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DUCKWEED FORUM

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International Steering Committee on Duckweed Research and Applications

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Duckweed-Fungal Interactions

Cover page

Duckweed-Fungal Interactions

A fungal strain of Penicillium infecting *Spirodela polyrhiza* (giant duckweed) being grown on fertilizer solution. Photo taken by Shawn Sorrels, graduate student in the Lam lab at Rutgers, the State University of New Jersey, New Brunswick, USA.

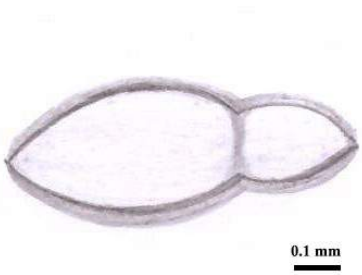
In this issue

The 4th International Steering Committee on Duckweed Research and Applications Members

- **Chair: Prof. Eric Lam**, Rutgers, The state University of NJ, New Brunswick, USA; ericL89@hotmail.com
- **PD Dr. Klaus-J. Appenroth**, Friedrich Schiller University of Jena, Germany; Klaus.Appenroth@uni-jena.de
- **Dr. K. Sowjanya Sree**, Central University of Kerala, Padanakkad, India; ksowsree9@cukerala.ac.in
- **Dr. Yubin Ma**, Ocean University of China, Qingdao, China; mayubin@ouc.edu.cn
- **Dr. Tsipi Shoham**, GreenOnyx Ltd., Tel Aviv, Israel; tsipi@greenonyx.biz

All prior Duckweed Forum issues: http://www.ruduckweed.org/

Science meets art: *Wolffia angusta* Landolt



It belongs together with *W. globosa* to the smallest duckweed and therefore are the smallest flowering plants (the meaning of "angusta" is small or confined) of all known till date. Its surface has a width of only 0.5 to 0.8 mm, which is pale green with intense green margins. It was reported from Australia and some countries in southern and eastern Asia, e.g. India (Rajasthan, West Bengal), Pakistan and Sri Lanka. Together with five other duckweed species, *W. angusta* was described for the first time in 1980 by Elias Landolt, ETH Zurich (www.e-periodica.ch/digbib/view?pid=gbi-002:1980:70::264#28). Beside other criteria, the small size and low number of stomata in this species speak in favour of belonging to the most derived, i.e. strongly reduced species, during duckweed evolution. It has the second largest genome size after *W. arrhiza* (Wang et al. 2011). Drawing by Dr. K. Sowjanya Sree, Central University of Kerala, India.

Letter from the Editor: Decade of Duckweed

Dear Readers,

As we embark on this new decade with our 28th issue of the Duckweed Forum, it is sobering to note the various major events that are on the top of the news cycle everywhere on our planet. From the Coronavirus Epidemic to Climate Change to International Conflicts, we see problems that are symptomatic of a world that is overburdened by burgeoning human population, activities and self-interest. The human drive for insatiable wealth and instant gratification must be tempered by sustainability and long-term planning in order to hedge off catastrophies in the near future. In this regard, I believe our duckweed community of researchers and practitioners could play a pivotal role in helping to translate the enormous potential of these tiny plants into a novel production system that can provide the essential sustenance for a greener world. This is a vision that I have for our future generations and I am sure many of you would likely share. At the recent Duckweed Workshop held during the *Plant and Animal Genomes* (*PAG XXVIII*) Meeting in San Diego (Jan. 11 – 15th, 2020), we decided to name this the "Decade of Duckweed" because we believe that this field has now gained the recognition by many researchers as a great model system and with all the talents and resources that exist now for duckweed, many new biology and applications will be forthcoming very soon and its impact will be felt in this decade. I am optimistic that this expectation will come true, but it will depend on all of you to contribute and to work together as much as possible.

In this issue of DF28, we continue to bring you various items of relevance to our field. These items ranged from the methods for creating your own herbarium of duckweed specimens to a Spotlight contribution by a young student interested to couple aquaculture to duckweed production. There is also a newsworthy item on updating the "real" number of species within the Lemnaceae family by Bog et al., which shrinks the duckweed family now from 37 to 36 species. Whether further fusion of other closely related species may occur in the future will have to wait for more genomic data to decide. A particularly important item is a proposal by the ISCDRA for a new registration/numbering system to manage newly isolated clones of duckweed. I hope all of you will take a look at this and provide your feedback to us as soon as possible. Especially, for labs who have collected and maintaining a significant number of duckweed strains, please send us your naming system so that we can collate them for the community in order to minimize redundancy in the future.

As always, I hope all of you enjoyed the effort that our team has done to put together this DF issue and I thank all the contributors for their efforts.

Best wishes to all,

Eric Lam

Chair, ISCDRA

What is the presently accepted constitution of family Lemnaceae?

A commentary on the number of duckweed genera and species

Manuela Bog[1], Klaus J. Appenroth[2], K. Sowjanya Sree[3*]

[1]Institute of Botany and Landscape Ecology, University of Greifswald, Greifswald, Germany, [2]Matthias Schleiden Institute, Plant Physiology, University of Jena, Jena, Germany, [3]Department of Environmental Science, Central University of Kerala, Kasaragod, India

There is a long-lasting discussion of whether duckweed should have the taxonomic level of a plant family (having the name Lemnaceae) or of a subfamily within the larger family Araceae (having the name Lemnoideae) (e.g. Nauheimer et al., 2012; Appenroth et al., 2013). Very recently Tippery and Les (2020) stressed that keeping duckweed at the taxonomic level of a family and as sibling to the families Orontiaceae (to be defined) and Araceae would be well in line with the taxonomic tradition and would solve several morphological problems created by uniting all three taxa to one family. They wrote: "We argue that family Lemnaceae deserves to continue as a unique angiosperm family, for the sake of nomenclatural stability and morphological clarity" (Tippery and Les, 2020).

Way back in 1999, the landmark work of Les and Crawford in the wake of research in molecular taxonomy of duckweeds introduced genus *Landoltia* to the plant family of Lemnaceae. This introduction had extended the status of genus to five that are as follows: *Spirodela, Landoltia, Lemna, Wolffiella* and *Wolffia*. The same group (Les et al., 2002) also showed that all of these five genera are monophyletic using plastidic sequences as markers for barcoding. Very recently, however, Tippery and Les (2020) mentioned that they still assume that all five genera are monophyletic because of the strict morphological differences. They pointed, however, also to the fact that nuclear markers do not very clearly support this theory (Tippery et al., 2015) and that concerning the genus *Wolffia* further investigations are required.

The number of species constituting these five genera is a persistent topic for discussions and the situation might remain so in the near future. Up until 1986 when "Biosystematics investigations in the family of duckweeds (Lemnaceae)" was published (Landolt, 1986), 34 species were identified based on morphological markers. In the following years, Landolt discovered four new species of duckweeds (Landolt, 1992, 1994, 1998), which shot up the number to 38. However, soon thereafter (Landolt, 2000), the species *Lemna ecuadoriensis* and *L. obscura* were synonymised leaving the number of officially accepted duckweed species to 37 (Appenroth et al., 2013; Sree et al., 2016).

After a decade, in 2010, investigating the genus *Lemna* by amplified fragment length polymorphism, we got the first hint that *L. yungensis* cannot be distinguished from *L. valdiviana* (Bog et al., 2010); we wrote: "The present analysis shows that they are genetically not sufficiently differentiated to prevent that clones from both species are mixed up in one cluster. Whether both species should be kept apart must be further tested by a larger number of clones and/or by including sequence data." Subsequent barcoding by sequence data using the plastidic markers *atpF-atpH* and *psbK-psbI* did not provide a clear separation of the two species (Borisjuk et al., 2015).

The delineation of these two species on the basis of quantitative morphometry (Bog et al., 2020a) did not yield any clear results and therefore, we decided to apply a broad array of advanced molecular methods in taxonomy to solving this issue (Bog et al., 2020a, b). We used fingerprinting by AFLP and barcoding with the markers *atpF-atpH* and *psbK-psbI* as before. In addition, we applied genotyping-by-sequencing (for the first time to Lemnaceae), and metabolomic profiling by matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MALDI-TOF-MS; used for the first time in case of higher plants). Results from all these applications taken together, we conclude that the clones of *L. valdiviana* and *L. yungensis* belong to just one species and that the name *L. yungensis* has to be considered as synonym of the older name *Lemna valdiviana*. Consequently, the number of currently accepted species of *Lemna* was reduced to 12 following the publication in the journal Taxon (Bog et al., 2020a) and the total number of duckweed species decreased to 36. The results of molecular taxonomy of Lemnaceae were also reviewed recently (Bog et al., 2019).

We are presently extending the application of the method genotyping-by-sequencing to the whole plant family of Lemnaceae.

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Useful methods: Herbarium of duckweed specimens

K. Sowjanya Sree[1] and Klaus-J. Appenroth[2]

[1]Department of Environmental Science, Central University of Kerala, Kasaragod, India, [2]Matthias Schleiden Institute, Plant Physiology, University of Jena, Jena, Germany

Herbaria of plant specimens can be used to identify plants, to certify their nomenclature, to validate scientific observations and many more other applications (Kew Herbarium). In order that these advantages are extended to the members of the plant family Lemnaceae, we prepared herbaria of duckweed species. A representative clone of each duckweed species was selected for this purpose. We have currently made 4 sets of these herbaria. One set has been presented to the Herbarium Hausknecht, University of Jena, another was recently presented to Prof. Dr. Marvin Edelman, Weizmann Institute of Science, Rehovot, Israel on the grand occasion of his 80th birthday in 2019 and the other two remain in the labs of Dr. K. Sowjanya Sree at Central University of Kerala, India and that of Dr. Klaus-J. Appenroth at Friedrich Schiller University of Jena, Germany. It is very pertinent to mention here that the Landolt's duckweed collection, Zurich, Switzerland hosts herbaria vouchers of many thousands of clones of duckweeds belonging to all species that were originally curated by Prof. Landolt himself during his career.

In the course of the preparation of our duckweed herbaria, we had to standardize several parameters and had established a standard protocol for the same. In this article we will share the protocol in detail which could be of help to anyone who would like to prepare similar herbaria for duckweed specimens.

Method:

The representative clones selected for preparing herbarium samples of each of the duckweed species were taken from the stock cultures at the University of Jena, Germany (Sree and Appenroth, 2020) and were cultivated under optimal conditions for four weeks by sub-culturing them each week. The plants from such well-grown cultures were used for herbarium sample preparation.

Using an inoculation loop, the plants were collected from the culture flask and placed on a thin, porous nylon sheet which was in turn placed on a blotting paper sheet. This allowed the removal of excess water or medium that may be stuck to the plant.

Using a thin paint brush, the plants were placed on the herbarium sheet. Note: Some plants were placed with their dorsal side up and some were placed with their ventral side up and so on. This allows all the features of the specimen to be viewed properly.

On top of the specimen, a parchment sheet (of same size as the herbarium sheet) was placed. Above this, 5-6 layers of blotting paper sheets (of same size as the herbarium sheet) were placed.

These layers were placed in a plant press (this comprises of two thin wooden planks secured by straps). The layers of the different papers as described above were placed in between the two planks and the straps are tightened equally on both ends to apply equal pressure on all sides.

About 10 to 15 specimens layered individually can be placed one above the other in the same press at a time. This set up was set aside in a cool dry place for 2 to 3 days.

After drying, the blotting sheets were removed. Most of the plants were stuck on the herbarium sheet in the process of drying. However, there were some which got stuck to the parchment paper that was placed above the plants. In this case, with a tooth pick dipped in a liquid glue, the plants from the parchment paper was picked up and transferred onto the herbarium sheet.

Each herbarium sheet was labelled with the scientific name of the species and the international four-digit ID number of the clone, following the tradition of Elias Landolt, as well as information concerning the origin of the clones.

This way, 37 specimens were conserved according to the available knowledge at the time (Sree et al., 2016). Meanwhile, the two species *Lemna valdiviana* and *Lemna yungensis* were combined more recently based on new data, retaining *L. valdiviana* as the valid name for all the clones designated under *L. valdiviana* and *L. yungensis*. Now, *L. yungensis* is a synonym of *L. valdiviana*. Therefore, the number of recognized duckweed species is reduced to 36 (Bog et al, 2020).

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Kew Herbarium. https://apps.kew.org/herbcat/gotoWhatCollectionFor.do

Discussion Forum: Registration of duckweed clones/strains-Suggestions by the ISCDRA

Eric Lam, Klaus J. Appenroth, Yubin Ma, Tsipi Shoham, K. Sowjanya Sree

4th ISCDRA

In the previous Issue of the *Duckweed Forum* (7 (4): 163-164 (2019)) we informed the community about the current status concerning the registration of newly isolated duckweed clones. Use of a 4-digit code, originally introduced by the late Elias Landolt for collection and curation of Lemnaceae clones, had been carried forward to be used at the international level. Currently, registration of duckweed clones is done at the Rutgers Duckweed Stock Cooperative (RDSC) as decided during the 1st International Conference on Duckweed Research and Applications in Chengdu, China (Zhao et al., 2012). In the following years, this had a significant impact on standardisation of the duckweed clone registration system, especially because only those clones were registered for which the species identity was determined in a reliable manner using a common molecular barcoding method. We hope that the number of misidentified clones has decreased significantly in the scientific literature by adopting this procedure. However, maintaining all the registered clones as live specimens in any one of the duckweed stock collections either at the RDSC (headed by Prof. Eric Lam) or at the Friedrich Schiller University in Jena, Germany (headed by Dr. Klaus-J. Appenroth), was overambitious as we underestimated the effort involved in keeping huge number of duckweed clones under axenic conditions over a long time.

Fortunately, in the last few years, a number of stock collections with several hundred duckweed clones were founded (Sree and Appenroth, 2020) and also the number of labs working with a small number of clones increased dramatically. This reflects the progress of the duckweed community. As a consequence, the workload for people responsible for registration and curation of the ever-increasing number of clones at the RDSC rose beyond the manageable limit.

The ISCDRA discussed the situation and decided to recommend the following path forward in terms of acceptable community standards:

1. Registration of new duckweed clones at the RDSC will no longer be mandatory before publication of work based on these material.
2. Private IDs for newly isolated duckweed strains should be created by independent research groups following a standardised ID system that would be recognizable by the international community. Our suggestion: a code structure beginning with two or three alphabets (representing the research group) followed by four digits. For instance, a clone in the collection of Dr. Klaus J. Appenroth at FSU, Jena, Germany would have the following code: KJA0001. Prior to their first usage, we recommend the research group to alert the RDSC of their intented nomenclature in order to avoid the use of the same abbreviations by two or

more groups. The RDSC will strive to maintain an up-to-date database of clone number systems being used by our community members on its website.

3. Registration of duckweed clones using the private ID system should be done exclusively on the basis of reliable identification of the species either based on morphology and whenever possible based on barcoding using proven plastidic barcodes (Borisjuk et al., 2015; Bog et al., 2020).
4. We request the researchers to deposit the barcode sequences used for species identification of the clones in any of a number of different publicly accessible databases such as the NCBI.
5. In most, if not all, cases, the accession numbers of the barcode sequences used for species identification should be cited in the relevant publication as a requirement before its acceptance.
6. In all publications, the method of identification of the species should be clearly stated and location of where it has been collected from identified with as much details as possible. Statements like "*Lemna minor* was collected from a pond in the neighbourhood of our Institute" should belong in the past.
7. The RDSC will continue to maintain new clones of special use and interest for the community that have clear and distinct phenotypes. In these cases, a Landolt 4-digit number will be assigned to the curated clone once they have been shared with the community by their deposition into the RDSC.
8. This suggested ID management system is now open for discussion and any input or suggestions should be sent to the members of ISCDRA by March, 2020.

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Student Spotlight: Tejaswini Dash

Department of Environmental Science, Central University of Kerala, Periye, India

(Email: tejaswini.dash835@gmail.com)

During my Masters course in Environmental Science, I always had an affinity towards plant research. However, this desire could be materialized only when I joined for Ph.D. in the lab of Dr. K. Sowjanya Sree at the Central University of Kerala in India. I had no idea about duckweed research when I joined but I was fortunate enough to almost immediately attend the 4th International conference on Duckweed Research and Applications as it was organized here in Kerala in 2017. I was fascinated to listen to all the exciting lectures and to learn the versatile character of duckweeds. To realize the vast potential of duckweeds was captivating. Attending this conference was not only a knowledge bank but it also provided a platform to personally meet and interact with many of the duckweed researchers from around the globe, to name a few: Late Prof. Dr. Satish C Maheshwari (University of Rajasthan, India), Dr. Klaus-J Appenroth (Friedrich Schiller University of Jena, Germany) and Prof. Dr. Eric Lam (Rutgers, The state University of New Jersey, USA). This conference gave me ample opportunities to kick start my research work.

Tejaswini Dash having a closer view at a pond with a well grown culture of *Spirodela polyrhiza* (left) and with duckweed cultures in the lab (right)

In the beginning, I could not believe that a tiny flowering plant could multiply so fast as the duckweeds do. Soon my focus was to put this property of duckweeds to a sustainable use. Kerala having a long coast line has several aquaculture ventures along its shore. Consumption of fish, other seafood and aquaculture products is a well-established food culture of the people living here. After surveying quite some of the shrimp culture facilities in the northern part of Kerala, I started to investigate the capacity of duckweeds to treat shrimp culture wastewater. Locally available species and clones of duckweed are being tested for this purpose. The bacteria associated with these duckweed clones are also being studied especially with respect to their wastewater remediation potential. I intend to develop a sustainable wastewater treatment system for aquaculture (to be more specific, shrimp culture) discharge where duckweed and their associated microbes will be used to clean the wastewater and the biomass thus produced could be utilized as a feed supplement in the same shrimp farm.

The increasing flow of knowledge in different areas of duckweed research is certainly a boon to the duckweed community. I feel fortunate to be associated with Dr. K. Sowjanya Sree who is my research supervisor. The brainstorming research discussions that we have from time to time and her passion to duckweed research is a motivation for young researchers like me. Interactions with Dr. Klaus-J Appenroth (who is currently visiting our lab) during the lab seminars is a great opportunity to develop and steer my research focus. I wish duckweed to take a lead role in sustainable development in the near future and I hope to give my best to contribute my bit to this success.

From the Database

Biochemistry

GC/MS-screening analyses of valuable products in the aqueous phase from microwave-assisted hydrothermal processing of *Lemna minor*

Kolb, M; Wichmann, H; Schroder, U (2019) SUSTAINABLE CHEMISTRY AND PHARMACY 13: Article Number: UNSP 100165

Microwave-assisted hydrothermal treatment of *Lemna minor* plant material was studied under subcritical conditions. The water phases of this process were extracted with polar and nonpolar solvents and the extracts were investigated by GC/MS screening analyses for value added products. The polar extracts were dominated by compounds known as flavouring agents and partly as platform chemicals, e.g. hydroxyacetone, 1-hydroxybutan-2-one, furfural, cyclotene and pyrrolidone. Pyridinol, an important educt for heterocyclic chemistry, was another interesting product. Benzaldehyde and oleamide were detected as main products in nonpolar n-hexane extracts. An essential aspect is that a large proportion of compounds like aldehyds and ketones were obtained when mild hydrothermal processing was applied.

Biotechnology

Duckweed-based clean energy production dynamics (ethanol and biogas) and phyto-remediation potential in Bangladesh

Nahar, K; Sunny, SA (2019) MODELING EARTH SYSTEMS AND ENVIRONMENT DOI: 10.1007/s40808-019-00659-y

This paper presents an overview of the prospect of biofuel resources in Bangladesh with second-generation (2G) aquatic energy crop-Duckweed. The review sheds light on growing duckweed as a promising feedstock to produce ethanol and biogas, as they can minimize dependence on limited crude oil and natural gas-fossil fuels that Bangladesh largely depends on. Current reserves are inadequate to meet the energy demand for long term economic growth. Biofuels, as nontoxic and biodegradable alternatives, could replace harmful fossil fuels as combustion of biofuel emits considerably less amount of CO_x, SO_x, hydrocarbons and particulate matter. Bangladesh has millions of hectors of potential sites across lakes, rivers and ponds, which can conduct high rates of nutrient uptake via phytoremediation, such as Nitrogen and Phosphorus, in industrial wastewater, and then be used to produce biofuels. The review explores different production processes and briefly charts future research agenda for the proliferation of clean energy in Bangladesh using locally available products.

Ecology

Sink trap: duckweed and dye attractant reduce mosquito populations

Cuthbert, RN; Coughlan, NE; Dick, JTA; Callaghan, A (2019) MEDICAL AND VETERINARY ENTOMOLOGY DOI: 10.1111/mve.12417

Duckweeds, such as *Lemna minor* Linnaeus (Alismatales: Lemnaceae), are common in aquatic habitats and have been suggested to reduce larval mosquito survivorship via mechanical and chemical effects. Furthermore, pond dyes are used increasingly in aquatic habitats to enhance their aesthetics, although they have been shown to attract mosquito oviposition. The present study examined the coupled effects of *L. minor* and black pond dye on the oviposition selectivity of *Culex pipiens* Linnaeus (*Diptera: Culicidae*) mosquitoes in a series of laboratory choice tests. Subsequently, using outdoor mesocosms, the combined influence of duckweed and pond dye on mosquito abundances in aquatic habitats was quantified. Mosquitoes were strongly attracted to duckweed, and oviposited significantly greater numbers of egg rafts in duckweed-treated water compared with untreated controls, even when the duckweed was ground. The presence of pond dye interacted with the duckweed and further enhanced positive selectivity towards duckweed-treated water. The presence of duckweed caused significant and sustained reductions in larval mosquito numbers, whereas the relative effects of dye were not evident. The use of floating aquatic plants such as duckweed, combined with dye, may help reduce mosquito populations via the establishment of population sinks, characterized by high rates of oviposition coupled with high levels of larval mortality.

Floating duckweed mitigated ammonia volatilization and increased grain yield and nitrogen use efficiency of rice in biochar amended paddy soils

Sun, HJ; A, D; Feng, YF; Vithanage, M; Mandal, S; Shaheen, SM; Rinklebe, J; Shi, WM; Wang, HL (2019) CHEMOSPHERE 237: Article Number: UNSP 124532

Biochar (BC) potentially accelerates ammonia (NH_3) volatilization from rice paddy soils. In this regard, however, application the floating duckweed (FDW) to biochar-amended soil to control the NH_3 volatilization is not studied up-to-date. Therefore, the impacts of BC application with and without FDW on the NH_3 and nitrous oxide (N_2O) emissions, NUE and rice grain yield were evaluated in a soil columns experiment. We repacked soil columns with Hydragric Anthrosol and Haplic Acrisol treated in triplicates with Urea, Urea + BC and Urea + BC + FDW. Total NH_3 losses from Hydragric Anthrosol and Haplic Acrisol were 15.2-33.2 kg N ha^{-1} and 19.6-39.7 kg N ha^{-1}, respectively. Urea + BC treatment recorded 25.6-43.7% higher ($p < 0.05$) NH_3 losses than Urea treatment, attributing to higher pH value of floodwater. Floating duckweed decreased soil pH and therefore significantly reduced ($p < 0.05$) the NH_3 volatilizations from the two soils by 50.6-54.2% over Urea + BC and by 34.2-38.0% over Urea treatment. Total N_2O emissions from Hydragric Anthrosol and Haplic Acrisol were 1.19-3.42 kg N ha^{-1} and 0.67 -2.08 kg N ha^{-1}, respectively. Urea + BC treatment increased N_2O emissions by 58.8-68.7% and Urea + BC + FDW treatment further increased N_2O emission by 187.4-210.4% over Urea treatment. Higher ammonium content of the topsoil, explained the N_2O increases in the Urea + BC and Urea + BC + FDW treatments. Urea + BC slightly reduced the rice grain yield and NUE, while the Urea + BC + FDW promoted both rice yield and NUE. Our data indicate that co-application of FDW along with BC in paddy soil could mitigate the NH_3 volatilization and enhance the rice grain yield and NUE.

Feed & Food

Production potential of greater duckweed *Spirodela polyrhiza* (L. Schleiden) and its biochemical composition evaluation

Sharma, J; Clark, WD; Shrivastav, AK; Goswami, RK; Tocher, DR; Chakrabarti, R (2019) AQUACULTURE 513: Article Number: UNSP 734419

The culture technique of greater duckweed *Spirodela polyrhiza* (L. Schleiden) was standardized in outdoor tanks using three different manures: manure 1 - cattle manure, poultry droppings and mustard oil cake, manure 2 - urea, potash and triple superphosphate and manure 3 - cattle manure, urea, potash and triple superphosphate. Significantly ($p < 0.05$) higher production was recorded in manure 1 compared to others. Manure 1 was subsequently selected for pond culture. In ponds, the production of duckweed was 2020 ± 150 kg ha^{-1} $month^{-1}$ dry weight basis. Protein content was significantly higher ($p < 0.05$) in duckweed cultured in manure 1. The amino acid profile study showed the presence of essential (37.4%), non-essential (58.2%) and free (4.5%) amino acids. Leucine, isoleucine and valine contributed 51.4% of total essential amino acids. Duckweed contained 7% lipid and α-linolenic acid (36-37%) was the major fatty acid. The study showed the nutritional value of duckweed as an animal feed ingredient.

Interaction with other organisms

Differential effects of synthetic media on long-term growth, starch accumulation and transcription of ADP-glucosepyrophosphorylase subunit genes in *Landoltia punctata*

Kittiwongwattana, C (2019) SCIENTIFIC REPORTS 9: Article Number: 15310

Murashige & Skoog (MS) and Hoagland's media were previously used for in vitro culture of *Landoltia punctata*. During subsequent ex vitro culture, the use of MS medium resulted in a higher growth rate, compared to Hoagland's medium. Thus, a higher starch content of *L. punctata* in MS medium was previously hypothesized. Here, *L. punctata* strain 5632 was isolated and characterized using morphological characteristics and the *atpF-atpH* intergenic region. During early cultivation stage, fresh weight and relative growth rate in MS medium were lower than Hoagland's medium. Conversely, starch content in MS medium was considerably higher than in Hoagland's medium. Medium effects on expression of genes coding for starch-biosynthesis ADP-glucosepyrophosphorylase (AGPase) were determined. Genomic fragments of small (LeAPS) and large (LeAPL1) AGPase subunits were characterized. Differential expression between each AGPase subunit genes was observed in both media. Additionally, in MS medium, the highest correlation coefficients between starch content and gene expression was found with LeAPS (0.81) and followed by LeAPL3 (0.67), LeAPL2 (0.65) and LeAPL1 (0.28). In Hoagland's medium, the coefficients of LeAPL3 (0.83) and LeAPL2 (0.62) were higher than LeAPS (0.18) and LeAPL1 (-0.62). This suggested different levels of contributions of these genes in starch biosynthesis in both media.

Physiology & Stress

Physiological and Transcriptomic Analysis Reveals Distorted Ion Homeostasis and Responses in the Freshwater Plant *Spirodela polyrhiza* L. under Salt Stress

Fu, LL; Ding, ZH; Sun, XP; Zhang, JM (2019) GENES 10: Article Number: 743

Duckweeds are a family of freshwater angiosperms with morphology reduced to fronds and propagation by vegetative budding. Unlike other angiosperm plants such as *Arabidopsis* and rice that have physical barriers between their photosynthetic organs and soils, the photosynthetic organs of duckweeds face directly to their nutrient suppliers (waters), therefore, their responses to salinity may be distinct. In this research, we found that the duckweed *Spirodela polyrhiza* L. accumulated high content of sodium and reduced potassium and calcium contents in large amounts under salt stress. Fresh weight, Rubisco and AGPase activities, and starch content were significantly decreased in the first day but recovered gradually in the following days and accumulated more starch than control from Day 3 to Day 5 when treated with 100 mM and 150 mM NaCl. A total of 2156 differentially expressed genes were identified. Overall, the genes related to ethylene metabolism, major CHO degradation, lipid degradation, N-metabolism, secondary metabolism of flavonoids, and abiotic stress were significantly increased, while those involved in cell cycle and organization, cell wall, mitochondrial electron transport of ATP synthesis, light reaction of photosynthesis, auxin metabolism, and tetrapyrrole synthesis were greatly inhibited. Moreover, salt stress also significantly influenced the expression of transcription factors that are mainly involved in abiotic stress and cell differentiation. However, most of the osmosensing calcium antiporters (OSCA) and the potassium inward channels were downregulated, Na^+/H^+ antiporters (SOS1 and NHX) and a Na^+/Ca^{2+} exchanger were slightly upregulated, but most of them did not respond significantly to salt stress. These results indicated that the ion homeostasis was strongly disturbed. Finally, the shared and distinct regulatory networks of salt stress responses between duckweeds and other plants were intensively discussed. Taken together, these findings provide novel insights into the underlying mechanisms of salt stress response in duckweeds, and can be served as a useful foundation for salt tolerance improvement of duckweeds for the application in salinity conditions.

Phytoremediation

Performance of *Lemna gibba* bioreactor for nitrogen and phosphorus retention, and biomass production in Mediterranean climate

Ennabili, A; Ezzahri, J; Radoux, M (2019) Journal of Environmental Management 252: Article Number: UNSP 109627

Lemna gibba (Lemnaceae) had been experimented in Morocco to develop macrophyte-based wastewater treatment systems adapted to the local climatic and socio-economic circumstances. This species growing on pre-treated urban wastewater, in a lagoon (*Lemna* bioreactor) operating in fed-batch, generates a net productivity of 28.39 t dw.ha^{-1} yr^{-1}, through regular harvest of the biomass produced. In wet seasons the roots of this macrophyte generally exceed 10 cm. The *Lemna* lagoon clearly reduces plankton production, especially during the vegetative period, when compared to the bioreactor without macrophytes (lagoon; chlorophyll-a concentration of 86.4 ± 168 µg l^{-1}). The *Lemna* bioreactor also removes more particulate nitrogen (N) and phosphorus (P), and shows a highly

significant total P and significant non-particulate P retention, in comparison with the lagoon. *L. gibba* can export daily the equivalent of 13.2% of N and 19.9% of P entering the bioreactor. The algal flora is dominated throughout the year by phytoplanktonic populations of Euglenophyceae and Chlorophyceae. Branchiopoda (Daphniidae), Insecta (Dytiscidae Chironomidae, Culicidae and Heteroptera), and Gastropoda are the main taxa of animalia developing in the *Lemna* bioreactor. In the Mediterranean climate, the *L. gibba* bioreactors would be more profitable in the tertiary wastewater treatment, especially P removal, provided regularly collect of the biomass produced.

Phytoremediation processes of domestic and textile effluents: evaluation of the efficacy and toxicological effects in *Lemna minor* and *Daphnia magna*

de Alkimin, GD; Paisio, C; Agostini, E; Nunes, B (2019) Environmental Science and Pollution Research International DOI:10.1007/s11356-019-07098-3

Phytoremediation has been proposed as a potential biotechnological strategy to remediate effluents before their release into the environment. The use of common aquatic plant species, such as macrophytes (e.g., *Lemna* spp.) as a clean-up solution has been proposed decades ago. However, the effectiveness of such processes must be assessed by analyzing the toxicity of resulting effluents, for the monitoring of wastewater quality. To attain this purpose, this work intended to quantify the efficacy of a *Lemna*-based wastewater phytoremediation process, by analyzing toxicological effects of domestic and textile effluents. The toxic effects were measured in *Lemna minor* (same organisms used in the phytoremediation process, by quantifying toxicological endpoints such as root length, pigment content, and catalase activity) and by quantifying individual parameters of *Daphnia magna* (immobilization, reproduction, and behavior analysis). Phytoremediation process resulted in a decrease of chemical oxygen demand in both effluents and in an increase in root length of exposed plants. Moreover, textile effluent decreased pigments content and increased catalase activity, while domestic effluent increased the anthocyanin content of exposed plants. D. magna acute tests allowed calculating a EC_{50} and Toxic Units interval of 53.82-66.89%/1.85-1.49, respectively, to raw textile effluent; however, it was not possible to calculate these parameters for raw and treated domestic effluent (RDE and TDE). Therefore, in general, the acute toxicity of effluent toward *D. magna* was null for RDE, and mild for the treated textile effluent (TTE), probably due to the effect of phytoremediation. Exposure to textile effluents (raw and treated) increased the total number of neonates of *D. magna* and, in general, both textile effluents decreased *D. magna* distance swim. Moreover, although both effluents were capable of causing morphological and physiological/biochemical alterations in *L. minor* plants, organisms of this species were able to survive in the presence of both effluents and to remediate them.

Nutrient removal efficiency by floating macrophytes; *Lemna minor* and *Azolla pinnata* in a constructed wetland

Muvea, FM; Ogendi, GM; Omondi, SO (2019) GLOBAL JOURNAL OF ENVIRONMENTAL SCIENCE AND MANAGEMENT-GJESM 5: 415-430

The use of constructed wetlands for purifying pre-treated wastewater is a cost effective technology that has been found to be more appropriate for many developing countries. The technology is also environmentally friendly with the wetlands being habitats for many water birds and other aquatic organisms. This study assessed nutrient removal efficiency of two floating macrophytes (*Lemna minor* and *Azolla pinnata*). The data generated was analyzed using both descriptive and inferential statistics. The significance level was maintained at 0.05. The results showed that the wastewater physicochemical parameters did not vary during the study period. The concentrations of nitrites and

nitrates increased over the experimental period in all the treatments (*Azolla pinnata, Lemna minor* and control), and the increase between the sampling occasions was statistically significant for the two nutrients (Nitrates: F=24.78, P= 0.00; Nitrates: F=198.26, P= 0.00). To the contrary, in all the treatments the concentrations of ammonia, total phosphorous, soluble reactive phosphorous and total nitrogen, decreased over the experimental period. The decrease in concentration for these nutrients between the sampling occasions was statistically significant (ammonia: F=195.57, p=0.00; total phosphorous: F= 56.50, p=0.00; soluble reactive phosphorous: F= 37.11, p= 0.00; total phosphorous: F= 104.025, p= 0.00). *Azolla pinnata* proved to be better than *Lemna minor* in the uptake of the nutrients particularly for the soluble reactive phosphorous (F= 35.18, P= 0.044). We conclude that the two macrophytes are good for wastewater treatment. It is recommended introduction and/or multiplication of *Azolla pinnata* in the constructed wetlands meant for wastewater treatment especially within the tropics.

Growth and nutrient removal efficiency of duckweed (*Lemna minor*) from synthetic and dumpsite leachate under artificial and natural conditions

Iqbal, J; Javed, A; Baig, MA (2019) PLOS ONE 14: Article Number: e0221755

Sustainable management of leachate produced from the dumpsite is one of the major concerns in developing countries. Aquatic plants such as duckweed have the potential to remove pollutants from wastewater which can also be cost-effective and feasible options for leachate treatment. Therefore, the objective of our present study was to examine the growth and nutrient removal efficiency of duckweed (*Lemna minor*) on leachate. Three tests were performed each by growing *Lemna minor* on synthetic leachate under controlled conditions and on dumpsite leachate under natural conditions. During each test, duckweed was grown in 300 ml plastic containers with a surface area of 25.8 cm^2. About 60 mg of fresh mass of duckweed was grown on 250 ml leachate at an internal depth of 9.5 cm. Results revealed that, in comparison to synthetic leachate, duckweed removed Chemical Oxygen Demand (COD), nitrogen (N), and phosphorous (P) more efficiently from dumpsite leachate under natural climatic conditions. However, the amounts of N and P absorbed into duckweed body mass were about 16% and 35% respectively more at synthetic leachate under controlled conditions. Maximum growth rate of duckweed (7.03 g m^{-2} day^{-1}) was also observed for synthetic leachate in comparison to the growth rate of 4.87 g m^{-2} day^{-1} at dumpsite leachate. Results of this study provide a useful interpretation of duckweed growth and nutrient removal dynamics from leachate under natural and laboratory conditions.

Phytotoxicity

Combined toxic effects of microcystin-LR and phenanthrene on growth and antioxidant system of duckweed (*Lemna gibba* L.)

Wan, X; Steinman, AD; Shu, XB; Cao, Q; Yao, L; Xie, LQ (2019) ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 185: Article Number: UNSP 109668

Microcystins and polycyclic aromatic hydrocarbons commonly co-exist in eutrophic freshwater environments. However, their combined toxicity remains unknown. The aim of this study was to evaluate the combined toxic effects of microcystin-LR (MC-LR) and phenanthrene (Phe) on duckweed (*Lemna gibba* L.) during a short-term exposure (7 d). *L. gibba* was exposed to a range of environmentally relevant concentrations of MC-LR (5, 50, 250, 500 μg/L) and Phe (0.1, 1, 5, 10 μg/L), both individually and in MC-LR + Phe mixtures (5 + 0.1, 50 + 1, 250 + 5, 500 + 10 μg/L). Subsequently,

biomarkers of toxicity such as growth, chlorophyll a, and antioxidant enzyme activity (catalase, superoxide dismutase, and peroxidase) were analyzed in *L. gibba*. Growth and the antioxidant system of *L. gib*ba were not significantly inhibited by Phe alone, whereas higher concentrations of individual MC-LR (≥ 50 μg/L) significantly inhibited growth and induced oxidative stress. Based on Abott's formula, their interaction effects were concentration dependent. Antagonistic effects were observed when exposed to combinations of lower concentrations of MC-LR and Phe (≤ 50 + 1 μg/L), while additive or synergistic effects were induced at higher concentrations of both compounds (≥ 250 + 5 μg/L). Moreover, higher concentrations of Phe (≥ 5 μg/L) increased the accumulation of MC-LR in *L. gibba*. Our results suggested that the toxic effects of MC-LR and phenanthrene were exacerbated only when they co-exist in water bodies at relatively high concentrations. Consequently, co-existence of MC-LR and Phe at low levels are unlikely to exacerbate ecological hazards to *L. gibba* in most aquatic environments, at least based on responses of this plant.

Toxicity of the herbicide flurochloridone to the aquatic plants *Ceratophyllum demersum* and *Lemna minor*

Zhou, J; Wu, Z; Yu, D; Yang, (2019) Environmental Science and Pollution Research International DOI:10.1007/s11356-019-06477-0

As a new and efficient selective pre-emergence herbicide, flurochloridone (FLC) has been widely promoted in recent years but readily results in residues in nature. As the primary producers and restorers of the water environment, aquatic plants are at risk of FLC exposure. In the present research, we studied the phytotoxicity of FLC in *Lemna minor* and *Ceratophyllum demersum*. The physiological and growth responses of these two aquatic plants exposed to different concentrations of FLC (0, 20, 100, 300, 1000, and 2000 μg/L) were measured. The results showed that FLC (≥ 20 μg/L) could cause serious photosynthesis pigment damage and bleaching in *C. demersum* and *L. minor*. Significant oxidative damage was observed in *L. minor* at 20 μg/L FLC, while there was no severe oxidative damage in *C. demersum*. At 100-300 μg/L FLC, peroxidase (POD) and superoxide dismutase (SOD) were activated to scavenge free radicals in *L. minor*, while POD acted as a protective enzyme in *C. demersum*. At higher concentrations of FLC (≥ 1000-2000 μg/L), *L. minor* reached less than healthy stability through the regulation of the antioxidant enzyme system and the chlorophyll a/b value. POD, SOD, and protein content returned to normal levels, and the growth parameters increased. However, in *C. demersum*, the enzymes POD and SOD and soluble protein were damaged, and oxidative stress reached the highest level at 1000-2000 μg/L FLC. Taken together, our results suggested that when treated with FLC, *L. minor* was more sensitive at lower doses (20 μg/L) and more adaptive at higher doses (1000-2000 μg/L) than *C. demersum*.

Humic acid regulation of the environmental behavior and phytotoxicity of silver nanoparticles to *Lemna minor*

Ding, YY; Bai, X; Ye, ZF; Gong, DQ; Cao, JJ; Hua, ZL (2019) ENVIRONMENTAL SCIENCE-NANO 6: 3712-3722

The environmental transformations of silver nanoparticles (AgNPs) are correlated with their behaviors and ecological risks. Humic acid (HA) is ubiquitous in natural water environments. However, the influence of HA on the environmental behavior, bioavailability, and toxicity of AgNPs is little known. This study investigated the processes of AgNP bioaccumulation in and toxicity to *Lemna minor* in an aquatic environment. AgNP effects were assessed in the presence of HA, which has been shown to interact with AgNP and modify its behavior and toxicity to organisms. Results showed that AgNP dissolution declined with the addition of HA. Carboxylate groups and C-O and C-O-C bonds were found to be involved in the HA and AgNP interaction, while XPS results suggested

that HA caused Ag^{+} to be reduced to $Ag^{(0)}$. The addition of HA inhibited the absorption and accumulation of Ag in *Lemna minor* roots and leaves. When exposed to AgNP treatments alone, the plant morphology and ultrastructure were damaged by root atrophy and a reduction in vessel number and cross-sectional areas (CSAs) was observed. However, the addition of HA mitigated AgNP toxicity, resulting in no significant difference in the chlorophyll biomass and concentration between the AgNP-HA and unexposed treatment groups. The addition of HA was found to inhibit the generation of ROS and the depolarization of mitochondrial membrane potential (ΔΨm) caused by AgNPs in root cells. Overall, these results establish the mechanism of the effect of HA on AgNP behavior and highlight the influence of HA on the uptake and toxicity of AgNPs to *Lemna minor*.

Original GC/EI/MS total ion chromatograms of *Lemna* (*Lemna minor* L.) treated or not with metribuzin, glyphosate, and their binary mixtures

Kostopoulou, S; Ntatsi, G; Arapis, G; Aliferis, KA (2019) Data in Brief 27:104591

The GC/EI/MS metabolite profiles of *Lemna minor* L. plants were recorded following treatments with sub-lethal concentrations of the herbicidal active ingredients (a.i.) metribuzin and glyphosate, and various of their binary mixtures. The raw GC/EI/MS total ion chromatograms (*.cdf format) of the *Lemna*'s endo-metabolomes were recorded, which are included in this article. Since *Lemna* is a model organism in ecotoxicological studies, the dataset could serve as a reference for *Lemna* metabolomics studies related to the investigation of the effects of phytotoxic compounds and their mixtures on its metabolism. Also, the dataset could be a valuable resource for the discovery of validated biomarkers of the toxicity of mixtures. The dataset support the research article "Kostopoulou etal., Assessment of the effects of metribuzin, glyphosate, and their mixtures on the metabolism of the model plant Lemna minor L.applying metabolomics. "Chemosphere 239, 2020, 124582."

Can duckweed be used for the biomonitoring of textile effluents?

Hocini, I; Benabbas, K; Khellaf, N; Djelal, H; Amrane, A (2019) EURO-MEDITERRANEAN JOURNAL FOR ENVIRONMENTAL INTEGRATION 4: Article Number: UNSP 34

In certain countries, it is important to monitor for poorly managed freshwaters in order to avoid ecosystem degradation. This can be achieved through biomonitoring involving the use of living organisms as ecological alert indicators. In this context, biotests of the duckweed *Lemna gibba* (*L. gibba*) were undertaken to assess the toxic effects of pollutants from the textile industry on a natural ecosystem. *L. gibba* was exposed to different concentrations (5-100 mg/L) of two dyes, namely Direct Red 89 (DR-89) and Vat Blue 20 (VB-20), under laboratory conditions. Our findings showed that at dye concentrations > 50 mg/L, visible damage to the duckweed (chlorosis and disconnection of fronds, leading to severe necrosis) appeared from the third day of toxicity testing due to the toxicity of the dyes. However, at dye concentrations ≤ 50 mg/L, the duckweed showed no visible signs of toxicity within an exposure time of 4 days, although these concentrations did significantly reduce the growth rate of *L. gibba* and its photosynthetic pigment levels. The dye concentration that reduced the growth rate of the plant by 50% (IC_{50}) was 36.3 mg/L for DR-89 and 26.9 mg/L for VB-20. The dye-concentration-dependent reductions in the growth and photosynthetic pigment levels of the duckweed demonstrated that this plant would be sensitive to the concentrations of these dyes in aquatic systems. The current findings therefore prove that physiological alterations occur in *L. gibba* following dye exposure, suggesting that this species is highly suited to use in the biomonitoring of textile effluents that contaminate water bodies.

The Ca^{2+} signaling, Glu, and GABA responds to Cd stress in duckweed

Yang, L; Yao, J; Sun, J; Shi, L; Chen, Y; Sun, JH (2019) Aquatic Toxicology 218: 105352

Cadmium (Cd) affects plants and animal health seriously. Ca^{2+} signals in plant cells are important for adaptive responses to environmental stresses. Here we showed that 50 μM Cd shock stimulated the Ca^{2+} signal via modifying the instantaneous Ca^{2+} flux from influx of 17 pmol·cm^{-2}·s^{-1} to the efflux of 240 pmol·cm^{-2}·s^{-1} at 100 μm from rhizoid tip. And the Ca^{2+} signal transferred to the vein and mesophyll cell. The Ca addition decreased the accumulation of Cd. The gene expression of glutamate receptor-like (GLR) proteins, which is activated by Glu and triggers Ca^{2+} flux, was increased significantly by 24 h Cd stress. Glu content was increased under Cd stress and exogenous Glu triggered the Ca^{2+} signal in duckweed, while Ca^{2+} addition caused no influence to Glu content. GABA, which is synthesized from Glu and acts as an inhibitory neurotransmitter, has been decreased with 24 h Cd treatment. GABA addition increased the abscission rate and Glu addition decreased the abscission rate during Cd stress, suggesting that the Glu/GABA ratio is important for responding to Cd. This research shows the sight of the Glu, Ca^{2+}, GABA signaling networks during Cd stress.

Uptake, Growth, and Pigment Changes in *Lemna minor* L. Exposed to Environmental Concentrations of Cylindrospermopsin

Flores-Rojas, NC; Esterhuizen-Londt, M; Pflugmacher, S (2019) Toxins 11: DOI:10.3390/toxins11110650

Cylindrospermopsin (CYN)-producing cyanobacterial blooms such as *Raphidiopsis, Aphanizomenon, Anabaena, Umezakia*, and *Lyngbya* spp. are occurring more commonly and frequently worldwide. CYN is an environmentally stable extracellular toxin, which inhibits protein synthesis, and, therefore, can potentially affect a wide variety of aquatic biota. Submerged and floating macrophytes, as primary producers in oligotrophic habitats, are at risk of exposure and information on the effects of CYN exposure at environmentally relevant concentrations is limited. In the present study, we investigated CYN uptake in the floating macrophyte *Lemna minor* with exposure to reported environmental concentrations. The effects were evaluated in terms of bioaccumulation, relative plant growth, and number of fronds per day. Variations in the concentrations and ratios of the chlorophylls as stress markers and carotenoids as markers of oxidative stress defence were measured. With exposure to 25 μg/L, *L. minor* could remove 43% of CYN within 24 h but CYN was not bioaccumulated. Generally, the pigment concentrations were elevated with exposure to 0.025, 0.25, and 2.5 μg/L CYN after 24 h, but normalized quickly thereafter. Changes in relative plant growth were observed with exposure to 0.25 and 2.5 mug/L CYN. Adverse effects were seen with these environmentally realistic concentrations within 24 h; however, *L. minor* successfully recovered within the next 48-96 h.

Investigations on the uptake and transformation of sunscreen ingredients in duckweed (*Lemna gibba*) and *Cyperus alternifolius* using high-performance liquid chromatography drift-tube ion-mobility quadrupole time-of-flight mass spectrometry

Seyer, A; Mlynek, F; Himmelsbach, M; Buchberger, W; Klampfl, CW (2019) Journal of Chromatography. A: 460673. DOI:10.1016/j.chroma.2019.460673

The uptake, translocation and transformation of three UV-blockers commonly employed in sunscreens, namely avobenzone, octocrylene and octisalate from water by *Lemna gibba* and *Cyperus alternifolius* was investigated. Reversed phase high performance liquid chromatography

coupled to drift-tube ion-mobility quadrupole time-of-flight mass spectrometry was used for analyzing the extracts from the selected plants after incubation with the UV-blockers for one week. For avobenzone several transformation products resulting from hydroxylation, demethylation and oxidation of the parent molecule could be identified by measuring accurate mass, performing MS/MS experiments and by determining their drift-tube collision cross sections employing nitrogen as drift gas. In addition, the plants were subjected to two commercially available sunscreens, providing similar results to those obtained for the standard solutions of the UV-blockers. Finally, a kinetic study on the uptake and transformation of avobenzone, octocrylene and octisalate was conducted over a period of 216 h, revealing that the UV-filters were mostly present in their parent form and only to a smaller part converted into transformation products.

Adsorption of Ni^{2+} and Pb^{2+} from water using diethylenetriamine-grafted *Spirodela polyrhiza*: behavior and mechanism studies

Qu, W; He, DL; Guo, YN; Tang, YN; Shang, J; Zhou, L; Zhu, RL; Song, RJ (2019) ENVIRONMENTAL SCIENCE AND POLLUTION RESEARCH DOI: 10.1007/s11356-019-06558-0

Novel adsorbent, diethylenetriamine-grafted *Spirodela polyrhiza* (DSP), was synthesized via modifying natural *S. polyrhiza* (SP) with diethylenetriamine by cross-linking with epichlorohydrin and applied to adsorb Ni^{2+} and Pb^{2+} from water. The effecting parameters on adsorption of Ni^{2+} and Pb^{2+} such as adsorbent dosage, pH, contact time, temperature, and initial concentration were studied through equilibrium experiments. The adsorption of Ni^{2+} and Pb^{2+} followed the pseudo-second-order model and the Langmuir isotherm adsorption model. The study discusses thermodynamic parameters, including changes in Gibbs free energy, entropy, and enthalpy, for the adsorption of Ni^{2+} and Pb^{2+} on DSP, and revealed that the adsorption process was spontaneous and exothermic under natural conditions. The maximum Ni^{2+} and Pb^{2+} adsorption capacities of DSP were 33.02 and 36.50 mg/g, respectively. The newly prepared materials were characterized through scanning electron microscopy (SEM), mapping analysis, and zeta potential analysis. Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) analyses indicated that functional groups (-OH and N-H) were involved in Ni^{2+} and Pb^{2+} adsorption. Notably, DSP can be easily regenerated and reused for multiple cycles. Therefore, DSP is a promising adsorbent for effective Ni^{2+} and Pb^{2+} removal.

Adsorption of thorium(IV) ions from aqueous solution by citric acid modified *Lemna minor*

Yang, SK; Huang, JJ; Xiang, KX; Wu, WL; Hou, XJ (2019) DESALINATION AND WATER TREATMENT 166: 186-192

The adsorption of thorium(IV) by the citric acid modified *Lemna minor* was study. The adsorption was found to be at a maximum with 97.49% of removal efficiency and 162.5 mg g^{-1} of adsorption capacity, thorium(IV) concentration 100 mg L^{-1}, contact time 60 min, pH 5.0, with 0.03 g dry *Lemna minor* biomass by citric acid treated. The results show that pseudo-second-order kinetic model was fit the data very well. The adsorption equilibrium was well described by Langmuir isotherm model. In addition, FTIR analysis indicates that hydroxyl, carbonyl and amino groups act as the important roles in the adsorption process.

Plastic particles adsorb to the roots of freshwater vascular plant *Spirodela polyrhiza* but do not impair growth

Dovidat, LC; Brinkmann, BW; Vijver, MG; Bosker, T (2019) LIMNOLOGY AND OCEANOGRAPHY LETTERS DOI: 10.1002/lol2.10118

We investigated the effect of nano- and microplastics on the freshwater duckweed species *Spirodela polyrhiza*, a vascular plant. *S. polyrhiza* was exposed for 120 h to concentrations ranging from 10^2 to 10^6 particles center dot mL^{-1}. We assessed effects on growth and chlorophyll production, and explored adsorption and absorption by way of confocal microscopy. For both nano- and microsized particles, no concentration-dependent effects on growth were found (expressed as fresh weight, frond, and root sizes). In addition, chlorophyll concentrations were not significantly affected. Confocal microscopy indicated that nanosized plastic particles adsorbed externally to the duckweed, especially to the roots. Internalized plastic particles could not be detected. Nevertheless, given their important role in ecosystems as a food source for a range of organisms, the adsorption of plastic particles to *S. polyr*hiza roots as detected in this study can result in the transfer of plastic particles to diverse herbivorous species within the ecosystem.

Degradation Studies of Selected Bisphenols in the Presence of beta-Cyclodextrin and/or Duckweed Water Plant

Kaleniecka, A; Zarzycki, PK (2019) Journal of AOAC International DOI:10.5740/jaoacint.19-0267

This research reports a multivariate experiment enabling observation of the potential application of macrocyclic compound [beta-cyclodextrin (beta-CD)] and/or duckweed organisms as the active factors for elimination of selected bisphenols A, B, and S from water samples. Target bisphenols selection was based on observation that such components can be present in food or environmental samples (e.g., vegetable/fruit juices, milk, drinking water, or treated wastewater). Biological research was carried out using aquatic organisms containing chlorophyll, particularly duckweed (*Lemna minor* L), that may work as an active biomass for the elimination or extraction of bisphenols micropollutants from water. Using such a system, we studied the potential encapsulation effect and removal efficiency of nontoxic macrocyclic oligosaccharide (beta-cyclodextrin) acting as an encapsulation reagent to promote the removal of selected bisphenols from liquid phase both with and without the presence of duckweed biomass. Experimental data have revealed that beta-CD or combined beta-CD/duckweed system has an effect on bisphenols elimination from water. The initial data set obtained from this preliminary experiment (and combined with supramolecular complex formation data calculated from chromatographic experiments, published previously) enables designing of further experiments focusing on the development of green chemistry technology. It is hoped that this may be used for the efficient removal of low-molecular-mass micropollutants using classical technological wastewater treatment processes modified by biomass and macrocyclic additives. This process needs to be optimized, but the results presented have revealed that such green chemistry technology, if successful, may be an interesting alternative for the selective removal of the micropollutants investigated from wastewater using classical adsorbents (e.g., carbons and carbon-related nanomaterials), particularly in terms of the worldwide problem with microplastic pollutants in the environment and food products.

Instructions to Contributors for the Duckweed Forum

The Duckweed Forum (DF) is an electronic publication that is dedicated to serve the Duckweed Research and Applications community by disseminating pertinent information related to community standards, current and future events, as well as other commentaries that could benefit this field. As such, involvement of the community is essential and the DF can provide a convenient platform for members in the field to exchange ideas and observations. While we would invite everyone to contribute, we do have to establish clear guidelines for interested contributors to follow in order to standardize the workflow for their review and publication by the Duckweed Steering Committee members.

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- Should be intelligible to DF readers who are not specialists in the field and should convey your essential points clearly.
- Should be short (no more than 150 characters including spaces) and informative.
- Should avoid acronyms or abbreviations aside from the most common biochemical abbreviations (e.g., ATP). Other acronyms or abbreviations should either:
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    - be clarified by use as a modifier of the appropriate noun (e.g., FOX1 transcription factor, ACC dopamine receptor).

Authors:

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Links for Further Reading

http://www.ruduckweed.org/ Rutgers Duckweed Stock Cooperative, New Brunswick, New Jersey State University. Prof. Dr. Eric Lam

http://www.InternationalLemnaAssociation.org/ Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants.

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