bacteria in phytoremediation strategies is becoming increasingly popular because endophytes can affect beneficially plant growth and contribute to a higher heavy metal tolerance. Many endophytes have been found to be equipped with various heavy metals tolerance mechanisms while they are able to take advantage of the specific niche provided by the plants. The microbial reduction of Cr(VI) to Cr(III) takes place intra-cellularly and has been demonstrated as a useful process. Therefore, the potential contribution of endophytic bacteria to plant detoxification from Cr(VI) deserves further investigation for phytoremediation applications to heavy metal contaminated environments.

This work is a part of EU's LIFE-ENVIRONMENT program where among other mitigation technologies, CWs with halophytic plants are being tested for Cr(VI) polluted groundwater remediation. More particular, the removal of Cr(VI) from polluted groundwater by constructed wetland planted with the halophytic plant *Juncus acutus* was investigated and the results revealed that the plant is a Cr(VI) tolerant and able to rhizofiltrate Cr(VI) with high efficiencies. As a second step the responsible mechanism for plant ability to accumulate and tolerate high concentrations of hexavalent Cr was examined through the investigation of the contribution role of plant's endophytic bacteria. For that purpose, Cr(VI) resistant endophytic bacteria were isolated from the halophyte and four isolated high Cr(VI) resistant strains were examined for their Cr(VI) reduction potential.

Resistance tests revealed that isolates from the leaf and the root were able to grow at high concentrations of hexavalent chromium. More specifically, the results of these experiments showed that six leaf isolates were able to tolerate up to 10 mg/L of Cr(VI) and two of them able to tolerate up to 100 mg/L of Cr(VI) (*Acidovorax* sp. strain U3 and *Ralstonia* sp. strain U36). Moreover, nine root isolates were found able to tolerate very high Cr(VI) concentrations (50 mg/L and 500 mg/L).

Furthermore, subsequent experiments were performed to test resistant strains ability to reduce Cr(VI) with two leaf (*Acidovorax* sp. strain U3 and *Ralstonia* sp. strain U36) and two root isolates (*Pseudomonas* sp. strain R16 and *Ochrobactrum* sp. strain R24). The data showed that all tested strains were found able to reduce Cr(VI) to Cr(III). For example, *Pseudomonas* sp. strain R16 was found able to completely reduce 100 mg/L of Cr(VI) to Cr(III) after 150 hours (figure).

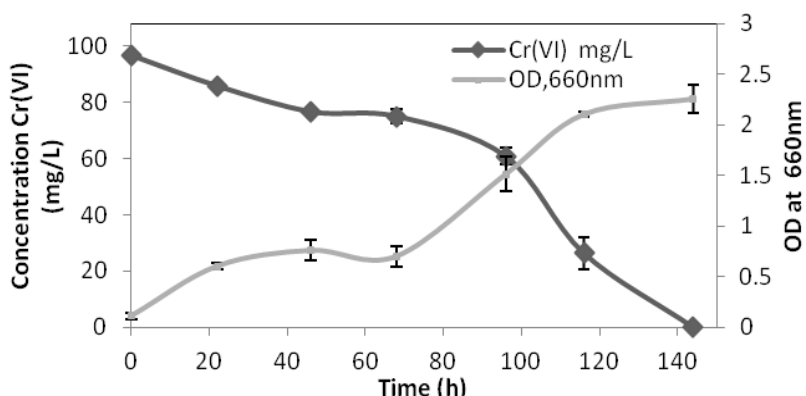


Figure. Cr(VI) reduction and growth curves of the root endophytic bacterium *Pseudomonas* sp. strain R16 at 100 mg/L Cr(VI) initial concentration. Error bars correspond to standard deviation.

**Acknowledgements:** This work was co-funded by the European Union in the LIFE10 ENV/GR/000601 project: CHARM - Chromium in Asopos Groundwater System: Remediation Technologies and Measures.



## IMPROVING PHYTOREMEDIATION OF CHLORENDIC ACID BY EXPLOITING PLANT-ASSOCIATED BACTERIA

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### ABSTRACT

The possibility to remediate an industrial site contaminated with chlorendic acid, a fire retardant and expected carcinogen, by applying phytoremediation using poplar (*Populus deltoides* x (*trichocarpa* x *deltoides*) cv. Grimminge) is investigated. Phytoremediation of organic pollutants is however often inefficient because most plants are not able to completely metabolize xenobiotic compounds, potentially resulting in new environmental problems, and eventually evapotranspiration of unmetabolized, volatile, toxic compounds. Enrichment with degrading plant-associated bacteria can overcome this problem, since bacteria can metabolize organic components to a greater extent, thus leading to an improved contaminant-degradation. Moreover, plant-associated bacteria can promote plant growth, thereby enhancing the efficiency of phytoremediation even more.

To find suitable bacteria for enrichment during phytoremediation of chlorendic acid, the cultivable plant-associated bacterial community of the chlorendic acid-contaminated site was isolated and genotypically characterized by sequencing of the 16S rDNA. The greatest diversity was found in the shoots with 9 different genera isolated, followed by 6 genera in the soil, 4 in the rhizosphere and 3 in the roots. *Bacillus* sp. is the only genus that could be found in all 4 compartments. Subsequently, the tolerance of the isolated strains to chlorendic acid was tested which resulted in 34 of the 100 isolated strains being tolerant to 50 mg l<sup>-1</sup> chlorendic acid. 33 of these strains even showed a tolerance to 500 mg l<sup>-1</sup> chlorendic acid. The degradation capacity of the tolerant strains is explored.

Meanwhile the isolated strains are screened for different plant growth-promoting characteristics (e.g. production of the phytohormone indole-3-acetic acid (IAA), nitrogen fixation, production of organic acids or siderophores which increase the uptake of iron). A consortium of bacteria will be selected, consisting of chlorendic acid-degrading and plant growth-promoting bacteria. The effect of inoculation of poplar with this consortium on the phytoremediation efficiency will be evaluated in a greenhouse experiment.

*Acknowledgements:* This project is funded by the Centre for Environmental Sciences, Hasselt University.





## MODIFICATION OF MICROORGANISM COMMUNITY IN THE GRASS GROWING IN SOIL CONTAMINATED WITH PAHs

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### ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are toxic to both humans and animals. Their significance increases with increasing number of aromatic rings. The most dangerous are the PAHs containing five and six aromatic rings, such as benzo[a]pyrene, showing a strong carcinogenic properties. They are also degraded very slowly, and therefore can remain in the soil for several years. Microorganism associated with roots of some plants can increase decomposition of PAHs in soil. Application microbial preparations containing the microorganisms capable to degrade PAHs or substance promote growth some microorganisms in soil can improve phytoremediation proces. High content of lignin-degrading enzymes in substrate used for the production oyster mushroom (*Pleurotus ostreatus*) fruit bodies causes that spent mushroom compost (SMC) can be used for bioremediation of soil. Formulation used for biological plant protection such as *Trichoderma harzianum* or effective microorganisms may have also positive impact to degradation PAHs.

The aim of this study was to estimate the effect of several microbiological formulation on composition of soil microorganism in rhizosphere of grasses recommended on the lawns along heavy traffic roads. In the experiment two dominant grass species on urban lawns was used: perennial ryegrass (*Lolium perenne* L. cultivar 'Solen') and red fescue (*Festuca rubra* L. cultivar 'Nimba') The plants were planted in 5 dm<sup>3</sup> pots containing a mixture of horticultural substrate with 1% of top soil and 2,5% of sand (pH = 6.8, EC = 486 ms). Substrate was mixed a mixture of four aromatic hydrocarbons: phenanthrene, chrysene, benzo[k]fluoranthene and benzo(g,h,i)perylene (Sigma-Aldrich) in amount 2 mg kg<sup>-1</sup> soil dry matter. The experimental factors were [1] endogenous microflora, [2] fungus *Trichoderma harzianum* T22 [3] effective microorganisms (EM) [4] waste from oyster mushroom production (SMC). Soil without plants was used as reference. To improve microorganism growth in every month grass has been cut off, shredded and left in pot. With the nested-PCR reaction amplified 16S rDNA region which were then separated by electrophoresis in a denaturing gradient gel electrophoresis (DGGE, Dcode Universal Mutation Detection System, BIO-RAD). Cut from the gel bands were immersed in deionized water and then re-PCR reactions were performed. The resulting products were sent for sequencing. Obtained sequences were compared with BLAST database.

Most of sequences have been identified as uncultured bacterial clone. The impact of all substances and preparations used was more pronounced in samples from the rhizosphere of grasses than in pots without plants. Inoculation *Trichoderma* contributed to reduction of bacterial diversity in soil, only one band has been identified as uncultured delta proteobacteria clone. Results suggest that the addition of *Trichoderma* contributes to stimulate growth of a specific bacterial population shifting the microbial community. Addition of EM and SMS to soil led to increase microbiological diversity. In combination with the SMS identified species was *Bacillus subtilis*. DGGE is a good method that allows the assessment of changes in the microbial population but because of the large variation in soil identification of the individual species is difficult. Assessment of these changes on the efficiency bioremediation process requires further studies.

**Acknowledgements:** The work was financially supported by Warsaw Plant Health Initiative of REGPOT in the frame of 7FP EU # 286093.



## RHIZODEGRADATION OF PHENANTHRENE BY A BACTERIAL STRAIN ISOLATED FROM AND REINOCULATED INTO THE RHIZOSPHERE OF A HALOPHYTE *SESUVIUM PORTULACASTRUM* LINN.

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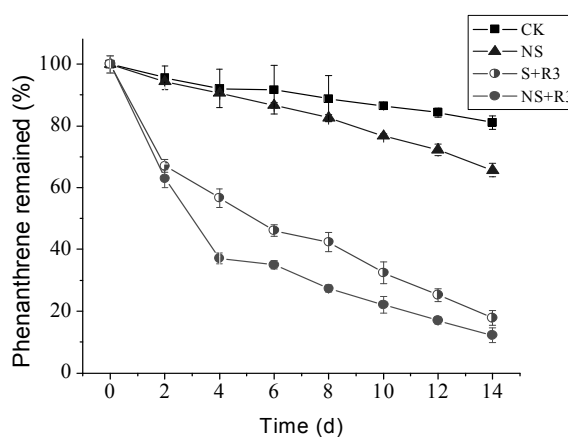
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### ABSTRACT

*Sesuvium portulacastrum* Linn., a halophyte has been used in constructed eco-floating bed system (CEFBS) for phytoremediation of polluted water in Yundang Lagoon (a saline lagoon in Xiamen City, southeast China). A bacterium strain (R3) which was identified as *Novosphingobium resinovorum*, with the capability to degrade 82.94% phenanthrene (initial conc. 100 ppm) within 7 days, was isolated from the rhizosphere of *S. portulacastrum* planted on the CEFBS in Yundang Lagoon.

To investigate the rhizodegradation capacity of *S. portulacastrum* on PAHs with R3 reintroduced into the plant root system, a well designed microcosm approach was carried out, in which 4 treatments (i.e., indigenous rhizosphere bacteria-removed plant, CK; indigenous rhizosphere bacteria-removed plant with R3, S+R3; indigenous rhizosphere bacteria-unremoved plant, NS; indigenous rhizosphere bacteria-unremoved plant with R3, NS+R3; ) with 3 replicates were set. The result showed that the phenanthrene (initial conc. 20 ppm) reduction ratio in each treatment was 18.9%, 82.2%, 34.3% and 87.8% for CK, S+R3, NS and NS+R3, respectively. PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis) and ETSA (Electron transport system activity) analysis indicated that R3 dominated the rhizosphere bacterial community and played the most important role in the process of phenanthrene rhizodegradation.



Although the metabolism of PAHs rhizodegradation hasn't been uncovered, our study suggested that co-metabolism may be a plant-microbe interaction important to rhizoremediation.

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## SELENIUM PHYTOTOXICITY IN CORN (*ZEA MAYS* L.): POTENTIAL ROLE OF MICROBES TO ALLEVIATE Se TOXICITY IN CEREAL CROP

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### ABSTRACT

Selenium is an essential micronutrient element for bacteria, animals, humans and for some lower plants. Although, Se is not consider essential for growth of higher plant but it can be toxic for plants at higher concentrations. In this study, we tested phytotoxic effects of elevated levels of Se (3 mg kg<sup>-1</sup> of soil) on corn plant growth, biomass, soluble protein and enzymatic content. Microbial community in rhizosphere of plant can play an important role to tolerate the environmental stresses. Plants were inoculated with Se resistant bacterial strains YAK-1 (Genbankacc # JX203248, showed 99% homology to *Bacillus foraminis* strain) to observe the effects of inoculation on plant growth as well as on Se toxicity. Such higher concentration of Se in soil caused significant reduction in plant shoot length (33%) and leaf soluble protein contents (22%), and significant increase in leaf acid phosphatase activity (30%) compared to control plants. Strain YAK-1 exhibited high resistance against Se (up to 20 mg sodium selenite mL<sup>-1</sup> of LB media) and showed following plant growth promoting characteristics; mixed organic acids production, ammonia production and hydrogen cyanide (HCN) production. In the absence of Se, bacterial inoculation resulted in increases in plant shoot length, plant dry weight, leaf acid phosphatase activity and soluble protein content. When Se-treated plants were co-cultivated in the presence of bacteria, plant shoot length increased significantly compared to only Se-treated plants, but it was not more than control plants. High concentration of Se in soil showed toxic effects on corn growth and inoculation with Se resistant rhizospheric bacteria can possibly alleviate Se toxicity in corn.

**Keywords:** bacteria, selenium, heavy metal resistance, plant-microbe interaction







## **ECOSYSTEM SERVICES & ECORESTORATION**



## SOIL ECOSYSTEM SERVICES FOR THE EVALUATION OF A METAL PHYTOSTABILIZATION PROCESS ASSISTED WITH PGPBs

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### ABSTRACT

Heavy metals are toxic to most plants at high concentrations, reducing plant development and, hence, compromising the efficiency of phytostabilization processes. One emergent way to overcome these limitations is the addition of endophytic plant growth-promoting bacteria (PGPB), which can positively affect plant growth and physiological status by, for instance, enhancing root development and increasing plant metal tolerance. Most importantly, the goal of any soil remediation technology must be not only to remove the contaminants from the soil or to render them harmless but to restore soil health. In this respect, microbial properties have been reported as most valuable bioindicators of soil health. Likewise, the human-centered concept of ecosystem services can be used to group these properties, facilitating the interpretation and providing long-term phytostabilization monitoring programs with the required stability through time.

In this study, we attempted to evaluate (i) the effect of applying endophyte PGPBs individually and in consortium (ii) the plant ecotype specificity of these endophytes and (iii) the combined effect of assisting the process with an organic amendment in a soil phytostabilization strategy through the measurement of soil ecosystem services. First, endophytic bacteria were isolated from a pseudometallophyte *Festuca rubra* native from an abandoned Pb/Zn mine tailing located in northern Spain, in order to examine their plant growth-promoting traits (ACC deaminase, IAA synthesis, siderophore production, phosphate solubilization, salinity tolerance and metal tolerance). Then, endophytic bacteria showing the best-performing traits were used (as individuals and in consortium) in a microcosm phytostabilization experiment with two different ecotypes of *Festuca rubra* (native and non-native of the mine tailing). Besides, half of the pots were amended with cow slurry. At the end of the growth period, plant biomass and metal contents in plant and soil were measured. Additionally, the effect of all these treatments on soil ecosystem services such as carbon storage, nutrient cycling and biodiversity preservation was determined through a variety of microbial properties with potential as bioindicators of soil health (soil microbial biomass, enzyme activities and community level genetic profiles, respectively).

The results obtained suggested that inoculated endophytic bacteria can have the potential to influence plant development and recovery of soil health in phytostabilization processes. Finally, soil microbial properties can be used to assess not only soil health but also the abovementioned ecosystem services.

**Acknowledgements:** The financial support by the Spanish Ministry of Economy and Competitiveness (FITOMINA project, AGL2012-39715-C03-02) is greatly appreciated.



## ECOLOGICAL ASPECTS OF PLANT TRACE ELEMENT (HYPER)-ACCUMULATION – SELENIUM AS A CASE STUDY

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### ABSTRACT

Plants that accumulate toxic trace elements are widely used in phytoremediation and biofortification. Such plants also occur in nature in areas that contain elevated levels of trace elements, either naturally or due to human causes. Some plant species even hyperaccumulate toxic elements to levels several orders of magnitude above those in surrounding vegetation. The ecological implications of these high levels of toxic elements in plants are important to study, particularly before large-scale phytotechnological applications. So far, there have been relatively few such studies. Here, an overview is presented of our studies on ecological aspects of selenium (Se) accumulation in plants.

Selenium (Se) is essential for mammals and a beneficial nutrient for plants. It is also toxic at higher levels, due to its similarity to sulfur and replacement of sulfur in proteins. Plants readily accumulate and assimilate Se via S transporters and enzymes. Some species native to seleniferous soils can even hyperaccumulate Se up to to 1.5% of their dry weight. We have studied the effects of plant Se on interactions with herbivores, detritivores, pollinators, neighboring plants, and mutualistic and pathogenic microbes, using a combination of field surveys and laboratory experiments.

It was found that plant Se accumulation can profoundly affect ecological interactions (for a review see [1]). Plant Se accumulation (and likely volatilization) protects plants against a wide variety of pathogens and herbivores, owing to both deterrence and toxicity. In naturally seleniferous areas, however, specialist herbivores and pathogens are present that have evolved Se resistance and can exclusively utilize Se hyperaccumulators as food source. It was also found that hyperaccumulators may use Se for elemental allelopathy against Se-sensitive neighboring plants, since they enhance Se accumulation in neighbors. Se-tolerant plant neighbors, however, profit from their enhanced Se levels next to hyperaccumulators, owing to herbivore protection. Selenium readily accumulates in flowers and seeds, and does not deter floral visitors. Honey bees and bumble bees were found to collect Se-rich pollen and to accumulate Se in their tissues, warranting more investigation on health effects of floral Se on pollinators. Furthermore, Se hyperaccumulators and their surrounding seleniferous soil harbor a wide variety of Se-tolerant fungi and bacteria that may affect plant Se speciation, accumulation and tolerance.

These findings may be useful for phytoremediation and biofortification, not only of Se but also of other plant-accumulated toxic elements that may have similar ecological effects. The findings also have intrinsic scientific value, as they give insight into the importance of hyperaccumulators in their local ecosystems. In seleniferous ecosystems, Se hyperaccumulators may significantly affect local species composition, through their negative effects on Se-sensitive ecological partners and positive effects on Se-resistant partners. They may also facilitate Se movement in the food chain and Se cycling in the local ecosystem. Hyperaccumulators of other elements likely function similarly.

### References:

1. El-Mehdawi A.F., Pilon-Smits E.A.H. (2012) Ecological Aspects of Plant Selenium Hyperaccumulation. *Plant Biology* 14, 1-10.

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## PHYTOAVAILABILITY AND ECOLOGICAL RISK ASSOCIATED WITH CADMIUM IN AN ORGANIC MATTER RICH SOIL

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### ABSTRACT

Bioavailability is an important factor influencing the applicability of phytoextraction and the potential for risk at contaminated sites. Low contaminant bioavailability to plants leads to ineffective phytoextraction, while high bioavailability can lead to considerable plant uptake which in turn may lead to receptor exposure to that contaminant. When contaminant bioavailability is low, risk to receptors may be negligible due to decreased contaminant exposure from the plant-based food chain, and if exposure is decreased enough to eliminate risk, site remediation may not be necessary.

Cadmium (Cd) is a xenobiotic metal that can cause adverse health effects to humans, plants, and animals. Soil can become contaminated with Cd through the land application of sewage sludge products like compost, as at a site in Peterborough, Ontario, Canada. The concentration of Cd ( $\leq 21.4 \mu\text{g}\cdot\text{g}^{-1}$  Cd) present in the soil at the site exceeds the standard set by the Ontario Ministry of the Environment ( $1.2 \mu\text{g}\cdot\text{g}^{-1}$  Cd) and might therefore pose ecological risk. A phytoextraction feasibility study was conducted for this site using native and naturalized plant species (*Brassica juncea*, *Chenopodium album*, *Helianthus annuus*, *Poa compressa*) in a control treatment ( $<1.0 \mu\text{g}\cdot\text{g}^{-1}$  Cd), a low Cd treatment ( $5.0\pm 0.3 \mu\text{g}\cdot\text{g}^{-1}$  Cd), and a high Cd treatment ( $16.5\pm 1.2 \mu\text{g}\cdot\text{g}^{-1}$  Cd) using soil collected from the site. None of the plants had a bioaccumulation factor  $\geq 1$  after 50 days of exposure and only *B. juncea* had a detectable concentration of Cd in its shoots ( $\leq 1.79 \mu\text{g}\cdot\text{g}^{-1}$  Cd). Low Cd phytoavailability was determined to be due to the high soil organic matter content of the soil. If receptor exposure is low enough to eliminate risk, then remediation is not necessary. Therefore, an ecological risk assessment was conducted to determine the potential for risk to ecological receptors. Data for receptor exposure to Cd from plants was taken from the phytoextraction feasibility study, and a worm bioavailability study was conducted to determine exposure from soil invertebrates. Worms (*Eisenia fetida*) had mean bioaccumulation factors of 6 and 3 in the low and high Cd treatments, respectively. The hazard quotient (HQ) for all seven receptors (*Scolopax minor*, *Turdus migratorius*, *Blarina brevicauda*, *Microtus pennsylvanicus*, *Sylvilagus floridanus*, *Procyon lotor*, and *Peromyscus maniculatus*) was  $<1.0$ , indicating no potential risk to any of these groups.

The results of this study demonstrate the advantages of conducting a risk assessment when phytoextraction is found to be ineffective at a contaminated site due to low contaminant bioavailability, as receptor exposure might be sufficiently low to eliminate ecological risk, and therefore the necessity to remediate. The results of this risk assessment are contrasted to those at another site in Ontario, where soil contaminated with PCBs exhibited high bioavailability to *Cucurbita pepo* in a phytoextraction feasibility study, and the results of an ecological risk assessment indicate that phytoextraction would result in risk ( $\text{HQ} > 1$ ) to all receptor groups evaluated.

*Acknowledgements:* NSERC CRD Grant to Drs. Barbara Zeeb and Allison Rutter



## NATURALLY SELENIFEROUS SOILS: POTENTIAL SOURCE OF SELENIUM FOR BIOFORTIFIED FOOD CROPS

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### ABSTRACT

Selenium (Se) is an essential micronutrient for human beings and considered an important preventative agent against different cancers, AIDS, and cardiac- and immune system-related disorders. Worldwide, Se deficiency is much more common than Se toxicity. Selenium-accumulating crop plants may be used to extract Se from seleniferous soils and to provide dietary adequate Se levels (55µg/day) in low-Se areas.

In this study Se accumulator Indian mustard (*Brassica juncea*) was tested for its ability to extract Se from naturally seleniferous shale rock-derived soil collected from Fort Collins, CO, USA. Two bacterial consortia, C1 and C2 (four highly Se-tolerant *Bacillus* strains in each, isolated from soil) were tested for their capacity to promote plant growth, Se accumulation, non-protein thiols in plants, photosynthesis rate, water use efficiency and stomatal conductance.

The Se concentration in the *B. juncea* plants was very high (711 mg kg<sup>-1</sup> DW in leaves and 358 mg Se kg<sup>-1</sup> DW in seeds). Plants inoculated with Se-resistant PGPR consortia C1 and C2 overall stimulated plant growth in this seleniferous soil. Plants inoculated with the C1 consortium showed significantly increased dry biomass, number of leaves, main inflorescence node length and seed weight in comparison to control plants. The C1-inoculated plants showed increased levels of non-protein thiols (antioxidants), enhanced photosynthesis and reduced stomatal conductance. Plants inoculated with C2 exhibited significantly enhanced non protein thiols, significantly reduced photosynthesis and reduced stomatal conductance compared to control plants. Selenium levels in the pod, leaf and seed tissue were enhanced in C1-inoculated plants and reduced in C2-inoculated plants, compared to control plants.

In conclusion, *B. juncea* may be used to extract Se from seleniferous soil for the production of Se-fortified plant material, and inoculation with bacterial consortium C1 further enhances the efficiency of this process.

**Keywords:** *Bacillus* spp., selenium, ICP, non protein thiols, photosynthesis



## PHYTOTECHNOLOGIES AND ECORESTORATION IN AGRICULTURAL MATRICES OF NEW ZEALAND

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### ABSTRACT

This paper evaluates the integration of phytotechnologies and ecological restoration to mediate the environmental footprint of agriculture in three contrasting landscapes in South Island, New Zealand. The agricultural matrix that accounts for 50% of the country's land area has occurred through its conversion from native vegetation cover in little more than the past 200 years. Earlier vegetation clearance and burning by Polynesians was followed by European introduction of ungulate grazing, cultivation, new plant species, fertilisation and irrigation. Within a relatively short time period, the original soil template that supported native species has been and continues to be profoundly modified. Restoration of native species on these soils presents new and different challenges both for plants and ecologists.

The aim of the research described in this paper is to understand the role played by biodiversity in soil biogeochemistry, rather than vice versa. Firstly, it is described how soil physico-chemistry has been modified through agricultural practices in both lowland and montane environments, with a focus on major nutrients, key trace element deficiencies and soil biota. Experimental results are then shown of how plant litters and rhizospheres of native plants substantially differ from forage crops in their modification of soil hydrology, pH, soluble and gaseous nitrogen and trace element dynamics. A significant interplay between soil biota and native plant rhizospheres also modifies soil biogeochemistry. Beneficial effects are demonstrated of native plants on marginal land, including riparian zones, hedgerows and paddock fence lines. Case studies are described that include a conversion of plantation forestry to intensive irrigated dairy, restoration of a coastal sand plain forest impacted by mining and agriculture, and an upland sheep farm.

The findings shows that selection of plant traits from New Zealand's unique biodiversity palette provides an opportunity to use phytotechnology to resolve some of the environmental constraints currently impacting agriculture, thereby future-proofing and adding value to agricultural production systems. This is introducing a new paradigm of incorporation of nature conservation into mainstream farm planning, in a country where more than 80% of the native flora and fauna of New Zealand is endemic and found nowhere else. Restoration of biodiversity into intensive agricultural systems may contribute advantageously to a primary industry with a branding based on its clean and green credentials.



## DECREASE IN SOIL ORGANIC MATTER FROM PALAEO TO PRESENT DAY SOILS IN SOUTH AFRICA – A CHALLENGE FOR PHYTOREMEDIATION

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### ABSTRACT

The recent geology of the last 65 Ma is known as the Cenozoic era which is subdivided into the quaternary and tertiary periods. The soils of the Cenozoic era contain the nutrients necessary to sustain flora, fauna and human life. Carbon, known as an indicator element in ecological and soil qualities, is present in the A-horizons of both present day and palaeo soils. The soil organic matter (SOM) content benchmarks the environmental status of that point in time when the specific horizon was exposed.

The objectives of this study were a) to compare contemporary and palaeo climatic conditions with geological deposits dated and characterized by means of C<sup>14</sup>, pollen and other methods, b) to determine the ecosystem health and status of different geological epochs, c) to use this data to predict future scenarios, and d) to discuss the use of long-term eco-management systems such as phytoremediation and precision farming in order to secure soil carbon.

Data for this study were obtained from the SOM content of different palaeo soil horizons exposed in the Florisbad Holocene deposit in South Africa. Sedimentary layers associated with varying climatic conditions of the last 400,000 years are represented at this site. These different layers were sampled and analysed for SOM by means of the Loss on Ignition (LOI) method. Data from soil organic matter analyses, together with pollen indicators and other dating information on the Holocene deposits were correlated with data from SOM analyses of the A-horizon of the current soils found in the same climatic zone and geological province. Results indicated that the SOM percentage in the present day A-horizon is lower than that found in the majority of the palaeo soils.

The palaeo deposits have a depth of 4.5 m and consist of five palaeo sedimentary layers, with the first as the top layer. Relative to the present day A-horizon in the area (not palaeo deposits) was used as a bench mark, a higher ecosystem functionality of 9.77% SOM was obtained for layer 1, 18.83% SOM for layer 2, 66.96% SOM for layer 3, and 16.87% SOM for layer 4. The bottom (fifth) layer had the lowest ecosystem functionality with a decreased value of 43.69% SOM relative to the present day A-horizon. The carbon sink in the present day soil surface is therefore lower than that found in the past. These decreased levels of carbon in the A-horizon may be due to agricultural activity, deforestation, overgrazing and/or adverse climatic changes.

To conclude: Based on these findings, phytoremediation could play an important role in ensuring soil carbon sequestration in disturbed areas (such as barren crop fields) or in anticipated global warming events. The negative impact of climate change in South Africa could be delayed by means of eco-restoration, re-forestation and habitat creation; this would facilitate an increase in SOM in present day A-horizons as well as improve the ecosystem functionality and quality of soils.

*Keywords:* cenozoic deposits, soil organic matter, palaeo soil horizons, carbon sequestration, phytoremediation programs

*Acknowledgements:* Financial support from the Technology and Human Resources for Industry Programme (THRIP) of the National Research Foundation (NRF) [South Africa] as well as from AGES (Potchefstroom) is greatly appreciated.



## PLANT ESTABLISHMENT, COMMUNITY STABILIZATION, AND ECOSYSTEM DEVELOPMENT ON OILS SANDS SOFT TAILINGS

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### ABSTRACT

The excavation of bitumen from the oil sands regions of western Canada has a disruptive influence on the natural ecosystems of the region and creates a significant challenge for reclamation efforts. In particular, the removal of the naturally occurring peatlands represents a loss of one of the world's largest carbon sinks. Working cooperatively with a major oil sands company, a project is underway to evaluate strategies for re-establishing peatlands on soft tailings from the oil sands mining. Sandhill Watershed is a 50 ha artificial wetland/upland complex north of Fort McMurray, AB. Within this watershed, a 17 ha area represents a constructed wetland where a layer of stockpiled peat has been established over the high saline oil sand soft tailings. The restoration trajectory of this fen complex is being monitored to evaluate the establishment and early performance of select native plant species established in the fen in comparison to plants growing naturally at reference sites.

Arrays of 20 native species were planted in plots in the fen complex in 2012 and 2013 while select sedge and grass species were planted throughout the remainder of the wetland subsection of the fen. Physiological performance of a subset of four plant species in the plots was evaluated in the summer of 2013. The four species evaluated were water sedge (*Carex aquatilis*), black-girdle bulrush (*Scirpus atrocinctus*), seaside arrowgrass (*Triglochin maritima*), and bog birch (*Betula glandulifera*). Physiological performance was assessed using a CI-340 portable photosynthesis system to quantify parameters such as net photosynthesis, evapotranspiration, and water use efficiency. Pore water was also sampled within each plot at depth intervals through the peat layer to the tailing interface to examine the vertical migration of base cations (particularly Na) in the wetland. The hydrological characteristics of the wetland plots were also determined and the plots grouped in categories based on the common soil moisture regimes. Comparable measurements were taken on plants of the same species growing at nearby reference sites.

Significant differences in physiological performance parameters were observed within each species in response to the moisture profiles across the plots. Parameters such as net photosynthesis were generally significantly different between the plots with consistent moisture and those that were drier (e.g., Fig. 1). Surprisingly, the range of data values obtained for the four species on the wetland plots were similar to the range observed for the comparable plants growing at the natural sites in the area. These results suggest that even under the worst moisture regime, the wetland fen plants were still physiologically active and that activity was comparable to plants at the benchmark sites.

This effort is one of only a few peatland reclamation efforts and one of the first to attempt to create a peatland directly on soft tailings. Additional monitoring is planned for the summer of 2014. This monitoring will examine plants established in 2012 and assessed in 2013 as well the new plants established in 2013. In addition, data will be collected on net productivity and organic matter production to assess how the physiological performance results relate to biomass production and organic matter accumulation. The results are expected to provide the oil sands industry with information on the trajectory of this reclamation and to provide information useful in planning and implementing future wetland fen reclamation efforts.

**Acknowledgments:** The financial support for this research provided through a grant from Syncrude Canada is greatly appreciated. We are also very thankful for logistic support from Jessica Clarke and Carla Wytrykush (Syncrude Canada).

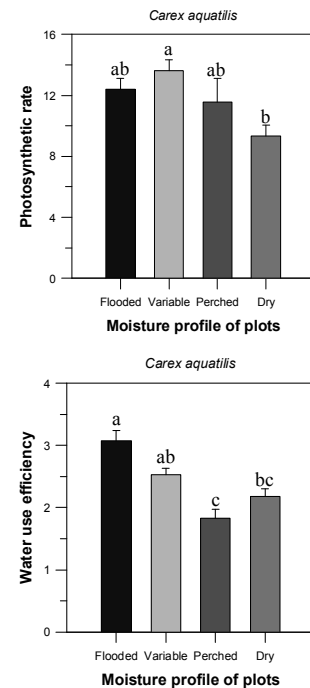


Fig. 1. Net photosynthesis rate and water use efficiency for water sedge (*C. aquatilis*) plants in the Sandhill fen wetland complex in response to the four moisture profiles across the plots.



## REVEGETATION OF MUCK DUMPING SITES IN INDIAN HIMALAYAN REGION

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### ABSTRACT

Eco-restoration of the muck dumping sites in command area of a hydro-electric power generation project is a matter of serious concern from ecological and social points of view. Realizing the importance of the technological interventions under such situation, CSIR-IHBT took up revegetation of 10 closed dumping sites (DS) of National Hydro-electric Power Corporation's (NHPC) Parbati Hydroelectric Project Phase-II in Kullu district, Himachal Pradesh, India, in 2010. These dumping sites are located at 1400 to 2220 m amsl covering about 9 ha. Owing to the steep slope of the dumping sites, a network of retaining walls made of stacked stone-filled gabions tied together with mild steel wire-net was made throughout the dumping sites for their stabilization. Considering the agro-climatic conditions of the dumping sites 11 tree species were selected for plantation. The saplings of the tree species were planted in gunny bags placed in pits at the sites containing garden soil, organic manure and peat moss. The consortium of selected plant growth promoting rhizo-bacteria (PGPR) formulated by the Institute was applied at collar level of the tree saplings. After plantation, pelleted seeds of potential herbs and shrubs were also spread on the surface of the dumping sites. About 1 year after planting, observations were recorded on rate of establishment of the saplings at each dumping site. The rate of survival ranged from 69.2 % (at DS-01) to 96.6 % (at DS-13b). The average survival rate was 85.4 %. Higher establishment and height was attained by *Robinia pseudoacacia* L.





## **PLANT-NANOPARTICLE INTERACTIONS**





## INVESTIGATING POSITIVE AND NEGATIVE IMPACTS OF ENGINEERED NANOPARTICLES ON PLANT GROWTH AND DEVELOPMENT

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### ABSTRACT

There are an increasing number of engineered nanomaterials emerging in the international market and a wider range of potential applications for those nanomaterials. Nanomaterials are being included in a variety of consumer products as well as industrial and agricultural chemicals in order to improve the stability and properties of the formulations and to improve their efficacy of those products and chemicals. Multiple benefits for such nano-enhanced products have been proposed. There are also concerns that broader use of nanomaterials, coupled with accidental spills and discharges into industrial and municipal waste streams, may facilitate the uncontrolled release of such materials into the environment. Such concerns have been magnified by studies showing adverse effects of some nanomaterials on plants and animals. In light of the expected growth of the nanotechnology industry, there is a pressing need to evaluate both positive and negative impacts of nanomaterials on living organisms. Collaborative research currently underway is examining the direct impacts of select nanomaterials on agricultural and food plants, as well as indirect impacts that may be caused by such materials.

Research on the potential positive effects of nanomaterials on plants have focused on potential benefits to TiO<sub>2</sub> and SiO<sub>2</sub> to the growth and yield of agronomic crops such as maize and soy as well as horticultural applications to promote plant establishment and reduce failure to pathogen infection during propagation. In the former case, studies have been conducted that examined the impact of foliar TiO<sub>2</sub> application to examine the physiological effect on photosynthesis and the repercussions for seed yield and seed quality. Studies to date have demonstrated a transient increase in photosynthesis in corn and soy in response to some foliar treatment strategies. Yield analysis is currently underway to assess the impact on seed production and the commercial characteristics of those seeds (e.g., oil and protein percentage). Similarly, plant propagation studies using wandering jew have shown that some nanomaterials reduce rooting of plant cuttings while other nanomaterials have no adverse effect. The effect of nanomaterial pretreatment on rooting, plant establishment, and pathogen resistance are being evaluated.

Research is also examining the adverse effects of nanomaterials, either directly to plants or indirectly to animals that might consume plants that have come in contact with nanomaterials. Experiments underway with an array of food plants are examining the phytotoxicity of nanomaterials. Recent results, for example, from root vegetables such as carrot and parsnip have shown that exposure to increasing concentrations of CuO or ZnO interfere with plant water relations (Fig. 1). Additional studies are examining the impact of nanomaterial exposure on root plasma membrane integrity and leakage (e.g., Fig. 2). Tissues from these and additional studies are being used in a physiologically-based extraction test to assess the release of the nanomaterials during human digestion to estimate the potential exposures that might result from consumption of such plant foods. An additional line of research is examining the interaction between specific nanomaterials and other soil contaminants (e.g., heavy metals). In particular, efforts are focusing on possible synergistic phytotoxicity of nanomaterials and heavy metals to plants. Additional efforts are focused on determining the extent to which nanomaterials increase the accumulation of metals and vice versa and the mechanism of interaction. The implications of the interaction between nanomaterials and heavy metals for dietary exposure to these compounds will also be assessed.

**Acknowledgments:** Financial support for this research was provided through USDA Grant 2011-06426.

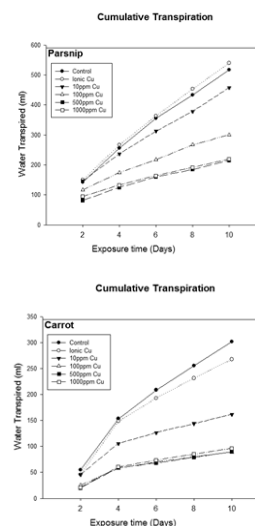


Fig. 1: Inhibition of evapotranspiration by carrot and parsnip in response to CuO.

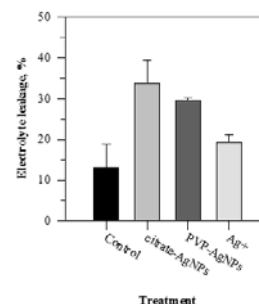


Fig. 2: Electrolyte leakage from lettuce roots after 60 days of watering with AgNPs or Ag<sup>+</sup>.



## PHYTOTOXICITY OF MULTIWALL CARBON NANOTUBES AND IMPACT ON THE UPTAKE OF COEXISTENT PESTICIDES BY LETTUCE (*LACTUCA SATIVA* L.)

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### ABSTRACT

The field of nanotechnology is projected to achieve a global market value of \$3 trillion by 2020, with engineered nanomaterials (NM) being currently used in a wide variety of commercially available products; including medical devices, plastics, textiles, cosmetics, electronics and agrichemicals. Carbon NM such as C<sub>60</sub>/C<sub>70</sub> fullerenes, single/multiwall carbon nanotubes (S/MWCNT), have been the focus of much interest with regard to agricultural applications, although the scientific literature reporting their phytotoxic effects is rather thin and conflicted. Besides, given CNT chemical and physical properties, interactions with coexisting organic chemicals can be anticipated. In this research study, the effect of non-functionalized and amino-functionalized multiwall carbon nanotube (CNT) (NF-MWCNT, NH<sub>2</sub>-MWCNT) exposure, as well as the impact of CNT presence on coexistent pesticide accumulation, was investigated in lettuce (*Lactuca sativa* L. cv. Green Grand Rapids). Lettuce seeds were sown directly into CNT-amended vermiculite (1000 mg L<sup>-1</sup>) to monitor phytotoxicity during germination and growth. During growth, lettuce seedlings were subsequently exposed to chlordane (cis-chlordane, trans-chlordane, trans-nonachlor) and *p,p'*-DDE (all at 100 ng/L) in the irrigation solution for a 19-d growth period. CNT exposure did not significantly influence seed germination (82-96%) or plant growth. Similarly, pesticide exposure had no impact on plant growth, total pigment production or tissue lipid peroxidation. After 19 d, the root content of total chlordane and *p,p'*-DDE was 390 and 73.8 µg g<sup>-1</sup>, respectively, in plants not exposed to CNTs; the shoot levels were 1.58 and 0.40 µg g<sup>-1</sup>, respectively. The presence and type of CNT significantly influenced pesticide availability to lettuce seedlings. Non-functionalized CNT decreased the root and shoot pesticide content by 88% and 78%, respectively, but amino-functionalized CNT effects were significantly more modest, with decreases of 57% in the roots and 23% in the shoots, respectively. The presence of humic acid completely reversed the reduced accumulation of pesticides induced by amino-functionalized CNT, likely due to strong competition over adsorption sites on the NM. These findings have implications for food safety and for the use of engineered NM in agriculture, especially with leafy vegetables.

**Keywords:** NF-MWCNT, NH<sub>2</sub>-MWCNT, pesticides, uptake, lettuce

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## TOXICOGENOMICS OF CdS QDs INTERACTIONS WITH *ARABIDOPSIS THALIANA*

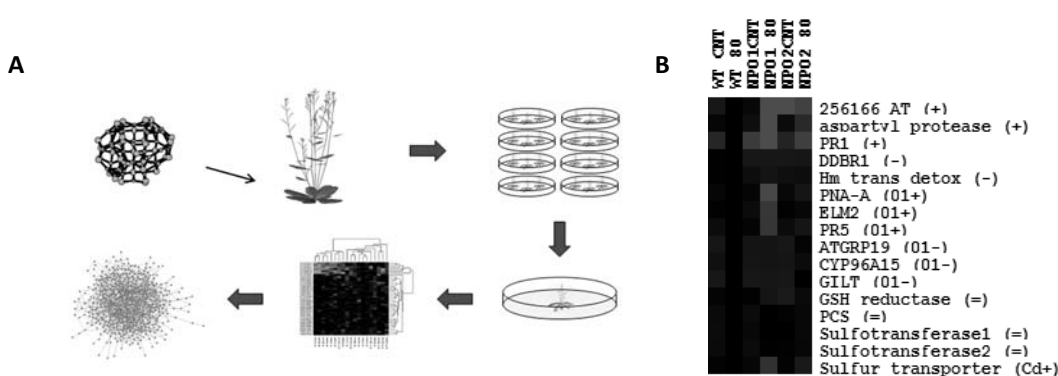
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### ABSTRACT

Nanotechnology is a rapidly growing industry and engineered nanomaterials (NMs) are applied in several areas such as electronics, biomedicine, pharmaceuticals, cosmetics, food production. The scarce knowledge about uptake, interaction with cells, toxicity, is hindering the full application of NMs, as shown by recent decisions of the European Commission and European Food Safety Authority. The aim of this work was to develop a toxicogenomics approach for risk assessment of NMs, focusing on quantum dots cadmium sulfide nanoparticles (CdS QDs) using *Arabidopsis thaliana* (L.) Heynh as model system. Two mutant lines of *Arabidopsis* have been selected as resistant to lethal concentrations of CdS QDs, and the phenotypes and genotypes have been characterized. The global gene expression profile in the two mutants have been analyzed using Affymetrix GeneChip *Arabidopsis* ATH1Genome Array, showing differences in the panel of induced and repressed genes not only among the mutants and the wild type, but also between the two mutants. Genes of particular interest in both mutants identified with the transcriptomic approach were validated by Real Time PCR. The results obtained from the two approaches (mutant-based and whole-genome) in plant could offer a possible hypothesis concerning the tolerance/resistance mechanism in which the CdS QDs are involved. Furthermore results found suggested that CdS QDs and Cd<sup>2+</sup> could exploit different pathways of tolerance/resistance. It was also observed a possible epistatic activity by which the up- or down-regulation of one or few gene could lead to a cascade of other genes influenced by the CdS treatments. The use of model organisms might offer new strategies for the risk assessment of NMs within the frame of environment and health. Therefore, plant-based model systems are able to provide information about genetic and physiologic targets of broad interest also in other organisms related to the risks posed by the exposition and contact with NMs. Our approach, which merges the classical top-down (from mutant phenotype to genotype) and bottom-up (from gene to function) approaches, allows to shed light on function and functionality of our targets: from observing phenotype to knowing what lays genetically behind that phenotype.



**A.** experimental set-up and result; **B.** RT qPCR heatmap resulted from validation of the genes isolated in microarray experiments.



## PLANT UPTAKE AND TRANSLOCATION OF RADIOACTIVELY LABELLED TiO<sub>2</sub> AND CeO<sub>2</sub> NANOPARTICLES

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### ABSTRACT

Nanotechnology and nanomaterials have been widely applied in the technological and consumer products during the last years. The addition of nanoparticles significantly improves quality of the many consumer products. On the other hand the fate of these nanoparticles in the environment after use is not well documented and their health risks are not clear. The fundamental problem of nanoparticles in the environment is the possible entry into a food chains of humans and animals preferably by the contamination of plant food and water sources. The investigation of nanoparticles plant uptake and translocation is complicated by the presence of naturally occurring nanoparticles which cannot be reliably distinguished from naturally occurring ones. For these purpose it seems to be unique the use of radioactively labelled nanoparticles for plant uptake studies. In these experiments the determined radioactivity originating from the tested nanoparticles can be measured unambiguously and with high sensitivity. The method is especially useful for the rapid testing of uptake and translocation of nanoparticles by agriculturally interesting plants.

The TiO<sub>2</sub> and CeO<sub>2</sub> nanoparticles labelled with <sup>48</sup>V and <sup>139</sup>Ce were prepared by direct cyclotron activation of corresponding nanomaterials using a Scanditronix MC-40 cyclotron as described previously. Phytoextraction of these labelled nanoparticles was tested using *in vitro* cultures of common cultivated plants (*Zea mays*, *Helianthus annuus*, *Cucumis sativus*). The nanoparticles were added to the 2-3 weeks old sterile seedlings cultures cultivated on half-strength Murashige-Skoog medium. After next 7 days the resulting plants were removed, root system gently washed with distilled water and the radioactivity measured using electronic autoradiography.

The results show that both used nanoparticles are adsorbed and/or absorbed by the root system and some translocation was observed using electronic autoradiography. Although about 99 % of radioactivity was typically found in the root area, the remaining activity was distributed between stem and leaf parts. This fact uniquely determined possibility of contamination of plants with nanoparticle material.

It can be concluded, that the nanoparticles can contaminate food chains and the health risk of these contamination must be evaluated. In these simple methodics we cannot yet distinguish if the contamination is caused with true nanoparticles or if the measured radioactivity is from dissolved ionic forms which could be produced by the interaction of the nanoparticles with some acidic compounds in plant tissues. Experiments with electron microscopy cannot distinguish between naturally occurring and artificial nanoparticles. For these purposes the advanced double labelled nanoparticles will be prepared and used in uptake experiments.

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## INTERACTION BETWEEN METALLIC NPs AND SALT MARSH PLANTS: IMPLICATIONS FOR PHYTOREMEDIATION

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### ABSTRACT

Increased use of metallic nanoparticles (NPs) raises the probability of these NPs to reach the environment. Health and environmental effects of common metals are well-known, however, when metals take the form of NPs, consequential hazards based on shape and size are yet to be explored. Although a lot of information exists regarding plants interaction with metals there is a lack of information among metallic NPs interaction with plants, including with salt marsh plants.

This work aims to study the interactions among a Cu metallic NPs (CuO) and the salt marsh plants *Halimione portulacoides* and *Phragmites australis* comparing with the interactions observed when the selected metal is in its ionic form. In addition, it aims to evaluate the potential of these plants for the phytoremediation of these NPs. The chosen plants have shown to uptake considerable amount of Cu when in its ionic form.

Experiments were conducted with sediment elutriate, a simplified natural medium, being the plant expose for 6 days to medium contaminated either with CuO or with Cu (II). To evaluate metal uptake, total metal concentrations were determined by atomic absorption spectroscopy in plant tissues at the beginning and at the end of the experiments.

Results indicate that *H. portulacoides* and *P. australis* had the ability to accumulate Cu in its roots, being the accumulation, respectively, 10 and 4 times lower when the metal was in the nanoparticle form. Regarding *H. portulacoides* no metal translocation was observed when NPs were added to the medium. But for *P. australis* metal translocation occur when the metal was added either in ionic or in NP form, indicating that interactions between plants and NP differ with the plant species.

These facts need to be taken in consideration when applying these plants for the phytoremediation of contaminated sediments.

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## CO-EXPOSURE TO ENGINEERED NANOPARTICLES ALTERS THE TOXICITY AND ACCUMULATION OF PERSISTENT PESTICIDES IN AGRICULTURAL CROPS

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### ABSTRACT

Although the use of engineered nanomaterials (NM) has increased, the fate and effects of these substances in the environment is poorly understood. As an emerging class of contaminants, there is significant potential for NM interactions with co-existing chemicals. Direct molecular interactions between NM and co-contaminants may change the availability and activity of either or both molecules in soils and water. In addition, NM may alter the ability of biota such as plants to tolerate or bioaccumulate co-existing chemicals. This uncharacterized level of co-contaminant interactions may be of particular concern in agricultural systems, where food chain contamination and subsequent human exposure may occur.

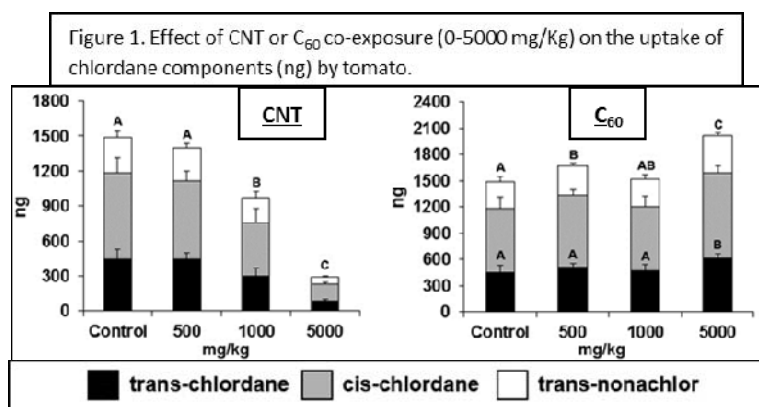
A number of studies have been initiated in which the impact of NM co-exposure on the toxicity and accumulation of persistent pesticides by plants has been evaluated. Exposure systems include both model media (vermiculite) with freshly added contaminants and soils with field-weathered pesticide residues. In one set of experiments, the accumulation of DDE by zucchini, soybean and tomato grown in fullerene (C<sub>60</sub>)-amended vermiculite increased by 30-65% compared to controls. In a second vermiculite-based trial, nonfunctionalized multi-wall carbon nanotubes (CNT) decreased the chlordane and DDE accumulation in lettuce by 83%, but amino-functionalized CNT effects were significantly more modest, with decreases of only 40%. Interestingly, humic acid completely reversed the reduced accumulation of pesticides induced by amino-functionalized CNT, likely due to strong competition over adsorption sites on the nanomaterial. However, in a third study Ag nanoparticles (NP) suppressed DDE uptake by zucchini and soybean grown in vermiculite. Interestingly, NP Ag resulted in greater suppression of DDE accumulation that did equivalent bulk or ion exposures and data suggests that Ag-mediated closure of aquaporins may be responsible for decreased uptake.

In soil with 0.20 mg/kg weathered DDE, C<sub>60</sub> co-exposure at 1000 mg/Kg on had little impact on residue accumulation by pumpkin. A second more comprehensive study involved corn, soybean, zucchini, and tomato being grown in soil containing weathered chlordane (2 mg/kg) and DDE (0.2 mg/kg) that was amended with 0, 500, 1000, or 5000 mg/Kg C<sub>60</sub> or CNT. CNT co-exposure consistently reduced chlordane and DDE accumulation by up to 80% in a

concentration dependent manner for all species, depending on plant type and CNT concentration. However, C<sub>60</sub> had species- and pesticide-specific effects on residue accumulation, ranging from complete suppression of DDE uptake (corn/tomato) to 35% increases in chlordane accumulation (tomato/soybean).

The finding that engineered nanomaterials can significantly alter the accumulation of pesticides in food crops may have significant implications for food safety, as well as for the movement of pesticides and other organic contaminants through the environment.

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## NANOPARTICLE EFFECT ON PHYSIOLOGY AND METABOLISMUS OF PLANTS

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### ABSTRACT

The use of nanoparticles (NPs) in commercial products and industrial applications has increased greatly in recent years although understanding of the interaction mechanisms at the molecular level between NPs and biological systems, is largely lacking.

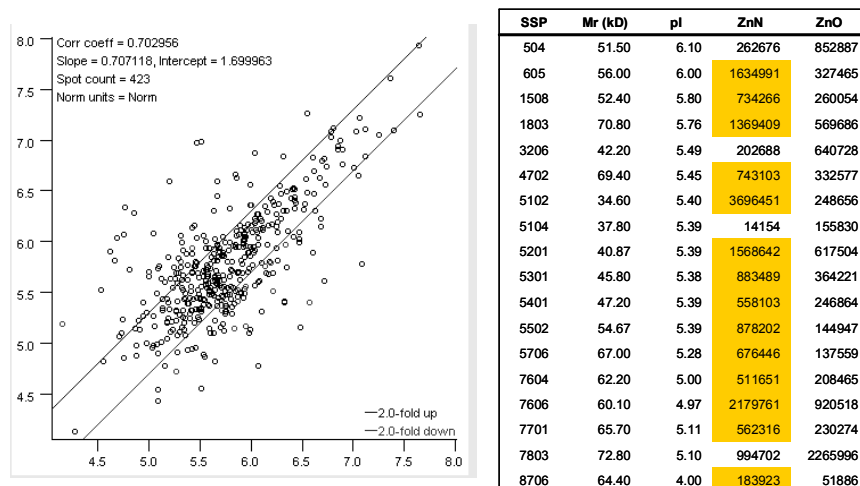
Our work is focused to the study of effect of nanoparticles to the higher plant metabolisms, both in laboratory and real conditions to elucidate potential of phytoremediation methodology for removing NPs from environment. In laboratory conditions nanoparticles of TiO<sub>2</sub>, ZnO<sub>2</sub>, AlO<sub>2</sub>, Fullerenes and Graphite fibers were tested using *Arabidopsis thaliana* as a model systems.

Generally, all nanoparticles decreases plant cells viability – the most toxic effect was found for ZnO<sub>2</sub>, where only 67% of starting viability was detected. All nanoparticles (with exception of graphite fibers) decreased the production of ethylene. Part of their unfavorable effects might be disturbance of defense pathways in tobacco cells, probably *via* disturbance of ion homeostasis.

All nanoparticles exhibit negative effect on cell division and stimulate various stress responses, e.g. antioxidant system and ethylene formation, while microarray data (1) confirm stimulation of antioxidant system as well as general stress response and down-regulation of genes related to cell division.

Proteomic study using DIGE methodology confirms overexpression of proteins, related to achieved microarray data.

Effect of selected nanoparticles to plant photosynthesis were studied too, preliminary data were achieved and will be discussed during presentation.



Effect of ZnO on *Arabidopsis thaliana* proteome

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- Landa, P., Vaňková, R., Andrlová, J., Hodek, J. Maršík, P., Štorchová, H., White, J. C. Vaněk, T. (2012) *Journal of Hazardous Materials* 241, 55-62

**Acknowledgements:** This work was supported by LH11047Myes and FR/TI3/778 project



## TROPHIC TRANSFER POTENTIAL OF RARE EARTH ELEMENT (REE) OXIDE NANOPARTICLES THROUGH TERRESTRIAL FOOD CHAINS

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### ABSTRACT

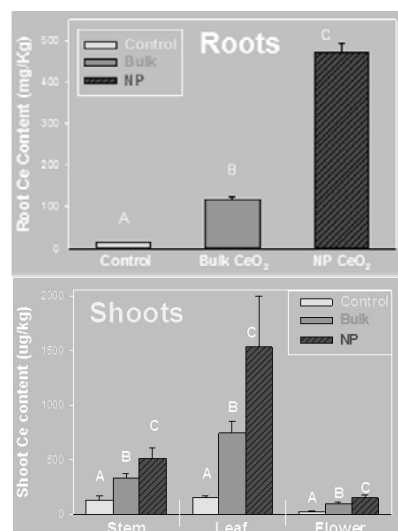
Although the use of engineered nanomaterials (NM) has increased dramatically, the fate and effects of these substances in the environment is poorly understood. Given that NM may represent an emerging class of contaminants, there is significant potential for NM interactions with terrestrial biota such as plants, as well as for bioaccumulation and biomagnification of these materials through food chains that may or may not include humans. In spite of this concern, very few studies have addressed the potential trophic transfer and biomagnification of NM in terrestrial environments. This lack of understanding on NM bioaccumulation may be of particular concern in agricultural systems, where food chain contamination and subsequent human exposure may occur.

Studies have been initiated in which 0 or 1000 mg/kg bulk or nanoparticle (NP) cerium oxide (CeO<sub>2</sub>) or lanthanum oxide (La<sub>2</sub>O<sub>3</sub>) was added to an agricultural loam. Zucchini was then grown in the cerium-amended or control soils for approximately 28 days; lettuce was grown in the lanthanum oxide-amended soils for 40 d. At harvest, the element content of the root and shoot tissue was determined by ICP-MS. Fresh shoot tissue that had been exposed to 0 or 1000 mg/kg bulk or NP CeO<sub>2</sub> or La<sub>2</sub>O<sub>3</sub> was used to feed herbivorous cricket populations for 14-21 days. Select cricket tissues were digested and the element content was determined by ICP-MS. Live crickets that had consumed zucchini or lettuce shoots exposed to 0 or 1000 mg/kg bulk or NP REE metal oxides were then used to feed carnivorous mantid or wolf spider populations. The Ce content of the carnivores was also determined by ICP-MS. Scanning/Transmission electron microscopy with energy dispersive X-ray spectroscopy will be conducted on select plant and animal tissues.

Zucchini plants exposed to NP CeO<sub>2</sub> had significantly greater Ce content than plants exposed to the bulk metal oxide (figure). Root Ce concentrations were 3.9 times greater in the NP-exposure; notably, all root tissues were rinsed with dilute HNO<sub>3</sub> to remove surface retained ceria prior to actual digestion and analysis for absorbed element. This data represents one of the few reports showing enhanced NP accumulation by plants under soil-grown conditions as compared to an equivalent bulk material. Crickets consuming NP-contaminated leaf tissue had approximately 20.0 ng/g Ce whereas those consuming bulk-contaminated tissue only had 10.3 ng/g. Although these levels are 10-100 times below the leaf tissue levels, the data suggest that trophic transfer does occur and that it may be particle size specific. Interestingly, cricket feces collected from insects consuming bulk and NP-contaminated leaves approached 240 and 650 ng/g, respectively, and increased over time. This suggests that significant Ce may enter the insect but that elimination may be effective. Although only limited mantid data has been obtained, individuals consuming bulk and NP-exposed crickets contained 3.2 and 7.6 ng/g, respectively. Again, these levels suggest trophic transfer, although not biomagnification. Interestingly, limited sampling of mantid feces showed Ce at levels up to 99 ng/g.

The finding that engineered NM are accumulated at significantly greater levels from soil than corresponding bulk materials and that particle-size specific trophic transfer may occur has significant implications for food safety, as well as for assessing the risk of NM released to the environment.

**Acknowledgements:** The financial support of USDA AFRI grant 2011-67006-30181 is greatly appreciated.





## IMPACT OF CERIUM OXIDE NANOPARTICLES ON THE PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF *BRASSICA RAPA* IN THREE GENERATIONS

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### ABSTRACT

The intrinsic ability of cerium oxide nanoparticles (CeO<sub>2</sub>-NPs) to form oxygen vacancy makes this material a popular component in a plethora of commercial products. Its application as a fuel additive in particular entails possible substantial release of this nanoparticle to the environment and warrants an intensive study on its environmental health and safety impact. Even though the short term impact of CeO<sub>2</sub>-NPs on the environmental health, including plant health has been evaluated, the long term, multi-generational impact of CeO<sub>2</sub>-NPs to plants is still unclear. The main aim of this study was to evaluate the potential impact of CeO<sub>2</sub>-NPs on a suite of physiological and biochemical processes of *Brassica rapa* after continuous irrigation of the plants with different concentrations of CeO<sub>2</sub>-NPs (0, 10, 100 and 1,000 mg/L) over three generations. The results showed that plants in the second and third generation displayed slower plant growth, smaller biomass, and fewer and smaller silique and seeds. Plants in the later generations also contained higher evidence of reactive oxygen species, specifically H<sub>2</sub>O<sub>2</sub>. An examination of the activities of three enzymatic antioxidants (SOD, CAT and GPX) involved in the generation and detoxification of H<sub>2</sub>O<sub>2</sub> indicated that the activities of these enzymes showed a generational pattern. Overall, the results implied that the second and third generations of plants experienced higher oxidative stress and the photosynthetic processes in later generations are more susceptible to the impact of CeO<sub>2</sub>-NPs than the first generation plants. This study provided the first evidence that that plant response to CeO<sub>2</sub>-NPs varied between different generations of plants and long term evaluation on the phytotoxicity of engineered nanomaterials to plants is critical.

**Keywords:** cerium oxide nanoparticle, oxidative stress, ROS, multigenerational impact



## GENE REGULATIONS AND ANTIOXIDANT ENZYME RESPONSES IN *ARABIDOPSIS THALIANA* TO NANOPARTICLE CERIUM AND INDIUM OXIDE EXPOSURE

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### ABSTRACT

Rare earth elements (REEs) nanoparticles (NPs) have been widely applied in various products such as polishing agents, cosmetics and catalyst. As the demands and use of nanoproducts increased annually, the released NPs could inevitably cause a series of environmental problems. However, the risk assessment of nanotoxicity in plants is still at the initial stage. In this study, the effects of cerium oxide (CeO<sub>2</sub>) and indium oxide (In<sub>2</sub>O<sub>3</sub>) NPs exposure on *Arabidopsis thaliana* were investigated. Our previous study has already demonstrated that CeO<sub>2</sub> NPs could induce several folds of MDA, an important indicator for plant's response to stresses. In order to fully understand defense system of *A. thaliana* to both NPs exposures, altered antioxidant enzyme activities, which are able to scavenge reactive oxygen species (ROS) induced by both NPs were measured at 250 and 1000 ppm treatments. Except superoxide dismutase (SOD), several antioxidant enzymes were highly induced to defend nanotoxicity. These antioxidant enzymes include catalase (CAT) and ascorbate peroxidase (APX), both of which mainly contribute to destructing ROS; phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD), which are involved to secondary metabolism in plants; glutathione S-transferase (GST) and glutathione reductase (GR), involved in sulfur assimilation and metabolism, were significantly induced at CeO<sub>2</sub> NPs exposure. At the molecular level, expression of genes central to the stress response such as the sulfur assimilation and glutathione (GSH) metabolic pathway was determined by qPCR. Meanwhile, expression of altered genes related to antioxidant enzymes, which have abilities of scavenging ROS, was also analyzed. Further experiments are currently underway to measure the ROS productions in response to CeO<sub>2</sub> NPs. DNA damages in *A. thaliana* caused by nanotoxicity is determined by analyzing the levels of 7,8-dihydro-8-oxoguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde). Last, but not the least, nutrient elements, especially cations, will be measured in order to indicate whether NPs could disrupt elements transportation at different exposure concentrations. These studies will be highly useful in understanding the fate, transport, and toxicity of manufactured NPs in the agricultural crops and to further develop strategies for mitigating the toxicity of these NPs in food crops.



## EVIDENCES OF GENOTOXICITY AND PHYTOTOXICITY IN ZEA MAYS AND HORDEUM VULGARE EXPOSED TO CeO<sub>2</sub> AND TiO<sub>2</sub> NANOPARTICLES

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### ABSTRACT

In recent years, remarkable progress has been made in developing nanotechnologies. This has been leading to a fast-growth of a commercial application which involves the use of a great variety of manufactured nanomaterials (NMs). So far, it is estimated that the research on the environmental health and safety of nanomaterials receives less than 5% of the funding spent to develop new nanomaterials [1]. There is a small but growing body of scientific studies showing that some NMs are toxic to commonly used environmental indicators such as algae, invertebrate and fish species. It was hypothesized that the plant uptake of NMs varies largely depending on the type of plant and the size and chemical composition of the NMs [2]. However, very few studies on NMs-plant interactions have been published, so far. For this reason there is great concern regarding potential NMs impact to agriculture and the food supply.

Potential early phytotoxic and genotoxic effects of CeO<sub>2</sub> and TiO<sub>2</sub> nanoparticles compared to bulk counterparts and control are currently investigated in barley (*Hordeum vulgare* 'Pilastro') and maize (*Zea mays* 'Synthesis') seeds/seedlings.

The early phytotoxic effects are measured by treatment of triplicate samples of 10 sterile seeds of barley and maize in Petri dishes with suspensions of CeO<sub>2</sub> (<50 nm) and TiO<sub>2</sub> (<25 nm) nanoparticles and the respective bulk counterparts at 0 (control), 500, 1000 and 2000 ppm for 7 days. At the end of treatment the relative seed germination inhibition, relative root growth inhibition and metal uptake by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in the roots and seeds with coleoptile are measured. Genotoxicity of nanoparticles are assessed by Randomly Amplified Polymorphism DNA (RAPDs) on seedlings previously germinated in sterile milli-Q water. Furthermore, TEM observations of root, seed and coleoptile tissues are performed. Mitotic index (MI) are measured in the same condition except for the exposure period which will be 24 hours.

The expected results will provide information about the potential early genotoxicity and phytotoxicity on CeO<sub>2</sub> and TiO<sub>2</sub> on two important crops.

### References:

1. Environ Toxicol Chem 2012 31, 3-14.
2. J Agric Food Chem 2011 59, 3485-3498

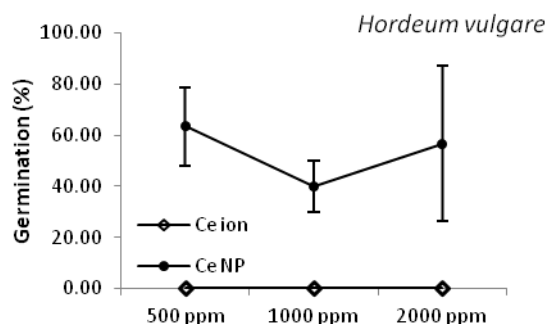


Figure 1: Germination percentage rate of barley seeds treated with Ce<sup>+</sup>/Ce NP.

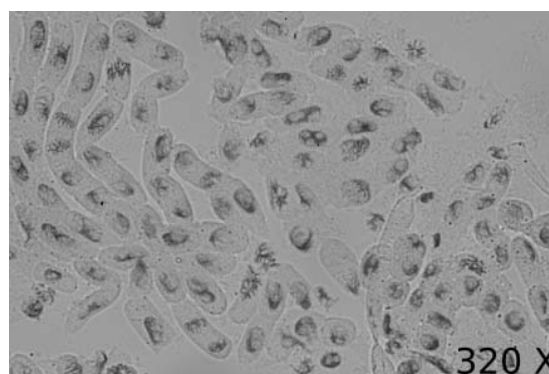


Figure 2: Meristematic cells of barley root tip treated with Ce NP 2000 ppm. In this picture there are 12 cells in mitotic phase.

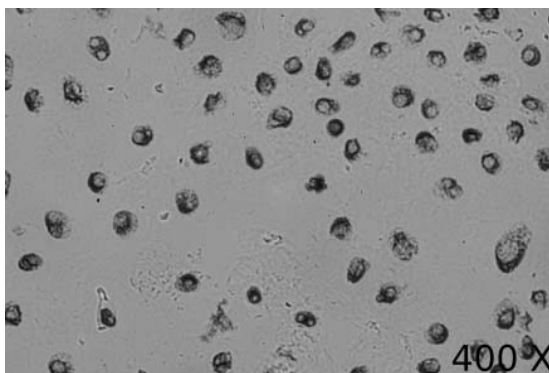


Figure 3: Meristematic cells of barley root tip treated with Ce<sup>+</sup> 2000 ppm. In this picture there are no cells in mitotic phase.



**BIOTISATION AND NANOTECHNOLOGY FOR PHYTOREMEDIATION**

**Turnau K.<sup>1,2</sup>, Ogar A.<sup>2</sup>, Wężowicz K.<sup>2</sup>, Plazuk E.<sup>2</sup>, Stojakowska A.<sup>3</sup>, Malarz J.<sup>3</sup>, Rozpadek P.<sup>2,4</sup>**

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**ABSTRACT**

The selection of the most efficient microbial consortia for the particular plant species grown in specific environmental conditions plays a key role in developing phytoremediation technologies. During recent years (within the project MAESTRO 2011/02/NZ9/137, financed by the National Science Centre, Poland) a wide variety of microbes were isolated from Polish flotation wastes containing high concentration of Zn and Pb. The collection of isolated microorganisms included bacteria and mycorrhizal fungi. They were tested using: *Verbascum* spp. *Helianthus annuus*, *Hieracium pilosella* and *Cichorium intybus* under greenhouse conditions. Differences in plant responses depended on plant species and fungal strains. For *Cichorium intybus* the most significant differences were found for quercetine and kaempferol derivatives. AM fungi have repeatedly been demonstrated to alleviate metal toxicity and reduce stress in plants. In the presence of arbuscular mycorrhiza a new antioxidant homeostasis is established. In chicory a decrease in SOD activity and an increase in the activity of catalase and peroxidase was observed, indicating the reduction in the production of superoxide. However, differences in the concentration of medically important compounds in M+ and M- chicory grown on polluted soil was not as evident. Fe nanoparticles and bentonite was added/supplemented into the substratum to verify their ability to improve *H. annuus* vitality.



## DIRECT TOXIC EFFECTS OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES ONTO MICROALGAL *CHLORELLA* SP.

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<sup>1</sup>School of Chemical Engineering, Universiti Sains Malaysia, Nibong Tebal, Penang 14300, Malaysia

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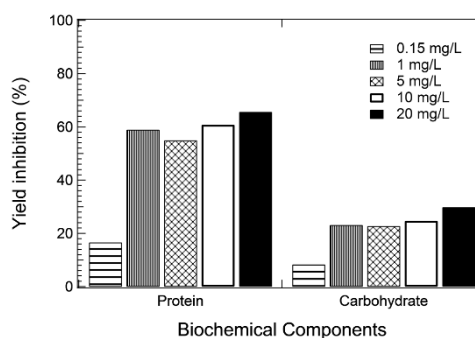
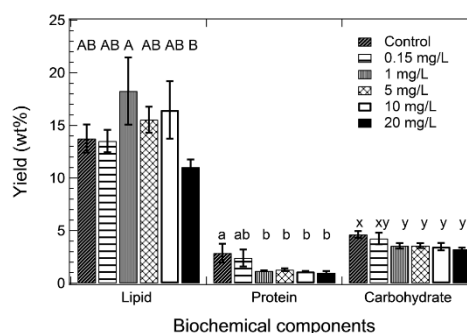
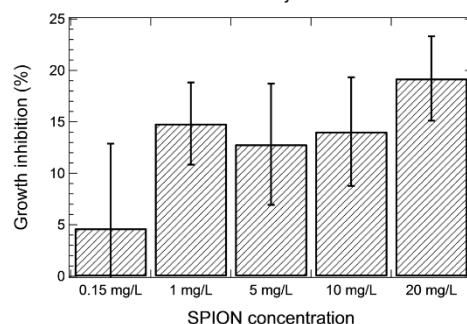
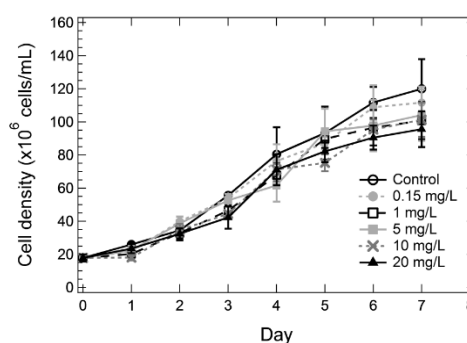
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### ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPION) have an increasing number of applications, such as in biomedical (Singh et al., 2010; Buyukhatipoglu and Clyne, 2010; Hoskins et al., 2012), wastewater treatment (Kong et al., 2012; Kakiyama et al., 2004; Yavuz et al., 2006) and also in microalgae harvesting through magnetophoresis (Xu et al., 2011; Lim et al., 2012; Toh et al., 2012; Liu et al., 2009; Prochazkova et al., 2012; Cerff et al., 2012; Hu et al., 2013). One of the attractive property of the SPION is that it exhibits magnetic responsive only after the introduction of magnetic field. However the largely utilization of SPION for environmental remediation tend to induce SPION losses to the surrounding, especially into the aquatic system, due to inefficient recovery system. In this study, *Chlorella* sp. microalga is used as the model system to evaluate the toxicity of SPION as microalgae are recognized as the indispensable base of food chain in aquatic system (Lavens and Sorgeloos, 1996). The study has showed the growth of the *Chlorella* sp. cells is inhibited by the dispersion of SPION throughout the culture medium. A 20 mg/L dosage of SPION showed a cell growth inhibition of  $19.2 \pm 4.1$  % with respect to the control group. The primary inhibition effect is due to the shading effect of SPION by blocking out the light illumination and inhibits the photosynthesis. The secondary toxicity comes from the internalization of SPION which induces cellular oxidative stress. It promotes lipid peroxidation and the disruption of cytoskeleton protein and carbohydrate in cells (Long et al., 2012; Singh et al., 2010).

The yield of total lipid, protein and carbohydrate at day 7th of exposure exhibit a reduction of 19.7 %, 65.7 % and 29.9 % respectively compared to the control group with a dosage of 20 mg/L SPION.

**Acknowledgements:** This work is supported by Fundamental Research Grant Scheme (FRGS) (Grant No. 203/PJKIMIA/6071271) and Research University Postgraduate Research Grant Scheme (USM-RU-PRGS) (Grant No. 1001/PJKIMIA/8045037) from Universiti Sains Malaysia and the Membrane Science and Technology cluster USM. Toh, P.Y. was supported by the My PhD scholarship from Ministry of Higher Education of Malaysia.



## RESPONSE SURFACE METHODOLOGY APPROACH FOR TRACE ELEMENTS AND NUTRIENTS ADSORPTION ONTO NANO-MAGHEMITE

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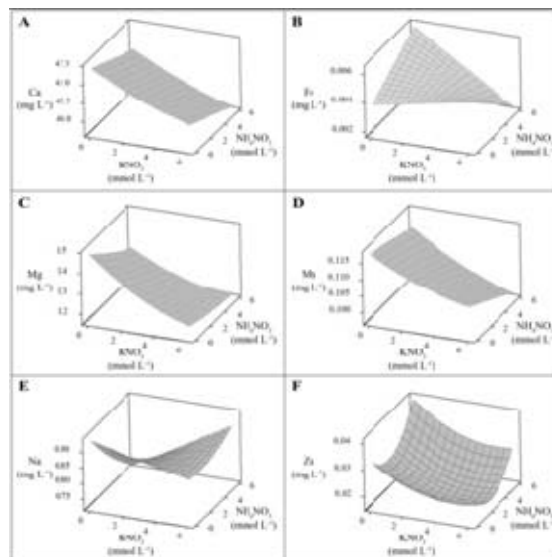
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### ABSTRACT

Iron nano-oxides are important scavengers of contaminants, being a good choice for contaminated soils because of their sorption properties [1]. Nano-maghemite (NM) has been investigated recently due to its abundance and effectiveness at removing As, Cu<sup>2+</sup>, Zn<sup>2+</sup>, and Pb<sup>2+</sup> from contaminated water, and as an in-situ remediation material.

An experiment was carried out to study the competition for adsorption between trace elements (TEs) and the nutrients K and N following the application of NM (iron nano-oxide; Fe<sub>2</sub>O<sub>3</sub>) to a soil solution (the 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extract of a TEs-contaminated soil). The soil was collected from the former mining area of Mokrsko (Czech Republic) affected by high TEs concentrations [2]. Using MINITAB software version 16.1.1. (MINITAB Inc., USA), response surface methodology (RSM) with a central composite design (CCD) of 13 runs (in triplicate) was designed for KNO<sub>3</sub> and NH<sub>4</sub>NO<sub>3</sub> (from 0 to 6 mmol L<sup>-1</sup>). After the nutrients were mixed with the soil solution in the different treatments, NM was added to obtain 1% w/v NM in the soil-nutrients-solution. After agitation (24 h in darkness), the pH was determined, samples were centrifuged and filtered using 0.45-µm nylon filters (efficient due to aggregation). Samples were analysed using ICP-OES (Varian, VistaPro, Australia). The CCD allowed to obtain statistically-significant quadratic models to predict the concentrations of TEs remaining in solution in the presence of NM and nutrients, simultaneously. Adsorption response surface plots were obtained to graphically illustrate their concentrations in the equilibrium within the experimental region (Figure).

According to the models, K<sup>+</sup> and NH<sub>4</sub><sup>+</sup> were important factors for Ca, Fe, Mg, Mn, Na, and Zn adsorption by NM (R<sup>2</sup><sub>adj</sub> > 95%, except for Zn with R<sup>2</sup><sub>adj</sub> = 87%), but KNO<sub>3</sub> had a dominant effect. Ca, Mg, and Mn concentrations showed a tendency to be lower in the presence of NM and nutrients. For Na adsorption, KNO<sub>3</sub> was not a statistically-significant factor at the 95% confidence level (p-value > 0.05), since it was unchanged in the presence or absence of KNO<sub>3</sub>. The available Zn depended inversely on the K<sup>+</sup> concentration and proportionally on the NH<sub>4</sub><sup>+</sup> concentration (Figure F), with a quadratic interaction (higher adsorption capacity of NM at 6 mmol L<sup>-1</sup> KNO<sub>3</sub> and 2.5 mmol L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>). So, the presence of K<sup>+</sup> could have facilitated the adsorption of Zn on the NM until reaching a limit, from which both NH<sub>4</sub><sup>+</sup> and K<sup>+</sup> equally decreased Zn adsorption. No significant differences were detected for pH, Al, As, Cd, or Cu (R<sup>2</sup><sub>adj</sub> < 0.8), and their quadratic models were not created. The interaction between nutrients and TEs in the soil solution may be crucial for the effectiveness of NM during the phytoremediation of contaminated soils.



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**Acknowledgements:** To Zuzana Michálková and Hana Šillerová for their help with analyses. Supported by Postdok ČZU (ESF/MŠMT CZ.1.07/2.3.00/30.0040), and by the Czech Science Foundation (GAČR 503/11/0840).



## PROTEOMICS OF *ARABIDOPSIS THALIANA* MUTANTS RESISTANT TO CdS QUANTUM DOTS (CdS QDs)

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### ABSTRACT

The industry of nanotechnology is a steeply growing and nanomaterials (NMs) have an application in many industrial fields, from electronics, to biomedicine, pharmaceuticals, cosmetics, and food production. The scant knowledge regarding uptake, interaction with cells, toxicity, is hampering the complete application of NMs, as shown by recent decisions of the European Commission and European Food Safety Authority. The aim of our work was to develop a toxicogenomics approach to assess the risk posed by NMs, focusing on quantum dots cadmium sulfide nanoparticles (CdS QDs) using *Arabidopsis thaliana* (L.) Heynh as model system.

In a previous study, two mutant lines of *Arabidopsis*, have been selected as resistant to lethal concentrations of CdS QDs (80 mg/L), and the phenotypes and genotypes have been extensively characterized both on molecular and physiological side (Marmiroli et al. 2014).

Proteomic analysis has been performed on crude protein extracts, obtained from whole 15-days seedlings of one of the foregoing mutants, *atnp02*, and on the wild type, grown on agarized MS, both treated with 80 mg/L CdS QDs and non-treated. Proteins with different isoelectric point (pI) and hydrophobicity have been separated by a 2D liquid chromatography technique (ProteomeLab PF2D, Beckman), and an analysis using MultiVue Software (Eprogen) has been carried out on qualitative/quantitative differentially abundant protein peaks between wild-type and mutant plants in both conditions. Proteins whose abundance was statistically different in response to the experimental conditions were identified by MALDI-TOF/MS to infer their possible role in the plant response to CdS QDs, in particular resistance.

One integrated study using a global proteomic approach to study stress response due to nanoparticles in *Arabidopsis* plants, together with physiological, biochemical and transcriptomic analyses, leads to a better understanding of some of the genetic, molecular and physiological mechanisms at the basis of nanoparticle stress response. The nature of the mutant resistance to CdS QDs can be clarified by comparing the transcriptomic analysis, which indicate the RNA abundance, with the actual quantity of proteins synthesized in the plant as effectors of the resistance phenotype.



## INVESTIGATION OF UPTAKE (ENDOCYTOSIS), AND TRANSLOCATION OF GOLD NANOPARTICLES IN PLANTS

**Meaghan Kern, Guangshu Zhai, Katherine Walters, Jerald Schnoor**

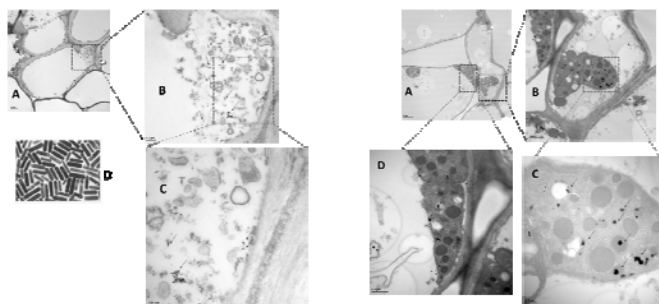
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### ABSTRACT

The exact mechanism by which nanoparticles enter and translocate in plant cells is still unclear. One potential mechanism is endocytosis. Previous research has shown the nanoparticles enter suspended plant cells through endocytosis and endocytosis can be inhibited by temperature (4°C) and the chemical inhibitor Wortmannin. In this study, we investigate endocytosis as the main nanoparticle uptake mechanism into whole plants of hydroponically grown *Helianthus annuus* and *Populus* spp. The inhibition of endocytosis is expected to inhibit the uptake of nanoparticles into *H. annuus* and a decrease or absence in gold nanoparticles is expected.

Preliminary research of TEM imaging on hybrid poplar has shown that gold nanorods enter and translocate from the roots to leaves of woody hybrid poplars (Figures 1 and 2). Gold nanorods were taken up and transported from roots to leaves (Figures 1 and 2). However, gold nanorods were found to maintain their shapes in the roots (Figure 1A-C) and to change their shapes during transport to leaves (Figure 2A-D). However, further objectives are to investigate the specific mechanism by which nanoparticles enter plant cells through attempting to inhibit endocytosis in woody and herbaceous dicots.



**Figure 1: TEM images of root after 6 day exposure of gold nanorods (10×34nm). A: Gold Nanorod in the roots; B: magnified panel A; C: magnified panel B; D: TEM of commercial gold nanorod (10×34nm).**

**Figure 2: TEM images of leaf after 6 day exposure of gold nanorods (10×34nm). A: Gold Nanorod (10×34nm) in the leaves; B: magnified panel A; C: magnified panel B; D: magnified panel A.**

*H. annuus* seeds were germinated in soaked paper towels and transplanted to an Erlenmeyer flask and grown in the growth chamber (16 hours daylight and 8 hours night). 0.10 Hoagland solution was used as the main hydroponic solution. Three different sets of experiments were set up for each of the three nanoparticle sizes (5, 25, 50 nm), with 2 replicates per treatment. Four different treatments were set-up: control with no nanoparticles, 4°C, 4°C with 120 μmol of Wortmannin, and control with nanoparticles. When seedlings of similar appearance have 4 true leaves, they were either dosed or not dosed with nanoparticles. Sunflowers with 4 true leaves were dosed with 4 mL of suspended gold nanoparticles. After 4 days, roots, stems and leaf samples were used for TEM imaging to look for the presence and absence of nanoparticles. In addition, ICP-MS analysis examines the metal concentration of the plant tissue of the woody and herbaceous plant material.





## UPTAKE AND ACCUMULATION OF ZnO AND CuO ENGINEERED NANOPARTICLES IN CARROT AND PARSNIP

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### ABSTRACT

With the advancement in the field of nanotechnology, the production and the usage of engineered nanoparticles (ENPs) has increased exponentially over the recent years. This has attracted global attention both in the industrial and scientific world and there are increasing efforts to investigate the release, toxicity and environmental fate of ENPs. The interaction of ENPs with plants is a subject of continued study and there is a growing body of research in this area. The present study investigated the phytotoxic effects of ZnO and CuO ENPs on carrot and parsnip and accumulation of metals in the edible portion of these plants.

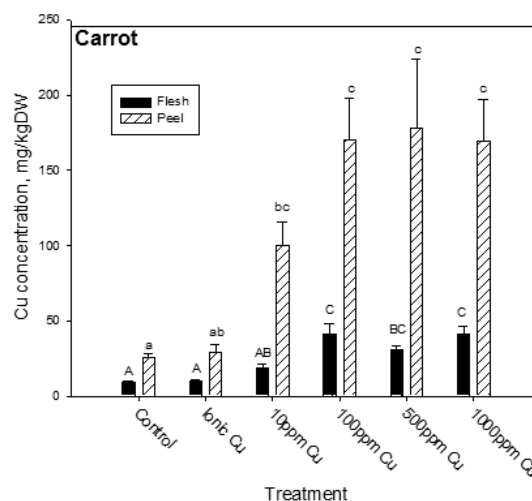
Six month old hydroponically grown plants of each species were treated for 10 days with increasing concentrations of one of the two ENPs at concentrations (of 0, 10, 100, 500, or 1000 ppm) in DI water. An ionic treatment, whose concentration was determined through prior efforts by quantifying the maximum dissolution of these ENPs in DI water at the aforementioned concentrations, of the plants with the corresponding ion ( $Zn^{2+}$  or  $Cu^{2+}$ ) was also included to distinguish between the nanoparticle and the ionic effects. Nutrients were supplied through foliar application during the treatment period and an array of physiological measurements was taken.

The plants accumulated the corresponding metals from ENPs in their storage organs. The metal concentration changed in a dose dependent manner and showed some saturation at higher concentration treatments. The peels for both the plant species accumulated significantly higher concentrations of metal than the flesh. There was no effect on the relative chlorophyll content of leaves or the biomass of the storage organ, but there was a significant difference in the total amount of water transpired during the treatment period suggesting an imbalance in water relations. Visually, the plants treated with some of the highest ENP concentrations showed a loss of turgor and leaves appeared were unable to support their own weight when compared to controls.

Overall, this study has provided information describing the impact of ENPs on these two plants and the accumulation of Zn and Cu. The results have potential implications for agriculture, remediation and human health.

Currently, efforts are underway to determine the spatial distribution and chemical form of these ENPs in the storage organs of these plants. Nutritional bioaccessibility will also be assessed using a simulated gastric extraction to assess the release of metals from the edible portion of these plants in the digestive tract of humans. The results will contribute to the ongoing assessment of the possible risk of these ENPs to human health from consumption of these plants.

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## IRON AND ZINC NANOPARTICLES IN MICROBIALLY ASSISTED BIOREMEDIATION OF Zn-Pb POST-FLOTATION WASTES

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### ABSTRACT

Almost 250 000 sites in Europe require urgent remediation. Nanoparticles have great potential to reduce the toxicity of heavy metals released to ground waters from post-flotation and industrial wastes. Although, remediation technologies are still in experimental or pilot stage, our recent studies, within the UMBRELLA EU project, provided several microorganisms that can support growth of plants on metal enriched substrata. Iron colloids can remove dissolved metals by immobilization/adsorption and transformation of elements on their surface. Nanoparticles can be produced either in chemical processes or naturally, by living organisms including plants, bacteria and fungi. In the experiment we used *Helianthus annuus*, *Iris pseudoacorus* and their symbiotic *Cadophora* sp. which is an endophyte isolated from plants inhabiting metal polluted sites and the water plant specific, mycorrhizal *Glomus spp.* In addition we tested cyanobacteria *Leptolyngbya frigida* isolated from a Zn waste in Sardinia for its ability to form Zn-based nanoparticles. Nanoparticle size determines its remediation potential. Visible/UV absorption spectra were recorded using a Hewlett-Packard HP 8452A diode-array spectrophotometer. The average size of nanoparticles reached about 100 nm.

The obtained data can be used for optimizing remediation technologies in artificial wetlands allowing efficient and sustainable metal removal and immobilization. This environmentally-friendly technology, based on plant-microbe consortia may be a useful alternative systems commonly used in environmental engineering.

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## NANO - HEAVY METAL INTAKE, TRANSPORT, ACCUMULATION AND TOXICOLOGY OF *FRAGARIA X ANANASSA*

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### ABSTRACT

Phytoremediation can be defined as the combined use of plants, soil amendments like solid waste disposal sites and agronomic practices to remove pollutants. This technique has many advantages compared with, other remediation procedures: low economic costs, the generation of recyclable residues, and the possibility of being applied to soils and waters, causing minimum environmental impact. Techniques involve the elimination of the pollutants from the soil, the toxic elements accumulating in the harvestable parts of the plants.

Nanotechnology is a fast emerging discipline not only in physics and chemistry but also in the field of biology. In view of the tremendous applications of nanotechnology, there is a motivation among scientists to carry out research in this most vital discipline. Chemists are highly interested in synthesizing nanoparticles of different dimensions employing many of the precious metals. Already scientists have started exploiting the bio-based synthesis of nano-metals using leaf extracts and microorganisms (bacteria and fungi).

This paper reports on the accumulation of nano-silver and its morpho-physiological effects in *Fragaria vesca* leaves shoots and roots. This research examines the effects of four nano-silver concentrations on the growth and chlorophyll contents of *Fragaria vesca* leaves shoots and roots, to evaluate the ability of young and mature laminae to take up nano-silver, to detect and partially characterize the nano-silver-binding biomolecules in the laminae, as a biochemical tolerance mechanism to nano-silver exposure.







## **HUMAN EXPOSURE & RISK**



## ECOLOGICAL BENCHMARK VALUES FOR Cd IN BRAZILIAN SOILS: A PHYSIOLOGICAL AND ECOTOXICOLOGICAL APPROACH

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### ABSTRACT

Trace elements, such as Cd, are naturally present in soils at different concentrations, with higher contents normally found in soils affected by anthropogenic activities. The increasing consciousness regarding soil pollution in Brazil has led the National Environment Council to develop a national legislation (Directive Conama 420/2009) proposing reference values for assessing soil quality. These values – ranked based on increasing risks – were set as “background”, “target” (ecotoxicological benchmarks), and “investigation”. Background values were supposed to be set individually for each State, while investigation values were defined nationwide based on human health risk assessments. Provisional target values, which are indicative of a level at which there is a sustainable soil quality, have also been set countrywide. Yet, each State was supposed to develop its own research in order to validate the proposed nationwide target value (e.g., 1.3 mg Cd kg<sup>-1</sup> soil). Based on this, we have established an experiment with plants in order to propose target value for Cd in Brazilian soils using a combined physiological and ecotoxicological approach for risk assessment. With that we intend to find out how high the concentration of this metal could go without, however, compromising soil quality and function, i.e., with negligible risks to the ecosystem.

The experiment was conducted in a greenhouse, using two representative soils from the State of Minas Gerais, as follows: Oxisol (clay 24%; sand 64%; pH 4.8; P 1.13 mg dm<sup>-3</sup>; K 32 mg dm<sup>-3</sup>; Ca 0.3 cmol<sub>c</sub> dm<sup>-3</sup>; Mg 0.1 cmol<sub>c</sub> dm<sup>-3</sup>; Al 0.6 cmol<sub>c</sub> dm<sup>-3</sup>; effective CEC 1.1 cmol<sub>c</sub> dm<sup>-3</sup>; V 9.6%; OM 1.6%), and Inceptisol (clay 31%; sand 47%; pH 5.3; P 2.6 mg dm<sup>-3</sup>; K 34 mg dm<sup>-3</sup>; Ca 1.6 cmol<sub>c</sub> dm<sup>-3</sup>; Mg 0.4 cmol<sub>c</sub> dm<sup>-3</sup>; Al 0.5 cmol<sub>c</sub> dm<sup>-3</sup>; effective CEC 2.6 cmol<sub>c</sub> dm<sup>-3</sup>; V 34%; OM 2.9%). Tests followed the OECD-208 normative, using four metal-sensitive plant species (*Zea mays*, *Oryza sativa*, *Phaseolus vulgaris*, *Raphanus sativus*). Plants were exposed to increasing doses of Cd (0.4; 0.72; 1.29; 2.3; 4.1; 13.6; 24.4 mg kg<sup>-1</sup>) during 21 days. In addition to the variables commonly evaluated in these kind of studies (e.g., fresh and dry shoot weight, or shoot height), we have also measured the following endpoints for the ecological assessment: germination, emergence, plant growth, enzymatic activity (antioxidant system), shoot Cd concentration, chlorophyll index, photosynthesis, stomatal conductance, transpiration, photosystem II efficiency, and relative rate of electron transport. Following these measurements, we calculated the effective concentration causing 50% inhibition of each evaluated endpoint (EC50). All accepted EC50 values (those following a normal distribution) were then plotted to derive a 5<sup>th</sup> percentile value (HC5), which is defined as that concentration expected to protect 95% of the assessed endpoints.

Toxicity symptoms were more severe for plants growing in the Oxisol, compared with the Inceptisol. *Raphanus sativus* did not germinate with 24.4 mg kg<sup>-1</sup> of Cd in the Oxisol and all other measured endpoints were also affected, yet with different intensities. While the plants cultivated in the Inceptisol showed mild toxicity symptoms, we have noticed significant variations in the enzymatic activity of the antioxidant system for plants exposed to Cd in this last soil. Using the accepted EC50 values, we could get HC5 values (mg Cd kg<sup>-1</sup> dry soil) of 2.15 (Oxisol), 10.35 (Inceptisol), and 2.65 (both soils together). The different values observed for HC5 are mainly associated with soil properties affecting the available fraction of Cd in the soil (e.g., pH, OM, and clay content). Finally, derivation of the new proposed target value was possible by adding the Cd background reference value for Minas Gerais State soils (0.4 mg kg<sup>-1</sup>) to the HC5, obtaining, as a result, a new proposed ecotoxicological screening benchmark soil value (target value) of Cd for soils of Minas Gerais State (mg Cd kg<sup>-1</sup> dry soil): 2.55 (Oxisol), 10.75 (Inceptisol), and 3.05 (both soils together). The last value is in accordance with a previous target value set for Cd in Brazilian soils (3.0 mg Cd kg<sup>-1</sup>, for the State of São Paulo).

We conclude suggesting that adding new physiological endpoints as additional variables to assess ecological risks of contaminated soils might be relevant for improving the sensitivity of assays with plants, thus helping us to define a more robust target value (ecotoxicological screening benchmark) for Cd in soils. Also, taking into consideration the high soil heterogeneity found in Brazil, it is recommended to have a range of target values based on different soil properties.

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## MOLECULAR AND BIOCHEMICAL CHARACTERIZATION AND MODE OF ACTION OF SAP13 IN PROVIDING TOLERANCE TO MULTIPLE ABIOTIC STRESSES

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### ABSTRACT

Global climate change and environmental stresses are the main reasons for the decline of crop production worldwide. In the past years, a major focus has been on improving plant species and their tolerance towards these stresses but not much has been achieved because of the limited knowledge of the gene/network of genes that might be involved in providing tolerance to such multiple abiotic stresses. Recently, members of Stress Associated Protein (SAP) family in plants have been shown to impart tolerance to multiple abiotic stresses. There are 14 SAP genes in *Arabidopsis thaliana* and these proteins contain A20, AN1 and C2H2 zinc finger domains. AtSAP13, a member of the SAP family carries two AN1 zinc finger domains and an extra Cys2-His2 domain. When overexpressed in *Arabidopsis* and *Brassica*, it has been shown to provide tolerance to multiple abiotic stresses such as toxic metals (As, Cd, Zn), drought, and salt. However, the mode of action of this SAP member in providing tolerance to multiple abiotic stresses is largely unknown. In-silico analysis of the promoter sequences upstream of ATG start codon of AtSAP13 using PLACE database predicted the presence of various abiotic stress related cis regulatory elements. We hypothesized that the expression of AtSAP13 gene might be regulated via the interaction of cis-elements present in the AtSAP13 promoter with abiotic stress related trans factors via protein-DNA interactions under different abiotic stresses. Through yeast one hybrid assay, we have proved this hypothesis and identified several transcription factors such as DREB, ERE, ZIP, HSE etc that are interacting with the AtSAP13 promoter. These interactions are being analyzed through Electrophoretic mobility shift assay (EMSA) to understand the molecular and biochemical functioning of AtSAP13. Candidate transcription factors interacting with AtSAP13 will be characterized further using both forward and reverse genetic approaches. Further, *Camelina sativa*, a member of Brassicaceae family and closely related with *Arabidopsis*, has been proposed as an ideal biofuel crop. In order to improve its adaptability to wider geographical ranges and marginal land, we aim to characterize and overexpress endogenous SAP13 in *C. sativa* in providing tolerance to various stresses. We have identified and cloned CsSAP13 in *C. sativa*. Resulting transgenic plants will be analyzed for enhanced biomass, seed and oil yield under multiple abiotic stresses. The knowledge and information gained will not only be applied on agricultural crops to engineer crops that will be better able to withstand such abiotic stresses and still produce sustainable yield but will also help to grow crops for food and biomass production on barren lands, thus making them more cultivable over time. Therefore, the proposed research could have a significant impact on global food security, biofuel production, and human and environment health enhancement.





## TOXIC ARSENIC COMPOUNDS REMAIN IN NATIVE PLANT SPECIES FROM ARSENIC POLLUTED SOILS

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### ABSTRACT

Arsenic is considered a toxic element for plants. However, the discovery of arsenic resistant and hyperaccumulating plant species has increased the interest in understanding the distribution of arsenic species in these environmental matrices. Plants are able to take up both arsenate (As(V)) and arsenite (As(III)) from soils, via phosphate transporters and some aquaporin channels, respectively, but both forms of arsenic are toxic to cellular metabolism of plants. Methylated arsenic species can be taken up by plants from soils as well, but generally less efficiently than inorganic species. Some authors have suggested that the synthesis of phytochelatins (PCs) is involved in As(III) detoxification in terrestrial plants. The chelating properties of these peptides lead to arsenic complexes which are less reactive and therefore less toxic for plants than the free arsenic ions [1]. Arsenic can also be present in plants as non-extractable arsenic species, strongly bound to lipids or to cell wall constituents. Nevertheless, the arsenic remained in plants as free inorganic or methylated ions has a greater interest due to its higher mobility and toxicity, and therefore, its ecological impact and risk to health.

The aim of this work consisted on the determination of the fraction of arsenic present as toxic forms (inorganic and methylated species) present in native terrestrial plants from polluted soils by former mining activities (Mónica mine, NW Madrid, Spain), with high total arsenic concentration levels (up to  $3,500 \mu\text{g g}^{-1}$ ) [2], due to their higher mobility and the risk associated to their reintegration into other environmental compartments. For this purpose, roots and aboveground parts were analysed separately, to assess possible transformations from translocation processes. Extractions were carried out with deionized water by microwave-assisted extraction, at a temperature of  $90^\circ\text{C}$  and three extraction steps of 7.5 min each. Total extracted arsenic concentrations were determined by ICP-AES, showing extraction percentages from 9 to 39%. Speciation studies were performed by HPLC-(UV)-HG-AFS [3], and they showed the main presence of As(V) (up to  $350 \mu\text{g g}^{-1}$ ), followed by As(III), in both plant parts. Monomethylarsonic acid (MMA) and trimethylarsine oxide (TMAO) were also found only in some plants. On the other hand, the use of  $0.5 \text{ mol L}^{-1}$  acetic acid as extractant led to higher extraction percentages (33-87%), but lower column recoveries, probably due to the extraction of arsenic compounds different to toxic free ions studied, which may come from biotransformation mechanisms carried out by plants to reduce arsenic toxicity. However, As(V) concentrations increased up to  $800 \mu\text{g g}^{-1}$  in acid medium, indicating the probable release of As(V) from organoarsenic compounds and therefore a higher potential risk for the environment [4].

From the easily soluble, water-extractable arsenic species concentration levels, it can be drawn that between 70 and 89% of the total arsenic in plants must have been biotransformed, so it is present under the form of different arsenic compounds. Some of these compounds are likely to be extracted with acetic acid, which seems to induce the release of As(V) from organoarsenic compounds, showing a higher potential risk for the environment. This high level of biotransformation of most part of arsenic could explain the survival capacity of the plant species studied, despite their high total arsenic concentrations. Still, elevated arsenic concentration levels remain as toxic forms, predominantly as arsenate, reaching up to  $190 \mu\text{g g}^{-1}$  considering roots and aboveground parts separately, and  $350 \mu\text{g g}^{-1}$  considering the sum of both plant parts (more than double in acid medium), which may constitute an environmental risk due to its possible reintegration to the environment. Therefore, the study and control of native plants growing in arsenic polluted soils is a relevant factor for environmental safe.

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## PHYTOREMEDIATION AND ENVIRONMENTAL RISK ASSESSMENT: A NEW APPROACH

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### ABSTRACT

Phytotechnologies applied to environmental remediation are considered green technologies. Nevertheless, despite the wide range of their potential applications and clear advantages, few research activities have been focused on their potential impacts. EPA has listed some of the possible adverse effects and highlighted the need of a Risk assessment applied to phytoremediation processes. Taking into account that phytoremediation is a continuum of different processes, the Environmental Risk Assessment (ERA) procedure applied to phytoremediation should be carried out on a case by case basis, meaning that its results would depend on the plants, the potential receiving environment(s) and the contaminant. At the same time the whole assessment has to be science based; it requires a complex procedure that needs to be transformed into an operational and standardized tool. In this paper we propose a methodology that aims to answer to these requirements. It has been developed starting from an Operating Model for the Environmental Risk Assessment (OMERA) applied to the environmental release of Genetically Modified Plants (M. Lener, V. Giovannelli, et al. 2013. Applying an operating model for the environmental risk assessment in Italian Sites of Community Importance (SCI) of the European Commission Habitats Directive (92/43/EEC), *Bulletin of Insectology* 66:257-267). It is mainly addressed to scientists, companies, regulators, environmentalists, also aiming to provide the stakeholders with a scientifically correct source of information. The ERA structure moves from the assumption that the occurrence of a risk is strictly related to the presence of four components: a) source b) diffusion factors c) migration routes d) receptors. Source is the site where the organism is released and/or enabled to express its harmful characteristics; diffusion factors are linked to biological characteristics of the plant; migration routes are linked to chemical, physical, biological characteristics of the receiving environment; receptors include humans, animals and ecosystems. The ERA structure comprises two main components: a conceptual model represented as a flowchart and an electronic Questionnaire (eQ) driven by a relational database (Microsoft Office Access). The eQ includes two kinds of question sets: the first is descriptive and allows the characterization of the topic to be analysed in each step; the second type is a so called on/off question, asking for a yes/no/don't know answer that can open or close specific paths of risk definition. According to a precautionary approach, when a "don't know" answer is given, highlighting a lack of knowledge, the system automatically selects the path that represents the worst-case scenarios. The on/off questions allow the user to follow the flow chart as a decision tree starting from the source, through all the components of the conceptual model, and leading to the identification of potentially affected receptors (if any) and related risks impacts (if any).

Principles end structure of OMERa have been modified to be adapted to phytoremediation. For example, the source characterization includes the contaminant, the phytotechnology applied and the potential use of amendments. In case of phytoremediation, the use of alien species is considered as a secondary source of risks and specifically analyzed. The resulting eQ is composed by almost 300 questions, which are not all activated on a case by case basis. Once the eQ is compiled the system will produce a report containing all the information and data inserted and a list of the identified potential effects. The OMERa methodology will be applied to different risk scenarios to be validated. It is useful to stress that the proposed model can be applied both *ex ante* and *ex post* release: in the *ex ante* phase to identify potential effects, in the *ex post* release phase to select Risk Management (RM) procedures, to set up monitoring activities, and to verify the success of the RM procedures applied.

Phytoremediation is a technology that meets requirements for a sustainable development, but as every human activity is not risk free; furthermore as for other issues it would be really useful and constructive to have the possibility to analyze and to collect research data and results in a standard way. The application of the OMERa method can be considered a tool for collecting and elaborating data in a standardized way, and would help the harmonization of the Environmental Risk Assessments performed by different actors.



## EUROPEAN AND NATIONAL LEGISLATION WITH REGARDS TO THE DIFFERENT STEPS IN A PHYTOREMEDIATION APPROACH FOR METAL-CONTAMINATED LAND

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### ABSTRACT

When applying phytotechnologies for management of metal-contaminated land, many questions are raised with regard to legislative issues. Within the REJUVENATE and GREENLAND projects we try to answer them using a flow approach in which each step when considering and applying phytotechnologies is examined: (1) describing the land status and use, (2) planting/sowing, (3) growing, (4) harvesting and (5) processing the crops, and (6) using the remainders. The focus is on European legislation as a basis for national rules (Directives) or as directly enforceable (Regulations), followed by legislation of the Member States and then, if relevant, regional legislation. Strategic communications of the Commission and proposals for new legislation are considered only when it is probable that these will become formal obligations in the near future. National legislation has been assessed in detail for all countries represented within the GREENLAND project.

In the first step, the status and use of the land, and the implications with regards to soil threshold values and the use of crops are defined. In the second step (planting/sowing), international regulation on crop selection, and more specifically on invasive and exotic species, and genetically modified organisms, as well as on soil management, *e.g.* soil amendments for stabilisation versus the enhancement of 'bioavailability' are considered. With regards to step three (growing), the principles of good agricultural practice are checked, *e.g.* concerning the use of pesticides. The classification of the harvested crop (step four) based on European and national legislation is complemented with an expert view. For the fifth step (processing), we focus on energy conversion of the biomass (*e.g.* pyrolysis) and on requirements with regards to input and output threshold values. In a final step we concentrate on the use or disposal of the remainders which contain metals in increased concentrations, such as the biochar.

The result of a complete analysis of legislation applicable throughout the entire phytoremediation cycle allows us to provide an objective overview to policy makers and regulators, who are now often overwhelmed and maybe even misled by the whole range of potentially related legislation.

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## DOUBLE WIN: PHYTOREMEDIATION SAFEGUARDS INCOME WHILE MINIMIZING HEALTH RISKS IN AN AGRICULTURAL REGION

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### ABSTRACT

In the Campine region (North-East of Belgium) a large agricultural area is contaminated with mainly cadmium, lead and zinc, and legal food threshold values and soil standards for cadmium are often exceeded. Uptake by crop plants is a significant route of cadmium transfer into the human food chain. Besides human health risks, there are economic concerns, i.e. for the farmers facing contaminated land who can no longer grow high income vegetables for the risk of their harvest being confiscated. Phytoremediation has often been suggested as an alternative remediation technology because it is a solar driven, low impact option that can be carried out at much lower cost than conventional methods. Its actual implementation lags behind because until now there does not exist a decision tool for the (gradual) integration of phytoremediation crops in an agricultural system. In this paper, food safety values are used to decide on the optimal introduction in time of different phytoremediation crops and vegetables based on phytoextraction capacities and economic benefits of these crops. The tool is then applied on the Campine region where about 280 km<sup>2</sup> is contaminated with mainly cadmium, while the region largely depends on agriculture. Results show that in the given region, (1) based on food threshold values, phytoremediation is chosen 15% over growing the allowed crop at the current cadmium concentration, and that (2) using the decision model based on food threshold values, safe use of the land including phytoremediation when economically optimal, results in costs that are twice as low as conventional remediation. Given the fact that in the Flemish region there are 85,000 potentially contaminated lands, we show that considering and applying phytoremediation when appropriate results in considerable cost savings, while minimizing human health risks.



## THE EFFECT OF SILICON ON THE UPTAKE AND TRANSLOCATION OF ARSENIC IN TOMATO (*SOLANUM LYCOPERSICUM* L.)

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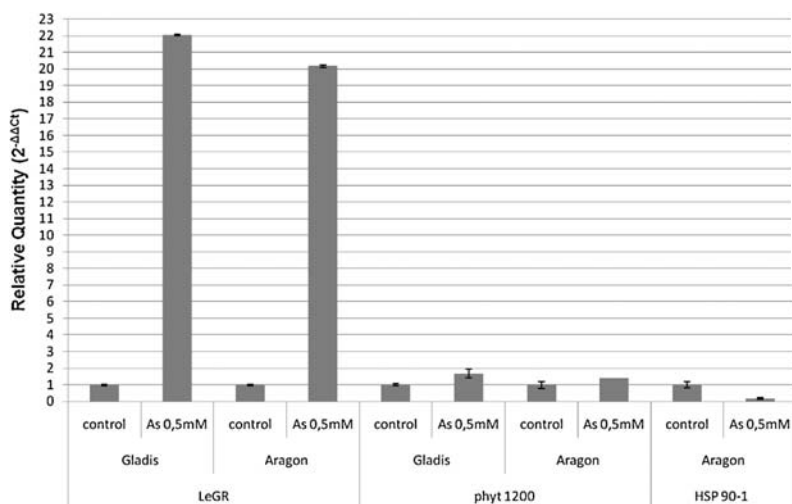
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### ABSTRACT

The metalloid element Arsenic (As) is toxic to most life forms; in humans, its ingestion has been associated with a whole series of pathologies leading to its classification as a carcinogenic. Si, the second most abundant element in the earth's crust occurs within soils mainly in the form of inert quartz or crystalline silicate; however, monosilicic acids are soluble and thus available to plants and microbes. Absorbed Si is beneficial for plant growth, largely because of its role in combating biotic and abiotic stress. Tomato is a major horticultural crop in both Europe and the US, but its fruit can be compromised by As contamination. Feeding of Si to tomato crops has been used to alleviate drought and salinity stress, but to date no attempt has been made to correlate Si supplementation with As uptake and its translocation to the aerial part of the plant. Here, we show that Si treatment can indeed influence As uptake in tomato. In addition, we demonstrate that tomato cultivars differ from one another with respect to their capacity to take up and translocate As, whether or not the plants are supplied with soluble Si. during previous experiments on eight different tomato cultivars we were able to discriminate that four of them translocate As to the berries, with and without Silicon treatments, in different concentrations according to the cultivar.

Transcription profiling related to three genes associated with the abiotic stress response was carried out for cvs. Aragon and Gladis, which were able to concentrate As inside the fruit, even when Si was administered. We choose the genes: Glutathione Reductase gene (LeGR), Heat Shock Protein gene (Hsp90-1) and Phytochelatin Synthase gene (Phyt1200), because in previous studies they were demonstrated to be involved in As stress response, for RT- q-PCR experiments. The relative abundance of each transcript was calculated from the average Ct of each amplification according to the  $2^{-\Delta\Delta Ct}$  method. Exposure to NaAsO<sub>2</sub> up-regulated Glutathione Reductase (LeGR) in the leaf to a greater extent in cv. Aragon than in cv. Gladis. The up-regulation of phytochelatin synthase (Phyt1200) in response to exposure to NaAsO<sub>2</sub> was less intense than for LeGR, and the two cultivars did not respond differentially. For LeHsp90-1, the presence of NaAsO<sub>2</sub> in the growth medium did not induce any change in transcript abundance in either cultivars.



Transcript abundance of selected stress-associated genes as assessed by qPCR. The data have been normalized to the abundance of the reference expressed sequence (exp (SGN-U346908) endogenous ) utilized as internal control







# **MOLECULAR ENGINEERING**





## USE OF POPLAR FOR REHABILITATION OF METAL POLLUTED SOILS

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### ABSTRACT

Phytoextraction is the use of plants to remove heavy metals from soils contaminated by industrial activities, such as smelting or disposal of high metal wastes. Poplar is well adapted for phytoextraction because it tolerates growth on contaminated soils and produces high biomass. In addition, poplar is also suitable for molecular genetic studies because its genome was sequenced in 2006 and it is amenable to transgenic technologies. However, previous reports indicated that metal accumulation was the highest in leaves causing metal return to the soil after leaf abscission. In order to circumvent this problem, genetic engineering can be used to limit metal accumulation in leaves or direct metal accumulation in poplar trunks using relevant metal transporter genes under the control of tissue-specific promoters.

We first focused our study on the functional characterization of suitable metal transporter genes. We selected candidate transporters potentially controlling metal storage in the vacuole, the main compartment where heavy metals are accumulated in plant cells. The NRAMP (Natural Resistance Associated Macrophage Protein) and the IREG (Iron Regulated Gene) metal transporter families, which are still poorly characterized in poplar, have been investigated. Based on the knowledge gained from their homologues in *Arabidopsis thaliana*, PtNRAMP3.1 and PtNRAMP3.2 are putatively involved in metal release from the vacuole while PtIREG1 is putatively involved in metal sequestration in the vacuole. We showed that both PtNRAMP3.1 and 3.2 are functional metal transporters but only PtNRAMP3.2 is targeted to the vacuolar membrane. In contrast, PtNRAMP3.1 is targeted to intracellular compartments. In addition, the function of PtIREG1 putative metal transporter gene was investigated by heterologous expression in yeast: PtIREG1 was shown to be able to complement the hypersensitivity of mutant strains to Ni but not to other metals, including Co, Cd, Fe, Mn and Zn.

Transgenic poplars overexpressing PtNRAMP3.1 or PtNRAMP3.2 are being assessed for metal tolerance and accumulation. When grown on non-contaminated soil, wild-type and transgenic poplar lines exhibit clear differences in leaf metal concentrations (Cu, Fe, Mg, Mn and Zn). Metal accumulation will also be measured in transgenic poplar lines grown on metal-contaminated soil. *Arabidopsis* and poplar plants overexpressing PtIREG1 as a fusion with the fluorescent protein GFP (PtIREG1-GFP) are being generated. This should allow identification of the subcellular compartment where PtIREG1 resides and analysis of the effect of PtIREG1 expression on metal tolerance and accumulation. Besides, PtIREG1-GFP under control of the CAD promoter, which targets expression in poplar xylem cells, was introduced in poplar to specifically drive metal accumulation in wood.

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## PREPARATION OF TRANSGENIC PLANTS STIMULATING BACTERIAL DEGRADATION OF PCBs

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### ABSTRACT

Polychlorinated biphenyls (PCBs) pose a serious problem as a widely spreaded contaminants. A possible choice for remediation can be the use of microorganisms, plants and their consortia. Some bacteria aerobically degrade PCBs using the pathway determined in biphenyl operon. Expression of biphenyl operon genes is inducible and it was found that several plant secondary metabolites including flavonoids and terpenes can serve as efficient inducers. In this work we investigated that plant secondary metabolite quercetin can serve also as an inducer of *bphA* gene, a gene of bacterial biphenyl operon. Concerning these results we decided to prepare transgenic plants of *Nicotiana tabacum* overexpressing gene for flavonoid-3'-hydroxylase (*AtF3'H*) from *Arabidopsis thaliana*, the enzyme converting plant indigenous kaempferol to quercetin.

Gene for flavonoid-3'-hydroxylase (*AtF3'H*) was fused with sequence for hexahistidine tail and the tagged gene was cloned under the root specific promoter in the pGreen0029 vector. Prepared construct was introduced into the *Agrobacterium tumefaciens* for plant transformation. Because of the root specific promoter the expression of *AtF3'H* gene would be targeted to plant roots with final overproduction of the quercetin in the root and its release to the rhizosphere. This should lead to stimulation of PCB degradation by rhizospheric bacteria. Prepared transgenic plants are nowadays under the investigation.

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## PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF N<sub>2</sub>-FIXING BACTERIA NODULATING LEGUMINOUS SPECIES ON SOILS FROM MINING AREAS

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### ABSTRACT

Mining soils are often devoid of natural recolonization, necessitating human intervention to restore a vegetation cover. A promising technique for rehabilitation of these areas is the phytoremediation. The success of phytoremediation is associated with microbial and their essential biological processes taking place in the rhizosphere or inside the plants, which benefits their growth and development. Among the microbial processes biological N<sub>2</sub> fixation (BNF) is highlighted, it is only performed by a group of prokaryotes able to convert the atmospheric N<sub>2</sub> into NH<sub>4</sub>. Among these prokaryotes, bacteria that establish mutual symbiosis with leguminous plants (N<sub>2</sub> fixing bacteria nodulating leguminous - NFBNL) are extremely important. The nodulated plants incorporate C and N to the soil, and increase their ability to absorb nutrients such as N, fixed by bacteria, which makes them more tolerant to environmental stresses.

Therefore, the aims of this study were (i) isolate, (ii) characterize phenotypically and (iii) identify NFBNL species from nodules of leguminous plants growing on gold mine soils, contaminated with arsenic, and zinc mine soils, contaminated with Zn, Cd and Pb.

The strains were isolated from nodules of *Crotalaria spectabilis* and *Stizolobium aterrimum* growing on gold mine soils, and nodules of *Leucaena leucocephala* growing on zinc mine soils, in Brazil. Then the strains were reinoculated in their host plants to check their nodule induction. Further, they were characterized phenotypically for their production of organic acids, indol-acetic acid, and siderophores, and 1-aminocyclopropane-1-carboxylate (ACC-deaminase), and also for their capacity to solubilize phosphate, and perform nitrogen fixation.

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## PREPARATION OF TRANSGENIC PLANTS WITH IMPROVED RESISTANCE TO STRESS

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### ABSTRACT

Osmotin was discovered in 1987 in cells of *Nicotiana tabacum* adapted to high level of salt. Later, it was found in other plant species, both monocots and dicots. This protein is involved in plant responses to biotic and abiotic stress. The antimicrobial activity against some fungi was already published as well as its cryoprotective and osmolyte role.

For the purpose of subsequent genetic manipulation, osmotin (*OSM*) gene was amplified using PCR with the genome DNA of Virginia tobacco (*Nicotiana tabacum*) as the template. The *OSM* fused with the oligonucleotide coding for hexahistidine tag (*His-OSM*) was expressed in *Escherichia coli* BL21 cells. After verification of the successful expression and localization, the recombinant osmotin was purified and renatured. The native conformation of the isolated protein was confirmed using MALDI TOF MS, infrared and Raman spectroscopy.

The recombinant osmotin was subsequently used for testing its antimicrobial activity against food and human pathogens. Significant antimicrobial activity was detected against the causes of candidiasis and otitis (*Candida parapsilosis* and *Candida tropicalis*), and against food pathogens *Debaryomyces hansenii*, *Torulaspota globosa* and *Saccharomyces ludwigii*, which cause for example souring of wines and musts. Simultaneously, the hemolytic activity and toxicity of osmotin against human embryonic kidney cell was examined. The results demonstrated harmlessness of osmotin to human cells at high concentrations.

The model plant species tobacco served as the second expression system. Coding sequence of *His-OSM* was inserted into the vector pGreen0029 under the control of cauliflower mosaic virus constitutive promoter. The obtained plasmid was transferred into *Agrobacterium tumefaciens* C58-C1 cells. The successful transient expression of *His-OSM* gene was verified by agrobacterial infiltration in both dicotyledonous and monocot plants. Transgenic plants bearing *His-OSM* gene were prepared by the agrobacterial cocultivation method. The presence of the transgene was detected on the DNA level and the transgene copy number was determined by quantitative PCR. The mRNA quantification demonstrated strong expression of the transgene.

The observation of seeds germination and chlorophyll content in the presence of NaCl showed higher tolerance of the transgenic tobacco plants to osmotic stress. Tobacco plants with increased production of osmotin can serve as a useful model for the preparation of agricultural crops with higher tolerance to biotic and abiotic stress. The first attempts to transformation of flax (*Linum usitatissimum* L.) and potato (*Solanum tuberosum* L.) plants by osmotin transgene have been performed.

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**BIOCHAR**



## INVESTIGATIONS WITH BIOCHAR AND HALOPHYTES IN THE PHYTOREMEDIATION OF A HIGH SALINITY LANDFILL

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### ABSTRACT

Soil salinity is an issue that affects the structure and productivity of nearly 1 billion ha of soil worldwide. The landfilling of cement kiln dust (CKD) represents an anthropogenic cause of soil salinity, and contributes to the creation of marginal salinized lands. Preliminary investigations of a CKD impacted landfill in Bath, Canada, indicate chloride concentrations nearly 1000 times that of average Canadian soils. The objectives of this research are to determine the ability of halophytic grasses to grow at a CKD impacted landfill as well as their ability to extract soil chloride. Greenhouse trials with CKD soil and a 10% (w/w) biochar amendment were conducted to evaluate the effects on the germination and growth of 13 warm and cool season halophytic perennial grass species. In addition, this research investigates the effects of biochar on high salinity soil and water through sorption experiments to determine its ability to reduce electrical conductivity (EC) and sodium adsorption ratio (SAR). Results from greenhouse germination and transplant studies along with two seasons of field work will be presented and discussed. Phytoremediation represents a low cost, low maintenance, *in situ* remediation option that could reduce the impact of industry induced soil salinity, while at the same time making use of marginal salinized soils to grow halophytic perennial grasses.

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## BIOCHAR IMMOBILIZES RISK ELEMENTS AND IMPROVES WILLOWS BIOMASS PRODUCTION ON HEAVILY CONTAMINATED SOIL

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### ABSTRACT

Currently, soil contamination is one of the most serious problems of soil protection, while the so-called old loads are the most significant threats to human health. Willows were described as suitable plants for phytoremediation, however extremely high content of risk element restrict biomass production of these trees [1]. The availability of risk elements can be possibly reduced by soil additives. Recently, the attention has been focused on the research of unconventional soil amendment additives - biochar. In the last decade, biochar ability to bind undesired contaminants, organic [2] and inorganic materials, heavy metals [3,4] has been widely described

The aim of this study was to observe the effect of elevated rates of biochar application on risk elements transport through the soil profile, then the effect of biochar amendment on plant growth as well contaminant accumulation in willow tissues was evaluated.

The experiment was established at greenhouse controlled conditions. To observe risk element content in leachate, the lysimeter (40 cm) pots were used. *Salix Smithiana* was chosen as an experimental crop. The leachate was analysed (ICP-OES) each 3 weeks during vegetation. The trees were harvested twice, firstly in July, secondly in early October to test maximum accumulation potential. The twigs and leaves were analysed separately. The experiment consists of 4 treatments: control (no applied biochar), and rates of 5%, 10%, and 15% of biochar from total mass of soil. At each treatment two willows cuttings were planted.

The results showed that 5% of biochar significantly increased biomass production; the increasing biochar rates didn't influence biomass production. Willows leaves at control treatment showed extreme phytotoxic signs. Biochar application significantly decreased lead uptake into plant tissues by 53% both in leaves and twigs when 5% of biochar was applied at first harvest, at second harvest by 77% in leaves. However zinc and cadmium uptake wasn't restricted significantly at 5 % treatment. Cd uptake significantly decreased by 32%, and 34% in leaf tissues at treatment of dose of 15% biochar amendment at first harvest. In leachate, both cadmium and zinc content decreased to values below detection limits at all biochar treatments and lead wasn't detected at these treatments.

Thanks to this study, we can summarize that biochar appears to be a very effective regulator of availability of observed risk elements and improver agent for biomass production of remediation plants.

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## EFFECTIVENESS OF BIOCHAR FOR INCREASING SOIL QUALITY AND REDUCING METAL BIOAVAILABILITY OF THREE DIFFERENT SOILS

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### ABSTRACT

Anthropogenic activities, including intensive agriculture and industrial activities, have led to a general decline of soil quality in the last decades. In this context, the loss of organic matter in soils and the contamination with heavy metals are two major problems. Since large areas are affected, low-cost and environmentally friendly approaches are needed for the remediation of degraded or polluted soils. The application of amendments may improve the soil quality, increase productivity and reduce the bioavailability of pollutants. Biochar has often been considered as an efficient and effective soil additive. In an on-going Austrian research project, several types of biochar are tested on two agricultural soils low in organic matter and one agricultural soil contaminated with Pb, Zn and Cd. For all three soils, the suitability of the biochar-amended soils for renewable biomass production using maize or *Miscanthus* is tested.

In a preliminary experiment, the following types of biochar were tested in different application rates: (i) cellulosic fiber sludge + cereal husks; (ii) Poplar wood chips biochar; (iii) *Miscanthus* biochar. All three types were produced by the company Sonnenerde and enriched with compost, and nitrogen ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>). On the contaminated soil, also lime and a 50:50 mixture of gravel sludge and siderite bearing material was used for comparison. The effectiveness was assessed by measuring plant biomass of *Lolium multiflorum* and nitrogen availability and uptake, and for the contaminated soil by additionally determining extractable metal concentrations in soil and metal uptake in plants (Cd, Cu, Ni, Pb, Zn). The three most effective treatments were subsequently also realised in two-year field experiments and in a parallel greenhouse pot experiment. Either maize or *Miscanthus* was planted in spring of 2013. Maize plants were harvested in October and *Miscanthus* plants were harvested in November. In parallel, soil samples were obtained for analysing C, N, and metals for the contaminated soil.

Preliminary results show that in case of the two soils initially low in organic matter, the nitrogen-enriched biochar were most effective in terms of increasing nitrogen supply, increasing organic matter content in soil and increasing plant biomass production. On one soil, the addition of nitrogen-enriched biochars led to a doubling of shoot biomass production compared to the untreated and unfertilized control.

On the contaminated soil, both the poplar biochar and the gravel sludge + siderite bearing material mixture were most effective in terms of reducing metal extractability and uptake in the plant biomass (*Lolium multiflorum*). In the case of Zn a significant decrease after lime, poplar BC and gravel sludge with siderite bearing material was detected. However, Zn remained above the phytotoxicity level of 200 mg kg<sup>-1</sup>; lime treatment reduced the Zn concentration in *Lolium multiflorum* to 513 mg kg<sup>-1</sup>, gravel sludge to 531 mg kg<sup>-1</sup> and poplar BC to 560 mg kg<sup>-1</sup> while in the control plot 713 mg kg<sup>-1</sup> Zn were measured.

The results of the field experiment and the parallel greenhouse test will provide further information on the effectiveness over a longer period (i.e. 2 years). So far, it has been found that a mixture of biochar and inorganic additives were most effective in increasing biomass productivity of *Miscanthus* on the metal-contaminated soil in comparison to an equivalent nutrient supply with mineral fertilizer (+80 %).

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## NANOSCALE INTERACTIONS BETWEEN ENGINEERED NANOMATERIALS AND BLACK CARBON (BIOCHAR) IN SOIL

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### ABSTRACT

The increasing manufacturing and use of engineered nanomaterials (NMs) has resulted in potentially excessive yet poorly characterized environmental exposure. In agricultural systems, exposure may occur directly through the application of nano-enabled agrichemicals or indirectly through the wide scale amendment of soils with NM-contaminated biosolids. NMs that have been released into these environments will interact with soil constituents, including black carbon (BC), that may subsequently affect particle fate and toxicity to soil-based biota such as plant and earthworm species. Biochar is a form of black carbon that is being proposed as an amendment to soils so as to improve soil fertility and enhance crop production. Due to the highly porous micro- and nano-structure of biochar, one can anticipate potentially significant molecular interactions with nanomaterials. However, the interactions of biochar with NMs and the subsequent effect on the bioavailability and toxicity of these materials to representative crop species (zucchini, soybean, corn, lettuce) and soil invertebrates (earthworms) is unknown.

Studies have recently been initiated in which biochar from several different feedstocks and synthesis conditions were added separately to an agricultural and residential soil at three amendment rates of 0, 0.5 and 5% (v/v). Dispersed cerium oxide (CeO<sub>2</sub>) or Ag nanoparticle solutions were added to containers to yield final concentrations of 0, 500, 1000 or 2000 mg/kg. Plant or earthworm species were subsequently added to the soils and were harvested after 28 days of exposure. To date, results from a single experiment have been analysed, although numerous additional experiments are currently underway. For soybean, there were minimal effects of pecan shell-derived biochar presence on Ce uptake or plant biomass; in several instances, the trend was for decreased NP uptake with char amendment, but there were no effects of statistical significance. Perhaps the most interesting finding was a significant decrease in pigment content/production in soybean upon nanoceria exposure, regardless of char presence. This is particularly interesting given the lack of biomass effects on soybean. Additional results focused on different types of biochar, plant species, and nanoparticles will be presented, as well as experiments focused on exposed earthworm species.

The results of these investigations will serve agriculture by providing a mechanistic foundation for the fate and bioavailability of NMs in biochar amended soil that will aid in the development of accurate nanotoxicity risk assessments.

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## OPPORTUNITIES AND DRIVERS FOR BIOFUELS AND BIOCHAR PRODUCTION BY VALORISING AGRI-FOOD SOLID PROCESSING RESIDUES AND WASTES

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### ABSTRACT

Food processing industry faces increasing regulatory pressure to reduce environmental impacts and provide sustainable management and use of wastes. The topic is gaining increased attention worldwide by the new advances in the integrated management of food processing waste of the global food & drink Industry, satisfying energetic needs of the production. However, most bioenergy systems are characterized by low efficiencies resulting in an increased cost of operation. Significant improvement in efficiencies could be achieved by developing systems that generate both power and heat. While, residues could be a useful energy source, they can also play a fundamental role in preserving soil fertility and contributing to carbon sequestration into the soils. For material and energy recovery efficiencies, a trade-off between the two targets should be accomplished. Small distributed gasification systems or pyrolysis systems could be a win-win solution. Gasification-based CHP integrated in a symbiotic way with the processing plant, can fulfil the needs on energy by using the residues while pyrolysis process can be optimized for the recovery of biochar and biofuels production. The application of biochar to the soil stabilizes a carbon fraction thus reducing the release of CO<sub>2</sub> in the atmosphere. This work presents a case study applied to an olive mill being a rural activity in the Mediterranean areas

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## USE OF BIOCHAR AS A POST ANAEROBIC DIGESTION TREATMENT FOR AMMONIUM-NITROGEN REMOVAL FROM PIGGERY SLURRY

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### ABSTRACT

In China, anaerobic digestion (AD) with biogas supply is the major livestock manure treatment procedure and the estimated amount of biogas residues from livestock biogas projects is more than 200 million tons every year. Farm spreading for land fertilization is the most common disposal method for the residual slurry, however, due to the high loads of nitrogen in the slurry; farm land carrying capacity is often exceeded especially in off peak cropping seasons. Unless novel research solutions are sought to recover nutrients and recycle water, there is a higher risk of adverse pollution from these wastes. The purpose of this study therefore was to investigate  $\text{NH}_4\text{-N}$  sorption capacity of biochar from piggery liquid manure after anaerobic digestion.

The biochar was produced from mixed wood cuttings (WDB) at temperatures of 500°C and risk husks (RHB) at 300°C. Proximate Analysis was used to determine the Moisture content (MC), Ash, Fixed carbon (FC) and Volatile content (VC) of the Biochar in accordance to the ASTM D1762-84 standard method for chemical of wood charcoal analysis (ASTM, 1990). The untapped bulk density was determined as the mass of uncompressed biochar that filled a volume of 10ml tubes whereas the pH and electro conductivity (EC) were determined after 2hours shaking of mixture of 1g biochar in 20ml of double distilled at a constant water bath temperature of 90°C using pH and EC reader (FE20 METTLER TOLEDO, Switzerland and Orion Model 115A, Thermal Fisher Scientific) respectively. The surface area and total pore volume of the biochar were determined using BET method with  $\text{N}_2$  absorption at 77K based on multipoint calculation technique. In addition, Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR) techniques were used to further characterize the biochar morphology and chemistry. We also characterized the slurry on some important parameters that could affect our study. The studied parameters were determined using the APHA (1998) standard for wastewater analysis and they included; pH, TSS,  $\text{BOD}_5$ ,  $\text{NH}_4\text{N}$ ,  $\text{PO}_4\text{-P}$  and COD. The bulky density, % MC, % ash, % VC and % FC of the WDB were; 0.4994g/ml, 4.3%, 5.87%, 8.92% and 85.21% for WDB whilst for RHB it were 0.298g/ml, 4.3%, 45.96%, 44.67% and 17.34% respectively. The BET surface area, total pore volume, pH and EC for WDB were  $273.63\text{m}^2\text{g}^{-1}$ ,  $0.176\text{mlg}^{-1}$ , 9.8 and  $411\mu\text{scm}^{-1}$  respectively whilst for RHB it were  $10.995\text{m}^2\text{g}^{-1}$ ,  $0.0308\text{mlg}^{-1}$ , 7.8 and  $300\mu\text{scm}^{-1}$ . The slurry TSS was 150-175mg/L, pH ranged from 8.0-8.5,  $\text{BOD}_5$  was 850-920mg/L, and  $\text{NH}_4\text{-N}$  was 1380-1450 mg/L, whereas  $\text{PO}_4\text{-P}$  and COD ranged from 15-25mg/L and 4000-4200 mg/L respectively. Ammonium sorption was studied using synthetic  $\text{NH}_4\text{Cl}$  [analytical grade] and the Anaerobic Digestion Slurry (ADS). From batch equilibrium and kinetics experiments the amount of  $\text{NH}_4\text{-N}$  absorbed as a function of biochar dosage, biochar particle size (0.25-1.25mm), initial effluent concentration, contact time, initial pH, and temperature was quantified after equilibration for 24 hours in an end-to end shaker with a constant water bath temperature of 318 K using 50ml of effluent solution (adjusted pH of 7.0) loaded onto 1g of biochar.

From the results, absorption was higher with pure  $\text{NH}_4\text{Cl}$  solution than with the slurry. The lower absorption with the slurry could be attributed to presence of other competing ions such metals present in the slurry. Furthermore, there was positive increase in absorption with pH, temperature, initial concentration, and contact time for two types of biochar. Wood biochar performed better than RHB with pure  $\text{NH}_4\text{Cl}$  but the difference with the slurry was not big as it would be expected considering the difference in BET surface area. The maximum adsorption of  $\text{NH}_4\text{-N}$  at 1400mg/L in the slurry was  $44.64 \pm 0.602\text{ mg}\cdot\text{g}^{-1}$  and  $39.8\text{ mg}\cdot\text{g}^{-1} \pm 0.54$  for WDB and RHB respectively. The Langmuir model fitted the sorption data better with  $R^2 = 0.973(\text{NH}_4\text{Cl})$  and  $0.897(\text{slurry})$  for RHB and  $0.986(\text{NH}_4\text{Cl})$  and  $0.8902(\text{slurry})$  for WDB. From the results, we conclude that biochar has potential to reduce nitrogen loading in piggery farm waste water and we suggest that biochar filters can be developed and used as the first line effluent recipients prior to release into lower N concentration degradation systems.



## BIOCHAR INFLUENCE ON CeNPs LEACHING AND PLANT UPTAKE IN *LEPIDIUM SATIVUM* FROM PACKED-LYSIMETER EXPERIMENT

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### ABSTRACT

The use of metal-nanoparticles (MeNPs) has been increasing over the last few years. Their presence in soil may be detrimental to the entire ecosystem; it has been shown that they may have negative effects on organisms' reproduction, mobility and survival. The magnitude of such impact depends also on the MeNPs mobility, other than the MeNPs bioavailability. Nanoparticles interact with the soil aggregates and both the physical and chemical properties of the soil influence the MeNPs mobility.

Porous media can interfere with the mobility of contaminants in soil, hence with MeNPs. Being the biochar a porous material with charged surface groups, it is expected to have an influence on the balance of the MeNPs transport in natural soils. Biochar notoriously changes the soil properties such as pH, EC and others that may have an impact on the MeNPs aggregation and mobility.

Biochar may immobilize the soil MeNP by increasing the cation exchange capacity but also by straining due to the high porosity. The difference in MeNPs mobility will be considered in soil amended with two biochars which differ only for the quenching process, that is, water or dry quenching. In the first case, we hypothesize that water quenching, by removing the ash from the pyrolyzed biomass and increasing the BET and porosity of the biochar, affects the interaction with the MeNPs and reduces the leaching.

Soil collected from a residential area in NE of Italy was mixed with Ce-NPs at 1,000 mg kg<sup>-1</sup> and amended with biochar from wood-pellets quenched with either water or without water at the dose of 5% (dw/dw). Twelve column-lysimeters packed with the soil mixtures will be seeded with watercress (*Lepidium sativum*). The leachates will be collected and analysed to evaluate the amount of NPs leached from the soil treated with the two biochars. At the end of the experiment, the plants will be harvested and analysed to measure the Ce uptake and the presence of CeNPs at cellular level as well as in the leachate will be verified by means of transmission and scanning electron microscopy.



## A STUDY ON THE CHARACTERISTICS OF BIO-CHAR WITH THE SLUDGE OF SEWAGE BY LOW TEMPERATURE HYDROTHERMAL CARBONIZATION

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### ABSTRACT

This study proposed a low temperature hydrothermal carbonization to treat and recycle sewage sludge. Such a reaction was used to identify the optimal conditions to produce bio-char. A chemical activation process was also introduced to develop the micro pores of the produced bio-char. To produce bio-char, the original material, sewage sludge, of 50 g was put in a reactor and experimented at 220, 230 and 240°C for 1, 2, 3, 5, 8, and 10 hours. In Batch 1., the optimal reaction temperature for the chemical activation of bio-char was determined by varying the reaction temperature at 500, 600, and 700°C for 60 minutes each. In Batch 2., based on the identified optimal activation temperature, the optimal reaction time for the chemical activation of bio-char was determined by doing an experiment for 30, 60, 90, and 120 minutes. As a result, the optimal production conditions of bio-char and activated bio-char were correlated with the iodine adsorption capacity. The iodine absorption capacity was assessed according to the experimental method prescribed in KS M 1802 of Korean Industrial Standard. The optimal conditions to produce bio-char and activated bio-char were 230°C and 8 hours and 600°C and 60 minutes, respectively. Those conditions were used to conduct an isothermal adsorption experiment for removal efficiency. Heavy metals such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn) and nickel (Ni) were analyzed under the standard method for waste in Korea. Lead recorded the highest heavy metal adsorption efficiency of bio-char, being followed by copper, cadmium, zinc, and nickel. Arsenic was hardly adsorbed overall. The heavy metal absorption efficiency of activated bio-char was shown more than 80% in all the heavy metals except for arsenic, which was not adsorbed relatively and recorded adsorption efficiency about 20~40%.

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## **GREEN ROOFS / COVERS / SUSTAINABILITY**





## ENERGY SAVING POTENTIAL BY THE APPLICATION OF GREEN ROOFS IN GREEK SCHOOL BUILDINGS

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### ABSTRACT

Green roof utilization is increasing in contemporary urban centers mainly due their numerous beneficial impacts that include microclimate improvement. However, their cooling and thermal insulating features have been studied mainly in either office buildings or hotels. The aim of the present study is to evaluate the contribution of green roof systems in the energy needs of school buildings, which constitute a special type of public buildings in Greece. Different types (extensive, semi-intensive) and construction options of green roof systems were studied on eight school buildings. The energy needs of the buildings were simulated with TRNSYS simulation software, before and after the installation of the green roofs, in order to quantify their potential energy savings. The results from the simulation confirmed previous research results that reported that all tested green roof types improved the energy savings for cooling. By contrast, the energy savings for heating were much less or even insignificant. It was also shown that the use of specialized materials, such as substrates with increased porosity or a drain layer with good thermal insulation characteristics are necessary to avoid heating penalties.



## THE ADAPTIVE APPROACH AS A MEANS TO INCREASE GREEN ROOFING THE MEDITERRANEAN BASIN

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### ABSTRACT

Green roof construction has been steadily increasing worldwide due to the numerous environmental and aesthetic advantages that they provide within the often monotonous and hostile landscape of contemporary cities. In city centers, which are mostly occupied by older buildings that can withstand only minimal additional weight loads, extensive green roof types seem to be the only applicable solution for green roof construction. However, in the absence of government incentives, a financial investment is frequently substantiated from the residents only if the green roof is an accessible and functional space.

In order to achieve sustainable management solutions, it seems necessary to deviate from the existing formulaic approach in concern to green roof typology towards to an adaptive approach. This deviation requires rigid data and knowledge of the most significant parameters for assuring green roof sustainability and accurately predicting resources inputs. These parameters include the type and the depth of the substrate, the necessary water inputs and the selected plant species.

Several substrate types will be presented along with their capacities as well as their effects on plant growth. The substrates commonly utilize coarse textured materials that are lightweight and possess an increased water holding capacity. In addition of significant importance is the source and percentage of the organic amendment of the substrate.

Substrate depth has been substantiated as one of the most important factors contributing to green roof sustainability. It is commonly accepted that as substrate depth increases the palette of plant selection as well as plant growth and their sustainability also increases. Substrate depth becomes of further importance in the challenging climatic conditions of the Mediterranean basin, especially in the shallow depths that are utilized in the extensive green roof types (2-15 cm).

However under an adaptive approach, a reduction of the substrate depth that otherwise would be intolerable for plant survivor, could be compensated by increasing water inputs. The exact prediction of the annual water inputs in an adaptive green roof system will depend on the selection of the plant species or plant phytocommunities.

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## VEGETATIVE COVER AS A SUSTAINABLE METHOD FOR REHABILITATING VARIOUS MINE TAILINGS THROUGH PHYTOSTABILIZATION

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### ABSTRACT

Phytostabilization is a useful tool in the rehabilitation of tailings storage facilities (TSFs); it involves the reduction of trace element mobility in a growth medium by means of absorption. In some cases it also refers to the physical stabilization of any surface, i.e. side slopes of TSFs. Vegetative material increases soil cover (cover [C-] factor in soil erosion equation) and consequently decreases soil erosion and the mobilization of any contaminants, e.g. trace elements. Leaching of contaminants is also reduced due to the hydraulic control exercised by plant roots, and the addition of soil organic matter by plants decreases the solubility of trace elements in a growth medium.

The objective of this study was to assess the suitability of different plant species for the phytostabilization of various mine tailings. Twenty-eight plant species (17 tree species and 11 grass species) were established in nine completely different types of tailings materials as well as in a control soil. Physical and chemical properties of tailings which are significant for phytostabilization were analyzed. Detailed monitoring was conducted over a period of 24 months to identify successes and failures. Monitoring criteria included the assessment of plant performance (plant physiology, plant stress factors) and changes in tailings chemistry (pH, electrical conductivity).

Final results indicate that the correlation matrix is a useful tool to assist in the recommendation of specific species for different tailings materials. Various successful tailings and species combinations were identified, e.g. *Bambuseae* sp., when planted in coarse coal discard material; and *Chrysopogon zizanioides* (Vetiver), when planted in low-pyrite gold tailings. These combinations showed minimal plant stress factors, good growth, and good canopy cover, basal diameter growth and root development.

Concluding remarks: Vegetative covers have proven to be a valuable method in tailings rehabilitation where successful tailings-species combinations are used; various improvements in the physical and chemical properties of tailings can be observed. Determining the success of specific species in given tailings materials early on in the planning phase of the rehabilitation programme is therefore essential.

**Keywords:** vegetative cover, tailings storage facilities, trace elements, soil erosion, phytostabilization, monitoring, cover factor

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## SEDUMS ROLE IN METAL RETENTION AND EMISSION BY GREEN ROOFS

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### ABSTRACT

To reach the ambitious objectives of good chemical states for waters that have been introduced in Europe with the Water Framework Directive 2000/60/CE, it is important to control pollutant fluxes. Urban runoff being recognized as one of the major sources of pollutants in surface water and rainwater harvesting spreading in the meanwhile, upstream stormwater management seems to be necessary.

Vegetative roofs are constituted by two materials currently used in water depollution, i.e. a "soil" and plants. Therefore, this technique is often considered as a potential solution to obtain a good roof water quality.

Nevertheless, vegetative roof impacts on water quality have proven to be mixed. The main reason is probably because vegetative roofs are not designed in order to purify rainwater. The objective of this presentation is to better understand plant impacts on metal storing and disposing in green roof to provide criteria for plant selection in order to improve roof water quality.

This study is mainly based on two complementary steps. The first one is the monitoring of green roofs built in 2010 near Nancy (France). Three plants associations have been used on eight different structures. The first one contains six species of *sedum* (*S. album*, *reflexum*, *spurium*, *floriferum*, *hispanicum*, *sexangulare*). This genus is widely used for green roofing for its ability to withstand harsh climatic conditions. The two other plant associations also contain some local plants such as *potentilla erecta*, *euphorbia polychroma* and *iris sibirica*. Plant development was studied every two months. Samplings were collected twice a year and metal content was analysed in root and aerial parts. Substrates were also sampled in 2010 and 2013 for total and extractible metal contents analyses and physico-chemical characterizations. Metal content in the fertilizer used when the plants were introduced was also evaluated. Atmospheric deposits and roof water were collected monthly and analysed to measure metal contents. Along with this in situ study, complementary experiments at meso- and microscopic scale were realized. Thin section (20 µm thickness) of substrates containing sedums roots were made in 2013 and observed thanks to a binocular magnifier. Roots and leaves ultra-thin sections (80 nm) were observed thanks to a transmission electron microscope equipped with an Energy-Dispersive X-ray microanalysis system used to detect metals in plant tissues.

The different plants showed comparable ability to retain metal, far from hyperaccumulator capacities. The global amount of metal stored in the plant compartment was mainly influenced by the biomass of the different species. It was also superior to the amount of metals brought by the atmospheric deposit, meaning that the use of hyperaccumulator may not be necessary. It was also noticed that metal contents in plants were highly correlated to metal contents in substrates, which are bigger than atmospheric deposits ones. This indicates that a part of available metals from the substrate is stored by the plants, which contributes to reduce metal lixiviation from the substrate by rain water and consequently to reduce metal concentration in green roof water. Cr content in fertiliser proved to be significant. A strong correlation was observed between Cr amounts brought by

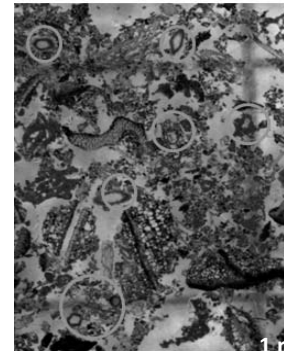


Fig. 1: Substrate section showing aggregates around *S. spurium* roots



fertiliser introduction and measured in plants. If this metal had not been retained by plants, fertilisation would have increased Cr content in roof water. Root plants seemed to foster aggregates formation (Figure 1), which can reduce small particles leaching. Organic matter content in substrate was also higher as plants were present than under bare surfaces. Both of these impacts indirectly improve water quality: the first one by reducing metal emission from the substrate and the second by improving the substrate ability to retain metals. Indeed, organic matter proved to have higher metal sorption ability than the mineral part of the substrate. However, plant roots also seemed to increase the availability of some metals in the substrate, such as Zn. This could have a negative impact on roof water quality if these elements are lixiviated by water before being absorbed by the roots. Ultrastructural observations of plant roots revealed that metals were mainly stored by cells containing condensed polyphenolic substances. Bacteria degrading these polyphenolic metabolites have also been noticed. This induces that storage of metal in green roof plants is only temporary. Consequently, if plants are introduced on green roof to improve water quality thanks to their ability to store metals, maintenance practice should be adapted to its characteristics. Seasonal mowing and collecting of aerial parts or periodical plants substitution could be considered.



## LAYER SOIL FORMATION ON TAILING HEAPS AFTER SOIL COVERS AND TREES AFORESTATION

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### ABSTRACT

As a result of mining activities and soft environmental regulations a sort of tailing heaps has been abandoned along the countryside in Mexico. Most of the treatments and managements of the tailing are too expensive to apply to millions of cubic meters of these residues. In addition, many of these materials are a cocktail of elements including toxic metals and metalloids. So, a proper disposal management such as electrochemical treatment, or dumping are not viable. One option to sort it out, followed by a mining company, was apply soil covers over the plateau of the damp and afforest with *Pennisetum clandestinum*, *Acacia farnesiana*, *Populus nigra*, *Casuarina equisetifolia* and *Eucalyptus camaldulensis* in different areas. Thirty years later a soil layer seems to be formed but some plant trees fall down or dry to died up right, no records about trace elements availability has been carried out on the soil layers formed during the evolution of the rhizospheric layer. Therefore the aim of this research was to follow the soil layer formation and trace elements availability depending of the plants set up.

We identified four layers in the soil profile: litter (organic matter), root development, transition between root and tailing, and tailing layer. *C. equisetifolia* formed a thick layer of organic matter (up to 10 cm), but did not allow the introduction of other pioneer plants, *P. nigra* formed a variable layer (2 – 20 cm) of organic matter but allowed the establishment of other plant species. *E. camaldulensis* behaved similarly to *P. nigra* in soil formation. *P. clandestinum* formed an organic matter top layer and a thick root layer. Trace elements concentration on the different layers formed varied depending the predominant plant species established, however generally speaking we cannot say that plant covers reduce metal availability in the top layer. Copper concentration for instance was low in the first 5 cm of soil were *C. equisetifolia* was growing, and increased in deeper layers, but in the area of *C. equisetifolia* and *P. nigra* the concentration was higher in the top layer (0-10 cm), decreased in the second layer and decreased again. Some tree plants fall down because their roots avoided growing down the tailing and keep on a surface layer, so when wind blows the roots are broken. Soil formation and trace element distribution in the soil profile vary between the predominant plants species established, possible explanation are discussed in this study.



## ADVANTAGES AND DISSADVANTAGES OF THE PRESENCE OF THE 1BL.1RS WHEAT-RYE TRANSLOCATION IN SUSTAINABLE WHEAT PRODUCTION

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### ABSTRACT

The 1BL.1RS wheat-rye translocation (i.e. a new B genome chromosome consisting of the long arm of chromosome 1B and the short arm of 1R replacing the normal 1B, Figure 1) is probably the most important one, used by many breeders. Although four sources of translocations and substitutions have been reported in wheat, the 1RS arm of the translocation, obtained by Riebesel in the 1920s, is originating from "Petkus" rye (Rabinovich, 1998). The first commercial cultivar (cv. "Salzmunder Bartweizen") carrying the 1BL.1RS translocation, was produced and released in Germany by Riebesel in 1957. However, in most current cultivars the translocation was transferred through the Russian cultivars Kavkaz and Aurora, which are progenies of the Riebesel lines. The translocated fragments of rye chromosome offer to the host cultivar a number of important agronomic traits, i. e. high yield potential, broad adaptation, resistance to drought and biotic stress conditions (diseases and insects) and increased regeneration capacity of the plants, contributing to a sustainable wheat production. More precisely, the short arm of 1R chromosome contains resistance genes against leaf rust (*Lr26*), stem rust (*Sr31*), stripe rust (*Yr9*) and powdery mildew (*Pm8*). The main disadvantages of the translocation are that the aforementioned genes are linked with decreased bread-making-quality, their action depends on the genetic background of the host cultivar and the prevailing environmental conditions, but most of all, they are no longer effective against new biotypes of the diseases. However, recent results demonstrate a considerable resistance of new Hellenic doubled haploid lines (lines obtained through anther-culture) not only to rusts and mildew but also to septoriosis under the conditions of Ukraine. It could be concluded from the results of various reports that the effects on yield and drought tolerance have been highly dependent on genetic background and environmental conditions of the host cultivar. For example, the 1BL.1RS translocation in spring cultivar 'Pavon' was correlated with the presence of a larger root biomass and higher grain yields especially under well watered conditions. For this, breeders should pay careful attention to the effects of genetic background and environment on drought tolerance of lines potentially conferred by rye translocations. New efforts (including introgression of new genes) are inevitable to broaden the narrow genetic background of cultivars carrying the translocation if its positive traits are to be exploited.

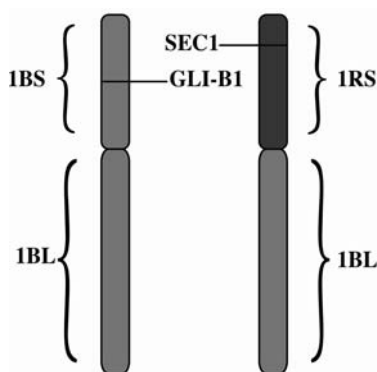


Figure 1. The normal 1B chromosome (left) and the translocated (right) and two corresponding storage protein genes.



## EFFECT OF THE 1BL.1RS WHEAT - RYE TRANSLOCATION ON THE ANDROGENIC RESPONSE IN BREAD WHEAT

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### ABSTRACT

Anther culture is the most convenient and effective technique to obtain doubled haploid (DH) plants and thus to accelerate the production of improved wheat cultivars. This androgenic response depends on culture medium, on cold or heat pretreatment and mainly on the genotype of the donor plants. In case of bread wheat (AABBDD) it was referred that the presence of the 1BL.1RS wheat-rye translocation could improve the embryoid production and green plant regeneration after anther culture. Thus, the present study was conducted to further investigate the effect of the aforementioned translocation on embryoid induction and green plant regeneration in bread wheat after anther culture. For this to be done, four bread wheat cultivars carrying the 1BL.1RS translocation (KVZ/Cgn, Acherontas, Elisavet, and Orpheus) and five bread wheat cultivars without the translocation (Acheloos, Vergina, Mykonos, Prelude and Chios) were used. Approximately 1500 anthers (derived from 30 spikes) from each cultivar, containing anthers in the mid (MU) to late uninucleate (LU) microspore developmental stage, were cultured after cold pre-treatment for 7 days at 4°C. The induction, regeneration, and rooting media used were the W14, 190-2 and basic MS medium respectively. The best anther culture response was recorded in two cultivars carrying the translocation (i. e. cvs Acherontas, 49.8 embryoids/100 anthers and KVZ/Cgn, 33.6 embryoids/100 anthers). Cultivar Orpheus (carrying the translocation) and Chios, (lacking the translocation) performed almost equally (23.8 embryoids/100 anthers and 22.1 embryoids/100 anthers respectively). More green plants were produced from the cultivars Acherontas (6.5 green plants/100 anthers) and KVZ/Cgn (5 green plants/100 anthers). No response was reported for the cultivars lacking the translocation 1BL.1RS except cultivars Acheloos and Chios. They produced 2.36 green plants/100 anthers and 2.9 green plants/100 anthers, respectively. It is concluded that the positive effect of the translocation 1BL.1RS observed on anther culture response of bread wheat cultivars cannot be attributed entirely to the presence of the translocation. This is because the genetic background of the cultivars carrying the translocation could be also important.





## EVALUATION OF PLANT SPECIES AND SOILLESS SUBSTRATES FOR MEDITERRANEAN SUSTAINABLE GREEN ROOF INSTALLATIONS

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### ABSTRACT

Background: The installation of green roofs and green facades on urban buildings has received a growing interest for the potential environmental benefits. One of the advantages of the green roofs is the reduction of the surface temperatures by the plant transpiration with mitigation of the “heat island” effect in the urban settlements [2]. In the continental regions the parameters of plant growth on green roofs have been object of study [1] but in the Mediterranean areas the development of the green roof technology still requires a research aimed at the evaluation of materials and plant species adapted to the seasonal climatic variations and the summer drought. The aim of this work is: 1) The selection of plant species and sustainable supporting substrates able to form a coverage for extensive green roofs in the climatic conditions of the Central Italy. 2) The collection of the environmental data on a green roof model for the prediction of the thermal insulation.

Materials and methods: 1) The species *Sedum acre* and *S. reflexum* were used for trials using a blend of soilless substrates (Agriterram™ and Agrilit 3, 50% each) and the addition of amendants or arbuscular mycorrhizal inoculum. The amendants were: Biochar from biomass and Compost from food waste, added at 10% Vol/Vol. The experiments were carried out on small containers (10 cm deep) under outdoor conditions during the four seasons. Periodically, the plant growth parameters were evaluated. 2) Containers of 7 m<sup>2</sup> each were made with layers of substrates (10 cm deep) and installed on a building terrace. The plant species *Sedum* spp. and *Sempervivum* spp. were transplanted at the density of 16 plants/m<sup>2</sup>. The trial included two indigenous accessions collected from Italian regions and two species from collections. The addition of compost to the substrate was evaluated also. Periodically, the coverage of each species and the inter-species interaction were evaluated. In addition, the temperature on the surface and the bottom of the system plant-substrate, the radiation, the moisture content and the climatic parameters were collected by sensors connected to a data collection system. The data were analyzed by a specific software in relation to the climatic parameters, in order to estimate the mitigation effect related to the plant coverage.

Results: The addition of the Compost to the substrate produced a significant effect on the plant growth parameters, endurance and ability to form a dense canopy over the four seasons. The inoculum with arbuscular mycorrhizae promoted the plant growth and the root development. The experiment on containers simulating the green roof conditions indicated the ability of indigenous Italian accessions of *Sedum* and *Sempervivum* to compete and form a consistent coverage when growing on mixed culture with collection accessions. The evaluation of the physical parameters of the systems plant-substrates indicated a mitigation of the surface temperature in the summer season, related to the plant transpiration.

Conclusions: The data indicated the good performance of a blend of species of Crassulaceae from collections and some indigenous Italian accessions for the realization of extensive green roof coverage, under the climatic conditions of Central Italy. The addition of Compost or mycorrhizal inoculum to a soilless substrate blend of 10 cm deep have shown an improvement of the plant growth parameters, under a limited fertilization rate. The model plant-substrate system has shown a reduction of the surface temperature indicating a mitigation effect in the summer season. A long term experiment is necessary to confirm the results under the variability of the climatic factors.

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## ANAEROBIC DIGESTATE AS A SUITABLE PLANT GROWTH PROMOTER IN SUB-ACID SOILS

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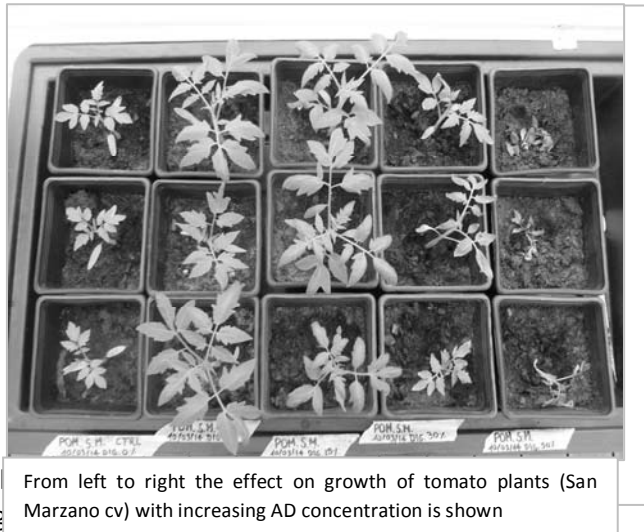
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### ABSTRACT

Biogas production is a suitable renewable alternative to fossil fuels and it is increasingly becoming of deep interest. One of the by-products of this process is the anaerobic digestate (AD), which can be obtained with an anaerobic digestion of animal or plants biomasses or with municipal waste, with different chemical properties and composition. From August 2012, Italian legislation has changed the definition of AD, and the rules for its use. Currently AD is classified as a by-product, instead of a waste. Many studies have assessed that, under controlled supply conditions, it has fertilising properties. Digestate can be used as a replacement for mineral fertilisers, reducing costs to farmers and minimising greenhouse gas emissions from cultivation.

The aim of this work is determining AD (methanogenic digestate, from anaerobic fermentation of swine manure, in which on average 80% of the total nitrogen is  $\text{NH}_4$ ) effect on horticultural model plants with different protocols. Germination index has been assessed in pea (*Pisum sativum*) and phytotoxicity in water cress (*Lepidium sativum*). Shoot elongation and leaf chlorophyll content have been measured in lettuce (*Lactuca sativa*) and endive (*Cichorium endivia*), with a special attention for the fresh/dry weights ratio and for the roots/shoots ratio. In barley (*Hordeum vulgare*) the roots elongation and the radical inhibition indexes were determined by processing the average weights and lengths of roots. Germination and phytotoxicity were assessed by *in vitro* experiment in agarized media, while shoots and roots elongation was assessed in pot experiments on soil.



From left to right the effect on growth of tomato plants (San Marzano cv) with increasing AD concentration is shown

Additional tests were performed using other horticultural plants: tomato (*Solanum lycopersicon*, two cultivars), pepper (*Capsicum annuum*, two cultivars) and geranium (*Pelargonium zonale*, one cultivar). Four different concentrations of AD diluted in a nutrient solution were supplied in order to determine the effects on growth rate, total biomass, leaves chlorophyll content, number of flowers, fruit size, harvest index. Different types of soil have also been tested.

In the experiments with different types of soil, it was shown that the AD from swine manure, with pH 8.0-8.5, had the effect of soil liming. Therefore, it can also be used to adjust the pH of acid and sub-acid soils, besides providing a fertilising effect.

The results of the combined tests on model plants and horticultural plants revealed the optimum concentrations of AD to be applied in order to maximise plants growth and soil liming.

Future perspectives concern the application of AD to several different tomato cultivars in metal polluted soils, to estimate the effect of AD on metal plant uptake.





## **BIOFUELS & BIOMASS FOR ENERGY**



## TEMPORAL EVOLUTION OF SOIL QUALITY UNDER SHORT ROTATION COPPICE AS COMPARED TO FOREST, GRASSLAND AND ARABLE PLOTS

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### ABSTRACT

Nowadays, agricultural soils are subjected to strong anthropic pressure, including their use for production of diversified energetic raw material competing with food production. Short Rotation Coppices (SRC) are an intensive crop of fast growing tree species intended to produce energy wood chips. Many studies have been led on SRC, but hardly any have as yet dealt with its impact on chemical/biological parameters and soil functions and publications on its impact on soil microbial communities are scarce.

We assessed the biological and physico-chemical properties of soils planted with SRC in situ for two years compared to agrosystem, forest and grassland soils. Samples were collected from field plots of the four land uses in the Aisne valley (France), in autumn and spring 2010 to 2012. Twenty-five different parameters involved in soil fertility, biological communities and activity were measured at four seasonal sampling times. Based on the parameters discriminating the four land uses, three soil quality indices were calculated to reflect fertility, biological activity and biological community.

For many parameters, SRC values were higher than agrosystem values, but lower than forest or grassland values. The calculated soil quality indices were relevant with the following gradient (arable field > SRC > grassland > forest). Six years after plantation, the SRC crop had increased the soil fertility and biological community indices compared to the agrosystem. However, biological activity indices were similar in the SRC and agrosystem plots although soil basal respiration and laccase activity were higher in the SRC than in the agrosystem. Our results demonstrate that Soil Quality Indices can be considered as valuable tools to improve agricultural management. Whether a balanced SRC ecosystem functioning will be maintained in the next 15-20 years will have to be verified.

*Acknowledgements:* We thank Philippe Lucas, the owner of the SRC plot. This project was supported by Kinomé company and ANRT (National Agency of Research and Technology) with a CIFRE (Industrial Convention of Research Formation) contract number 2009/13/07. ADEME (« Agence de l'Environnement et de la Maîtrise de l'Energie ») sponsored and supported the research (contract number 0975C0095).



## ENERGY USE AND GREENHOUSE GAS EMISSIONS IN ORGANIC AND CONVENTIONAL PEACH AND KIWI ORCHARDS

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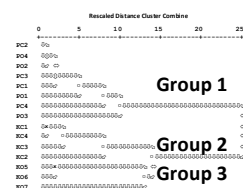
### ABSTRACT

Energy flow in peach and kiwi orchards can help to determine first the best management strategies for each species and second the possibility of using the one having best environmental advantages. Conventional and organic peach and kiwi orchards found in the Prefecture of Pella, northern Greece, were selected in order to (a) determine energy flow of the farming systems, (b) reveal groups of orchards on the basis of their similarities to energy inputs, (c) estimate gas emissions and carbon footprint.

Fifteen farms [four conventional and three organic kiwi orchards (KC1, KC2, KC3, KC4 and KO1, KO2, KO3); four conventional and four organic peach orchards (PC1, PC2, PC3, PC4 and PO1, PO2, PO3, PO4)] were selected during the years 2010-2013. The energy encapsulated by the crop was estimated using the duration of each operation, the number of machines and laborers, all field operation inputs (irrigation, fertilizer application, harvesting etc.) and production coefficients (plant protection products, fertilizers etc.). Non-renewable (fuels, chemical fertilizers and pesticides) and renewable energy inputs (human labour, animal manure) are the components of total energy inputs. Carbon dioxide, CH<sub>4</sub>, and N<sub>2</sub>O and CO<sub>2</sub>-equivalent emissions were estimated for all agricultural practices, soils, fuel and fertilizers [1,2]. The Hierarchical Cluster Analysis method was applied using seven production coefficients' variables (fertilizers, diesel, herbicides, fungicides, insecticides, labor, machinery) in order to reveal groups of farming systems [3,4].

Hierarchical cluster analysis revealed three groups of the studied orchards (Fig. 1). Group 1 (PC1, PC2, PC3, PC4 and PO1, PO2, PO3, PO4) had the lowest total and non-renewable energy inputs. Group 2 (KC1, KC2, KC3, KC4) had intermediate total energy inputs and the highest non-renewable energy inputs, while the opposite was observed for Group 3 (KO1, KO2, KO3). The highest contributors in cluster formation were human labour, shoring of branches, herbicides, fungicides, and machinery.

The largest contributors for gas emissions were fuels and fertilizers. Organic kiwi orchards (Group 3) tended to have the lowest gas emissions. Local management practices mainly affected the grouping of the studied orchards revealing their importance for the development of agro-environmental plans. Finally agricultural policies should regulate the local agro-environmental balance.



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## PHYTOREMEDIATION WITH MISCANTHUS PRODUCED FOR BIOENERGY

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### ABSTRACT

Optimal use of land for food, energy, and materials, and phytoremediation of degraded lands are both prominent sustainability issues. Miscanthus, a C-4 perennial grass, has high biomass productivity with good water use efficiency and low nutrient demands. It grows well on some contaminated and marginal lands that are not being used for food crops. It has the potential to improve soil quality by adding organic matter to soil, while as a perennial grass, it has advantages to reduce water and wind erosion. It can be harvested and used directly for combustion heating in a pelleted form, or potentially be processed for liquid biofuels and biochar. This system was recently reviewed [1].

There are many locations in the world where land is not being used for crop production because of past degradation, contamination, and/or a failure to be farmed profitably. Lands contaminated by the Chernobyl disaster, Ukraine, and brownfields in Slovakia are examples of where miscanthus has been used for attempted recovery. In the US, mine spoils and depleted farmlands are being tested for large scale production of miscanthus. Research in progress in Ukraine, Slovakia, and the United States by the authors tries to address the need for phytoremediation of sites contaminated by past military activities. Heavy metals, especially Pb, are present at the identified field sites, from gasoline spillage and combustion, small arms firing ranges and diverse other activities. These are likely combined with diverse organic contaminants. During 2013, environmental monitoring in Kamenetz-Podilsky, Ukraine, showed Co, Mn and Pb as contaminants at one site and Cu, Zn and Cd at another. At Fort Riley, Kansas, Pb contamination on former practice ranges covers hectares of land.

Current research includes testing the productivity of miscanthus on soils from the contaminated sites, as well as the effect of the miscanthus on soil quality and contaminant fate. Pot studies in Ukraine show that miscanthus grows on soils from the contaminated site and there is slow uptake of the heavy metals observed during two seasons of growth. Potential insect pests and diseases are being monitored. Rates and extent of contaminant uptake are critical factors in considering whether to use the biomass for energy production. Pot studies in Kansas show that soil amendments, principally organic matter, are important in establishment of miscanthus in poor soils where heavy metal contamination is present. Examples of results with effective use of amendments will be included. Hydroponic growth tests have been used to determine nitrogen requirements of miscanthus, and its tolerance of salts. Miscanthus has been effectively established and monitored several seasons under natural climatic conditions in large field plots at K-State, near Fort Riley, KS.

Safe and effective use of miscanthus in phytoremediation systems must examine how contaminants affect miscanthus growth, and also the extent to which miscanthus accumulates or excludes contaminants. Natural climatic conditions, diseases, and pests must also be considered during establishment, and for long-term maintenance.

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## GROWING *LEMNA MINOR* IN HUMAN AND SYNTHETIC URINE FOR BIOMASS PRODUCTION, NUTRIENTS AND ANTIMICROBIALS REMOVAL

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### ABSTRACT

Constructed wetlands with aquatic plants such as the duckweed *Lemna minor* have been widely used for tertiary wastewater treatment. Moreover, in recent studies *Lemna minor* has been used as a feedstock for biofuel production as well as a good resource of proteins and starch. In domestic wastewater, more than half of nutrient content originates from urine. As a result, there are some recent studies using urine for microalgae cultivation. According to them, human urine has the potential to become a good medium for biomass production of microalgae with high content in nitrogen, phosphorous and protein. In recent studies, the cultivation of duckweeds in swine wastewater represent high amounts of biomass production and starch content depending on harvest time, nutrient content etc. To the best of our knowledge, no information is available for the cultivation of *Lemna minor* using urine.

Based on the above, the main objective of this research was to achieve *Lemna minor* cultivation using human and synthetic urine. For this reason, the effect of several parameters (urine dilution rate, temperature, addition of microelements) on duckweed growth rate was investigated. The ability of *Lemna minor* to remove selected nutrients (NH<sub>4</sub>-N, P) and selected antimicrobials (Cefadroxil, Metronidazole, Trimethoprim, Sulfamethoxazole) from urine were also studied.

Duckweed *Lemna minor* was grown on diluted human (fresh, hydrolysed and stored for 1 day) and synthetic urine. Batch experiments were initially done using different dilution factors (1:2, 1:5, 1:10, 1:25, 1:50, 1:100, 1:150, 1:200, 1:250) of human and synthetic urine in order to calculate growth rate of *Lemna minor* under different conditions. Experiments were also done at different temperatures (12 °C, 18 °C and 24 °C), different initial mass of duckweed, addition of organic carbon sources and different concentrations of trace elements such as Cu, Fe, Mn, Mg etc. Afterwards, the best urine solution was selected and the crude protein, starch and lipids content of biomass were determined. Finally, the removal rates of selected antimicrobials compounds commonly found in urine were calculated.

According to the results, the combination of duckweed *Lemna minor* and human/synthetic urine can provide a valuable solution for removal of nutrients and antimicrobials compounds from urine. The production of duckweed biomass can also serve as a biofuel feedstock due to high starch and protein content.

**Acknowledgements:** This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: **THALES**. Investing in knowledge society through the European Social Fund. ([www.aegean.gr/environment/watermicropol](http://www.aegean.gr/environment/watermicropol)).





## ANAEROBIC CO-DIGESTION OF SEWAGE SLUDGE WITH GRAPE RESIDUES FROM WINE PRODUCING PROCESS

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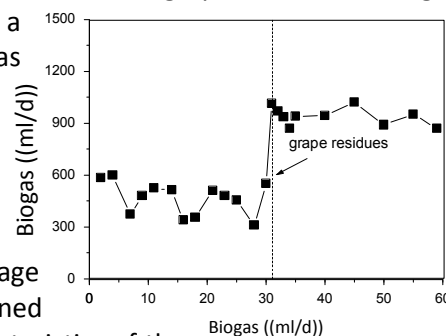
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### ABSTRACT

The grape agro-industrial sector is of major economic importance in many countries worldwide. Wine producing process generates large amounts of by-products which are totally unexploited and in some cases dangerous for the environment. Greece has produced an annual yield of about 350 kt of must corresponding to 140 kt of wine-grape residues. Anaerobic digestion of biomass is a well-known biological process producing biogas and biofertiliser. A significant number of biogas plants have been built, and now the concept is spreading all over the world. Biogas plants treat various types of organic residues including sewage sludge, food industry residues and manure. Wineries have a specific, seasonal product and the amounts of grape residues generated may not be sufficient to make digestion cost-effective. However, the addition of grape residues in a centralized facility digesting sewage sludge would ensure the viability of the process.

Two type of influent feedstock were utilized: 100% sewage sludge (SS) and a 5% of grape residues and 95% sewage sludge in order to investigate the biogas production of the grape residues – sludge co-digestion. The continuous experiments were carried out in a 3L digester constructed from stainless steel. The digester was operated at 35°C with a total feeding volume of 125ml daily and hydraulic retention time of 24 days. Feedstock was added four times daily (every 6 hours). Biogas was collected by displacement of water. Initially, the reactor was inoculated with anaerobic sludge originating from the Municipal Sewage Treatment Plant of the city of Heraklion (MSTPH). Also sewage sludge was originated from MSTPH. Grape residues were obtained from a local winery (Alexakis SA) in Heraklion, Crete. The characteristics of the feedstock are summarized in Table 1.



The initial feed was sewage sludge and the bioreactor was operated using this feed for 30 days. Grape residues were then added to the feed so that the reactor was fed continuously with 95% sewage sludge and 5% food waste. The reactor treating the sewage sludge produced  $463 \pm 109$  ml/d biogas before the addition of grape residues  $940.50 \pm 53$  ml/d biogas after the addition of food waste (5% v/v in the feed).

**Acknowledgements:** This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund (Acronym: EXISTING).



## HARVESTING OF MICROALGAE THROUGH MAGNETOPHORESIS: INHIBITORY EFFECT OF IONPS TOWARD BIOFUEL PRODUCTIVITY

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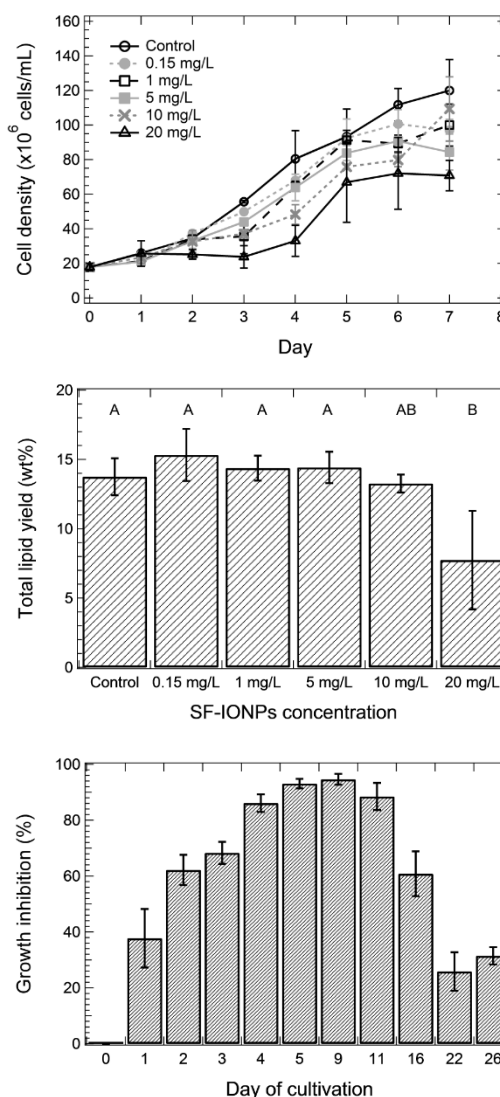
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### ABSTRACT

Microalgae are suggested as the potential third generation renewable fuel sources due to its high oil yield (Johnson, 1999). Magnetophoretic separation of microalgae has been proven as a feasible harvesting method to achieve up to 99% cell separation efficiency (Lim et al., 2012) which yield a biomass estimated at \$180/ton (Toh et al., 2012). A previous study showed a highly effective attachment of the surface functionalized iron oxide nanoparticles (SF-IONPs) onto the *Chlorella* sp. cell surface via the electrostatic-mediated-attachment (Toh et al., 2014). In industrial application, the recycling of the supernatant after the microalgae harvesting is necessary to minimize the water utilization for cost cutting purpose and reutilize the remaining nutrients to avoid eutrophication after discharge to the environment. However there will be some SF-IONPs residuals in the supernatant due of its high colloiddally stable property (Yeap et al., 2012). Even though there are a number of studies about the toxicity effect of iron oxide nanoparticles (IONPs) in the biomedical application (Singh et al., 2010; Buyukhatipoglu and Clyne, 2010; Hoskins et al., 2012), however, there is still limited study on the toxicity effect of the IONPs toward the cultivated microalgae. In this study, the effective attachment of SF-IONPs onto *Chlorella* sp. cells has inhibited the cells growth. The growth inhibition is directly proportional to the dosage of SF-IONPs in culture media. The 20 mg/L of SF-IONPs has retarded the growth of cells up to  $37.02 \pm 14.06\%$  with respect to the control group. Total lipid yield was observed to reduce significantly up to 43.70 % with respect to the control group. Therefore residual SF-IONPs of more than 10 mg/L present in the supernatant is highly advised not to be recycling since it will retard the oil yield and elevate the production cost of the biofuel. However, in the continuous culture of cells which contained 50 mg/L of SF-IONPs up to 26 days, the grow inhibition effect from the SF-IONPs was observed in the first 9 days. The microscopy viewing found that the cells attached with the SF-IONPs will finally die and become colourless cell debris. However, the cells that are free from the attachment of the SF-IONPs will growth continuously and performed cell division. The growth of new cells in the medium diluted the concentration of SF-IONPs and eliminates the growth inhibition effect toward the cells.

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## ASSESSMENT OF *JATROPHA CURCAS* AS A POTENTIAL FEEDSTOCK FOR BIOFUEL PRODUCTION IN SOUTH MEDITERRANEAN COUNTRIES

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### ABSTRACT

*Jatropha curcas* L. is a perennial shrub or small tree of the Euphorbiaceae family, characterized by low input requirements and high adaptability in harsh environments. This plant species is naturally grown and cultivated in tropical and sub tropical regions producing high quality bio-oil, which can be easily converted to biodiesel.

The EU funded JatroMed project ([www.jatromed.aua.gr](http://www.jatromed.aua.gr)) is evaluating the ability of this crop to be cultivated in South Mediterranean countries, aiming at the reinforcement of the development of poor rural areas by covering the energy needs of local communities. In order to reach this goal, five countries, namely Greece (coordinator), Italy, Egypt, Morocco and Algeria have successfully planted eight worldwide selected genotypes of *Jatropha curcas* in three low income rural sites of Egypt (Borg El Arab in Alexandria governorate), Morocco (Hard Draa in Essaouira region) and Algeria (Adrar wilaya). On 2012, in each target country four genotypes were established (two common for comparison reasons and two different). Each genotype is cultivated in one hectare and is treated under different levels of fertilization, irrigation and pruning.

The plantations are well established, the first fruits are harvested and oil is produced and analyzed. A harvesting device is under development by modifying existing olive harvesters and validating the results with field tests. The economic analysis of the production chain (from sowing to bio-oil production) is performed by applying Discounted Cash Flow (DCF) and Activity Based Costing methods. Social aspects are considered, such as acceptability from the farmers, number of jobs created, implication of women, etc.

The results so far showed that the best performed genotype for Egypt is Michoacán (non-toxic from Mexico), for Morocco is JCLMax (toxic from India) and for Algeria is Veracruz (non-toxic from Mexico). In conclusion, *Jatropha curcas* seems to be a promising energy crop for bio oil production in South Mediterranean countries.

**Keywords:** *Jatropha curcas*, biofuels, energy crops, agronomic practices, harvest, socio-economics

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## PHYTOREMEDIATION POTENTIAL OF SELECTED ENERGETIC PLANTS (*MISCANTHUS GIGANTEUS* AND *PHALARIS ARUNDINACEA*) IN DEPENDENCE ON FERTILIZATION

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### ABSTRACT

Phytoremediation is the process of using plants to clean soil contaminated with heavy metals and other hazardous compounds, and also allows to restore degraded soils utility and natural values. Degraded lands are usually excluded from the food production; however, may be suitable for the cultivation of energy crops such as *Miscanthus giganteus* (miscanthus) or *Phalaris arundinacea* (reed canary grass). These plants due to the ability to collect and accumulate heavy metals are excellent for phytoremediation. Depending on the plant species, metals may be absorbed in larger amounts in the roots or aerial parts. Both, miscanthus and reed canary grass, due to a very well developed root system, may meet another additional function, namely anti-erosion, which further supports the possibility of using them in the process of phytoremediation.

This paper presents the influence of fertilization in cultivation *Miscanthus giganteus* and *Phalaris arundinacea* in the process of phytoremediation of Cd, Ni and Zn. There were two types of fertilization – organic with the use of sewage sludge and non-organic with the use of mineral salts.

Research show that after first year of vegetation both Cd ( $0.344 \text{ mg kg}^{-1} \text{ d.m.}$ ) and Zn ions ( $29.47 \text{ mg kg}^{-1} \text{ d.m.}$ ) were the most accumulated by the biomass of miscanthus grown on soil fertilized with municipal sludge (OW). However, in the case of the reed canary grass, the correlation can be attributed to the Ni ( $3.76 \text{ mg kg}^{-1} \text{ d.m.}$ ) and Zn ( $122.6 \text{ mg kg}^{-1} \text{ d.m.}$ ). In turn, after the second year of vegetation all of the tested metals are characterized by a lower absorption as compared to the first year. In the case of reed canary grass Cd and Zn content in the biomass after the second year of vegetation was similar to the control, while the Ni concentration was lower in relation to the control. In the case of miscanthus, it was observed that the concentration of Ni and Zn in the biomass after the second year of vegetation was similar to the control, while the Cd content varied in dependence on the applied fertilizer.

Concluding, both miscanthus and reed canary grass are able to accumulation of heavy metals, although the quantity of absorbed ions were depended on fertilization and the time.

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## USE OF TE CONTAMINATED SITES FOR THE PRODUCTION OF BIOMASS FOR BIOCHAR

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### ABSTRACT

Trace element (TE) contamination of soils is a worldwide problem. However, although not considered safe anymore for food production without clean-up, many of these soils may still be used to produce biomass for non-food purposes such as biochar. In a first step we investigated the suitability of the tree species willow (*Salix sp.*), poplar (*Populus sp.*), and birch (*Betula pendula*) which grew on a TE contaminated site in France. Birch revealed significantly lower leaf Cd concentrations (1.2 – 8.9 mg kg<sup>-1</sup>) than willow and poplar (5 – 80 mg kg<sup>-1</sup>), thus posing the lowest risk for TE contamination of surrounding areas. It also displayed lower bark Cd, Zn and Pb concentrations than poplar and willow making it suitable for biochar production. Thus, biochars were produced by pyrolysis at temperatures 450 and 700 °C from birch wood growing on the TE contaminated site in France and birch wood originating from non-contaminated soil. During the pyrolysis, in contrast to Cu, Fe, Mg, K, Mn and P, the elements Cd, Pb, S and Na volatilized. The produced biochars were applied as an amendment on a non-contaminated low-fertility soil, and its effect on the growth and metal accumulation of ryegrass (*Lolium perenne*, var. *Calibra*) as well as soil microbial abundance was investigated. The root biomass of the biochar treated plants was lower than that of the non-amended plants, while that of the shoot was higher. Plant shoot K and Zn concentrations were increased significantly by up to 7- and 3.3-fold respectively, owing to the application of TE-contaminated biochar. For P, Mg, Mn, Fe and Cu no significant increase in concentration could be detected. Neither the TE-contaminated biochar, nor the non-contaminated biochar had adverse effect on the bacterial community of the soil. The results show that if the contaminated sites are chosen according to their soil TE, e.g. Cd and Zn, the produced biochar could be used to biofortify crops which grow on soils with low Zn concentrations.



## MICROALGAE FOR WASTEWATER TREATMENT, CO<sub>2</sub> MITIGATION AND BIOFUELS: DREAM OR SUSTAINABLE MAID FOR ALL WORK?

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### ABSTRACT

Over the last decade, intensive research has been carried out all over the world to explore the huge potential of microalgae for biofuel production, which could be coupled to the use of industrial flue gas to enhance CO<sub>2</sub> fixation as a greenhouse gas mitigation strategy and to remove pollutants from various wastewaters. However, large-scale microalgae production for such purposes is not yet technically and economically feasible. Challenges are thus to increase the efficiency of both algae production and conversion into biofuel. The following parameters are critical for sustainable microalgal biomass cultivation: CO<sub>2</sub> concentration; possible use of wastewater streams; light intensity and quality; photobioreactor design; batch, fed-batch or continuous growth conditions.

The objective of our research was thus to assess the influence of these parameters on the quantitative and qualitative production of biomass, lipids and added-value chemicals, taking into account the probable antagonism between biomass and lipid production, often depending on the nitrogen supply. Several microalgal species were grown at laboratory scale to compare the yield of biomass and lipid production. In addition to the potential use of oil for biodiesel production, we are currently working towards demonstrating the technical feasibility of an innovative process, for syngas production via hydrothermal processing of microalgae. The process is envisioned as a closed-cycle with respects to nutrients, water and CO<sub>2</sub>, that are separated and reused for microalgae growth. The possible effect of increasing injection of CO<sub>2</sub> in the growth medium was also investigated, with the aim to recycle CO<sub>2</sub> from flue gas and enhance biomass production.

On the other hand, to achieve the economical and ecological sustainability of algal biofuel production, it will be necessary to integrate it with the extraction of high-value products in a biorefinery process. Carotenoids have been proposed as added-value compounds that could contribute to make microalgal biofuel production economically feasible. Therefore, the viability and sustainability of extracting carotenoids before the hydrothermal treatment of the remaining biomass to produce syngas were investigated.

Finally, harvesting and dewatering microalgae cells remains a technical challenge and typically contributes to 20-30% of biomass production costs and can represent up to half of the total cost of algal biofuels. The potential of co-culture of filamentous fungal species with microalgae in a lichenization process as a strategy to reduce the cost and energy consumption of harvesting and of the whole process seems promising and was thus investigated. In submerged cultures, filamentous microorganisms actually aggregate and grow as loosely packed pellets or compact granules. Microalgae cells were immobilized in these pellets and easily removed as an aggregate with the fungal cells. Pellet formation is strain specific and highly dependent on operational conditions during cultivation. This study was especially focused on lichen pellet formation during the co-culture of *Chlorella sorokiniana* and of an unidentified filamentous fungus, which was eventually characterized. While algae growth was optimal between pH 6 and 10, the highest pellet formation was observed in the pH range of 4-7, thus requiring a strict control of pH during the whole cultivation. The dense formed pellets did sediment in submerged cultures when the agitation was stopped and were easily harvested. The effect of such a co-cultivation on the production of added-value chemicals like carotenoids and on the biofuel potential of the remaining biomass is under evaluation, and will be compared to results obtained with cultivation of microalgae only.

The proposed strategies may significantly reduce energy demands with regards to harvesting and dewatering of microalgal biomass and improve the sustainability of the whole process, but should be assessed at larger scale.

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## TECHNICAL, ECONOMIC, ENVIRONMENTAL AND SOCIAL SUSTAINABILITY OF GASIFICATION-BASED BIOENERGY SYSTEMS FOR THE MEDITERRANEAN AGRO-INDUSTRIAL SECTOR

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### ABSTRACT

Small-scale gasification-based bioenergy systems coupled could be innovative alternatives for CHP production when fueled with agro-residues, providing parallel benefits related to residues management and sustainable agri-food production and agriculture. In this study, an indicator-based estimation of sustainability, considering not only economic but also environmental and social issues was attempted for a gasification-based bioenergy system. The outcomes of the study indicate that small decentralized units could be used in a symbiotic way with industrial agro-companies or agricultural associations in an eco-efficient way to cover their needs in heating and electricity. However, commercial success depends on capital reduction instruments such as subsidies, electricity feed in tariff and biomass price. The use of agro residues and food wastes provides the benefit of zero cost feedstock and the mobile character of the unit the zero cost of biomass logistics.



## SRWC POTENTIAL BIOENERGY MARKETS IN NORTH CAROLINA, USA

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### ABSTRACT

Woody biomass will be a major feedstock for energy production, including biofuels, to secure the future of domestic energy production in the United States. Fast-growing, purpose-grown tree plantations, or short rotation woody crops, are one of the most promising methods to meet future woody biomass demands. Whereas considerable research has been done in the U.S. on SRWCs, relatively little has been done in North Carolina. Over the last two years, we have begun a research and extension program to develop an understanding of potential SRWC production in North Carolina in order to meet immediate woody biomass market demands for wood pellets and potential biofuel demand for ChemTex. Our programmatic objectives are to develop recommendations of species or clones for specific site conditions; to develop planting, management; and harvesting guidelines for site conditions; to estimate growth rates, harvest yields, and feedstock characteristics; to estimate production and harvesting costs on various lands; to understand potential pest and disease issues; and to evaluate wood chemistry of species and clones specific to energy wood use. We will report on performance results from randomized-block establishment trials at coastal to mountainous sites for select clones of *Populus*, native hardwoods, and eucalyptus. Site trials vary by land type from cropland, managed forests, degraded lands, and marginal lands.

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## DEVELOPING A BIOMASS TO ENERGY UTILIZATION MANAGEMENT SPATIAL DECISION SUPPORT SYSTEM - IT TOOL FOR THE ISLAND OF CRETE

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### ABSTRACT

There are more than 1,000,000 tones of biomass that are produced in an annual base in Crete, the majority of which are currently disposed improperly (the least), where could be used in renewable energy production, either through anaerobic digestion or incineration systems. Support system will focus on biomass appropriate for incineration units. Their collection and utilization was (and still is) non-economical due to a number of reasons as for example their physiochemical characteristics (high moisture content), their seasonal production and their dispersion in a vast and mountainous terrain.

The main aim of the support system – IT TOOL is to collect, analyze and evaluate a series of information (new knowledge) that will result in the development of a scheme, that would allow the collection of biomass that is currently not collected or utilized in the island of Crete and it's transformation into a fuel that could be used either in central heating systems of private households and public buildings or as fuel in renewable energy producing units, in an environmentally and economically profitable way.

IT system combines all geographical datasets collected and will be able to dynamically convey the information in order to support decisions of the biomass investors. IT tool use inherent capabilities of GIS which provides better analytic capabilities and facilitates decision-making to a greater extent for the stakeholders. IT can be extremely helpful in deriving the most meaningful information necessary for better decisions. IT tool allow users to interact in a comprehensive manner by changing parameters and making different scenarios.

IT tool that allows the easiest and more useful representation of the data collected meaning investments in environmentally friendly technologies among of which renewable energy production systems. Is estimated that more than 1.0 billion euros will be invested in biomass units, since until 2020 more than 350 MWe units must be installed and operate. Since more than 50 % of the will be incineration units their need will be well above 1,500,000 tn of dry biomass. In this case systems that would allow the economical and environmentally friendly collection, treatment and transportation of biomass will provide a huge benefit to the local economy.

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## DEVELOPMENT OF A PRODUCTION AND UTILIZATION SYSTEM OF WOODY BIOMASS FOR HEATING MUNICIPAL BUILDINGS – BIOMASS

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### ABSTRACT

Municipalities in both Greece and Cyprus pay tens of thousands per year for pruning and cleaning of gardens and parks. In most cases these materials led the funeral along with other municipal solid waste, with a significant economic burden on each municipality. The «BIOMASS» proposes to address all these plant residues not as a problem but as an opportunity. Lead partner in the project is the Municipality of Festos, while partners are the Technological Educational Institute of Crete, FODSA of Samos and Community Council of Voroklini.

The project «BIOMASS» has as its main objective: Utilization of woody debris produced by urban and agricultural activity and converts them into solid biofuel (pellets) for heating public buildings. Finally the specific objectives of the project are to develop a system for collecting the plant residues urban, develop a system for collecting the plant residues from agricultural crops, the development of a plant pellets for each operator, the development of a network of burners in public / municipal buildings will allow the use of pellets produced, environmental and economic assessment of the process, and technical education personnel to other Municipalities and individuals on how to develop and operate the related systems. The main expected results are reducing the amount of plant residues and petroleum consumption from stakeholders for heating public buildings and at the same time develop at least two to three new full-time jobs for the operation of each of these systems.

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## COMPARATIVE STUDY OF H<sub>2</sub>-PRODUCTION UNDER SULFUR DEPLETION BY THE GREEN ALGAE *C. REINHARDTII* AND *S. OBLIQUUS*

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### ABSTRACT

Photosynthetic hydrogen production by green algae was first reported by Gaffron and Rubin [1]. Under anaerobic conditions, electrons are transferred through the photosynthetic electron transport chain to ferredoxin, and subsequently to hydrogenase, which converts protons to molecular hydrogen. Photosynthetic hydrogen production can take place either by a PSII dependent or a PSII independent pathway; in the first case water is the electron donor, whereas in the second case electrons originate from the catabolism of organic substrates through the NAD(P)H-dehydrogenase.

The expression and activity of hydrogenase are both very sensitive to the presence of oxygen. In the PSII-dependent pathway, the photolysis of water results to production of molecular oxygen, which inhibits hydrogenase activity and thus hydrogen production. An effective way for establishing oxygen depletion in *Chlamydomonas reinhardtii* cultures is the removal of sulfur from the culture medium [2]. Sulfur depletion results in partial deactivation of PSII; under these conditions consumption of oxygen in a closed system was found to be much higher than oxygen production. These growth conditions are appropriate for hydrogenase activation and the production of molecular hydrogen.

In the present contribution we compare the ability of two green algae (*Chlamydomonas reinhardtii* and *Scenedesmus obliquus*) to produce hydrogen under sulfur depleted conditions. Thermal conductivity hydrogen measurements using gas chromatography have clearly demonstrated that of the two species only *Chlamydomonas* cultures can produce high amounts of hydrogen under complete sulfur deficiency. Further experiments in *Scenedesmus* cultures using different sulfur concentrations in the growth medium clearly showed that low concentrations of sulfur are necessary for hydrogen production.

The above-mentioned different response of *Chlamydomonas* and *Scenedesmus* cultures to sulfur deficiency could be the key for better understanding photosynthetic hydrogen production. Comparative studies using the two algal species, by proteome analysis and EPR characterization of PSII, could allow us to explore the hydrogen production mechanism of *Chlamydomonas* under sulfur deficiency. In addition, optimization of the conditions for hydrogen production by *Chlamydomonas* cells under sulfur deprivation took place by changing growth parameters, such as the initial cell concentration, the initial culture volume in the hermitically closed bottles, the initial glucose concentration and the presence or not of shaking.

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## PREDICTION OF FORAGE ENERGY POTENTIAL THROUGH THE REGIONAL DIFFERENCES IN GROWING CONDITIONS AND ENERGY POLICY

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### ABSTRACT

Recent development of renewable energy sources (RES) and production of biofuels in the Czech Republic was significantly modified by a system of quotas and subsidies, which does not sufficiently respond to all consequences of this development. A tension occurs between the renewable energy on the one hand and natural resources, biodiversity, food safety and protection of local climate on the other hand.

The only independent evaluation of all available sources and their need from local level can give valid answer which source of bioenergy is suitable with the respect of above mentioned limitation. Potential of RES (photo-, wind, water, biomass), existing energy production facilities including RES applications, energy utilizable wastes, wood harvesting residual potential are gathered into large databases within the LIFE+ project Regional Sustainable Energy Policy (ReStEP). The most complicated evaluation is in the case of biomass production and utilisation. There are several limitations affecting biomass production including natural ones, legislation and sustainable development.

Recently, the focus has been given on the most productive crops such as maize however these wide-row crops could have some negative impact on environment. Other forage crops including legume's monocultures, legume-grass mixtures and permanent

Species	Yield t.ha <sup>-1</sup>	Energy content MJ.kg <sup>-1</sup>	Energy production GJ.ha <sup>-1</sup>
Silage maize	12 – 20	17.0 – 18.7	210 – 370
Forage sorghum	10 – 18	17.1 – 18.6	170 – 330
Alfalfa	8 – 13	17.0 – 18.3	140 – 230
Red clover	7 – 12	16.9 – 18.1	120 – 210
Legume-grass mixture	5 – 14	16.9 – 18.5	85 – 250
Permanent grassland	3 – 11	16.7 – 18.6	50 – 200

grasslands should be used as a bioenergy source. Unlike to maize forage crops also provide some important benefits for environment such as nitrogen fixation, improvement of soil fertility and protection against soil erosion [2], as well as phytoremediation of contaminated land [3].

The objective of this study is to show differences in the biomass and energy production of main forage crops with respect of climate, soil fertility, degradation and erosion, as well the limits for energy use of agricultural residues.

Productivity in terms of energy output is expressed as a calorific value determined by the adiabatic calorimeter IKA C 5000 according to ISO 1928 (2009). This value represents an energy potential for direct combustion of biomass and is used for calculation of energy balance, important for comparison of different species or management practices [1]. The simple comparison of forage crops and their groups by the potential yield, the calorific value and the energy output is given in the table. Wide margins of all parameters presented there show the highest energy production by maize at fertile soils under suitable conditions. Comparable energy content of all crops and environmental advantages of forage ones supports the strategy, that proper crop management, up to some extent of less productive grasslands can substitute maize as energy source in less favourable areas.

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**MONITORING**



## PHYTOMONITORING OF AIRBORNE PCB CONTAMINATION FROM TWO LOCAL POINT SOURCES IN THE CANADIAN ARCTIC

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### ABSTRACT

Saglek Bay (LAB-2), located on the coast of Labrador is a former Plevault station operated from 1953 to 1971 when it was abandoned. In 1996, it was determined that the site was contaminated with polychlorinated biphenyls (PCBs) with concentrations in soils far exceeding the Canadian Environmental Protection Agency (CEPA) regulation of 50 µg/g. This led to remediation work carried out from 1999 to 2004 to remove and/or isolate all PCB-contaminated soil exceeding 50 µg/g and to further remediate parts of the site to <5 µg/g PCBs. In this study, spatial and temporal trends of PCB concentrations in the new-year growth of dwarf birch (*Betula glandulosa*) and willow (*Salix* spp.) were investigated over a period of fourteen (1997–2011) years in an effort to track ecosystem recovery following the removal of the point source of contamination.

Concentrations of PCBs in plants were measured before (2001–2002), during (2003–2004), and for six years after (2005–2010) the screening and containerization of PCB-contaminated soils. PCBs were measured as Aroclor 1260 equivalents (dry weight) by gas chromatography with an electron capture detector (GC/ECD) using U.S. EPA method 680/8270. During the remediation activities, ambient air PCB concentrations were measured using active air samplers for comparison to the passive plant samplers (i.e. the dwarf birch and willows). PCB concentrations measured by the active samplers reached a maximum of 0.037 µg/m<sup>3</sup> which was below the project criteria of 0.15 µg/m<sup>3</sup> indicating minimal source emissions. During the same time period, PCB concentrations in the passive samplers (i.e. plants) showed significant increases of 2–14 fold compared to the baseline data from previous years.

The birch data also showed significant changes between monitoring events within the 2003 and 2004 sampling seasons (June to September) and decreases when ambient air concentrations were low, indicating the sensitivity of new-year growth to reflect net accumulation and ambient conditions at a temporal scale of approximately two weeks. In the third year following remediation, concentrations decreased to below baseline levels reflecting the overall remediation and source removal at the site.

The BAF-5 radar site located on the summit of Resolution Island, Nunavut, tells a similar story. As part of the Distant Early Warning (DEW) Line, large quantities of PCBs were left when the site was abandoned in the 1970's. Approximately 4000 kg of pure PCBs were removed in 1999, while lower level PCBs were remediated and or disposed of using a variety of techniques between 1999 and 2005. Plant biomonitors accurately tracked airborne PCBs during and after remediation. Together, these data show that we can effectively use plants to phytomonitor terrestrial ecosystem recovery following removal of PCB point sources at remote Arctic sites.

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## TERRESTRIAL PLANT BIOCONCENTRATION FACTORS FOR ORGANIC CHEMICALS: EXPERIMENTAL VALUES DATABASE AND SCREENING-LEVEL MODEL EVALUATION

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### ABSTRACT

Quantifying and predicting the transfer of chemicals from the soil environment into terrestrial plants is important for assessing human and ecological risks, evaluating the use of plants as biomonitors of environmental contamination and predicting the effectiveness of phytoremediation. Empirical data describing chemical uptake by plants roots are generally expressed as ratios of chemical concentrations in the plant compartment of interest (e.g. shoots, roots, xylem sap) to that in the exposure medium, (soil, soil pore water, hydroponic solution) measured at the time the samples are collected. These ratios, that may or may not reflect equilibrium, are generally referred to as bioconcentration factors (BCFs) but have also been named for the specific plant tissue sampled such as root concentration factors, stem concentration factors or transpiration stream (xylem sap) concentrations factors. Plant-soil BCF values have been used directly as models to provide estimates of plant tissue concentrations from measured exposure concentrations and have also been used as input into mechanistic models and in risk assessments. However, the development of such models is dependent on quality experimental data that provides insights into the underlying processes of chemical uptake and biotransformation and physical losses within plants. We collected and reviewed measured data from over five hundred published papers to develop a terrestrial plant bioaccumulation database that was used to evaluate several screening-level plant uptake models. The database includes plant bioconcentration factors for 360 organic chemicals and 100 plant species. The review shows the lack of experimental data collected in a consistent, reproducible manner confounds comparisons between studies and limits model evaluation and development. Key parameters describing plant growth and health were often missing. Additional experimental data for ionic and ionogenic organic chemicals are needed along with information on the biotransformation of chemicals within plants. Until standard protocols for the measurement of plant uptake data are available and used to generate consistent, high quality data, models will be only able to provide order of magnitude estimates of root uptake and resulting tissue concentrations. Case studies of well-conducted plant studies are summarized to provide guidance for future standard protocol development.

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## PHYTOSCREENING FOR PERCHLORATE: RAPID ANALYSIS OF TREE SAP

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### ABSTRACT

Perchlorate is an emerging contaminant increasingly encountered at contaminated sites, found in groundwaters of 49 states. The highly mobile pollutant can transport rapidly, making site assessment difficult and costly. A method to rapidly assess in-planta perchlorate concentrations was developed. Tree sap collected from tree cores via centrifugation at 30,000g was used to evaluate the perchlorate contamination. The sap sample was directly analyzed by an UFLC-MS/MS method after isotope labeled perchlorate internal standard addition and filtration. A hydroponic greenhouse study of willow trees was conducted to fully evaluate the analytic techniques and demonstrate that tree sap perchlorate concentrations were proportional to dosing solution perchlorate concentrations. The method was applied at the Longhorn Army Ammunition Plant in Karnack, Texas, USA to delineate a perchlorate plume that extends into a wooded area. At the field site, analysis of ~200 tree cores revealed plume boundaries similar to those obtained by traditional groundwater monitoring methods. Phytoscreening offered higher spatial resolution and detail than the limited groundwater sampling, for considerably less cost than the groundwater sampling efforts. This research demonstrates the potential for phytoscreening to extend beyond the traditionally studied organic compounds, providing a low-impact, rapid method for delineating perchlorate plumes in groundwater.



## COMPARISON OF TREE CORING AND SOIL GAS SAMPLING FOR SCREENING OF CONTAMINATED SITES

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### ABSTRACT

Site characterization is often time consuming and a financial burden for the site owners, which raises a demand for rapid and inexpensive (pre)screening methods.

Phytoscreening by tree coring has shown to be a useful tool to detect subsurface contamination, especially of chlorinated solvents. However the application and dissemination of the method is still limited. On the other hand, soil gas sampling for mapping of volatile organic compounds in the subsurface is a common and commercially applied method. Both methods are semi-quantitative, low-invasive and inexpensive, which makes them suitable as initial screening methods for site characterization.

The aim of this study is to compare tree coring and soil gas sampling to evaluate to which extent tree coring may supplement or substitute soil gas sampling as a site contaminant screening tool. And where both methods are feasible, evaluate when (with respect to compounds, soil properties, and locations) one method is preferred over the other.

Fields sampling was performed at European sites contaminated with fuel components or chlorinated solvents from former site activities (industrial production, gas stations, air base or gas plant) in fall 2012 and 2013. Samples from different tree species such as willow, asp, oak, birch and pine were collected and analyzed by headspace GC-MS.

The soil gas measurements were conducted by consulting engineering firms as part of the site characterization prior to the tree core sampling events. Results obtained both by tree coring and soil gas sampling are compared and held up against quantitative results obtained by groundwater- and/or soil sample analysis.

Significant correlation between the methods is not always the case. However, both methods can detect contamination in the shallow subsurface and then identify high risk areas. The uptake of BTEX into trees varies to a greater extent with the tree species and the site conditions than chlorinated solvents, which lead to greater uncertainty.

Both methods have their advantages and disadvantages. Hence, the methods supplement each other.

Based on results from these initial screening methods, other more advanced/quantitative and cost-intensive methods can be focused, with the overall goal to make site characterization more complete and/or efficient.

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## PERSPECTIVES OF WOODY PLANT IMPLEMENTATION IN PHYTOREMEDIATION OF TOXIC ELEMENTS IN INDUSTRIAL CITIES

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### ABSTRACT

Currently, more than 70% of the world population lives in large cities. Most Russian megalopolises are characterized as developed industrial complexes providing serious impact on the environment. Woody plants are the major representatives of the biota that are the biofilters of toxic compounds and fixators of carbon dioxide and sources of oxygen and organic matter in urban ecosystems. In our research as a model urban ecosystem was chosen a city of Tula, a large industrial capital of the region, bordering Moscow, Lipetsk, Orel, Kursk, and Kaluga regions. The city is known for the presence on its territory of a number of ferrous and nonferrous metallurgical enterprises producing pig iron, ferro-manganese, vanadium and chromium compounds, armory, machine building and defense enterprises. The results of our investigations showed that the soil of the city by 40% subject to polymetallic contamination with Fe, Cu, Zn, Pb, and As. In the air, an increased content of Fe and Cu is observed. Sampling was carried out in the buffer zone of metallurgical enterprises and in the sanitary protection plantations along the main city highways.

Analysis of biogeochemical activity of woody plants under high anthropogenic impact pollution conducted by means of two complementary analytical techniques – atomic absorption spectrometry (AAS) and instrumental neutron activation analysis (INAA) – revealed promising species of woody plants for phytoremediation of the environment:

*Populus nigra* - Mn, Fe, Ni, Zn, Cd, Pb

*Caragana arborescens* - Fe, Sb

*Betula pendula* - Mn, Fe, Ni, Zn, Cd, Pb

*Philadelphus coronarius* - Pb, Sb

*Aesculus hippocastanum* - Ni, Cu, As, Pb

*Spiraea japonica* - Cd, Cu

*Larix sibirica* - Mn, Fe, Pb

*Forsythia intermedia* - Cd, Cu

*Acer platanoides* - Mn, Fe

*Syringa josikaea* - Cd, Sb

*Tilia cordata* - Fe, Cu

*Syringa vulgaris* - Fe

*Sorbus aucuparia* - Fe

*Cornus alba* - Fe

*Cotoneaster lucidus* - Mn, Fe, Pb, Cd, Cu, Ni

*Crataegus sanguinea* - Ni

*Crataegus monogyna* - Fe, Ni

Data on biogeochemical activity of species and their adaptive capacity in terms of polymetallic pollution permits competently select woody plants for greenery planting of sanitary protection zones of the technologically polluted urban ecosystems and a combination of species will allow maximal remediation of the environment from toxic elements.

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## THE REPETITIVE-NON CODING DNA OF BRYOPHYTES, A DIFFERENT, SIMPLE, FAST, AND INEXPENSIVE KIND OF "EARLY GENETIC INDICATOR"

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### ABSTRACT

Studies over the last years showed that the highly repetitive /non-coding DNA, which was once considered useless for the life of the organism, to the point of being called "Junk DNA" or "Selfish DNA", it is vice versa important for the adaptation of the organism to the environment. In fact, the external stimuli, such as temperature variations, pollutants, or even mechanical stimuli or viral infections, are able to induce quantitative changes just in this peculiar fraction of the genome. It is not about DNA modifications "harmful", but rather about a sort of "language" that the genome uses to respond to environmental stimuli: in fact the modifications of the non-coding DNA bring in turn, via a "conformational" effect, to an amendment of the higher structures of the double helix of DNA, thereby affecting, albeit indirectly, the transcription of the coding DNA.

Our idea is to propose the observation of the behavior of highly repetitive DNA as a tool to test the effect of the environmental pollution. In our opinion, in fact, such different kind of technological approach could provide a breakthrough in such field of research. Indeed, being the modulation of repetitive non-coding DNA one of the first responses of the genome to the environmental stresses, such a methodology allows us to test the effects of environmental pollution in a time earlier to that of other indicators. Also, given that the modifications of repetitive/non coding DNA are a response only "preparatory" to other, potential, genetic events, such different techniques could detect a potential risk of pollution also in the cases in which the natural defense reaction of the plant makes ineffective other kind of indicators.

In this context, we suggest to observe the behavior of repetitive DNA in answer to stresses in the genome of Bryophytes: in fact, the experimental data obtained by our group in both, aquatic and terrestrial species of these plants, experiments conducted either directly within the cell, by cytochemical analysis of the nuclear genome (characterization of DNA with A + T and G + C specific fluorochromes, *in situ* DNA hybridization, Electron Microscopy Analysis, etc.), or indirectly, through appropriate characterizations of the extracted DNA (kinetics of dissociation and recombination, *in vitro* hybridization, Southern Blotting, etc.), have shown that, differently to what happens for the other plants, the Bryophytes have a peculiar structure of the nucleus that consent accurate and immediate observation of the behavior of repetitive-non coding DNA, in a simple, fast and inexpensive way.

**Keywords:** environmental pollution, early genetic indicators, repetitive/non coding DNA, bryophytes

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## CHANGES IN THE LEVELS OF HSP70 IN RELATION TO HEAVY METALS EXPOSITION IN *LEMNA MINOR*

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### ABSTRACT

The heavy metal pollution is one of the most widespread pollution on Earth. In the last decade many attempts have been made to find suitable biological plant indicators and appropriate markers witnessing the levels of stress produced by heavy metals.

In our study we use *Lemna minor* as a bio-indicator, and HSP70 as a protein marker to evaluate the effects of heavy metals.

Specifically, we investigated the occurrence and abundance of HSP70 protein depending on different heavy metals, by changing both levels and time of exposure of specimens cultured *in vitro*.

We used different HSP70 antibodies able to recognize cytosolic, chloroplastic or mitochondrial isoforms, in order to recognize which of them is/are specifically involved in response to heavy metals stress, by conventional and 2-D western blotting.

The possible relationship(s) between the presence of different isoforms, their abundance, and of the changing heavy metals stress exposition and concentrations, are discussed in order to define this method as a useful tools to assess and monitor heavy metals' pollution in plants.



## AQUATIC MACROPHYTES AS POTENTIAL PHYTOREMEDIATORS AND BIOMONITORS OF POLLUTED WATER BODIES IN THE MIDDLE URAL

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### ABSTRACT

The Middle Ural is a highly urbanized region and the most water bodies are contaminated by a wide spectrum of pollutants, including heavy metals (HMs). Aquatic macrophytes are important components of freshwater ecosystems. Some species have expressive ability to bioconcentrate and bioaccumulate both nutrients and toxic elements in their green parts. Therefore they're actively used as biomonitors and phytoremediators [Borisova et al., 2014; Krems et al., 2013; Pratas et al., 2011]. Nevertheless the physiological and biochemical basis of metal hyperaccumulation and the mechanisms of tolerance in plants are not still studied enough.

In response to HMs stress in the plants leaves develop effective defense systems, including synthesis of thiol-enriched proteins and peptides – metallothioneins and phytochelatins [Prasad, 1999; Malec et al., 2009]. It is known, that the latter are not only able to bind the excess of HMs, but also help to neutralize toxic reactive oxygen species (ROS), generated under the HMs stress (Thornalley and Vasak, 1985). The aim of this study was to determine the accumulative ability of some macrophyte species and investigate the level of thiol-enriched compounds in comparison with HMs contamination of polluted water bodies in the Middle Ural.

The study objects were *Elodea canadensis* Michx., *Potamogeton perfoliatus* L., *Ceratophyllum demersum* L., *Batrachium trichophyllum* (Chaix.) Bosch. and *Lemna gibba* L. The studies were carried out on watercourses of Sverdlovsk region (Middle Ural, Russia) that differ in their extents of pollution: rivers Chusovaya (the Kama River tributary), Iset' and Pyshma (Tobol River tributaries), and other water bodies within the mentioned above river basins. Ikbulat Lake, Sysert' and Reshetka rivers have been taken as "conventionally clean" since these water bodies are subjected to the lowest anthropogenic impact. The plants were sampled in July during the period of blooming. The water samples were simultaneously collected. Integrated samples of leaves were collected at  $\geq 10$  plants. The HMs content in the leaves of aquatic macrophytes and in nonfiltered water was determined using atomic absorption spectrometry (AAS Vario 6, "Analytik Jena", Germany). The content of soluble and membrane-bound thiols was estimated according to Ellman (1959). The non-protein thiols (NPT) were estimated according to Nagalakshmi, Prasad (2001). Protein content was determined according to Bradford (1976), using bovine serum albumin (Sigma) as a standard. The parameters were determined in three replicates. The statistical significance of the results was determined using nonparametric Mann-Whitney U-test,  $p < 0.05$ .

The most polluted with copper was Pyshma River (0.042 mg/l), nickel – Revda and Pyshma rivers (0.988 and 0.146 mg/l, respectively), zink – Iset' River (0.040 mg/l), manganese – Pyshma River (0.241 mg/l), iron – Chusovaya River (0.912 mg/l). *B. trichophyllum* and *C. demersum* showed the highest potential for accumulation of HMs (especially for manganese, zink, iron and copper). Others species exhibited more selective accumulative ability. A high ability to accumulate manganese and zink was also found in *E. canadensis* and *P. perfoliatus*, iron and zink – in *L. gibba*. The order of values of the biological accumulation of HMs in the most studied species was as follows: Ni < Cu < Fe < Zn < Mn. The metals content in the leaves of aquatic macrophytes growing in impact water environment could be several times higher than in unpolluted plants. For example, in the leaves of *L. gibba* from a watercourse subjected to increased anthropogenic load (the Chusovaya River), the content of Cu was 7.2 times higher and Zn was 4.3 times higher than with plants from "conventionally clean" water bodies (the Sysert' River).

The increased accumulation of HMs in plant tissues upon environmental pollution was accompanied by changes in the level of thiols. It was found, that the content of nonprotein thiols, soluble and membrane-bound thiols in macrophyte leaves depended on HMs impact. Plants that lived in waters with high anthropogenic pollution had a significant increase of thiols compared with the "conventionally clean" ones. Besides the content of soluble and membrane-bound proteins was also significantly raised. This indicated that the macrophytes could be used as a good bioindicators in continuous long period monitoring of contaminated water ecosystems.

Thus, it might be supposed that some species of aquatic plants can be more successful bioaccumulators and show high potential in possible use as phytoremediators. At the same time the plants tolerance to the stressful environmental conditions is determined by their ability to develop different protective mechanisms, among which is the most specific synthesis of nonprotein and protein thiols, that can neutralize the toxic effects of HMs.



## LONG-TERM MONITORING OF CHLORINATED SOLVENTS IN TREES

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### ABSTRACT

Phytoremediation, while an attractive low-impact technology for site remediation, poses difficulties regarding assessment of contaminant removal and long-term efficacy. Direct measurements and calculation of pollutant removal rates by trees onsite provide a clearer picture of phytoremediation efficacy in contrast to indirect measurements, such as groundwater monitoring wells. However, the removal rate is dependent on environmental conditions, resulting in variable *in planta* concentrations and contaminant removal rates. This project used *in planta* solid-phase microextraction (SPME) coupled with gas chromatography (GC) to measure chlorinated solvent concentrations in four trees at a contaminated site in Rolla, Missouri over a four year period. A single measurement was made in less than twenty minutes, with ten minutes of that time required for transportation to and from the GC. The sampling ports in each tree have provided more than 100 samples per tree over the four-year period. Concentrations in the tree exhibited a seasonal trend, with highest concentrations observed during the summer months, when translocation of groundwater was highest. Peak concentrations measured in the summer were approximately one order of magnitude higher than minimum concentrations measured in the winter and were correlated with calculated evapotranspiration (ET) based upon local weather. Contaminant removal rates were generated using calculated ET and *in planta* concentrations. This modeling approach and *in planta* measurements offer a new method to project contaminant mass removal rates for phytoremediation of organic contaminants.









## **HALOPHYTES**



## PHYTOREMEDIATION OF HEAVY METALS FROM COASTAL WATERS AND THEIR MOLECULAR MECHANISMS

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### ABSTRACT

Heavy metal pollution in coastal marine waters is a serious global problem that needs to be properly assessed and mitigated. It poses negative long-term implication on the health of human and also the ecosystem at large. Here we describe the causes and effects of heavy metal pollution on coastal waters. Phytoremediation of heavy metals is discussed with emphasis on Cadmium, Arsenic, Lead, Copper, Chromium, Manganese, Nickel, Vanadium and Zinc. Located between marine and terrestrial environments, mangroves are transitional coastal ecosystems which are found mostly in the tropical and subtropical regions. The phytoremediation potential of several mangrove species is discussed. Finally, the different molecular mechanism involved in phytoremediation of metals is described. There has been significant progress in determining the molecular basis for metal accumulation, which provides a strong scientific basis to outline several strategies for phytoremediation of metals from coastal waters. The metal transporter genes that are involved in hyperaccumulation of metals and biotechnological approaches including the transgenic plants are elucidated.

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## PRODUCTION OF THE HALOPHYTE FORAGE *ATRIPLEX AMNICOLA* FROM HEAVY METALS CONTAMINATED SOILS

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### ABSTRACT

Most of the studies about *Atriplex* plants grown on contaminated soils focused on *Atriplex halimus* while the genus of *Atriplex* contains more than 100 species. There is little information available about river saltbush (*Atriplex amnicola*) grown on contaminated soils also its response to organic fertilization. This study aims to determine (i) heavy metals accumulation in the shoot tissues of river saltbush; (ii) toxicity of heavy metals in the stems and leaves of river saltbush and (iii) impact of compost on reduction of heavy metals uptake.

Field experiment was conducted out at Ellwan, Assiut Governorate, Egypt (located at Longitude and Latitude 27 11 43 N and 31 04 19 E respectively). Untreated sewage waste water was used to irrigate the soil for more than 50 years and that soil considered to be moderately heavy metals contaminated soil [1]. One month-old seedlings of river saltbush were transplanted in the field at 1×1 m spacing and the plot area was 2×5 m<sup>2</sup>. Compost at rates of 0, 15 and 30 ton ha<sup>-1</sup> were applied and mixed with the soil before planting.

From our study we found that *Atriplex amnicola* plants were affected significantly by compost treatments. Increasing compost application rates to 30 ton ha<sup>-1</sup> increased the stems and leaves fresh weight by 9 and 14 %, dry weight by 14 and 9%, crude protein (CP) increased by 29 and 25% and ash content by 39 and 15% respectively. The high rate of compost reduced the Zn and Pb concentrations in stems by 11% and 32%, while in leaves by 6 and 38% respectively. Despite the extremely high total heavy metals concentrations in the studied soil, plants of river saltbush were able to maintain shoot elements content below toxic level. Toxicity levels of Zn, Cu, Pb and Cd are 100-400, 20-100, 30-300 and 5-30 mg kg<sup>-1</sup> dry weight of plant respectively [2]. The above numbers about toxicity levels are belong to the plant health but metal concentrations are also important for animal health. Permissible limits of heavy metals in animal's forages are 10 mg kg<sup>-1</sup> for Cd, 10-100 mg kg<sup>-1</sup> for Pb, 15-500 mg kg<sup>-1</sup> for Cu and 250-1000 mg kg<sup>-1</sup> for Zn as reported by [3]. In examining the obtained data, it appears that river saltbush metal accumulation did not exceed these limits, and it is may be a source of forage for livestock production in contaminated soils. Crude protein (CP) in the leaves of *Atriplex amnicola* ranged between (20.1-25.2%). Na and K concentrations in the leaves of *Atriplex amnicola* were higher than those in traditional forage crops but this do not prevent using of them as forage [4]. The current study shows the ability of *A. amnicola* to reduce HMs accumulation in their aerial parts. This shrub contained more crude protein and ash content compared to the traditional forage crops and the produced plant material is suitable as safe animal forage.

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## USE OF HALOPHYTES FOR PHYTOREMEDIATION OF ROAD RUNOFF CONTAMINATED BY DEICING SALTS

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### ABSTRACT

In winter maintenance, between 200,000 and 2,000,000 tons of deicing salts are spread on the French road network every year. The main deicing salt used in France is sodium chloride (NaCl), and its impacts on water streams, soils and biota are reported in the literature of the countries using this product. Even though 95% of the applied road salt ends up in the environment, there is almost no specific treatment reported in the literature. The high loads of dissolved NaCl in road runoffs are only diluted by their passage through retention ponds used for road runoff treatment in France.

Halophytes have been successfully tested for phytoremediation of soils polluted by trace metals and for phytodesalination. The use of halophytes on a biofilter treating road runoff could therefore be a promising technique to reduce the environmental impacts of deicing salts.

The objective of this study is to demonstrate that halophytes are suitable for phytodesalination of road runoff. The bioaccumulation of NaCl and other road pollutants (such as trace metals) are therefore tested for several plants.

Samples from a retention pond in France were collected and analyzed in order to measure deicing salt and trace metal concentrations encountered in the field. The measurements performed on those samples showed that Na<sup>+</sup> concentrations ranged between 10 and 2900 mg/L in the retention pond water input under study, and Cl<sup>-</sup> concentrations ranged between 5 and 4000 mg/L. In the pond output water, Na<sup>+</sup> concentrations ranged between 10 and 1100 mg/L, and Cl<sup>-</sup> concentrations between 5 and 500 mg/L.

These data permitted the preparation of phytodesalination bioassays and the choice of halophytes suitable for our experimental site (*Armeria maritima* and *Atriplex hortensis*). Tests of the halophyte germination, growth and pollutant bioaccumulation have been led. The halophytes have been hydrated with brines of concentrations between 0 and 10 g/L (reflecting those measured in the field) under strictly controlled conditions (temperature, nutrition, hydration, light, substrate quality, etc.). Two types of sodium chloride were used for the preparation of brines: chemically pure NaCl and the deicing salt (containing, besides NaCl, 5% of impurities and additives). After two-month-long bioassays, the pollutant bioaccumulation will be measured: analysis of Na<sup>+</sup>, Cl<sup>-</sup>, and trace elements will be performed on the halophytes (in stems, leaves and roots), and on the substrate after plant harvest.

So far, the growth of the two species under study was analyzed and both *Armeria maritima* and *Atriplex hortensis* have shown a relatively low growth. Although *Atriplex hortensis* showed relatively higher fresh mass compared to *Armeria maritima*, dry mass/fresh mass ratio was significantly higher for *Armeria maritima* suggesting higher water uptake for *Atriplex hortensis*, which could be attributed to a better tolerance of salinity increase.

The analysis of Na<sup>+</sup>, Cl<sup>-</sup> and trace metals accumulation in the plants is still in progress.

**Acknowledgements:** The financial support by CEREMA project DEFROUHPROU and by GEMCEA is greatly appreciated.

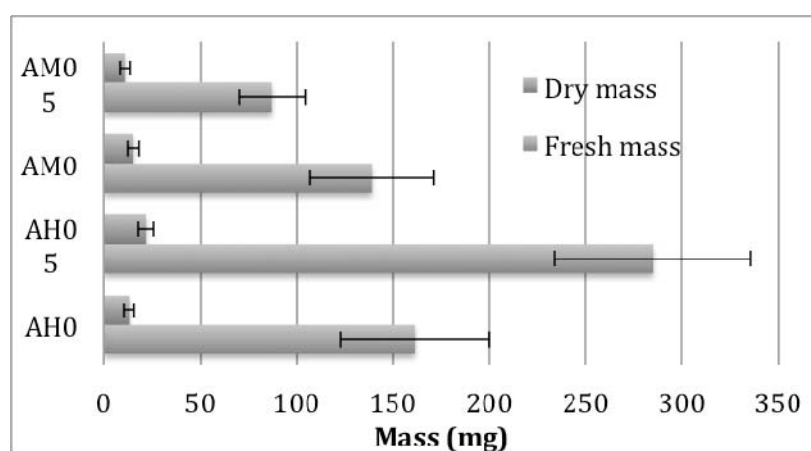


Figure 1. Growth of *Armeria maritima* (AM) and *Atriplex hortensis* (AH) treated with deionised water or brine with 500 mg/L of pure NaCl



## MIXED HEAVY METAL TOLERANCE AND ACCUMULATION IN THE WETLAND HALOPHYTE *JUNCUS ACUTUS* L.

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### ABSTRACT

Halophytic species are widespread in Mediterranean basin and are known to tolerate various environmental stresses including heavy metals. *Juncus acutus* L. which is a perennial wetland halophytic plant with a wide ecological range has proven before to be tolerance to various environmental stresses including heavy metal stress by zinc and hexavalent chromium. In this study, the mixed metal tolerance and accumulation ability of the plant is investigated in order to explore its potential for mixed heavy metal phytoremediation.

For that purpose, a 5 months (October to April) pot experiment was conducted (six pots per treatment) with *J. acutus* plants collected from the Souda Bay in Chania and planted to the same amount of a typical surface soil collected from an agricultural area in Chania. After a sufficient adaptation period, the experiment started with the addition of 10 ppm Cd, 150ppm Ni, 500ppm Zn and 500ppm Pb, in one dose as aqueous solutions. The experiment took place in an open air area under natural light conditions with temperatures ranging from 5.3 to 31.1 °C. The soil was always over-saturated, as the pots were irrigated with water to achieve a water layer above the soil surface in order to simulate wetland conditions. At the end of the experimental period, measures of metals concentrations in plant tissues and in water of the soil pores and measurements of plant biomass, chlorophyll content, shoot water content and shoot proteins were performed. Moreover, plants were, also, assayed for activities of antioxidant enzymes such as the guaiacol peroxidase (GPX) and catalase (CAT).

The results suggest that *J. acutus* is not a hyperaccumulator of the examined metals (Table); however, it showed high tolerance to mix heavy metal pollution since all plants showed no visible

Table. Metal concentration in *J. acutus* tissues (ppm). Values represent means  $\pm$  standard error (n=6).

		Cd	Pb	Ni	Zn
Control	Shoots	n.d.	1,25 $\pm$ 0,08	n.d.	15,66 $\pm$ 2,3
	Roots	n.d.	5,98 $\pm$ 0,9	12,78 $\pm$ 6,6	28,64 $\pm$ 5,6
Metal treatment	Shoots	1,59 $\pm$ 0,6	84,94 $\pm$ 30,7	100,22 $\pm$ 18,7	200,28 $\pm$ 39,5
	Roots	12,77 $\pm$ 1,9	626,35 $\pm$ 106,5	259,65 $\pm$ 36,4	476,14 $\pm$ 55,9

toxicity symptoms such as chlorosis, necrosis, or root inhibition. Moreover, shoot proteins, chlorophyll content and shoot water content were not found to be statistically affected by the presence of metals and in addition, plant' s biomass was increased in the presence of metals. Furthermore, GPX activity and CAT activity of exposed plants to metals was not statistically elevated in comparison with the controls.

All the above suggest that *Juncus acutus* L. is a heavy metal tolerant plant that could be used in phytoremediation strategies for revegetation of mixed heavy metal polluted areas.

**Acknowledgements:** This work was co-funded by the European Union in the Work Programme 2012 "COOPERATION" FP7-KBBE-2012-6 project - "Integrating biotreated wastewater reuse and valorization with enhanced water use efficiency to support the Green Economy in EU and India" (WATER4CROPS, Grant No. 311933).



## ALTERNANTHERA BETTZICKIANA A POTENTIAL PHYTOREMEDIATOR: GROWTH, METAL UPTAKE AND OXIDATIVE STRESS

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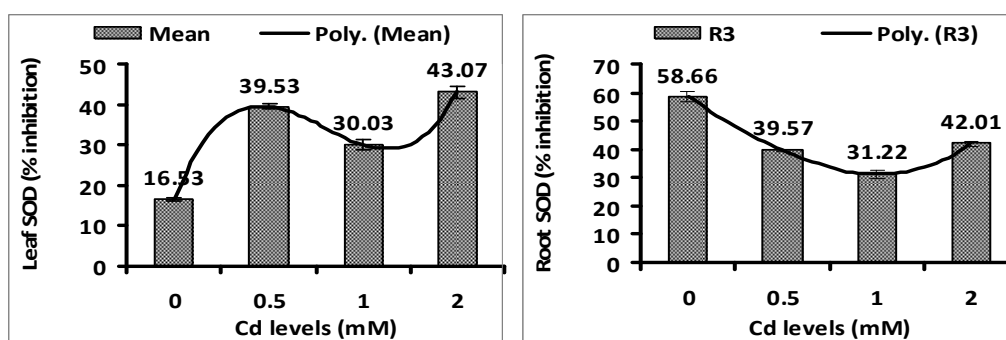
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### ABSTRACT

Industrialization and urbanization have heavily contaminated irrigated soils by heavy metals which upset the ecosystems including surface and ground waters, human health, food and cause many drastic issues for food chain. Phytoremediation is a vital and inexpensive technique used to remediate heavy metal contaminated soils with textile effluents, tannery waste and wet lands. Plants that show high metal accumulation often show slow growth rate and little biomass production when grown on soils contaminated with metals. Exploration and cultivation of heavy metal tolerant plants with a better growth is a very effective method for the purpose of phytoremediation of different mediums contaminated with heavy metals.

In this investigation, varying physiological and biochemical attributes of ornamental plant *Alternanthera bettzickiana* due to its tolerance to high salinity levels as reported in literature. So, keeping in view the resistance of this plant against high salinity levels, the present experiment was performed to find out the potential of *A. bettzickiana* to different regimes of cadmium (Cd) in maintained in soil. Different levels of cadmium treatments (0 mM, 0.5 mM, 1 mM and 2 mM) were applied in solution form after the establishment of the cuttings. CdCl<sub>2</sub> was applied as a source of Cd. For the estimation of varying physiological and biochemical attributes the plants were harvested after 10 weeks of planting the plant stem cuttings. Potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), urea (CH<sub>4</sub>N<sub>2</sub>O) and diammonium phosphate [(NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>] were used as fertilizers @ 2.19:1.36:2.4 mgL<sup>-1</sup> after every 15 days to maintain the NPK ratio in the soil. Plant height and biomass increased significantly with the application of Cd stress and the maximum gain in biomass was obtained when Cd was applied @ 2 mM concentration. No visual symptoms of metal toxicity were observed on plants leaves or roots at all Cd regimes. Of different enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities were also affected significantly in Cd treated plants but the increasing or decreasing trend was plant part specific. SOD activity in roots decreased while increased in leaf due to Cd stress and the maximum increase in leaf SOD was recorded at 2 mM Cd level. However, the opposite trend was true for CAT activity in root and leaf. While the POD activity increased in both root and leaf due to Cd stress. Leaf and root MDA content as indicator of lipid peroxidation also increased due to Cd application in both root and leaf and more increase was at 2 mM Cd level. Furthermore, soil Cd application increased its internal content in both the roots and leaves and the maximum Cd content was in plants treated with 2mM Cd level. From the results it was concluded that *A. bettzickiana* plants accumulated high concentrations of Cd when grown under cadmium stress and showed tolerance these levels. Thus we can conclude that *A. bettzickiana* seems to be valuable for phytostabilisation strategies.



## PHYTOREMEDIATION OF A HYPERSALINE SOIL IN KUWAIT BY RHIZOSPHERIC AND PHYLLOSHERIC MICROORGANISMS ASSOCIATED WITH ORGANS OF A HALOPHILIC WILD PLANT

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### ABSTRACT

Hydrocarbonoclastic microorganisms associated with roots and aerial organs of *Halocnemum strobilaceum*, a wild, halophytic plant growing in a hypersaline coastal area in Kuwait, were counted and isolated. The NaCl concentration of this coastal area ranged between 2 M and 4 M. Up to  $8 \times 10^4$  colony forming units (CFU)  $g^{-1}$  of extremely halophilic, hydrocarbonoclastic microorganisms were counted in the rhizosphere, and several hundreds of CFU  $g^{-1}$  in the phyllosphere of this plant. According to their 16S rRNA-gene sequences, the rhizospheric microorganisms were affiliated with the two archaea, *Halobacterium* sp. and *Halococcus* sp., and with the five extreme halophilic bacteria, *Brevibacillus borstenlensis*, *Pseudoalteromonas rutherfordii*, *Halomonas sinaensis*, *Pseudomonas stutzeri*, *Mycobacterium bolletii* and *Panibacillus polymyxa*. The phyllospheric microorganisms were affiliated with the extreme halophilic bacterium *Ochrobactrum* sp. and the halophilic dimorphic yeast *Candida utilis*. All individual organisms, when used as inocula could consume, in batch culture, crude oil and pure aliphatic and aromatic hydrocarbons in mineral media, with 2 M salinity. Also the total rhizospheric and phyllospheric microflora associated with the plant when used as inocula were effective in hydrocarbon removal at high salinity. It was concluded that this wild, halophytic plant species is a suitable agent for the phytoremediation of hypersaline environments polluted with oil.

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## **CICHORIUM SPINOSUM AS A PHYTOREMEDIATION SPECIES**

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### **ABSTRACT**

Increased salinity is an ever-growing environmental problem that severely affects agriculture by limiting crop production and by causing loss of agricultural land. According to the United Nations Food and Agriculture Organization approximately 20-30% of all agricultural lands are becoming saline, whereas Global Assessment of Soil Degradation (GLASOD) reports that about 8% of all salt-affected soils are human-induced.

In the present study, *Cichorium spinosum* plants, which are native to the island of Crete, were grown under medium to high salinity levels (4-8 dS/m) by adding NaCl to irrigation water, in order to evaluate their productivity and mineral composition, and were compared to control treatment (1.8 dS/m). From the results it is observed that *Cichorium spinosum* is tolerant to medium salinity levels (4 dS/m), since the number of leaves, fresh weight of leaves and total leaf area is significantly higher than that at high salinity levels (8 dS/m), without differ significantly from the control treatment (1.8 dS/m). In addition, chlorophyll content was not affected by salinity level, whereas leaf thickness increased significantly for plants grown under medium salinity levels (4 dS/m). Regarding mineral composition, Na<sup>+</sup> content increased significantly whereas Mg<sup>2+</sup>, Mn and K<sup>+</sup> decreased with increasing salinity. Ca<sup>2+</sup> and Fe<sup>3+</sup> content was not affected.

Therefore *Cichorium spinosum* could be considered a species with tendency to accumulate Na<sup>+</sup> without its productivity being affected. In this context, the cultivation of wild native species such as *Cichorium spinosum* could be a useful means to alleviate problems of saline soils by cultivating it for phytoremediation purposes, whereas the final products could be marketed as edible herbs. In addition, this species could be cultivated in areas where irrigation water is of low quality due to high content of NaCl (coastal areas or areas where ground water is saline), allowing farmers to exploit these areas and gradually improve their soils.







## **EMERGING ISSUES**



## SUSTAINABLE REUSE OF NITROGEN-LADEN PROCESS WATER IN POPLAR TREE VADOSE ZONES AT VARIOUS SCALES

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### ABSTRACT

Process water from industrial-scale preparation of food products from potatoes, onions and similar produce contains relatively high levels of organic carbon and nitrogen. Large processing facilities generate over 5 million gallons of process water daily with land irrigation being one of the disposal methods of choice. Irrigation to produce a commodity crop, such as alfalfa or sugar beets, represents a beneficial reuse of this process water. But, over application of process water, particularly in sandy soils, can cause nitrate contamination of shallow aquifers at levels approaching 10 times the Federal standard for drinking water. The critical question then is:

*How can the reuse benefits of this nitrogen-laden process water be maximized in a way that protects groundwater while maintaining the economic viability of the food processor?*

Our central hypothesis was that deeply rooted poplar trees increase the vadose zone dwell time, resulting in enhanced nitrogen removal. A pilot-scale system (Figure 1) was utilized to test the impact of poplar tree root zones on dwell time and on reactive transport of organic carbon and nitrogen that was applied via an intermittently-dosed synthetic wastewater. The results from the pilot-scale system were used to calibrate a 2-dimensional conservative and reactive transport model (Hydrus 2D/3D) configured with the Constructed Wetlands 2D biological processes module. The Hydrus model was then used to predict the impact of nitrate on groundwater using various year-round irrigation scenarios with consideration of spatial and temporal effects inherent to the full-scale application of this enhanced vadose zone nitrogen removal approach.

Pilot-scale data indicated a significant increase in dwell time attributable to poplar tree roots in sandy soils. This dwell time enhancement led to a commensurate increase in denitrification during dormancy ( $4.5 \pm 2^\circ\text{C}$ ). While significantly less nitrate was measured in the effluent of rooted test cells, the nitrate concentration in all test cell effluents increased over time to levels exceeding  $70 \text{ mg-N L}^{-1}$ . The synthetic wastewater contained less than  $1 \text{ mg-N L}^{-1}$  nitrate; therefore, the steady increase of effluent nitrate was attributed to hydrolysis of accumulated, slowly degradable organic nitrogen followed by rapid nitrification. A nitrogen mass balance analysis was performed to estimate the dwell-time specific, first-order rate coefficients for hydrolysis and denitrification at the pilot-scale.

The Hydrus model was able to accurately fit ( $R^2 > 0.94$ ;  $\text{RMSE} < 0.052$ ) van Genuchten shape, saturated hydraulic conductivity, dispersivity, and immobile water content parameters from pilot-scale bromide tracer studies. The biochemical parameters considered were dissolved oxygen, organic matter (readily and slowly degradable and inert), ammonium, nitrite, nitrate, nitrogen gas, inorganic phosphorus, and heterotrophic and autotrophic organisms. Heterotrophic bacteria were assumed responsible for hydrolysis, OM mineralization, and denitrification. Autotrophic bacteria were assumed responsible for a two-step nitrification process ( $\text{NH}_4^+$  to  $\text{NO}_2^-$  and  $\text{NO}_2^-$  to  $\text{NO}_3^-$ ). Initial values for these parameters were obtained from [Langergraber & Šimunek, 2005] and over 50 model iterations were performed to determine optimal values. Once optimized, simulations of 720 hours of intermittent dosing were run for both the rooted and non-rooted scenarios. The effluent nitrate concentration in the rooted simulation was  $6.4 \text{ mg/L}$  compared to  $12.0 \text{ mg/L}$  in the non-rooted system. The simulated impact of roots on nitrate was consistent with the effluent nitrate concentrations measured in early pilot-scale testing cells with roots ( $1.0 \text{ mg/L}$ ) and without roots ( $5.1 \text{ mg/L}$ ). The study provides strong evidence that less nitrate escapes a deeply rooted vadose zone than a non-rooted, or shallow rooted, system even during dormancy ( $4.5^\circ\text{C}$ ). Results from Hydrus simulations at larger spatial and temporal scales are forthcoming.

*Reference:* Langergraber, G., & Šimunek, J. (2005) Modeling variably saturated water flow and multicomponent reactive transport in constructed wetlands. *Vadose Zone Journal*, 4(4), 924-938. doi:10.2136/vzj2004.0166

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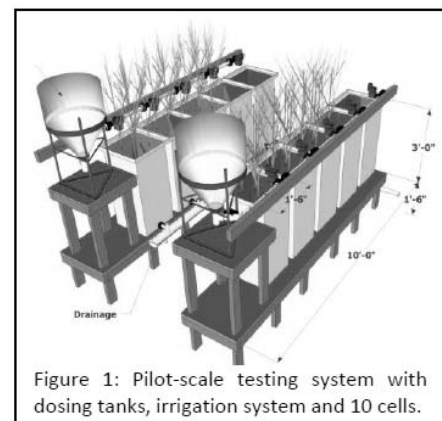


Figure 1: Pilot-scale testing system with dosing tanks, irrigation system and 10 cells.



## REMOVAL OF CHROMIUM (VI) FROM THE AQUEOUS SOLUTION USING PERSIMMON TANNIN GEL

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### ABSTRACT

Chromium is used in many items, such as textile, leather tanning, electroplating, metal finishing industries, wood treatment, corrosion control, oxidation, and anodizing industries. In the case of absorption was done at high levels, chromium can generate serious problems and, when concentration reaches 100  $\mu\text{g/g}$  body wt., it can ultimately become lethal. Recently, the main processes for elimination chromium are adsorption, reverse osmosis and chemical reactions that contain reduction and precipitation. Among them adsorption has been shown as a feasible alternative method for removing traces of chromium from wastewater. Many different adsorbents were tried to remove chromium from wastewaters.

We have reported that microorganisms could remove many kinds of toxic and useful metals, such as lithium, uranium, thorium, rare earths, and gold from the aqueous solution. Additionally, immobilized persimmon gel removed gold (III) from hydrogen tetrachloroaurate(III) solution. However, microorganisms could remove little amount of chromium from the chromium (VI) solution. Therefore, the removal of chromium from aqueous chromium (VI) solution using immobilized persimmon gel in this study was examined.

The removal of chromium using persimmon tannin adsorbent was strongly affected by the pH of the solution. The amounts of chromium (VI) removed were quantitatively under pH 2, however, those were decreased with increasing the pH of the solution. The amount of total chromium removed was maximal at pH 2, however, those were decreased with increasing or decreasing the pH of the solution. It seems that the positively charged protonated hydroxy groups of persimmon tannin and negatively charged hydrogen chromate ions are bonded at low pH region. Additionally, the amount of reduced chromium was increased with decreasing the pH of the solution.

The removal of chromium using immobilized persimmon tannin adsorbent was strongly affected by the chromium concentration of the solution. Immobilized persimmon tannin adsorbent can remove chromium quantitatively from the solution containing lower 5 ppm chromium (VI), however, the ratio of the amount of chromium (VI) removed toward that of initial chromium (VI) was decreased with increasing the initial chromium (VI) concentration.

The amount of chromium ( $\mu\text{mol/g}$  dry wt. adsorbents) was increased with increasing the initial chromium (VI) concentration of the solution. When the initial chromium (VI) concentration was 1700  $\mu\text{M}$ , 4860  $\mu\text{mol}$  of chromium (VI) was removed. The amount of chromium removed was fitted with *Langmuir's* isotherm. The amount of reduced chromium was maximal from the solution containing 400-800  $\mu\text{M}$  chromium (VI).

The removal of chromium using immobilized persimmon tannin adsorbents was strongly affected by the adsorbent amounts. The ratio of the amount of removed chromium (VI) per that of initial chromium (VI) was increased with increasing the amount of adsorbent, however, the amount of chromium removed ( $\mu\text{mol/g}$  dry wt. adsorbent) was decreased with increasing the amount of adsorbent. The amount of total reduced chromium was increased with increasing the amount of adsorbent, however, the amounts of reduced chromium ( $\mu\text{mol/g}$  dry wt. adsorbent) was maximal using 10-15 mg of adsorbent (dry wt. basis).

Adsorption of chromium (VI) was very rapidly and reached equilibrium within two hours. Reduction of chromium (VI) was a little slower than adsorption. The time course of chromium removal was fitted by an adsorption model using time-dependent *Langmuir equation*.

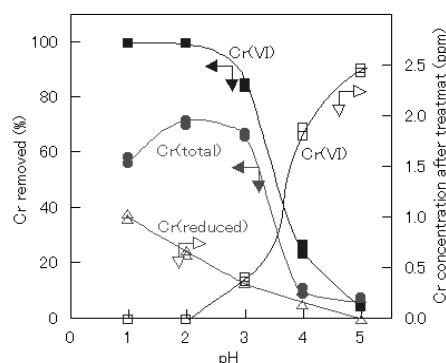


Fig. 1. The effect of pH on the removal of Cr by persimmon tannin gel.

15 mg (dry wt. basis) of the gel were suspended in 100 ml of the solution containing 2.5 ppm of chrome for 1h at room temperature.



## THE IMPORTANCE OF PLANTS WHEN USING *IN SITU* CARBON SORBENTS FOR THE REMEDIATION OF ORGANIC CONTAMINANTS

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### ABSTRACT

Risk based remediation technologies for organic contaminants are gaining in popularity. An example of such a technology is the use of carbon sorbents, specifically biochar and activated carbon (AC), which immobilize the contaminant *in situ* and thereby reduce contaminant uptake in plants and invertebrates. To date, most studies have focused on AC and in sediment systems and there is less data available for biochar, which is a greener more cost-effective material capable of improving plant growth and sustainably sequestering carbon. Our previous work demonstrates that the addition of 2.8% biochar and AC to highly PCB-contaminated soil (>50 µg/g, Aroclor 1254/1260) reduces the phytoavailability of PCBs by ~70%. These carbon amendments also reduced the bioavailability of PCBs to *Eisenia fetida* (worm) (average 57% reduction) and restored the microbial community structure and function to that of a remediated site ([PCB]<sub>soil</sub> < 3 µg/g). However, when applying this technology to a different organic contaminant, DDT, neither AC nor biochar were successful in minimizing DDT phytoavailability. These findings are in contrast to the existing literature which reports that AC reduces DDT bioavailability by greater than 90%. Many of these studies originate from researchers studying sediment systems. Generally, these studies have utilized equilibrium passive sampling devices based on polyoxymethylene (POM) (a polymer) to estimate the effectiveness of carbon sorbents, and have largely neglected the role that plants play in contaminant mobility. For this reason, we included both passive POM sampling and worm bioavailability studies in our two-year plant trials with carbon amendments. We found that carbon amendments significantly reduced the uptake of contaminants into the POM samplers by 32% and into worms by 50%, while no significant reductions in plant uptake were achieved. Thus, although passive samplers may be adequate for predicting contaminant availability to invertebrates such as worms, further research is required using plants to determine the actual potential of carbon amendments in soil systems as a remediation technology.



## ASSESSING THE AMENDING POTENTIAL OF LOW CARBON FOOTPRINT MATERIALS IN Zn MINING AREAS

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### ABSTRACT

Mining activities are essential for society's development. Yet, they cause environmental impacts, which must be minimized so that the benefits of this activity can overlap the likely risks it might pose. Open-pit mining often involves the removal of the soil overlaying the ore body. At the end of the exploration process, the areas contain materials with heterogeneous soil physicochemical characteristics, often unsuitable for plant growth. Improving soil chemical, biological, and physical conditions is a requirement for recovering most of these areas. For that, it is often necessary to apply suitable soil amendments in order to alleviate the potential phytotoxicity and/or provide plant nutrients. Such amendments must be chosen not only based on their efficiency and cost, but also according to their carbon footprint, which is currently a trend for any "green remediation" proposal. This study aims at evaluating the amending potential of biocompounds, limestone, sewage sludge, and biochar in a substrate collected from Zn-mine degraded areas. Our main goal is to select the best soil amendment for the recovery of these areas – with a focus on low-cost, locally produced materials –, using *Andropogon gayanus* as cover crop for a phytotechnology project.

The experiment was conducted under greenhouse conditions with soils samples collected from a zinc mining area located in the State of Minas Gerais, Brazil (clay 560 g kg<sup>-1</sup>; sand 145 g kg<sup>-1</sup>; pH 5.9; P 3,5 mg dm<sup>-3</sup>; K 14.8 mg dm<sup>-3</sup>; Ca 0.9 cmol<sub>c</sub> dm<sup>-3</sup>; Mg 0.7 cmol<sub>c</sub> dm<sup>-3</sup>; Al 0.4 cmol<sub>c</sub> dm<sup>-3</sup>; effective CEC 3.8 cmol<sub>c</sub> dm<sup>-3</sup>; Zn<sub>total</sub> 530 mg L<sup>-3</sup>; Cd<sub>total</sub> 1.6 mg L<sup>-3</sup>; Pb<sub>total</sub> 1,253 mg L<sup>-3</sup>). We tested four amendments and five increasing rates for each material, with three replicates: a wood-based biochar residue (0; 2.5; 7.5; 15; 30 g kg<sup>-1</sup>), a sewage sludge (0; 1.25; 2.5; 5; 10 g kg<sup>-1</sup>), a by-product limestone (0; 0.5; 1; 2; 4 g kg<sup>-1</sup>), and a biocompound (0; 1.25; 2.5; 5; 10 g kg<sup>-1</sup>). All amendments were collected at sites nearby the degraded area, being locally produced or occurring as by-products of neighboring industrial activities. The materials were added to the soil and incubated for 60 days with soil humidity at 60% of field capacity. After incubation, soils were tested for pH (in water) and phytoavailable levels of Cd, Pb, and Zn and subsequently sowed with 15 *Andropogon gayanus* seeds, from which we evaluated germinability, plant survival and shoot height.

Increasing limestone and biochar rates caused soil pH to increase up to 2.5 units, hence reducing phytoavailable Cd (~32% limestone and ~57% biochar) and Zn (~28% limestone and ~41% biochar), with little effect upon the availability of Pb (~5% biochar and ~3% limestone). Adding sewage sludge and biocompound had little effect on soil pH, yet they still reduced the availability of Cd (~7% sewage sludge and ~14% biocompound), while causing Zn phytoavailability to decrease (biocompound ~24%) or increase (~14% sewage sludge). Phytoavailable Pb increased slightly following the application of sewage sludge (~8%) and biocompound (~10%).

Germinability was affected differently due to the treatments, with little effect caused by biochar and a trend of reduction caused by adding sewage sludge, limestone, and biocompound. Nevertheless, despite the observed reductions in the germination of *Andropogon gayanus* seeds, all treatments increased plant survival, with the largest increases in *A. gayanus* survival being observed with the addition of 5 g kg<sup>-1</sup> of sewage sludge (~122%) and 10 g kg<sup>-1</sup> of biocompound (~138%). Similarly, shoot height was also positively affected by the addition of all soil amendments, with best results observed following the application of 1 g kg<sup>-1</sup> limestone (~90%), 10 g kg<sup>-1</sup> of biocompound (~70%), 15 g kg<sup>-1</sup> biochar (~54%), and 5 g kg<sup>-1</sup> of sewage sludge (~51%). This positive effect is possibly a result of improvements in nutritional status.

Considering their local availability and low cost and due to the improvements caused in soil chemical attributes, as well as on plant survival and shoot height, we conclude that all evaluated materials could be used as soil amendments for the studied Zn mine area, being suitable for further testing under field conditions. Additional research is underway to assess if the combined use of the tested soil amendments could result in enhancement of the beneficial effects.

**Acknowledgements:** The financial support provided by FAPEMIG, CAPES and CNPq.





## LED LIGHT QUALITIES AS AN ARTIFICIAL STIMULUS IMPROVE THE GROWTH OF TWO MEDITERRANEAN SPECIES CULTIVATED INTO A CONTROLLED ENVIRONMENT

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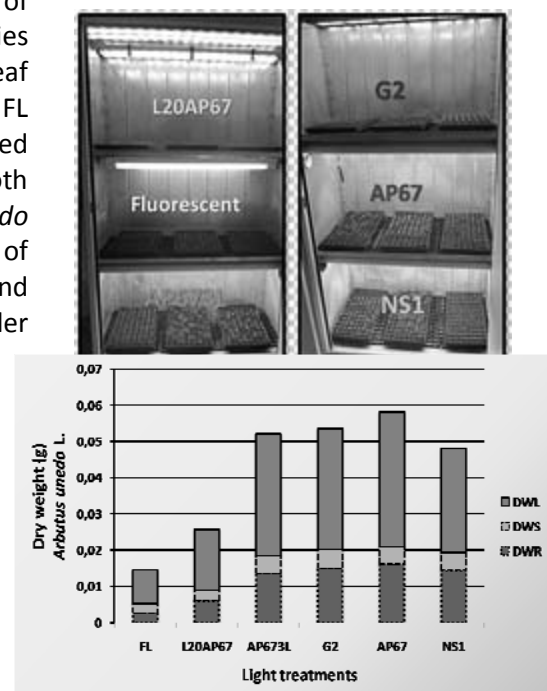
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### ABSTRACT

Plant illumination is particularly important in a controlled environment. Light-emitting diodes (LED) is a promising light source used in plant physiology research. However there have been very few investigations on the application of LEDs in plant growth chambers. In the present study we evaluate the effects of different LED or fluorescent lights on the growth traits of two Mediterranean species, *Arbutus unedo* L. and *Myrtus communis* L. seedlings cultivated in mini-plug containers fulfilled with stabilized medium soil substrate. Seedlings were uniformly grown into two growth chambers for seven weeks under light-emitting diodes (LEDs) (L20AP67, AP673L,G2, AP67, NS1 -Valoya) or Fluorescent light (FL). LEDs used emitted a continuous spectrum thanks to a mixture of ultraviolet, blue, green, red, far-red and infra-red in various percentages. Furthermore root growth potential (RGP) was used as a performance attribute for seedling quality evaluation.

During the seven week experimental period the effect of different light treatments was significant and species dependent by means of height growth rate, the leaf colour rating and the leaf number. L20AP67 LED and FL light induced significantly taller seedlings but showed less beneficial effect on the root development for both species compared to the rest of LEDs. *Arbutus unedo* seedlings showed significantly lower dry weight mass of leaves, shoots and roots under the illumination of FL and L20AP67. While *Myrtus* seedlings better adapted under the AP67 LED illumination for the same parameters tested. Root-to-shoot ratio for both species obtained significantly lower under the FL light than LEDs. After 31 days into the RGP bath, *Arbutus* seedlings pre-cultivated under the G2 LED better predicted the new root length and dry weight thus almost doubled. For *Myrtus* seedlings that pre-cultivated under the FL no significant effect found for the new root length, while G2 LED obtained the heaviest roots among all treatments.



These results confirm and extend the results of other studies indicating that light quantity and quality interact to determine plant morphology. A thorough understanding of this interaction is essential to the development of light sources for optimal plant growth and development.

**Acknowledgements:** The financial support by FP-7 project REGENFOREST contract No. 286067 is greatly appreciated.



## PHYTOEXTRACTION AND ECOLOGICAL CATALYSIS: SYMBIOSIS FOR FUTURE

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### ABSTRACT

Phytotechnologies such as metals phytoextraction are very attractive solutions for the reclamation of contaminated brownfields. However, the economic potential of these technologies remains poor, due to a lack of credible outlets for metal-contaminated biomass. Strong efforts are still necessary to recycle metals from contaminated-biomass, so as to develop the full economic potential of phytoextraction. This is particularly true in a context of resource depletion, with increasing metals prices and market volatility.

In this communication, we aim to present a new outlet for metal contaminated-biomass. Phytoextraction generates plant wastes, which are valued through an innovative concept of ecological recycling. Because certain plants are able to hyperaccumulate primary or strategic metals as Ni<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup> and Pd<sup>2+</sup> can be directly used as catalysts in organic fine chemical reactions. Ecocatalysts derived from hyperaccumulators are useful for the synthesis of molecules with high added value in fine and industrial chemistry (aroma and cosmetics with the «natural» label, drugs and oligomers with biological interests, highly fonctionalized aromatic heterocycles, chiral structures, key intermediate in various industrial chemical process and biopesticides). They allow the development of substitutive reagents to oxidants forbidden by REACH too. The new catalysts represent very efficient alternative to catalysts derived from metallurgy, with a three dimension domain of chemistry/ecology/environment.

This original approach brings the first perspective of enhancing the unique biomass and establishes a new field of Green Chemistry: Ecocatalysis. It is already a Green Revolution in Chemistry. Phytoextraction are source of innovation in organic synthesis and organic synthesis is the driving force of developed phytotechnologies.

These results are the first use of metal-rich biomass in organic synthesis and constitute a strong encouragement for the economic development of phytoextraction programs for metal-bearing soils.

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## **SOCIAL AND ENVIRONMENTAL ASPECTS FROM THE IMPLEMENTED RENEWABLE ENERGY PARKS (SOLAR, AIR, BIOMASS) IN CYPRUS**

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### **ABSTRACT**

Renewable energy sources are, nowadays, not only a necessity but also a choice. Many technologies to exploit them have been established and operate all over the world, producing energy and offering multiple benefits. However the renewable energy projects are not always without negative impacts to the environment and the society, which must be effectively evaluated and confronted, in order only the positive impact to remain. However the research question is does this happen in all cases? The aim of this paper is to evaluate from a social and an environmental aspect, renewable energy projects which has been established in Cyprus previous year, in order to find out if they are licensed and constructed effectively, if the necessary preventive measures are implemented against social and environmental impacts during the projects operation and which kind of renewable energy sources is implemented more efficiently, socially and environmentally. The object is also to infer conclusions for the renewable energy sources sector in Cyprus. A number of techno economic, institutional, environmental and social criteria have been appointed, according to which the evaluation of renewable energy projects is performed. Data from three different established renewable energy projects (solar park, air park, Biomass Park) have been collected. Firstly it is tested if their social and environmental performance presents variation. Afterwards the projects are compared according to the above criteria, in order to identified, which project is more efficiently. Based on the results and of literature review, a SWOT analysis was prepared for the renewable energy sector in Cyprus. The results indicated that the projects do not differ from each other and that they run efficiently, environmentally and socially. Based on the multicriteria analysis the photovoltaic park emerges as the more socially and environmentally efficient, with the wind parks coming second and the biogas unit third. Finally a significant number of strengths and opportunities, and a comparatively smaller number of weaknesses and threats are concluded for the renewable energy sector in Cyprus.



## DEVELOPING A PRACTICAL DECISION SUPPORT TOOL (DST) FOR THE APPLICATION OF PHYTOTECHNOLOGIES

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### ABSTRACT

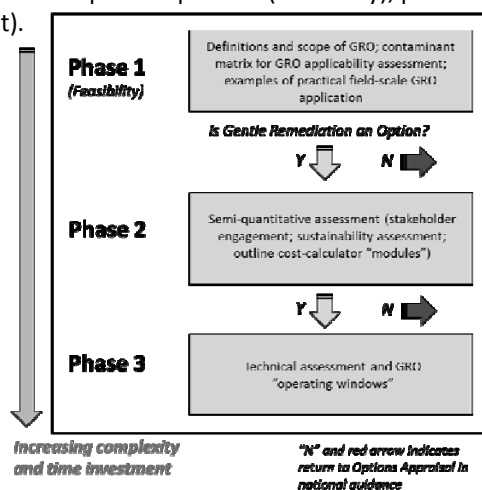
A range of systems and tools have been proposed to support decision making within the contaminated land arena. A critical review, coupled with stakeholder feedback collected during the European Commission FP6 ERANET project SUMATECS and presented in Onwubuya et al (2009), has highlighted a general lack of stakeholder knowledge of these systems and tools, and specifically of those which can be used to support the selection and application of phytotechnologies. Based on stakeholder feedback, existing tools were regarded as too general (containing insufficient detail on the range of remediation options available), or alternatively were too complicated for regular or widespread use by decision makers in selecting and applying phyto-based (and indeed other) remediation technologies. Following the SUMATECS project, the European Commission FP7 GREENLAND project was established to overcome a range of barriers to the practical application of phytotechnologies and other gentle remediation options (GRO). A key component of this project was the development, using data from long-term (> 5 year) phytotechnology site trials across Europe, of a practical and simple decision support tool (DST) focused on the wider contaminated land community. This DST is presented here.

The GREENLAND DST is a simple Microsoft Excel-based tool targeted on non-specialists which follows (in line with existing national decision support guidelines) a tiered approach (below, right). The tool is designed to interface with existing national guidance at the options appraisal stage, although the DST also has applicability at earlier (site planning) stages. The tiered approach used consists of three phases: phase 1 (feasibility), phase 2 (semi-quantitative assessment) and phase 3 (technical assessment).

Each phase terminates in a decision point (Yes = proceed to next phase; No = return to options appraisal), with increasing complexity and time investment requirements from phase 1 to 3. Phase 1 includes: (a) a (qualitative) contaminant matrix, in the format of the UK's CLR-11 remediation option applicability matrix, which forms a decision checklist for the site to be remediated, and consists of a list of phytotechnologies which have been checked against a list of Contaminants of Concern (CoC) through detailed literature review in order to provide evidence that the methods have previously been used in the remediation of the specified contaminants on (at least) a research scale; and (b) examples of practical, large-scale phytotechnology applications.

Phase 2 contains modules on: stakeholder engagement (including guidelines for stakeholder engagement and criteria for the identification of different stakeholder profiles/ categories); sustainability assessment (drawing on SURF-UK sustainability indicators, and tables showing potential economic, environmental and social benefits); and economic assessment (using a simplified "cost calculator" for quantifying (within limits) site costs and local and wider economic benefit). Phase 3 contains detailed technical information for practical remediation project design and implementation, and outlines "operating windows" for phytotechnology application. The DST has been populated and "calibrated" using data from long-term European site trials within the GREENLAND site network, and a major redevelopment project in east London, UK. Ongoing work is aimed at finalising the DST using an iterative approach involving validation by a range of experts and potential end-users.

**Acknowledgements:** The authors are grateful for financial support from the European Commission under the Seventh Framework Programme for Research (FP7-KBBE-266124, GREENLAND).



## EFFECT OF ANTIOXIDANT RICH SPICES, CLOVE AND CARDAMOM EXTRACTS ON THE METABOLIC ENZYME ACTIVITY OF *LABEO ROHITA*

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### ABSTRACT

**Background:** Herbs, spices, and various plant extracts have received increased attention as possible replacement of antibiotics. In this view, aromatic substances and essential oils extracted from various plants became interesting due to their antimicrobial [Guler, *et al.*, 2005], antioxidant [Dragland, *et al.*, 2003] effects and their stimulating effects on animal performance [Ciftci, *et al.*, 2005, Guler, *et al.*, 2005b] and digestive enzymes [Lee, *et al.*, 2003]. These plant extracts are useful in reducing stress.

**Objectives:** Studies indicate that clove and cardamom have a number of medicinal properties and is a potent antioxidant, the present study was undertaken to evaluate their anti-stress effect in *Labeo rohita*.

**Methods:** Spice extracts, Experimental design and diets, Enzyme analysis, Statistical analysis.

**Results:** The present study was conducted to evaluate the protective effect of clove (Clo) and cardamom (Cdm) extracts as natural antioxidants in the diet of *Labeo rohita* fingerlings. Both the spice extracts were mixed separately or in combination at the level of 0.5 and 1.0% in the fish feed. Thus, seven experimental diets were the following Clo-0.5, Clo1-1.0, Cdm-0.5, Cdm-1.0, CC-0.5 and CC-1.0 and control with no extract. The results revealed that, SOD and CAT activities were significantly ( $p < 0.05$ ) higher in Clo-0.5 compared to the control. AST, ALT, LDH, MDH and G-6-Pase activities in liver and muscle decreased significantly ( $p < 0.05$ ) in all treated groups as compared to the control. Significantly ( $p < 0.01$ ) lowest activities was observed in treatment group Clo.-0.5%.

**Conclusion:** The results suggest that the dietary administration of clove extract supplementation at a concentration of 0.5% level possess good anti-stress activity.

**Acknowledgements:** The first author is highly thankful to the Director/Vice-chancellor, Central Institute of Fisheries Education, Mumbai, India for providing facilities for carrying out the work.



## PROCESSING OF PLANT BIOMASS HARVESTED AT TRACE ELEMENT-CONTAMINATED SITES MANAGED BY GENTLE (PHYTO) REMEDIATION OPTIONS

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### ABSTRACT

Depending on the GRO (phytoextraction, in situ immobilization and aided phytostabilization) set up on a polluted site and the type of plant used, harvested plant parts may contain concentrations of trace elements (TE) that may be higher than those found in similar vegetation grown on uncontaminated soils (background). To render such technologies economically attractive and feasible, harvested biomass should be valuable and enter valuation pathways. On contaminated lands, plants may serve to provide feedstocks for bioenergy, non-food products and biofuels and, thus, may contribute to achieve the EU aim by 2020, i.e. to get 20% of its energy from renewable sources. As a potential advantage, these plants will not compete with plants grown on uncontaminated lands as contaminated lands are not suitable for food production. In GREENLAND (<http://www.greenland-project.eu/>), one task aimed at testing various conventional and innovative technologies of biomass valorisation, such as combustion, anaerobic digestion, pyrolysis and solvolysis, and determining the fate of the TE in the resulting products of each conversion process. Assays were carried out on a wide range of plant species cultivated at the field trials of the GREENLAND partners.

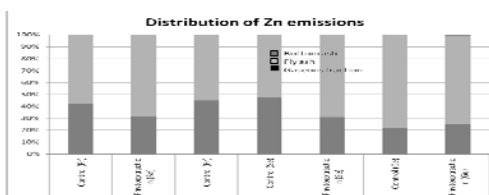
Figure 1 shows the distribution of Zn in the emissions, i.e. bottom ash, particulate fraction (fly ash) and gaseous fraction of the flue gas, as a result of combustion assays performed in a biomass boiler of 40kW on willows and poplars cultivated for phytoextraction purposes and the comparison with corresponding virgin wood (control). For all assays, Zn occurred mainly (> 50%) in the fly ash. The bottom ash represents the second compartment for the occurrence of Zn whereas the gaseous fraction of the flue gas represents a minor compartment for Zn emissions. The distribution is not depending on the initial burnt wood, i.e. virgin wood (control) or Zn enriched wood (phytoextraction). The use of TE enriched wood in biomass boilers seems possible if they are equipped with efficient filters to avoid air pollution. Results are in accordance with [1,2]. Anaerobic digestion, pyrolysis and solvolysis assays were performed in batch reactors. Results from all these conversion technologies will be presented and discussed as well as the possibility to use end products of the processes.

The valuation of plants produced by GRO seems possible if TE do not disturb the functioning and the performance of the process and if such plant use complies with current regulation.

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**Acknowledgements:** This work was financially supported by European funds through GREENLAND (FP7, KBBE-2010-4, 26124).



## SEMI-CONTINUOUS TREATMENT OF PENTACHLOROPHENOL BY BIOLOGICAL FENTON REACTION

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### ABSTRACT

**Introduction:** Environmental pollutants such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) have been detected in aquatic environment. Recent studies demonstrated EDCs can be treated by plants, plant tissues, and/or plant enzymes. We proposed a biological Fenton reaction (BFR), by which organic pollutants are oxidized with hydroxyl radicals produced from endogenous hydrogen peroxide ( $H_2O_2$ ) in the presence of ferrous ions ( $Fe^{2+}$ ). However, BFR was investigated only in batch treatments, and continuous or semi-continuous performance has not been known. Here we applied BFR to semi-continuous treatment of a recalcitrant pollutant, pentachlorophenol (PCP). In addition, in order to verify the oxidation performance, electron spin resonance (ESR) technique was employed to detect hydroxyl radicals produced by BFR.

**Methods used:** Aquatic plants used in this study were floating plants, Amazon frogbits (*Limnobium laevigatum*). Semi-continuous experiments were designed to repeat a fill and draw treatment 5 times, where solution in vessel was changed to feed solution every 3 days. Plants (20g fresh weight (FW)) were cultivated in a 2.5 L glass vessel and were maintained under intermittent illumination at 3000lx provided by white fluorescent lamps with light periods of 16 hours and dark periods of 8 hours. Concentrations of PCP and  $Fe^{2+}$  in the feed solution were set at 0.38 in first two cycles and 0.76  $\mu M$  in the rest cycles, and 0.18mM, respectively, and measurements were made for PCP and endogenous  $H_2O_2$  concentrations. Control tests using no aquatic plant with iron compounds and aquatic plants with no iron compound were conducted. ESR analyses were made using DEPMPO (5-(diethoxyphosphoryl)-5-methyl-pyrroline-N-oxide) as a spin trapping agent in the presence and absence of iron compounds; and observed results were compared with that of Fenton reagents (0.05mM ferrous sulfate heptahydrate and 0.2mM  $H_2O_2$ ). Aquatic plant samples (0.3g-FW) were ground using a mortar and pestle with 0.05mM  $FeSO_4 \cdot 7H_2O$  and 0.2mM DEPMPO. The mixture was centrifuged at 12,000 $\times g$  and 4°C for 5 minutes and the supernatant was used for the measurement of ESR.

**Results and discussion:** Figure 1 shows the result of semi-continuous experiments. Significant difference of PCP removals was observed repeatedly in the different conditions. Moreover, addition of iron compounds facilitated the removal of PCP in comparison with the other two conditions. Figure 2 compares three ESR spectrums for (1) plant with  $Fe^{2+}$ , (2) plant without  $Fe^{2+}$ , and (3) Fenton reagent. Six ESR spectrums peaks specific to DEPMPO-OH adduct, which are shown by the inverted triangle marks in the result for Fenton reagents, were detected in BFR, while no clear peaks were observed in the absence of iron compounds. Thus, hydroxyl radicals can be produced by the addition of iron compounds to aquatic plants. Measurement of endogenous  $H_2O_2$  showed that hydrogen peroxide tended to decrease in the presence of  $Fe^{2+}$ , but was kept at least around 0.1mM in plants during semi-continuous experiment.

**Conclusions:** The possibility of a biological Fenton reaction (BFR) was demonstrated through a semi-continuous experiments and ESR analysis. A further study on operating conditions and long-term experiments will be needed to evaluate precise performance.

**Acknowledgements:** This study was supported in part by Grant-in-Aid for Challenging Exploratory Research (#23656334) and for JSPS Fellows (#25767).

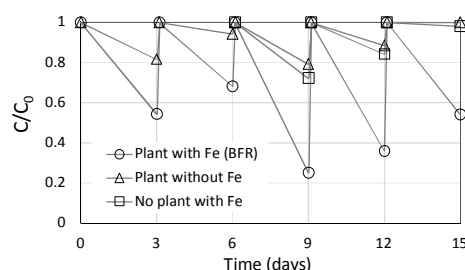


Figure 1: Time course change of PCP concentration under different conditions

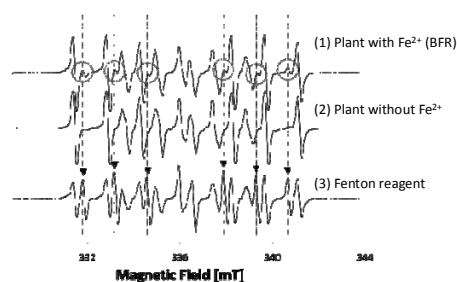


Figure 2: ESR spectrums for three conditions



## THE USE OF POLYPHENOLIC COMPOUNDS OBTAINED FROM OLIVE OIL MILL WASTE (OMWW) AS ECOLOGICAL PLANT PROTECTION AGENT AGAINST SEVERAL FUNGAL PATHOGENS ON TOMATO PLANTS. STUDIES *IN VITRO* AND *IN VIVO*

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### ABSTRACT

The assessment of different concentrations and forms of polyphenols (free and encapsulated) from olive oil mill waste (OMWW) as plant protection materials against economically important plant pathogenic fungi in *in vitro* and *in vivo* tests is the main research area of this work. In the first stage of the experimental process, it was assessed the zone of inhibition in mm of several fungal pathogens using the methods of disk diffusion assay, and well diffusion assay. In the second stage of the evaluation, the effect of polyphenols against 14 fungal microorganisms was examined in order to determine the MIC / microbicidal concentration (MIC / MFC). In a later stage *in vivo* evaluation of liquid polyphenols (LFP) obtained from OMWW as natural bio-chemicals against several fungal pathogens on tomato plants was carried. The evaluation of the results obtained by determining the MIC and MLC, demonstrated that the fungus *Aspergillus flavus* appeared highly resistant to the LFP concentration required minimum sample rate > 35-40 % for inhibition and killing effects respectively. In order of major protection resulting from the use of polyphenolic compound against major diseases, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Ascochyta lentis* gave the most promising results. Moreover, the use of low concentration of LFP at 5 and 10 % could control in some cases fungal pathogens. However, higher concentration of LFP (20 and 30%) appeared possible phytotoxic effects.





## BIOMANAGEMENT OF PESTICIDE CONTAMINATED SOILS: MICROBIOLOGICAL METHODS FOR FEASIBILITY ASSESSMENT AND MONITORING

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### ABSTRACT

There is rising public concern as a wide variety of toxic organic chemicals are being introduced deliberately into the environment. Pesticides are one common example of these chemicals, which enter the soils frequently in recent years. Chlorinated phenols (CPs) are a group of ionisable organic compounds of major environmental concern. Chlorophenols, particular those with three or more chlorine atoms, have gained an increasing use as fungicides, herbicides and insecticides [1]. Pentachlorophenol (PCP) from the category of CPs generated harmful effects in aquaculture or soil systems [2]. Thus, the cleanup PCP contaminated soils is imperative and it is critical to develop efficient approaches to remove multiple contaminants from soils.

The need to remediate these natural resources has led to the development of new technologies that emphasize the destruction of the pollutants rather than the usual approach of disposal. Bioremediation is the use of biological interventions of biodiversity for mitigation of the noxious effects caused by environmental pollutants in a given site [3]. Bioremediation with divers' aspects more recently, phytoremediation has emerged as one of the alternative technologies for removing contaminants from the soil. Furthermore, monitoring entire microbial communities in soil has traditionally been a very time-consuming task.

In this contribution we used phospholipid fatty acid (PLFA) profiles, one based on the extraction of lipids directly from soil, indicating the effects of Pentachlorophenol on soil microbial community structure. Four native plant species, white clover, ryegrass, alfalfa, and rapeseed, were studied, in order to confirm which species of plants improved the biodegradation ability for chlorinated pollutant.

After 60 days cultivation, white clover, raygrass, alfalfa and rapeseed all significantly enhanced the degradation of PCP in soils. The content of the biomarker PLFAs was significantly higher in the rhizosphere samples than the unplanted samples ( $p < 0.05$ ). Significant difference of the microbial community structure was observed in samples of alfalfa from other rhizosphere and unplanted samples, indicating the rhizosphere of alfalfa enhanced the tolerance of soil microorganisms to pentachlorophenol significantly.

Phytoremediation appeared to have great potential for the remediation of PCP contaminated soil. The presence of vegetation significantly promoted the dissipation of PCP in the soil environment. Remediation capacity varied greatly among plant species. The difference was attributed not just to the effect of higher overall biomass yields but to different rhizosphere microbial community structure.

**Keywords:** soil, pentachlorophenol, phytoremediation, phospholipid fatty acids, endoenzyme activity

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## OPTIMIZATION OF CADMIUM ( $\text{Cd}^{2+}$ ) REMOVAL FROM AQUEOUS SOLUTIONS BY NOVEL BIOSORBENT

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### ABSTRACT

In this research, Dead leaves of a common ornamental plant, *Dracaena draca* known also as dragon tree was used as a biosorbent for the removal of Cadmium ( $\text{Cd}^{2+}$ ) from aqueous solutions using a full  $2^3$  factorial experimental design. Three factors were investigated at two different levels, metal ion concentration ( $X=10$  and  $100\text{ppm}$ ), hydrogen ion concentration ( $\text{pH}=2$  and  $7$ ) and biomass dose ( $\text{BD}=0.1$  and  $0.5\text{g}$ ). Experiments were carried out in duplicates with  $50\text{ ml}$  of  $\text{Cd}^{2+}$  solutions at room temperature.

$\text{Cd}^{2+}$  concentrations in the solutions were determined using Varian Inductively Coupled Plasma (ICP-AES). When comparing observed values (experimental) with calculated values (model), they were set closely together that allowed suggesting a normal distribution where ( $R^2=0.9938$ ). Characterizations of the biosorbent were done by FT-IR and SEM. The FT-IR analysis of biomass before and after biosorption indicated the involvement of hydroxyl, carboxylic acids, alcohol and carboxylate groups in biosorption of  $\text{Cd}^{2+}$ .

Results also showed that the most significant effect for  $\text{Cd}^{2+}$  biosorption was ascribed to ( $X$ ). The interaction effects of ( $\text{pH BD}$ ) and ( $X \text{ pH}$ ) were found to have significant influence on  $\text{Cd}^{2+}$  removal efficiency. The highest  $\text{Cd}^{2+}$  removal percentage attained by  $79.60\%$  at  $X=10\text{ ppm}$ ,  $\text{pH}=7$  and  $\text{BD}=0.5\text{g}$ . The reusability of the biosorbent was tested in three desorption cycles and the regeneration efficiency was above  $99.7\%$ .

*Acknowledgements:* The financial support by Young Scientist Award, National Man and Biosphere Egyptian Committee (UNESCO) is greatly appreciated.



## CONCENTRATIONS OF PERFLUOROCTANOATE AND PERFLUOROCTANE SULFONATE IN SEDIMENT OF WESTERN CAPE RIVERS, SOUTH AFRICA

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### ABSTRACT

In this study, we report for the first time, concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) in sediments from a South African environment, particularly for the largest catchment areas of the Western Cape, South Africa. This area is associated to the largest agricultural sector, of which the produce and end-products are intended for the international markets. Therefore, worldwide these compounds have been investigated and documented, but in South Africa in general, and in the Western Cape Province in particular, this still yet to be done. Thus, the aim of this study was to determine the concentration of PFOA and PFOS in riparian sediments of the three rivers of the Western Cape; and the objectives were: a) to analysis and quantification of PFOS and PFOA contamination levels in sediments from the three rivers; b) to determine sediment's physico-chemical characteristics that may facilitate PFOA and PFOS sorption on sediments, c) to correlate PFOA/PFOS distribution in the three rivers to domestic, industrial and agricultural areas coverage of the three rivers. Sample preparation and analysis were based on the ISO 25101: 2009(E) method, using solid phase extraction (SPE) followed by liquid chromatography combined with electrospray tandem mass spectrometry (LC-MS/MS). Sediment granulometry core water characteristics and percent total organic carbon were also quantified.

From the results, PFOS and PFOA were observed in all the river sediment samples and were found in concentrations up to 19 ng/g and 187 ng/g for Salt River, 121 ng/g and 772 ng/g for Diep River, and 75 ng/g and 193 ng/g for Eerste River, for both PFOS and PFOA, respectively. Additionally, it was observed in this study that PFOA sorption onto the sediment was high at a higher pH (> 8), high %TOC in smaller grain size (< 0.1mm), and high salinity, instead of low salinity including pH (< 8) as observed for the Diep River, while the prevalence of PFOS concentration was lower under similar conditions. For the Eerste River, PFOS concentration in the sediment samples was lower at a lower pH (> 7.95), %TOC (> 16% and 20% w/w) for sediment grains < 0.1mm and > 0.5mm, respectively, with low nitrate, phosphates and ammonium concentrations. For the Salt River, although high salinity was measured as 418 mg/L with a high %TOC in grain sediment of size > 0.5 mm, and a high phosphate content, PFOS were below the detection limit in several samples with PFOA and PFOS concentrations being high in other sample as ionic organic chemicals, such as PFOA and PFOS, salinity and pH largely increase their extent of sorption onto solid matrices.

Some of the PFOA and PFOS concentrations are higher than those previously reported in similar studies in various countries; this suggests there is cause for concern. Although sediment has a poor sorption capacity for both PFOS and PFOA in comparison with plants, the prevalence of PFOS and PFOA in sediment samples from rivers from which irrigation water is sourced for agricultural purposes, indicates a risk of agricultural produce contamination.

With such an insight, it is obvious that PFOA and PFOS in South African rivers pose a significant threat to communities using river water in riparian areas.

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## TRANSPIRATION TO THE *PTERIS VITTATA* L. ARSENIC ENRICHMENT BY SEVERAL DIFFERENT TEST METHODS

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### ABSTRACT

Biological factors (leaf area, stomatal function, root quantity and distribution) and abiotic factors (climate, soil moisture, etc.) joint action in plant transpiration. Transpiration rate closely relationship with stomatal opening and closing about, when fully open stomata, transpiration rate can be increased by 20% to 40%.

Generally, the regulation of transpiration based on different methods: (1) by adjusting the relative humidity in artificial climate chamber to regulate different transpiration rates. ZHANG Yong et al found that using this method, high transpiration of tomato seedlings under Cd and Pb content than low transpiration increased by 1.47-1.73 times and 1.25-1.75 times. Sharif Ali Salah showed that with this method, *Triticum aestivum* wheat varieties under high transpiration rate of Cd, Zn accumulation increased significantly lower than the low transpiration rate. (2) use of alternate root osmotic stress, this method reduces transpiration under the premise of maintaining normal growth of plants, half of the roots into the nutrient solution, and the other half roots in nutrient solution + PEG6000 (polyethylene glycol, penetrant manufacturing water Stress) to a cycle of alternating 12h. (3) Some of exogenous hormones. As cytokinins (cytokinin, CK) can induce stomatal opening. (4) shading.

Studies have shown that plant transpiration process of absorption of heavy metals play an important role. Plants absorb pollutants to the performance of the water soluble contaminants can reach the root showed that diffusion through channels and freedom apoplast, after being absorbed by roots, part of the root store down the rest of the transpiration stream through the xylem vessels with rising together from the root transport in the blade.

This experiment by *Pteris vittata* L. as object, studies the high transpiration rate (artificial climate box regulation), low transpiration rate (artificial climate box regulation) and dry land treasure of *Pteris vittata* L. arsenic enrichment regulation function. Research results show that: although HR and LR has influence to the *Pteris vittata* L. biomass, but under the condition of different arsenic treatment is different, Under the condition of without arsenic, high transpiration rate (HR) and low transpiration rate (LR) compared to the total biomass, aboveground biomass, underground biomass increased obviously, under the condition of arsenic, HR and LR treatment compared to the total biomass increases, the difference is not big, especially the underground biomass change is not obvious. The difference of transpiration conditions lead to the *Pteris vittata* L. arsenic concentration difference is obvious, Arsenic and without arsenic, HR are better than arsenic concentration under the condition of LR have obvious growth, under As100 treatment HR is increased by 22.7% than LR conditions, under As200 treatment HR is increased by 27.1% than LR conditions. Transpiration of *Pteris vittata* L. arsenic accumulation contribution rate is extremely low; the contribution rate of different transpiration or different arsenic treatment is small differences. Dry land treasure, can obviously inhibit the *Pteris vittata* L. transpiration, especially the T2 processing, the effect is much better. Don't add dry land treasure but arsenic treatment, the *Pteris vittata* L. also received obviously inhibits transpiration, when arsenic treatment, the transpiration of the *Pteris vittata* L. is restrained. Without arsenic, biological enrichment factor has certain change, Plus arsenic, transfer coefficient and biological enrichment factor change is big, Especially under the treatment of the T2 and arsenic is very significant, transfer coefficient and the concentration coefficient is decreased by 27.8% and 36.4% respectively, it proves that combination of dry land treasure and arsenic has an important effect of *Pteris vittata* L. transshipment and concentration of arsenic.

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## ESTIMATION OF BIOGENIC EMISSIONS OF VOLATILE COMPOUNDS FROM A MINE SOIL UNDER VARIOUS BIOREMEDIATION TECHNIQUES

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### ABSTRACT

In the frame of a regional research project studying bioremediation techniques for Andalusian mining soils, focusing on revegetation with appropriate plant species, one of the objectives was the evaluation of possible emission of volatile organic compounds (VOCs) to the atmosphere, after the application of amendments like sewage sludge or irrigation with wastewater. The application of these organic amendments to degraded soils requires quantifying the balance of organic emissions to the atmosphere, because they have been reported to contain harmful compounds and precursors of other atmospheric pollutants, like aromatic and halogenated hydrocarbons.

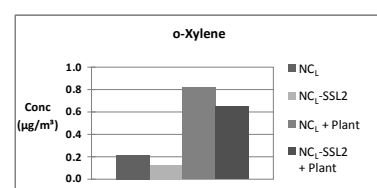
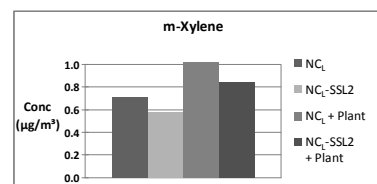
An acid mine soil from the Riotinto mine area (Iberian Pyrite Belt), which constitutes one of the world largest massive sulphide deposits (mainly Cu-Pb-Zn), was selected for this study, after treating it with a liming agent (Carbocal) to raise soil pH to physiological values. Triplicate pots (250 g) containing limed Nerva soil (NC<sub>L</sub>, control) or NC<sub>L</sub> amended with 2% stabilized sewage sludge (NC<sub>L</sub>-SSL2), from the wastewater treatment plant of Sevilla (SW Spain), were employed. Tomato plants (*Lycopersicon esculentum*, Mill.) were selected as plant species due to the ease of cultivation and short period of development. Tomato seedlings ( $\approx 7$  cm) were individually planted in the pots, which were then irrigated with wastewater to 80% field capacity. Pots were placed in an isolated small glass greenhouse. Volatile organic compounds (VOCs), including BTEX (benzene, toluene, ethylbenzene and xylenes), styrene and tetrachloroethylene, were retained on glass tubes containing Tenax, from which they were analysed by GC-MS, after thermal desorption.

Pots were sequentially sampled: First NC<sub>L</sub> or NC<sub>L</sub>-SSL2 without plant and then with plant. Results indicate that pots with plants released higher VOCs concentration than when the plant was absent. This effect was more marked for *o*- and *p*-xylene (Figures show the results for *m*- and *o*-xylene) and reflects the plant ability to release volatile compounds available in the soil solution.

Pots added with sewage sludge (NC<sub>L</sub>-SSL2), with or without tomato, released in general lower VOCs concentration (see Figures). VOCs may arise from the organic pool present in soil and from the organic amendments (SSL and wastewater). However, previous experiments with amended pots showed that only SSL, and not irrigation with wastewater, resulted in a significant increase of soil organic carbon. The observed results could be explained by an increased retention of VOCs in soil due to SSL addition, as has been shown for different organic compounds, as pesticides.

In summary, tomato plants constitute an effective interface in the transfer of volatile compounds from the soil to the atmosphere, increasing the release of volatiles regarding the only-soil emissions. In contrast with this negative effect, organic amendments added to soil, such as treated sewage sludge, reduce the release of these biogenic pollutants due to an increase of their retention on soil.

**Acknowledgements:** Junta de Andalucía and FSE funds (P10-RNM-5814 project) have supported this work.



## VICIA-MICRONUCLEUS TEST AS A TOOL TO ASSESS SOIL GENOTOXICITY POTENTIAL

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### ABSTRACT

The assessment of the environmental impact by anthropological activities is an important challenge for the 21st century. Since the industrial revolution, the contamination of water, soil and air by heavy metals or organic pollutants indeed speeded up. Although chemical analyses reveal the typology of pollution in a given matrix, they do not give information about the real ecotoxic potential of the matrix, which takes into account the bioavailability of pollutants. This information requires the development of biological tests, and especially with plants that present indeed a particular interest in ecotoxicology due to their (i) immobility, (ii) important roots network, and (iii) fundamental role in ecosystems as primary producers [2].

Ma [4] described higher plants as the most sensitive organisms for the detection of mutagens and genotoxic effects of environmental pollutants. Although ecologically relevant for soil toxicity assessment, plants are surprisingly not the most commonly used organisms for genotoxicity tests [1,6].

Genotoxicity - simply defined as the toxicity on the genome - is an indicator of dysfunctions appearing at sub-lethal concentrations. An easy endpoint to observe is the formation of micronuclei that are small nuclei appearing whenever a chromosome fragment or a complete chromosome is not incorporated into the nuclei during mitosis [5]. It therefore reveals a break of genetic material (clastogenic effect) or a dysfunction of mitotic spindles (aneugenic effect).

The aim of this study was to assess the genotoxic potential of six different soils contaminated by heavy metals. In this context, the recently standardized *Vicia*-micronucleus test (ISO-29200) [3] was used with a direct exposure approach. Results showed that four soils strongly induced genotoxicity. When dealing with phytotechnology experiments on polluted soils, one important goal is to compare soil genotoxicity potential before and after treatment. Two metal-polluted sites were further selected and sampled in more details. These sites were used to grow poplar for bioenergy production over a period of seven years. This phyto-management reduced the soil genotoxicity potential. Further results obtained from this test combined with soil data characteristics (metal contents, ...) will be presented and discussed on the poster.

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## TACKLING POLLUTION BY ORGANIC FARMING IS CAPABLE OF INCREASING FORTIFIED FOODS

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### ABSTRACT

The situation of global pollution is poisoning the biosphere and causing global temperatures to rise inevitably. Urge a drastic change in the way man is relating with Nature. One change that may produce more beneficial effects on the biosphere and human health, is the use of organic farming to produce food in a more integrated way with Nature, and to increase the capacity of man's own response compared to the toxicity of current biosphere environment. In this paper it is shown that another way to deal with environmental contamination is possible. Indeed it is shown that despite the opinion of many experts, organic farming is capable of increasing the ability of man to fortify foods. Specifically Bodegas Dagon, after more than 20 years of growing grapes under this discipline is able to achieve concentrations of stilbenes such as resveratrol, wich become a world record. Specifically Versus 14.3 mg/l reported.,wines from "Bodegas Dagón" contain (HPLC and UV-spectroscopy),  $1611.73 \pm 72.66$  mg/l of resveratrol. Thus wine from Bodegas Dagon becomes the world's healthiest reported to date.

The anthropocentric view of humanity, has considered, in all these activities, to the Nature, in many cases, as an enemy: animals which eat crops, weeds growing where they should not grow, drought or flood, that should not occur, etc. The result of this vision and way of acting, is that man is no longer integrated as an element in Nature, and has become a "stranger" to it. Nature appears that wants to dislodge the man, from his body, using earthquakes, volcanic eruptions, Tsunamis, etc. At this time, the man needs two imperative things: first, delete toxicity in his environment and, in turn, increase its ability to respond to pollution. Second, obtain products more nutritious for feeding, to make them a health ally against disease.

Among the natural compounds, which have drawn more attention to scientists in different fields (agriculture, food, health, cosmetic, etc.), are found the so called,- stilbenes. Resveratrol (the most important) is considered a phytoalexin, that is, an antimicrobial substance, that once synthesized de novo by plants, accumulates rapidly, at areas of pathogen infection. Specifically the phytoalexins can be biosynthesised in grapevines as a defense to fungal diseases, such as *Botrytis cinerea*. However, resveratrol may be synthesized in response to more than one pathogen, therefore may be synthesized in plants undergoing biotic stress, but also, undergoing many environmental stresses (abiotic stresses).

TPerhaps the reason for the presence of such concentrations of resveratrol unprecedented, has to be found, especially in the role of this molecule as an antioxidant. Nevertheless, understanding the antioxidant capacity in a broader physic- chemical sense, and not just as a molecule antiradical. Strictly speaking an antioxidant is a molecule that decreases the tension in the system when, it is forcing to boot, or share electrons. Free radicals are molecules tension-creating, as it is the oxygen. However there may be molecules that make a function in both directions, that is, reduce the tension to lose electrons, but also to win them, behaving as anti-reducers. The results are molecules that make a buffering function of the electron density surrounding.

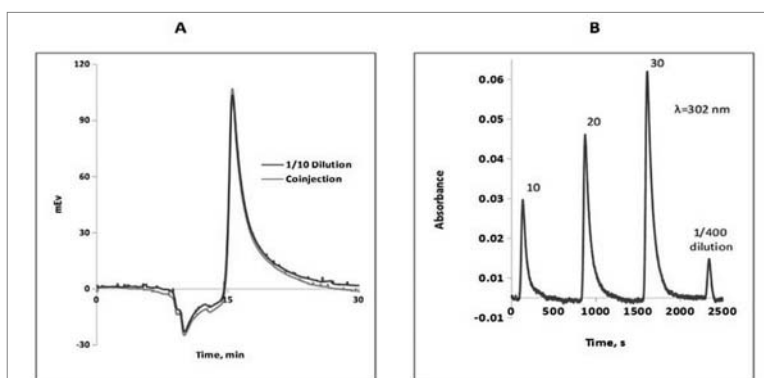


Fig 1: Identification and quantification of resveratrol in "Dagon" wine by HPLC. A. Identification by coinjection of pure resveratrol in wine samples using an IR detector. To a 1/10 dilution of pure wine, was added a sample of pure resveratrol of known amount. B. Determination of the concentration of



## SOCIAL MEASURING IN THE FRAMEWORK OF SUSTAINABLE WASTE PREVENTION ACTIVITIES

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### ABSTRACT

Through a LIFE+ Project which focuses on the Development and Demonstration of a Waste Prevention Support Tool for Local Authorities WASP Tool (*Project LIFE10 ENV/GR/000622, that is co funded from EU*), the Municipality of Paralimni in Cyprus will develop its Prevention Strategy concerning the waste management. The quantification of waste reduction is also difficult because total and per capita waste generation or composition rates are on the rise. Waste reduction should be considered in terms of reduction below future rates as well as below existing rates. In addition, certain source reduction programs, particularly education programs, may not lead to changes in individual purchasing and waste generation behaviour until a few years after initiation. It takes time for residents to develop new purchasing practices, and manufacturers time to redesign products. States and communities would benefit by expanding the type of source reduction programs offered as well as by improving their methods of quantifying such achievements. Few communities have established comprehensive source reduction programs, partly because source reduction is more difficult to measure than waste diversion through recycling and composting.

However how easy is to change citizen behaviour remains unsolved research question. The paper focuses on the social behaviour and how easy is to change those behaviour through specific prevention activities like (a) home composting (b) reuse of material (like shopping backs and refilling bottles for water), (c) food. The research indicated that continual public awareness remains one of the main aspects to chain citizen behaviour. The results indicated that at least 20% of the participants (100 participants) have change their behaviour and they have used the compost bins instead to send green waste and food waste to landfill as well as the usage of the refilling bottle for water in primary schools (as target group equal to 1250 student) was more than 75%.





## ANAEROBIC DIGESTION OF ZEA MAYS AFTER PHYTOREMEDIATION: IMPACT OF TRACE ELEMENTS ON BIOGAS YIELD AND DIGESTATE USE

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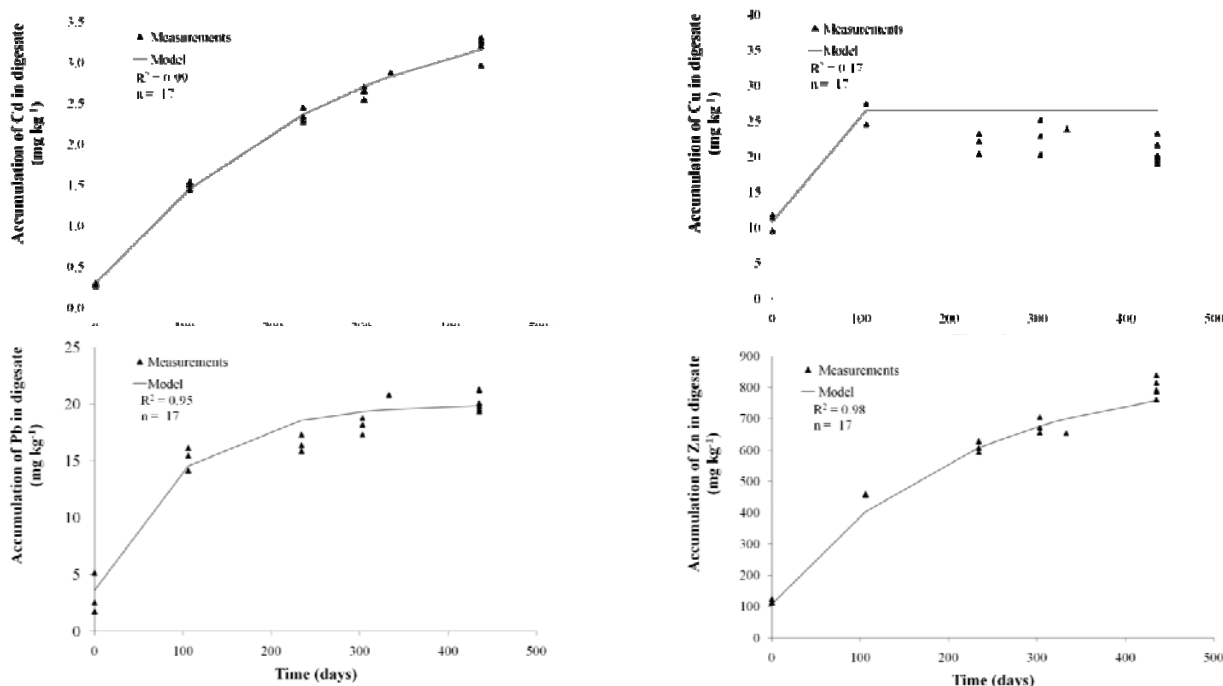
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### ABSTRACT

Energy maize is an attractive option for phytomanagement of trace element-contaminated land. Not only is its biomass productivity promising but also the agronomic knowledge on and economic feasibility of this non-food crop are favourable. However, what can and has to be done with the biomass after harvest has not been studied before. We study the potential economic (biogas production) and environmental (digestate use) impact of anaerobic digestion of contaminated maize using a multidisciplinary approach. Laboratory scale tests have shown that anaerobic digestion of maize with increased concentrations of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) reaches a biogas production potential of  $191 \pm 32 \text{ Nm}^3 \text{ ton}^{-1}$  fresh matter (fm). A semi-continuous test of 435 days delivers similar results ( $195 \pm 61 \text{ Nm}^3 \text{ ton}^{-1}$  fm). Both results are comparable with the biogas potential of anaerobic digestion of maize originating from non-contaminated land. The accumulation of trace elements in the reactor did not negatively affect biomethanisation processes. Trace element concentrations in the resulting digestate were 3-5 times higher than concentrations of trace elements in the input material. A comparison between trace element concentrations in the digestate and legal threshold values for different uses of the digestate was made and the same options as for 'regular' digestate remained.



Evolution of the measured and the modulated concentration of Cd (A), Cu (B), Pb (C) and Zn (D) mg kg<sup>-1</sup> dry matter (DM) at several times during the semi-continuous batch test

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## IMMUNOMODULATORY AND GROWTH RESPONSE OF *L. ROHITA* TO DIETARY FORTIFICATION OF CLOVE AND/OR CARDAMOM EXTRACT

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### ABSTRACT

**Background:** Though antibiotics provide a useful means to control many bacterial diseases but development of antibiotic resistance is a major problem. As an alternative, feeding many plant extracts through diet protects the fish against chronic oxidative stress-related diseases (Sakai, 1999). Many plants contain different compounds in their natural extracts, generally rich in antioxidants (El Saleh, Al Sagair and Al Khalaf, 2004). Enormous reports are available regarding the immunomodulatory role of different plant extracts in fish (Al Jishi, and Abuo Hozaifa, 2003).

**Objective:** In this context, it was hypothesized that fortification of these spice extracts in fish feed, which exhibited good antioxidant activity, must have immune-modulatory properties. Hence, the extracts of clove and cardamom were taken as isolation or in combination to study the growth and immune-modulatory effect in a tropical fish *L.rohita*, which has not been reported elsewhere.

**Methods:** Extraction of spices, Growth performance, Challenge study, Sampling, Determination of superoxide anion (NBT), Total serum protein, albumin and globulin, Lysozyme activity, Plasma superoxide dismutase (SOD), Catalase (CAT), Statistical analysis.

**Results:** Present study was conducted to evaluate the immuno-modulatory and growth response of clove and/or cardamom extracts in the diet of *L.rohita*. The solvent extracts (ethyl acetate) of clove and cardamom were selected based on the highest antioxidant properties and antimicrobial activities exhibited by these two extracts out of eleven commonly used spices screened for the same. Both the spice extracts were mixed separately or in combination at the level of 0.5 and 1.0%. Thus, six experimental diets were Cl-0.5%, Cl-1.0%, Cd-0.5%, Cd-1.0%, C.C-0.5% and C.C-1.0%. Highest growth rate ( $P<0.05$ ) was recorded in the Cl-0.5 group. Hepatosomatic index (HSI), gastro somatic index (GSI) and protein efficiency ratio (PER) was also highest ( $P<0.05$ ) in Cl-0.5 group. Lowest mortality was recorded in the Cl-0.5 group after challenged with *Aeromonas hydrophila*.

**Conclusion:** It can be concluded that clove extract at 0.5% in the diet promotes growth, enhances antioxidant activity and protects the immunity of *L.rohita* challenged against *A.hydrophila*.

**Acknowledgements:** The first author is highly thankful to the Director/Vice-chancellor, Central Institute of Fisheries Education, Mumbai, India for providing facilities for carrying out the work.



## SUSTAINABLE BEACH AND COASTAL MANAGEMENT IN INSULAR COMMUNITIES UNDER WARM CLIMATE CONDITIONS

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### ABSTRACT

Coastal areas, thanks to their unique morphological structure, which combines land, sea and air, have been and still are considered highly sensitive ecosystems as well as irresistible attractions for most people. Strong indicative of their particular significance constitutes the fact that, although the coastal zones around the world occupy a total 15% of the total surface of the planet, the 60% of the total world population was proven to reside in them. In Europe, the percentage of the urban population residing in coastal areas (up to 50km from the coast) reaches an approximate 30%, while in Greece, a 33% of the total population of the country resides at a distance shorter than 2 kilometres from the coastline. The economic significance of the coastal areas is rendered obvious thanks to their unique characteristics which enable a wide variety of activities and attract a large number of people, thus facilitating tourism. The main purpose of this article is to illustrate the need to prepare a management model which will enable the viable, sustainable management of coastal areas and beaches (sustainable beach – taking into consideration all three dimensions of sustainability: economy, society and environment) both in Greece and in the general Mediterranean area. The objective of this model will be the optimal satisfaction of all guests-bathers of a beach and the simultaneous sustainable development and environmental protection of coastal areas and beaches. The model will be easily adopted and applied both by individual coastal areas-beaches of certain municipalities or regions as well as by all beaches of each municipality or region. It will also be instantly applicable by beaches and coastal areas “belonging” to private entities and businesses such as hotel units.



## LIGHT-EMITTING DIODES USE FOR *OCIMUM BASILICUM* L. CULTIVATION

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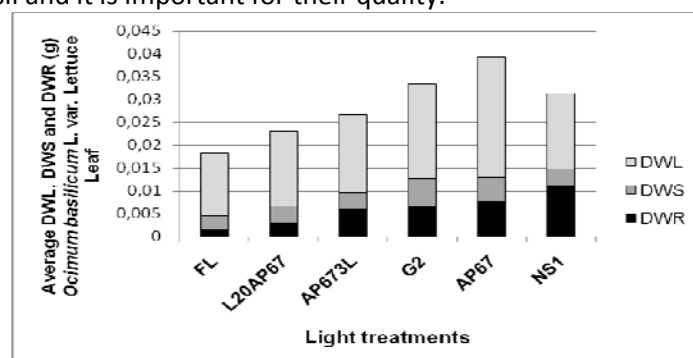
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### ABSTRACT

The use light-emitting diodes (LEDs) in production of regeneration material is cost and environmental friendly approach. The objective of this research is to analyze the effect of LEDs in physiological and morphological characteristics of two *Ocimum basilicum* L. varieties (Lettuce Leaf and Red Rubin-mountain Athos hybrid) during pre-cultivation in mini-plug containers. Fluorescent light (FL) as control and five LEDs (G2, AP673L, L20AP67, AP67, NS1 -Valoya) emitting light with differing spectrum mixing ultraviolet, blue, green, red, far-red and infra-red were used. Seedlings were cultivated into a growth chamber with set conditions for four weeks.

According to the results, Lettuce Leaf seedlings formed significantly more leaves under AP67 light than under the FL and L20AP67. However, the leaf number of the hybrid was not significant. No significant differences were found for both varieties regarding the leaf colour. Lettuce Leaf showed a light green colour while Red Rubin-mountain Athos hybrid showed purple-green colour. Furthermore, L20AP67 and AP673L lights showed an increase in Lettuce Leaf seedlings' shoot height and root length, respectively. For Red Rubin-mountain Athos hybrid means for shoot height and root length were found significantly increased under G2 and NS1 lights respectively. Seedlings illuminated under LEDs showed significant increase in dry mass of leaves, shoots and roots compared to FL for both varieties. Especially root dry mass favored under the NS1 light quality. Root/Shoot ratio was found higher under LEDs than the control. Moreover, for the Lettuce Leaf variety FL induced statistically significant larger leaves compared to all LED treatments while L20AP67 and FL lights induced the larger leaves for the hybrid.

The present results indicate that LEDs appear to have more potential on the growth and quality characteristics of *Ocimum basilicum* L. than fluorescent light. Therefore, such LEDs can be used for the cultivation of basil and it is important for their quality.



**Acknowledgements:** The financial support by FP-7 project REGENFOREST contract No. 286067 is greatly appreciated.



## BIOLOGICAL AND BIOCHEMICAL EFFICIENCY OF LYOPHILIZED CRANBERRY EXTRACT ON REGULATION OF ANTIOXIDANT DEFENSE SYSTEM IN NONALCOHOLIC STEATOHEPATITIC RATS

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### ABSTRACT

The risk of toxic liver damage has markedly increased in recent years due to the exposure to environmental toxins, pesticides and chemotherapeutics. Many compounds, including useful drugs, can cause liver cell damage through their metabolic conversion to highly reactive substances and the generation of free radicals. Nonalcoholic steatohepatitis (NASH) is an increasingly recognized condition that may progress to end-stage liver disease. It is characterized by fat infiltration of the liver, inflammation, hepatocellular damage (ballooning) and fibrosis, with NASH patients at higher risk of developing cirrhosis, terminal liver failure, and hepatocellular carcinoma. Cranberries are small, dark red fruits that are widely consumed as juice. It contains significant amount of phenolic compounds which have antioxidant properties and other health benefits. Studies have shown that supplementations with berries were effective in reducing oxidative stress associated with aging. Further, cranberries have been reported to possess anti-inflammatory and anti-mutagenic properties and provide cardio-protection. In this experiment, rats were classified into four groups as follows: normal control group, NASH rats fed high fat diet, nonalcoholic steatohepatitic rats fed high fat diet and received lyophilized powdered cranberry suspended in 0.5% CMC, group fed basal diet and received lyophilized powdered cranberry suspended in 0.5% carboxymethylcellulose CMC. The model of NASH rats elicited significant increase in serum lipid parameters: total cholesterol, total lipids, tricylglycerols and phospholipids, liver antioxidant enzyme activities with concomitant significant elevations in liver enzymes and glucose-6-phosphate dehydrogenase, in association with a reduction in reduced glutathione, glutathione peroxidase, serum total protein and direct and total bilirubin. Administration of cranberry to NASH rats produced significant increases in tested antioxidant enzyme activities, G6PD and serum total protein, direct and total bilirubin concomitant with significant decreases in the levels of serum lipids as well as liver enzymes AST, ALT, ALP, GST and  $\alpha$ -GT. In addition, it was noted that NASH rats exhibited a degree of DNA fragmentation; however, oral administration of cranberry extract partially inhibited the DNA fragmentation.

*Keywords:* nonalcoholic steatohepatitis, cranberry extract, lipid parameters, antioxidant enzymes, DNA fragmentation



## BIODIVERSITY OF BARREN SOIL AND BIOREMEDIATION POSSIBILITY

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### ABSTRACT

Coal was known as burning rock and is used primarily for producing electricity and by heavy industries. Except of benefits and economic development, mining activities have brought great damage to the ecosystem. Also, mining activities generate a large amount of spoil material and waste rocks which deposited aboveground. Area strip mining moves over a ecosystem continuously, with overbunded material from one part of coal mine deposited into the previously mined part. This will lead to the formation of barren soils, with negative environmental consequences and deterioration of local flora. Because the plants and microorganisms are important parameter of restoration of mine degraded soils, the aim of this study was to describe the biodiversity of barren soil in coal mine field "Kakanj" (Bosnia and Herzegovina), in order to estimate the bioremediation potential of selected area.

Research was performed in summer of 2011, 2012 and 2013. During the three years, plant diversity of barren soil was analyzed using the methodology of Swiss-French phytocenological school. Chemical and microbiological characterization of barren soil was performed in summer of 2013. The basic chemical characteristics were analyzed using the standard methodology, while organic pollutants using the GC/MSD and GC/FID technique, respectively. Microbiological characterization was conducted using the dilution-plating method. After isolation of bacteria, two most abundant strains were identified by API and APIWEB system, and by sequence analyses of 16S rDNA.

The results showed the absence of plants in barren soil in summer of 2011, presence of one plant in 2012 (*Amaranthus albus* L.), and 10 species belonging to 5 families (*Amaranthaceae*, *Chenopodiaceae*, *Convolvulaceae*, *Poaceae*, and *Polygonaceae*) in 2013. These plant species had significant effect on restoration of natural vegetation.

The chemical analysis showed the very acid pH reaction, absence of carbonates, high C/N relation, poor content of available P and Mg, and high content of available Fe, Ca and Ni. Content of PAHs and PCB was poor, while high content of hydrocarbons C<sub>10</sub>-C<sub>40</sub> was detected.

Microbial activity in barren soil was low. Total number of bacteria was 3.1 CFU\*10<sup>5</sup>/g, ammonification bacteria 3.3 CFU\*10<sup>5</sup>/g, while abundance of other groups of bacteria was lower. Absence of *Azotobacter* sp. and very low presence of actinomycetes was recorded. Microbial diversity in surface layer of barren soil was higher compared with subsurface layer. Enzyme activity (dehydrogenase, phosphatase, arylsulphatase) was also higher in surface layer of barren soil.

On 0.1xTS agar plates, two types of colonia were observed. One of these, named 13k, formed small single spherical whitish colonies, while other strain, named 19k, formed small to single spherical whitish to yellowish colonies. Both strains were gram-positive and spore forming. After incubation on API 50CH and using the APIWEB technique, results showed the maximal similarity of 13k strain with *Bacillus thuringiensis*, and strain 19k with *Paenibacillus alvei*. The obtained sequences of 16S rDNA of both strains were analyzed by BLAST. Compared with sequences available in database showed the maximal similarity with 16S rDNA sequences of *Bacillus* sp.

The main object of ecorestoration in coal mine areas is reconstruction of ecosystem according to the developing sequences of coal mining, using the results of plant and microbial diversity. It is evident that biodiversity could be used as complementary assessment criteria to estimate the rehabilitation status of coal mine-affected sites. In the future, researches will be performed on examination of role of vegetation and plant-microbial interacion on structure of microbial communities and biological reclamation of barren soil.

**Acknowledgements:** The financial support by EU Comission project AREA, contract No. 316004, and project of Ministry of Education and Science of the Republic of Serbia No. TR31080



## STUDY OF LIGHT EMITTING DIODE (LED) IN ACCELERATING THE GROWTH AND QUALITY OF *PICEA ABIES* SEEDLINGS FOR INDOOR CULTIVATION

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### ABSTRACT

The light sources generally used for indoor plant cultivation are fluorescent lamps. To select a more efficient light source, the present study evaluated the effects of different light qualities on the growth and seedling quality of *Picea abies* Karst. pre-cultivated in mini-plug containers fulfilled with enriched peat soil substrate. Seedlings were uniformly grown into two growth chambers for five weeks under light-emitting diodes (LEDs) (G2, AP67, AP67-ARCH, L20 AP67, NS2 -Valoya) or Fluorescent light (FL). LEDs used emitted a continuous spectrum thanks to a mixture of ultraviolet, blue, green, red, far-red and infra-red in various percentages.

Our results showed that the different light treatments had an effect on the height growth rate only for the first week of the whole experimental period therefore no significant differences were found. However the highest average height increment was found for the seedlings grown under the L20 AP67 and AP67 LEDs. Seedlings grown under the FL light formed less needles compared to LEDs and significant differences found especially with those grown under the G2 and AP67 lights during the last two weeks of the experimental period. Among LEDs the G2 light induced significant more needles compared to NS2 during the first three weeks. AP67 light enhanced the dark green colour of needles than the rest of light qualities. Furthermore, FL and L20 AP67 lights promoted a significant increase in shoot height contrast to the NS2. On the other hand AP67, G2 and AP67-ARCH LEDs significantly promoted the root length compared to the FL light. LED qualities such as G2, AP67, AP67-ARCH and NS2 significantly increased the dry weight mass and the R/S ratio of *Picea* seedlings contrary to FL light. Especially shoot dry weight mass favored under the G2 irradiation shown a significant increase compared to all the light treatments. Also among LEDs, L20 AP67 had significantly less beneficial effect on the seedlings for the same physiological parameters.

Light treatments	400-500 nm	500-600 nm	600-700 nm	700-800 nm	R:FR ratio
FL	34.8%	24.1%	36.7%	4.4%	5.74
L20 AP67	10.5%	26.2%	48.9%	14.4%	2.91
AP67- ARCH	11.9%	19.3%	60.5%	8.3%	5.56
G2	7.7%	2.4%	64.4%	25.5%	2.51
AP67	13.8%	15.1%	53%	18.1%	2.77
NS2	20.2%	38.9%	35.7%	5.2%	8.16

The present results demonstrate that LEDs and especially the AP67 and G2 better promote growth and quality characteristics of Norway spruce than fluorescent light regarding it as potential planting stock material.

**Acknowledgements:** The financial support by FP-7 project REGENFOREST contract No. 286067 is greatly appreciated.



## APPLICATION OF EXOGENOUS ORGANIC MATTER PROMOTE SOIL FERTILITY AND CARBON SEQUESTRATION

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### ABSTRACT

The effects of application of five organic amendments compost of manure and olive pomace (C), a palm leaf based compost (CP), a green manure derived from olive pruning waste (GW), an olive mill wastewater (OMW) and ovine manure (OM) on the rate of mineralization, sequestration of C in soil as well as on the microbial biomass content and level of selected enzymatic activities were studied. A single dose of 39 mg of organic carbon/ 100 g of soil was tested. These soils in addition with the control soil were incubated at 28 C° and a 12% of humidity for 56 days. Soil respiration, organic C and microbial biomass were measured.

Soil respiration increased in the amended soils on the first week of incubation the highest values of all studied parameters were found when OM and GM were applied. Increases in CO<sub>2</sub> emission resulting from OM and GW amendments were strongly associated with microbial biomass content. The highest number of fungi was observed in the OM amendment while the number of bacteria was most important when GM was applied

Organic carbon and microbial biomass (fungi and bacteria) were also increased in amended soils compared to the control.

Most of the parameters studied were found to be highly correlated, indicating a balanced enhancement of soil biological activity after the application of organic residues.

In conclusion, organic amendment can be a technique that sequesters carbon in soils that enhanced carbon storage in soils.





## THE EFFECT OF pH IN MICROALGAL CULTURES USING BICARBONATE-ENRICHED MEDIUM

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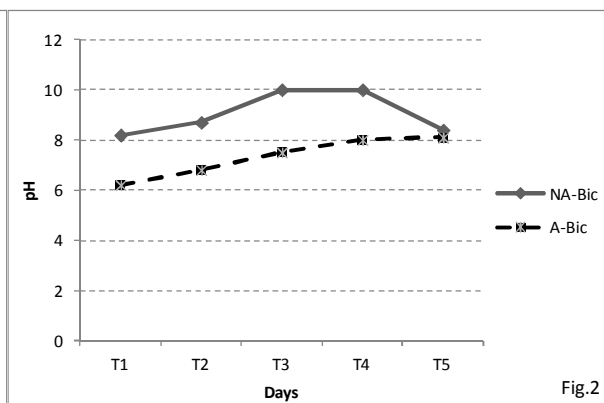
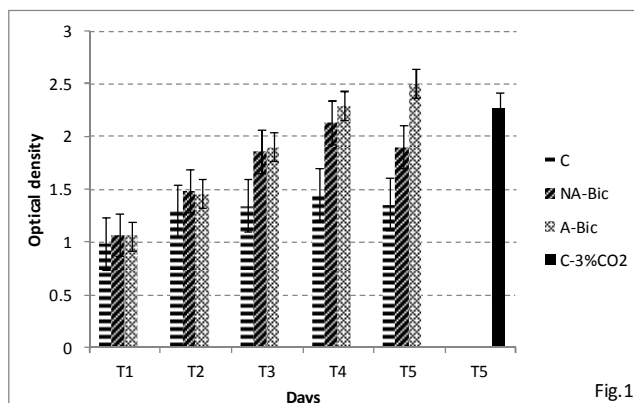
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### ABSTRACT

One of the most important environmental problem is the increased concentration of CO<sub>2</sub> in the atmosphere and its effect as greenhouse gas. Different strategies to reduce this gas were suggested. One approach is the CO<sub>2</sub> capture through bio-mimetic traps of carbonic anhydrase which product is a bicarbonate solution. The aim of this work is to verify the behaviour of one of the most common microalgae, *Chlorella sorokiniana*, at the consumption of carbon dioxide captured in this manner and to evaluate the best conditions to encourage the use of this form of carbon. Moreover the effect of partial acidification of a common culture medium suitable for this species was investigated.

The *Chlorella* was cultured in Kuhl medium at a constant temperature of 25 °C, under constant agitation (90 bpm), and continuous light. The experimental group were: Control with 3% CO<sub>2</sub> (C-3%CO<sub>2</sub>), Control without carbon (C), Not Acidified culture with bicarbonate added (NA-Bic), Acidified culture with bicarbonate added (A-Bic). Growth was followed daily through optical density for 5 days. The inoculum density was 10<sup>6</sup> cells/mL. The pH of Kuhl medium for group A-Bic was 3.8 before inoculum and 6.5 after inoculum. The pH of the other groups was 8.1. Cultures of NA-Bic and A-Bic groups received for 5 days in medium, 50 g/L of bicarbonate solution for 5 min/h for 4 h/day.

Fig. 1 shows OD mean values during the experimental period. The obtained results showed that all cultures at the end of considered period had a significant growth. When Carbon was added to the culture, the growth increased and in particular *Chlorella* in NA-Bic medium always presented lower growth than *Chlorella* in A-Bic medium, with values not different from Control with 3%CO<sub>2</sub> (C-3%CO<sub>2</sub>) (0.02>P>0.05). Even the culture in A-Bic medium showed values like C-3% CO<sub>2</sub> group.



In conclusion, at this experimental conditions the cultures of microalgae *C. sorokiniana* added of carbon in different form (3%CO<sub>2</sub> and A-Bic) reached final OD values that revealed equal carbon uptake. The pre-acidification of medium (A-Bic) has reduced the most stressful increases of pH resulting from the addition of inorganic Carbon as bicarbonate (NA-Bic) (P<0.02) (Fig. 2).

**Acknowledgements:** The financial support by PON03\_00157 *Smart Generation - Sustainable Systems and Technologies for the energy generation* is greatly appreciated.



## EFFICACY OF PRD APPLICATION ON OLIVE TREE IN ARID CLIMATE

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### ABSTRACT

The current situation in Tunisia is constrained by an increase in water demand from the agricultural sector. Water resources are limited, both in quantity and quality. Scarcity of water available for irrigation is one of the most serious problems for agriculture in arid and semi arid regions. This is the situation of the Center and South of the country, where 70% of surface water has a salinity content of more than 1.5 g/L. With regard to the state orientation policy (intensification and creation of new olive orchards under drip irrigation), Deficit irrigation system seems to be an adequate technique to improve water efficiency and to promote the irrigated-olive sector. Moreover, the regulated deficit irrigation (RDI), and especially the partial root drying (PRD) irrigation systems, maintain a good yield and improve product quality and water use efficiency.

In this context, we applied in the region of Sfax (Tunisia) a partial root-zone drying (PRD) strategy to olive trees (*Olea europaea* L., cv. Chemlali) planted at 4x4 m spacing. Three irrigation treatments i.e. PRD1 (where irrigation was applied every two weeks); PRD2 (where irrigation was applied every month); and FI (full irrigation) compared to a rainfed control. Ten trees were investigated per treatment. Yield components of the adult olive trees under the different treatments were then evaluated especially changes in endogenous sugars, Relative water content (RWC) and mineral content (Na, P, K, Ca, Cl).

Our results showed a significant difference in leaves sugars concentration varying from 29 to 106 (mg/g) respectively for FI and rainfed treatment. Moreover, high calcium concentration (3.4 %) for the PRD2 treatment was detected. Leaves phosphorus contents varied from 0.093% to 0.113% and potassium content was almost 1.03 % for all treatments.

The RWC is among the important parameters of water status of the plant and it ranged between of 79% - 81%.

*Keywords:* olive, PRD, water, soil, mineral nutrients, irrigation, soluble sugars



## GROWTH AND NUTRIENT REMOVAL KINETIC IN WASTEWATER OF *SCENEDESMUS OBLIQUUS*, *NEOCHLORIS OLEOABUNDANS* AND A NATURAL MICROALGAE BLOOM

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### ABSTRACT

Domestic wastewaters from tertiary treatment seem to be suitable for algae growth [1], and these microorganisms have potential to play an important remediation role at this stage of the process.

The aim of this study was to compare the kinetics of growth and nutrient removal from several species of microalgae ubiquitous in urban wastewater and the receiving waters. The freshwater microalgae *Scenedesmus obliquus*, *Neochloris oleoabundans* and a natural bloom of microalgae were selected for the screening. The natural bloom did not consist of isolated species from the wastewater, but a collection of them. It is perfectly conceivable that a natural bloom whose natural habitat is the wastewater should grow and consequently remove nutrients adequately on wastewater.

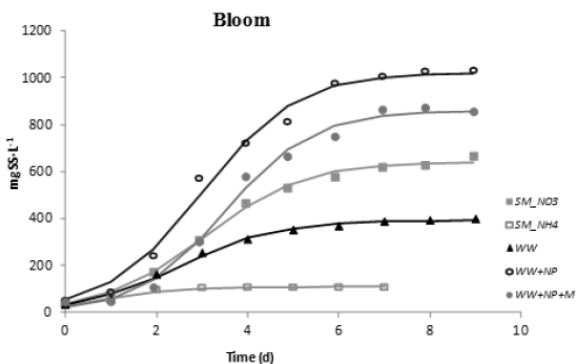


Figure 1. Biomass evolution of the algal bloom in different culture media. Symbols are experimental data and solid lines represent the predicted data according to Verhulst model.

Five culture media have been tested in this work: (1) a synthetic medium Combo two-fold (named as SM\_NO3); (2) a modified Combo two-fold medium, which composition is identical to Combo two-fold medium excepting the use of NH<sub>4</sub>Cl (SM\_NH4); (3) urban wastewater from a secondary effluent of a conventional wastewater treatment plant (Arcos de la Frontera WWTP, southern Spain, 25,000 inhabitants, 36°6.56"N, 5°47'37.12"O) filtered through a glass fiber filter of 0.45 μm pore diameter (WW); (4) wastewater cited in (3) enriched in NaNO<sub>3</sub> and K<sub>2</sub>HPO<sub>4</sub> to make the nitrogen and phosphorus final concentration similar to that of Combo two-fold medium (WW+NP); and (5) modified wastewater described in (4) with the addition of micronutrients (minerals, metals and vitamins) from synthetic medium Combo two-fold (WW+NP+M). Biomass, nitrogen and phosphorus evolution were monitored on a daily basis and the Photobiotreatment Model [2] was satisfactorily used to interpret and compare the results.

Different biomass productivities were obtained in wastewater for the species tested: 0.14, 0.06 and 0.07 g SS L<sup>-1</sup> d<sup>-1</sup> for *S. obliquus*, *N. oleoabundans* and the natural bloom respectively. Total nitrogen and total phosphorus were completely removed in all the experiments, excepting in those reactors where pH inhibition took place. Total phosphorus removal (2 – 5 days) occurred in shorter time than total nitrogen (3 – 7 days). The natural bloom and *S. obliquus* presented the highest specific nutrient uptake rates. Moreover, *S. obliquus* was the species with the highest lipid productivity for all the culture media. Wastewater has demonstrated to be a suitable culture medium for *S. obliquus* and the natural bloom, being comparable, or even better, to synthetic medium.

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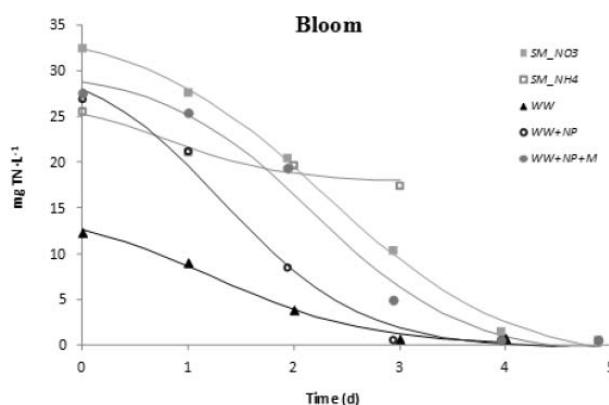


Figure 2. Removal of total nitrogen from different culture media by the natural bloom. Symbols are experimental data and solid lines represent the Photobiotreatment Model.



## ABILITY FOR PM ACCUMULATION BY BLACK ALDER TREES GROWN IN THREE SITES DIFFERING IN THE LEVEL OF EMITTED CAR EXHAUST

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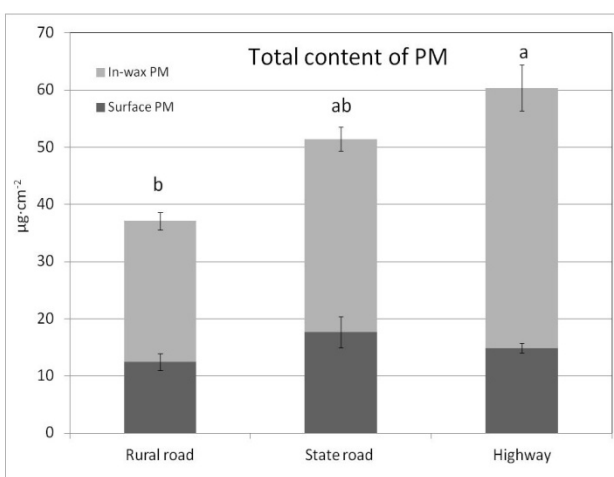
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### ABSTRACT

Increasing urbanization contributes significantly to the reduction of air quality. One of the most dangerous inhaled pollutants is particular matter (PM) especially, when on PM of diameter less than 3  $\mu\text{m}$  heavy metals (Zn, Mn, Ni, Cd, Pb, Fe, etc.) and organic pollutants as dibenzofurans, polycyclic aromatic hydrocarbons (PAHs) or polychlorinated biphenyls (PCBs) deposit. Increased level of PM in inhaled air affects negatively human well-being, health and even may cause death. According to European Environment Agency life expectancy of Europeans is, on average, reduced by 8.6 months but in Benelux, Silesia and the Po Valley even up to 12–36 months. If pollutants have been emitted to the outdoor atmosphere only possible method to clean it is *via* environmental biotechnology - phytoremediation. It involves growing plants, on the surface of which PM is accumulated therefore plants are acting as biological filters. In this work we focused on an assessment of the potential of black alder trees (*Alnus glutinosa* L.) to accumulate in/on leaves particular matter, heavy metals and Cl.

Leaf samples for assessments were collected in autumn of 2013 from trees growing three sites located near MIAST within NATura 2000 area. Trees were growing nearby roads differing in the level of car exhausts emitted to the atmosphere in results of different car traffic intensity: highly polluted (**highway S8**), medium polluted (**state road 62**) and clean site (**rural road**). Amount of PM accumulated on leaves were measured in two categories and three size fraction ( $10\text{--}100\ \mu\text{g}\cdot\text{cm}^{-2}$ ,  $2.5\text{--}10\ \mu\text{g}\cdot\text{cm}^{-2}$  and  $0.2\text{--}2.5\ \mu\text{g}\cdot\text{cm}^{-2}$ ). First the leaves were rinsed with water to wash off PM (surface PM), which under natural conditions can be washed away by rain. Next the samples were rinsed with chloroform to dissolve and wash off epicuticular waxes with immobilized particulates (in-wax PM, phytostabilized in waxes). Solutions were filtered through 3 types of filters of varying pore size. Amount of PM and waxes were determined gravimetrically and expressed in  $\mu\text{g}\cdot\text{cm}^{-2}$ . In another experiment leaf samples were ground to powder and analyzed for: Br, Cr, Zn, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, and Cl concentration using XRF spectrometer Alpha 4000 (Innov-X System, USA). The potential ability of whole trees for accumulation of pollutants was estimated using allometric equations.

Amount of PM accumulated on leaves of examined trees depended on sites where they were growing being highest in those collected from trees nearby highway and lowest collected from trees nearby rural road. In all locations wPM were in greater amount than sPM. It was estimated that trees growing in distance of  $\sim 100$  m from source of exhaust emission accumulate nearly twice much more than trees (of comparable crown size) growing near by the rural road ( $17.9\ \text{gcm}^{-2}$  vs of total  $10.4\ \text{gcm}^{-2}$ ). Trees growing near by the highway deposited more waxes than in the two other locations.



There were also significant differences in amount of heavy metals and Cl accumulated on/in trees leaves depending on pollution level due to car traffic intensity and in trees growing nearby highway their content was in most cases highest.

**Acknowledgements:** The work was financially supported by Warsaw Plant Health Initiative of REGPOT in the frame of 7FP EU # 286093.



## UPTAKE OF ANTIMICROBIAL AGENT TRICLOCARBAN (TCC) IN VEGETABLE PLANTS

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### ABSTRACT

TCC is antimicrobial chemical used in numerous personal care products such as soaps, lotions and toothpastes. The presence of this chemical in Waste water treatment plant (WWTP) and subsequently in biosolids and irrigation water used in agriculture has raised concerns in unintentional exposure of human health. This research aims to study the fate of TCC in vegetable plants and soil, using radio-labelled and regular TCC. The experiments were conducted for 60 days' time duration in soil columns using 5 vegetable plant; onion, potato, chili, cabbage and tomato. The TCC concentration found in biosolids and effluent WWTP water were spiked in the soil column. Radio labelled and regular TCC were tracked using Liquid scintillation column (LSC) and Liquid Chromatography -Mass Spectrophotometer (LC-MS) respectively. Prior to LSC and LC-MS, the samples will be combusted in a sample oxidizer (SO) and the resulting <sup>14</sup>C from CO<sub>2</sub> was quantified in LSC. At the same time sample from the Accelerated solvent extractor (ASE) were used to analyze the concentration of both TCC in LC-MS and LSC in its original form. The LC-MS and LSC result showed a similar kind of trend in TCC accumulation when analyzed separately for various plants, with highest accumulation in roots but however more accumulation of TCC was found in various species across the plant analyzed on LSC.

*Keywords:* biosolids, WWTP, TCC, <sup>14</sup>C-TCC, SO, ASE, LC-MS

*Acknowledgements:* The financial support by USDA-NIFA foundation program & MSU AgBioResearch and This project was initially started with a CWS venture Grant.



## MICROALGAE TUBULAR AIRLIFT PILOT PHOTOBIOREACTOR AND HIGH RATE ALGAL POND AS TERTIARY TREATMENT OF URBAN WASTEWATER

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### ABSTRACT

Legislation concerning wastewater treatment is gradually becoming stricter and conventional treatments cannot perform the removal needed of certain pollutants. These new regulations are leading to enlargements and installation of new processes in wastewater treatment plants. This is the case of the regulation of nitrogen and phosphorus concentration in effluents, being needed advanced processes to accomplish the required removal and to avoid the risk of eutrophication in the receiving waters. However, the biological nutrient removal processes are costly, complex and immensely energy intensive. This technology accounts for 60–80% of energy consumption during wastewater treatment [2].

The aim of this study was to simultaneously operate and compare at pilot plant scale, the performance of a 380

liters airlift tubular photobioreactor (TPBR) and 533 liters high rate alga pond (HRAP) located in a wastewater treatment plant (WWTP), with the main objective of treating the effluent as a tertiary treatment for nutrient removal while enhancing algae biomass generation.

TPBR and HRAP were operated in the WWTP of Arcos de la Frontera (Southern Spain, 25,000 inhabitants, 36°6.56"N, 5°47'37.12"O) at a extreme climatic conditions for the region, from 24th October 2011 to 29th March 2012 (157 days) with the main objective of the tertiary treatment of the effluent (nitrogen and phosphorus removal) below the most restrictive limits of the European Directive [1] while generating a valuable microalgae biomass.

Regarding the areal productivity ( $P_a$ ;  $g\ SS\ m^{-2}\ d^{-1}$ ), significant differences between TPBR and HRAP were appreciated, ranging between 2.5 and 3.5-folds higher in TPBR than in HRAP depending of the period of operation. The maximum  $P_a$  reached

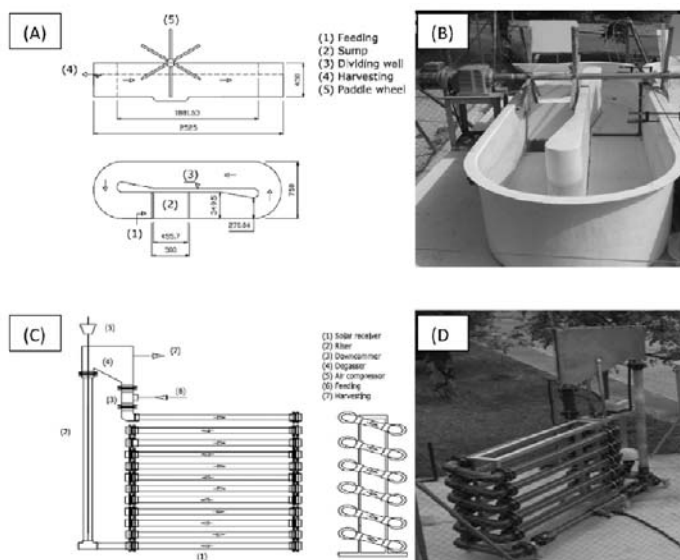


Figure 1. Pilot photobioreactors used in this work. A and B high rate algal pond (HRAP); C and D airlift tubular photobioreactor (TPBR).

were  $8.26 \pm 1.43$  and  $21.76 \pm 0.3\ g\ SS\ m^{-2}\ d^{-1}$  for HRAPS and TPBR respectively.

TN removal efficiency (TNRE) during all the continuous mode period were higher in TPBR than in HRAP, being significant the differences during the different climate conditions. The average TNRE were  $89.68 \pm 3.12$  and  $65.12 \pm 2.87\%$  for TPBR and HRAP respectively.

Soluble N and P concentration in the TPBR effluent were during all experiment below the most restrictive limits of discharge of European Directive (1998) ( $1\ mg\ L^{-1}\ P$  and  $10\ mg\ L^{-1}\ N$ ), while HRAP only reached both limits when photosynthetic activity increased. Under the same conditions, the photosynthetic activity of TPBR was between 2 and 2.5-folds higher than HRAP. HRAP was greatly influenced by the radiant flux density, confirming the greater light limitation. TPBR suffered inhibition at low temperatures. The major operating disadvantage of TPBR was severe biofouling that appeared in TPBR and affected negatively biomass generation and nutrient removal.

### References:

1. European Commission Directive, 1998. 98/15/EC of 27 February. Official Journal of the European Communities
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## A SUSTAINABLE APPROACH FOR *ACTINIDIA* PEST MANAGEMENT

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### ABSTRACT

Current strategies to contain emerging diseases in growing kiwifruits involve the massive use of pesticides. Their high doses applied increase the risk of pollution with consequences to the soil fauna and human health. In order to reduce the negative impact on the environment, the European Economic Community adopted a new legislative framework on pesticides to foresee the replacement of chemical substances with natural ones. Following this European trend, the aim of this work is to improve the quality production of *Actinidia* by growing plants able to better resist pathogen infection through the use of chitosan, a natural and biodegradable, well-known elicitor of Systemic Acquired Resistance (SAR).

Two years old plants of yellow (*A. chinensis* Planch.) and green (*A. deliciosa* (A. Chev.) Liang & Ferguson) kiwifruits were inoculated with *Pseudomonas syringae* pv. *actinidiae* (PSA), an important and emerging threat of kiwi plants in Italy, 24 hours after soil chitosan treatment and maintained in a quarantine greenhouse. The SAR's onset was determined 2 weeks after the bacterial inoculation with the detection of plant response to the infection by biochemical analyses. In particular, to evaluate the link between chitosan and SAR, the activities of enzymes involved in biotic stress response were determined, i.e. antioxidant enzymes guaiacol peroxidase (G-POD) and ascorbate peroxidase (APX), and those of phenolic compounds pathway (phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO)).

According to the results, both species treated with chitosan counteracted the pathogen attack in a similar way. In fact, after elicitor application the activities of antioxidant enzymes (G-POD and APX) and of enzymes involved in strengthening plant defence barriers (PAL and PPO) were increased in both species. Moreover, the higher phenol amounts observed after the treatment further confirmed chitosan's role in eliciting plant responses against the pathogen.

Further tests on chitosan's potential as an elicitor of SAR in kiwifruit plants are in progress. A rapid method to evaluate its effect has been adopted. Following a screening of possible SAR molecular markers, two members of Pathogenesis Related Protein families (PR1 and PR5) were chosen and two primers pairs were designed for measuring *PR* gene expression. A quantitative method such as qRT-PCR will be used to quantify the elicitation induced by chitosan at the molecular level during different developmental stages of kiwi plants.

This study has shown the reliability of chitosan treatment in eliciting plant defence response and its potential in the protection against PSA. Thus chitosan might be considered a sustainable and eco-friendly alternative to the traditional and hazardous pesticides for kiwi pest management, in order to reduce the number of chemical treatments.



## VARIATION OF SOIL pH AND SOIL LOSSES WITH NEUTRALIZING AGENT IN ACIDIC SLOPE AREAS

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### ABSTRACT

The soil pH of mountainous areas usually indicates 4 to 5 as acidic pH in Korea. The soil losses could cause to low productivity of crops and severe acidification in mountainous areas. Moreover, turbid water by soil losses from slope can be the 2<sup>nd</sup> problem as water pollution. Therefore, this research would be showed to effect for decreasing soil losses and increasing soil pH when waste lime as neutralizing agent was applied in slope areas ; generally, neutralizing agent could be added the aggregation and decreased acidification of soil. Following table shows to the experiment condition for this research.

Table. Experimental condition of this research

Plan		Basis of calculation
Flux	30 mm/hr	Average rainfall intensity of heavy rain period (pH 5.9)
Sprinkling Time	72 min	Sprinkling time considered pressure loss of pump and hose (1 time : 60 L/72 min = 0.83 L/min)
Sprinkling frequency	3 times	0.83 L/min * 3 times * 72 min (Total 180 L)
Angles	45°	Critical angle of overturning
Soil weight	0.5 ton	Lysimeter volume(0.4 m <sup>3</sup> ) × soil bulk density (1.25 ton/m <sup>3</sup> ) ※ experimental group are combined neutralizer 2% (10 kg)
Monitoring and Sampling	30 day	runoff : Performed daily during 0 ~15 days, Thereafter performed once in two days soil : 0, 10, 20, 30 day (4 times)
Measurement items		runoff : pH, EC, SS, Turbidity soil : pH, EC

In the result of pH from outflowing water, control group which was filled with acidic soil without waste lime showed sub-acid pH about 4~5. On the other hand, experimental group which was filled with acidic soil with waste lime indicated over pH 6. In case of soil loss test, control group had 6.4% of soil losses while experimental group had 4.57~4.87%.

*Acknowledgements:* This research has been maintain with project "Development of acid soil neutralization and amelioration technology" from Korea Mine Reclamation Corporation.







## **FATE & REUSE**



## UNDERSTANDING THE CONTAMINANT FATE OF LANDFILL LEACHATE BIOREMEDIATION USING SRC WILLOW

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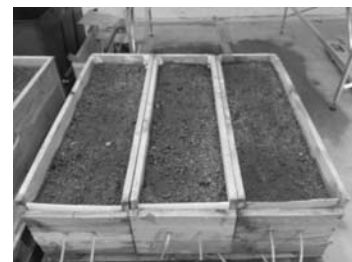
### ABSTRACT

Landfill leachate is formed by the percolation of rainwater through both municipal and industrial wastes. Usually containing a range of both dissolved and suspended contaminants; landfill leachate can have detrimental impact on the environment, especially aquatic systems. Northern Ireland has a particular problem with landfill leachate due to heavy precipitation combined with numerous landfills. Although recycling initiatives have reduced the amount of waste going to landfill, many landfill sites can produce leachate many years after closure. Leachate requires extremely expensive treatment to decontaminate enough to discharge. Alternative methods of leachate disposal such as phytoremediation are emerging as a more cost-effective and environmentally sustainable approach.

A three year study investigated the potential of using Short Rotation Coppice (SRC) willows (*Salix* spp.) to bio-remediate landfill leachate. The primary objective was to identify genotypes suitable for the bioremediation of leachate. A set of 120 commercial and non-commercial willow genotypes were screened in leachate at the equivalent to 45 N ha<sup>-1</sup> yr<sup>-1</sup> dosage rate. The growth rate of willows was measured, as was weights of above and below ground biomass. Many of commercial genotypes e.g. Nimrod and Olaf produced high yields both above and below ground with the addition of treatment; however a select number of non commercial genotypes such as 970111 produced equally healthy plants.

Once suitable genotypes were known, the next objective was to identify an appropriate dosage rate. Eight willow genotypes (Olaf, Jorrun, Germany, Endeavour, Nimrod, RR05196, 97111 and 80404) were selected to test the impact of leachate concentration using dosage rates equivalent to 0, 75, 150, 225 and 300kg N ha<sup>-1</sup> yr<sup>-1</sup>. Plants were assessed as before with the addition of plant tissue (leaves and stems) and the soil analysed (using ICP Emission Spectroscopy) for key nutrients (N, P, K, Ca, Mg, S, and C). Six of these genotypes showed high yields at 225 and 300kg N ha<sup>-1</sup> yr<sup>-1</sup> and were further treated with a higher concentration (600kg N ha<sup>-1</sup> yr<sup>-1</sup>).

With suitable genotypes and appropriate dosage rates known, a large scale runoff and leaching experiment was constructed. The objective was to address the monitoring of the amount and composition of the secondary leachate and surface runoff in the presence of willow. The data collected from this larger scale greenhouse trial will show the likelihood of environmental risks occurring when treating willow with leachate. Analysis of the runoff and leachate will indicate the fate of the leachates main contaminants. A series of boxes were constructed with the ability to collect samples, these were allocated three levels of treatment (the equivalent to 0, 300 and 600kg N ha<sup>-1</sup> yr<sup>-1</sup>) on two types of plots. Plot type one consisted of just bare earth and plot type 2 contained willows (Olaf). All 3 treatments were given to both plot types in a randomized experiment. A simulated rainfall event occurred at monthly intervals and leaching and runoff samples were collected. Parameters analysed were pH, E.C, TON, nitrite, ammonia, phosphate, sulphate and suspended solids using a photometric analyzer which is a colorimetric endpoint analysis.



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## WASTE WATER ECOMANAGEMENT THROUGH (ZERO ENERGY SEWAGE TREATMENT PLANT) Z.E.S.T.P.

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### ABSTRACT

ZESTP (Zero Energy Sewage Treatment Plant) is a good solution to treat the sewage water. Such treatment system are well known for their high nutrient absorption capacity, simplicity, low construction, operation and maintenance cost and process stability. The dissolve oxygen level increased (72.72 %) due to photosynthetic activity. According to monitoring study, concentration based physico- chemical parameters removal efficiencies for the ZESTP was as pH (9.41%), Turbidity (84.21%), Conductivity (45.70%), Salinity (42.73%), Acidity (73.93), Free CO<sub>2</sub> (69.31%), BOD (72.97), COD (43.80%), TSS (69.45%), TDS (16.80%), Total Hardness (62.42%), Chloride (70.70%), Cd (59.08%), Fe (50.75%) and Cu (76.68%). The development of Phytoremediation technologies for the cleanup of contaminated site is plant based and use of plant's natural ability to contain, degrade or remove toxic chemicals and pollutants from sewage water. Therefore plant can be introduced into and environment and allowed to absorb contaminates into leaves and roots. ZESTP is a cost effective and echo-friendly strategy, which can compliment or replace conventional approaches. On the basis of nature of pollutants and sensitivity of plant, three aquatic macrophytes (*Eichhornia crassipes* (Mart.) Solms, *Pistia stratiotes* L. and *Hydrilla verticillata* Casp.) were selected for the present investigation. Macrophytes based wastewater treatment system have several advantage compared to conventional treatment system i.e. low operating cost, low energy requirement, they can often be established at the site where the wastewater is produced.

*Acknowledgements:* The financial support by Rockefeller foundation U.S.A./G.E.A.G. Gorakhpur-India is greatly appreciated.



## EVALUATING WASTEWATER EXPORT TO LOCAL STREAMS FROM A FORESTED-MUNICIPAL WASTEWATER LAND-APPLICATION FACILITY

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### ABSTRACT

Land application of wastewater to managed forests is an important treatment and disposal practice of municipal wastewater in North Carolina (USA); however, there is limited research on the net contribution of wastewater from these facilities to surface waters as well as wastewater residence time and transport pathways. This study uses stable isotope analysis of oxygen and hydrogen, chloride concentrations, hydrological gauging, and spatial watershed analyses to examine the contribution of irrigated wastewater to local stream systems. Preliminary data from a 2,000 ha wastewater treatment site in Jacksonville, NC (USA), show higher chloride concentrations and consistently more positive isotopic signatures in wastewater lagoons than precipitation and reference surface waters which provides a source signature to track through export pathways. The isotopic signatures for surface water and groundwater draining irrigated areas are more positive and contain higher chloride concentrations than areas without irrigation. Establishing water budgets for irrigated and non-irrigated catchments at these facilities is necessary to develop contaminant budgets for potential contaminant export of personal care products and pharmaceuticals in order to ultimately determine potential health risks to humans and wildlife. This system discharges to sensitive estuary surface waters on the coast of North Carolina (USA). A separate study is evaluating the presence of these chemicals in irrigated and non-irrigated areas at this site.

*Acknowledgements:* NCSU Department of Forestry and Environmental Resources, North Carolina Department of Agriculture.







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