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Are *Lemna* spp. Effective Phytoremediation Agents?

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ABSTRACT

Lemna spp. of the family Lemnaceae have been widely studied for their potential application in phytoremediation. A few *Lemna* species are already being adopted to enhance natural attenuation for both organic and inorganic pollution in polishing ponds of wastewater treatment facilities, and constructed wetland designed for decontamination of metal pollution. In view of this growing interest, we review in this article the potency and limitation of *Lemna* species as effective phytoremediation agents. We find that *Lemna* species have many unique properties ideal for phytoremediation plants species: they are have fast growth and primary production; high bioaccumulation capacity; ability to transform or degrade contaminants; ability to regulate chemical speciation and bioavailability of some contaminant in their milieu; resilient to extreme contaminant concentration; and can be applied on multiple pollutants simultaneously. In addition, they have properties significant for public health likewise livestock production and aquaculture, and ecological function. However, we also find a few important limitations of *Lemna* as an ideal phytoremediation agent. The plants are small in size and floating in nature. Hence, they are easily blown off the water surface resulting in transferring contamination to uncontaminated sites because *Lemna* biomass degrades easily thereby readily releasing the contaminant back into the water pathway. This also results in both low sedimentation and contribution to humic material in the benthic. Further, *Lemna* has very high wet-dry biomass ratios which may be deceiving to believe that they have high bioaccumulation on one hand, while on the other, the energy required to dewater the biomass may be equivalent to conventional treatment plants. Nevertheless, *Lemna* species remain one of excellent plants for studying process in phytoremediation, and a good phytoextraction agent for application in shallow and small polishing ponds.

Keywords: aquatic system, bioaccumulation, bioavailability, bioremediation, biotransformation, BOD, heavy metals, hormesis, organic pollutants, phytoextraction, trade off, water pollution

CONTENTS

INTRODUCTION

The legacy of rapid urbanization, industrialization, fertilizer and pesticide use has resulted in major pollution problems in both terrestrial and aquatic environments. In response, conventional remediation systems based on high physical

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Invited Review

and chemical engineering approaches have been developed and applied to avert or restore polluted sites (Schnoor *et al.* 1995; Schnoor 1997; Singh *et al.* 2003; Goulet *et al.* 2005; Pilon-Smits 2005). Much as these conventional remediation systems are efficient, they are sparsely adopted because of some economical and technical limitations. Generally, the cost of establishment and running deter their use and meeting the demand particularly in countries with a weak economy. Logical, this high cost technology can neither be applied justifiably where: (1) the discharge is abruptly high for a short time but the entire average load is relatively small, (2) the discharge is very low but long-term (entire load of medium range) nor (3) the discharge is continuously decreasing over a long duration (Dudel *et al.* 2001, 2004; Mkandawire *et al.* 2004a). Thus conventional remediation approaches are best for circumstances of high pollutant discharge like in industrial, mining and domestic wastewater. Recently, it is evident that durability restoration and long-term contamination control in conventional remediation is questionable because in the long run the pollution problem is only suspended or transferring from one site to another.

In view of this, there has been growing interest in the search for alternative remediation technology that is effecttive, durable and also cost-effective. One such technology is phytoremediation, the use of plants and associated microbes for environmental cleanup. The technology is allegedly cost-effective because it is natural energy driven and requires minimal capital and running costs. It is a non-invasive alternative or complementary technology for engineering-based remediation methods (Salt *et al.* 1995; Schnoor *et al.* 1995; Adler 1996; Miller 1996; Schnoor 1997; Sadowsky 1998; Pilon-Smits and Pilon 2002; Singh *et al.* 2003; Pilon-Smits 2005). It is a cutting edge area of research in the contemporary field of environmental and remediation technology. Earlier research in phytoremediation focused on screening plants species for phytoremediation potential. The focus is drifting towards engineering the phytoremediation systems for efficiency and responsiveness to contamination loading. Plant species with potential for phytoremediation should posses the following properties: (1) they should extract and accumulate, transform, degrade, or volatilise contaminants at levels that are toxic to ordinary plants; and (2) The plant species must have fast growth and high yield. Additionally, a good phytoremediation species should be applicable to remediate multiple pollutant simultaneously because pollution rarely occurs as a single chemical (Ochs *et al.* 1993; Horst 1995; Schnoor *et al.* 1995; Dakora and Phillips 2002; Miretzky *et al.* 2004; Tu *et al.* 2004).

Currently, a few plants species are know to possess these properties that qualify them to be good phytoremediation species for terrestrial and aquatic environments (Fairchild *et al.* 1997, 1998; Cossu *et al.* 2001; Hume *et al.* 2002; Sooknah and Wilkie 2004). Among species identified for aquatic phytoremediation are species from the genus *Lemna*, a free-floating tiny macrophyte (Salt *et al.* 1995; Carvalho and Martin 2001; Wang *et al.* 2002; Mkandawire *et al.* 2004c; Goulet *et al.* 2005; Stout and Nusslein 2005). *Lemna* species commonly grow naturally in wetlands including some highly contaminated water bodies (Landolt 1982). *Lemna* species are highly advocated for application in wastewater treatment facility, constructed wetland and even in restoration of contaminated water bodies (Adler 1996; Zayed *et al.* 1998; Wang *et al.* 2002). However, there are many questions that arise over the real efficiency and applicability of *Lemna* sp. in phytoremediation. If *Lemna* spp. really possesses the phytoremediation properties reported in the literature, in addition to their abundance and availability in most contaminated water bodies, why is water pollution a big problem and issue? To answer this question, we reviewed the potency and limitation of *Lemna* spp. as an effective phytoremediation agent, and we report findings from the review process in this current article. This review is on the whole *Lemna* genus despite that *Lemna minor* and *Lemna gibba* are prominent because they are the most reported in literature and probably the most researched in phytoremediation.

Table 1 The family *Lemnaceae* after Landolt (1980, 1986) and Landolt and Kandeler (1987).

Family	**Lemnaceae**			
Sub-family	**Lemnoideae**		**Wolffioideae**	
Genus	***Lemna***	***Wolffia***	***Spirodela***	***Wolffiella***
Species	*L. aequinoctialis*	*W. arrhiza*	*S. polyrrhiza*	*W. lingulata*
	L. gibba	*W. borealis*	*S. punctata*	*W. oblonga*
	L. minor	*W. brasiliensis*		
	L. minuta	*W. columbiana*		
	L. trisulca	*W. globosa*		
	L. turionifera			
	L. valdivana			

SYSTEMATIC POSITION AND ECOLOGY OF *LEMNA*

Lemna is a genus of monocotyledonous free-floating aquatic macrophytes in the Lemnaceae family (**Table 1**), which is commonly known as duckweed. They commonly grow in stagnant or slow-flowing, nutrient-enriched waters throughout tropical and temperate zones. Their growth conditions include temperatures range of 6-33°C, a wide pH range with optimal growth between pH 5.5 and 7.5 (Mkandawire and Dudel 2005a, 2005b). *Lemna* spp. form a Lemnatae type of macrophyte communities, a quartz "mono-specific" plant association in which they are the dominant primary producer (Landolt 1980, 1982, 1986; Les *et al.* 2002). Unlike most terrestrial and aquatic angiosperms, *Lemna* spp. repro-

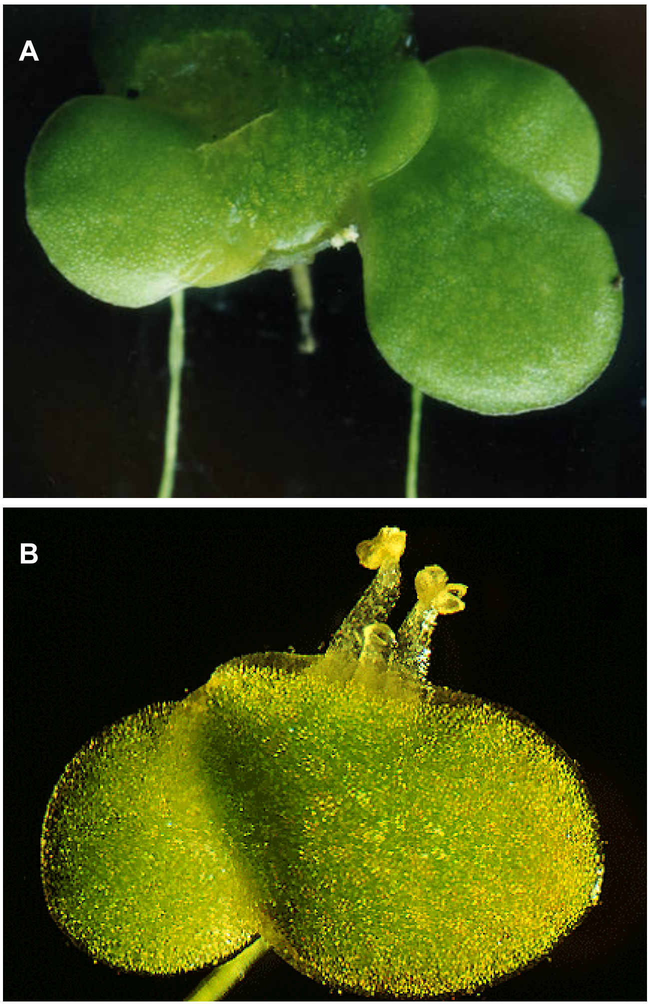

Fig. 1 Dorsal view of *Lemna gibba*. (**A**) vegetative state (reproduced from Mkandawire 2005); and (**B**) flowering stage showing style and two stamens protruding from lateral budding pouch (Courtesy of Oregon State University Herberium in USA).

duces almost exclusively asexually despite being flowering plants, thereby allocating almost all their resources to vegetative growth (Landolt 1980, 1986; Landolt and Kandeler 1987). Anatomically, they are a diffuse unit known as a frond which is composed of leaflets and a root-like structure (**Fig. 1**). From the phylogenetic point of view, *Lemna* spp. are in the evolutionary path of secondary simplification of a former complex and highly differentiated vascular plants (Les *et al.* 1997).

Their relatively simple but advance anatomical and physiological structure has scientific and engineering significance. These properties allow easy handling, and manipulating under laboratory conditions. Consequently, they are considered a model plant – representative of higher plants – for a number of chemical and biogeochemical studies involving regulation of element assimilation in higher plants. Apart from phytoremediation studies and use, the *Lemna* spp. are among the most standardised test organisms in aquatic ecotoxicology (EPA 1996; DIN 2000, 2001; Eberius 2001; ISO 2001; OECD 2002).

POTENCY OF *LEMNA* SPP. AS A PHYTOREMEDIATION AGENT

Primary production

The efficiency of carbon assimilation (primary production) in decontamination and detoxification from the water pathway has many aspects which include: (1) generation of biomass for accumulation and immobilisation of contaminants; (2) production of organic carbon in the form of litter for durable fixation depending on biomass quality and stability against decay; (3) quick removal of excess nutrients from the medium to fuel fast growth and primary production; and (4) production of a *Lemna* mat that has bio-redox advantages by reducing gas exchange, and harbouring microflora (Salt *et al.* 1995; Ensley *et al.* 1996; Zayed *et al.* 1998; Carvalho and Martin 2001; Wang *et al.* 2002; Mkandawire *et al.* 2003, 2004a; Goulet *et al.* 2005). No doubt growth and primary production is an important deciding property for selec-ting phytoremediation species.

Generally, *Lemna* species are considered as very fast growing, thereby a high turnover and yield (Landolt 1980, 1986). Most *Lemna* species have a mean specific growth rate range of 0.2-0.3 d^{-1} with a doubling time in the ranging between 0.7 and 2 days (Landolt 1980, 1986; Bergmann *et al.* 2000; Cheng *et al.* 2002a; Körner *et al.* 2003; Prytz *et al.* 2003). However, *Lemna minor* and *Lemna gibba* can reach specific growth rate of about 0.6 d^{-1} under ideal conditions, rich in nutrients (Reid and Stanly 2003; Mkandawire *et al.* 2004c). Maximum relative growth rates (RGR) of 0.73 to 0.79 d^{-1} have been observed in *Lemna aequinoctialis* Welw and *Wolffia microscopica* (Griffith) Kurz, which correspond to doubling times between 20 and 24 h (Körner *et al.* 2003). Lowest maximal growth rates are observed in submerged species (Landolt and Kandeler 1987). In general, Körner *et al.* (2003) find RGR values of *Lemna* spp. comparable to angiosperm herbaceous plants which range between 0.03 and 0.37 d^{-1}, whereas algae grow at rates between 0.26 and 2.84 d^{-1}.

Table 2 Some reported annual yield of *Lemna* spp. under near-optimal conditions in the field.

Location	Yield in dry biomass (t/ha/yr)	Source
Thailand-Vietnam region	60-145	Landolt and Kandeler 1987; Anh and Preston,1997; Khang 2000
Israel	36-51	Landolt and Kandeler 1987; Leng *et al.* 1995; FAO 2001
Russia	7-8	Landolt and Kandeler 1987; FAO 2001
Uzbekistan	7-15	FAO 2001
Germany	22-34	Mkandawire *et al.* 2004b; Mkandawire *et al.* 2004c; Mkandawire and Dudel 2005a
India	30-70	Landolt and Kandeler 1987; Leng *et al.* 1995; FAO 2001
Egypt	>30	Landolt and Kandeler 1987; Leng *et al.* 1995; FAO 2001
Different regions of USA	57-185	Landolt and Kandeler 1987; Leng *et al.* 1995; FAO 2001

Landolt and Kandeler (1987) estimate annual mean yield for Lemna species of 73 tons ha^{-1} yr^{-1} dry biomass. Since maximum growth rates as well as yields of Lemnaceae are species and clone specific, **Table 2** provides a selection of measured yields of *Lemna* spp. in different parts of the world. Some yield of above180 tons ha^{-1} yr^{-1} dry biomass have been recorded (FAO 2001). The yield of *Lemna* spp., when compared to algae in aquatic systems is relatively high. The average yield of *Lemna* reported in literature lies between 25 and 50 g m^{-2} d^{-1}dry biomass in natural uncontaminated water bodies, even though daily yield of close to 200 g m^{-2} d^{-1} have been estimated under laboratory cultures and in some tropical regions. Thus, *Lemna* species are estimated to have 41% and 75% of biomass-related extraction potential for metals. For instance, Mkandawire *et al.* (2004c) estimated that *L. gibba* biomass can extracted arsenic and uranium in the magnitude of 751.9 ± 250 and 662.7 ± 203 kg ha^{-1} y^{-1} representing extraction potential of 48.3 ± 15.1 and 41.4 ± 11.9% under ideal laboratory condition – optimal steady state condition with unlimited growth. **Fig. 2** shows estimated biomass yield of *Lemna* spp. through annual seasons in tropics (Thailand) and temperate (Germany)

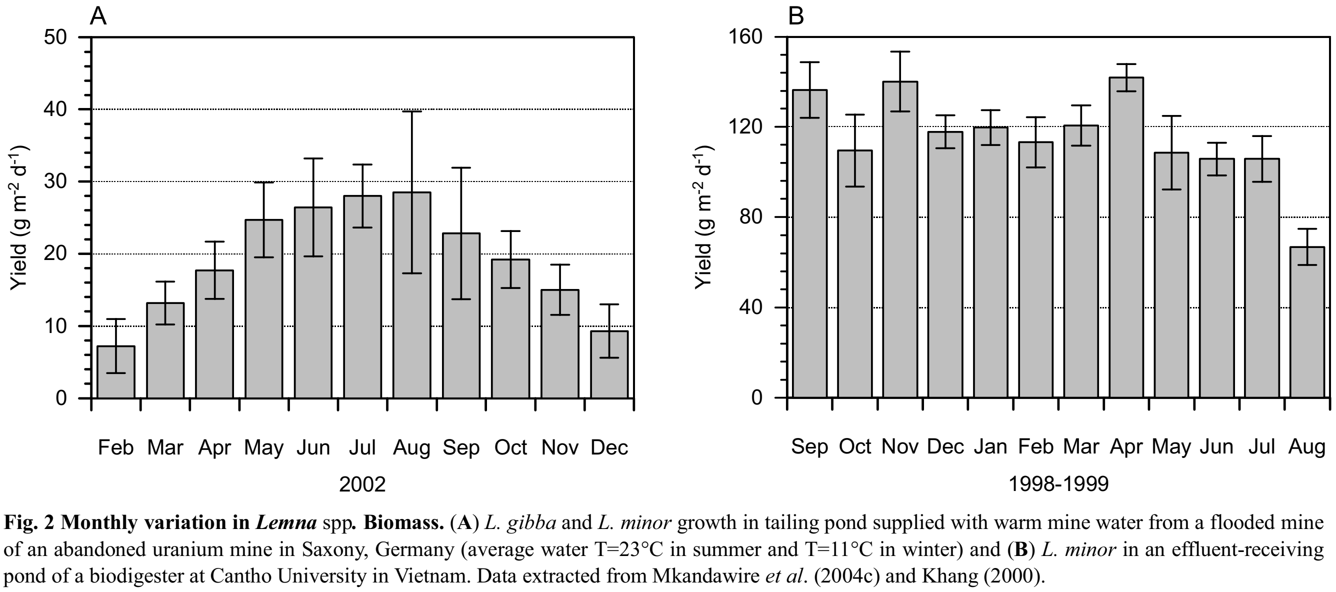

Fig. 2 Monthly variation in *Lemna* spp. Biomass. (**A**) *L. gibba* and *L. minor* growth in tailing pond supplied with warm mine water from a flooded mine of an abandoned uranium mine in Saxony, Germany (average water T=23°C in summer and T=11°C in winter) and (**B**) *L. minor* in an effluent-receiving pond of a biodigester at Cantho University in Vietnam. Data extracted from Mkandawire *et al.* (2004c) and Khang (2000).

Table 3 Removal potential of nutrients by *Lemna* spp. from wastewater ponds (calculated and modified from values reported in literature).

	Mean concentrations in wastewaters ($mg\ L^{-1}$)		**Elimination capacity (%)**
	Influent	**Effluent**	
COD	500	320	30-40
BOD	50	30	60
Total N	40	20	50
NH_3	17	2	80-90
Total P	6	3	50-60

Table 4 Some mineral compositions of *Lemna* spp. and their potential to remove minerals from water bodies (calculated from the literature).

	Fractions		**Elimination potential ($kg\ ha^{-1}\ y^{-1}$)**
	Culture medium ($mg\ L^{-1}$)	***Lemna* dry biomass ($mg\ kg^{-1}$)**	
N	0.75	60,000	6000
P	0.3-3.0	5000-14,000	560-1400
K	100	40,000	4000
Ca	360	10,000	1000
Mg	72	6000	600
Na	250	3250	320
Fe	100	2400	240

region. Firstly, the message is that despite reduced growth at different seasons of the year, *Lemna* species are capable of growing throughout the year and thereby can provide the required biomass to take up contaminants from the aquatic system.

Capacity in wastewater treatment

Since the early 1970s, considerable work has been done on the use of *Lemna* spp. as a means of treating wastewater of both agricultural and domestic origin (Oron *et al.* 1987; Buckley 1994; Hammouda *et al.* 1995; DIN 2001; Schneider *et al.* 2001; Al-Nozaily and Alaerts 2002; Cheng *et al.* 2002a; Obek and Hasar 2002; Valderrama *et al.* 2002; Zimmo *et al.* 2002, 2004; Goulet *et al.* 2005). **Table 3** presents the cleaning capacity, while **Table 4** presents mineral removal potential of *Lemna* species, both calculated from the literature. Almost a decade ago, Koles *et al.* (1987) described the guidelines for the use of *Lemna* spp. to remove ammonia and phosphorus from water. A *Lemna*-covered wastewater treatment system in practice work optimal within depths between 30 and 150 cm (Koles *et al.* 1987). Smith and Moelyowati (2001) have also developed guidelines for designing a Lemna spp. based wastewater treatment system. The guidelines have a design program that suggests that a combination of anaerobic ponds, *Lemna* spp.-based treatment systems and maturation ponds can minimise land requirements associated with wastewater treatment using only phytoremediation procedure. Vatta *et al.* (1995) developed models for *L. gibba*-based wastewater treatment plants. They developed a comprehensive process model which simulates the behaviour of a waste-water treatment system based on *L. gibba*. The model accounts for the main chemical and biochemical phenomena involved in a natural waste-water treatment system. Their predictions are quite reliable especially in mini-ponds and in real-size treatment.

Both tables show that contamination cleaning potential and capacity of *Lemna* for mineral contamination is very high. The fast growth and turnover of *Lemna* spp. is fuelled by the ability to rapidly take-up high amount of nutrients from their medium. Thus, the high values of nutrient elimination potential are a direct result of rapid growth rate and high turnover. This is one of several properties exploited to remove surplus nutrients from effluents in wastewater treatment systems. Therefore, harvesting of excess *Lemna* biomass is a common practice in wastewater treatment systems to overcome space limitation and finally to remove surplus nutrients especially nitrogen and phosphorus, while at the same time maintaining a growth steady-state. *Lemna* spp. grow very densely in nutrient-rich environments in which layers of fronds grow one on top of another to form a mat that can be as much as 10 cm thick. This thick mat creates an anaerobic environment in the water on which this mat floats, which promotes anaerobic digestion and denitrification of wastewater (Landesman 2000; Cheng *et al.* 2002a; Landesman *et al.* 2005). Therefore, *Lemna* spp. can also be part of constructed wetland systems, either in the wastewater-receiving or to polishing ponds in wetland-treated effluents. Polishing is one of the last steps in wastewater treatment used where residual nutrient, organic and suspended solids are removed either aerobically or facultative. A general illustration of the process mainly involved degradation of organic pollutants in wastewater is presented in **Fig. 3A**. The elimination capacity for organic material in terms of biological oxygen demand (BOD) and chemical oxygen demand (COD) is lower in comparison to other vascular plants and rich in cellulose but emerse growing macrophytes in constructed wetland. Nitrogen removal is at the same level or even higher (Gérard *et al.* 2002; Vymazal 2005). P-elimination is higher in halophyte than Lemna-dominated treatment systems because phosphates are usually fixed on the gravel beds in the benthic zone.

Bioaccumulation potential

There are several studies that have shown that most *Lemna* spp. show an exceptional capability and potential for the uptake and accumulation of heavy metals, radionuclides as well as metalloids, surpassing that of algae and other aquatic macrophytes (Körner and Vermaat 1998; Szabo *et al.* 1999; Vidakovic-Cifrek *et al.* 1999; Zimmo *et al.* 2000; Axtell *et al.* 2003; Zimmo *et al.* 2004). **Table 5** presents some selected metals reported in the literature which shows high accumulation capacity in some *Lemna* species. For example, the zinc concentration in frond tissue was 2700 times higher than that of its medium (Sharma *et al.* 1995). Under experimental conditions, *L. minor* is a good accumulator of Cd, Se, and Cu, but a moderate accumulator of Cr, and a relatively poor accumulator of Ni and Pb (Zayed *et al.* 1998). *Lemna* spp. have also shown potential in attenuation of uranium as well as arsenic in surface waters of decommissioned uranium mining (Mkandawire *et al.* 2004a, 2004c; Mkandawire 2005). The uptake rates of Al by *Lemna* spp. is estimated between 0.8-17 mg g^{-1} d^{-1} (Goulet *et al.* 2005). Therefore, practical utilisation in phytoremediation is using more than one *Lemna* spp. but also in complement to other aquatic macrophytes.

The accumulation of metals and metalloids in *Lemna* takes advantage of quality biomass for biosorption on the cell surface, and high metabolic mediated incorporation of contaminants into the cells. The metabolic mediated incurporation is regarded as a more permanent sink of pollutants, while biosorption can be either temporary or permanent depending on the biosorption mechanism process involved (Mkandawire *et al.* 2003). Biosorption is a property of certain types of inactive biomass to bind and concentrate metals by acting as a chemical substance, or an ion exchanger of biological origin (Dudel *et al.* 2004). We investigated the biosorption of both uranium and arsenic on *L. gibba* biomass (Mkandawire *et al.* 2003), while other groups have also studied biosorption of different metals and radionuclide on the biomass of *L. minor* extensively (Cecal *et al.* 1999; Palit *et al.* 1994; Singh *et al.* 2000). We agree, along with other researchers that *L. minor* and *L. gibba* dry biomass are excellent biosorbents for a few metals and metalloids (Dushenkov *et al.* 1997; Axtell *et al.* 2003; Mkandawire *et al.* 2003). Apart from removing contaminants from the water's pathway, their biosorption shields toxicity by fixing contaminants in dead biomass (Mkandawire *et al.* 2003, 2005). A few physiological studies have found metals and metalloids in the vacuole and cell in the cellulose of the cell wall. Once toxic metals and metalloids are incorporated into *Lemna* cells, they are compartmentalised through enzymatic-mediated sequestration. The processes involved in sequestration of contaminants in *Lemna* cells is summarised in **Fig. 3B**.

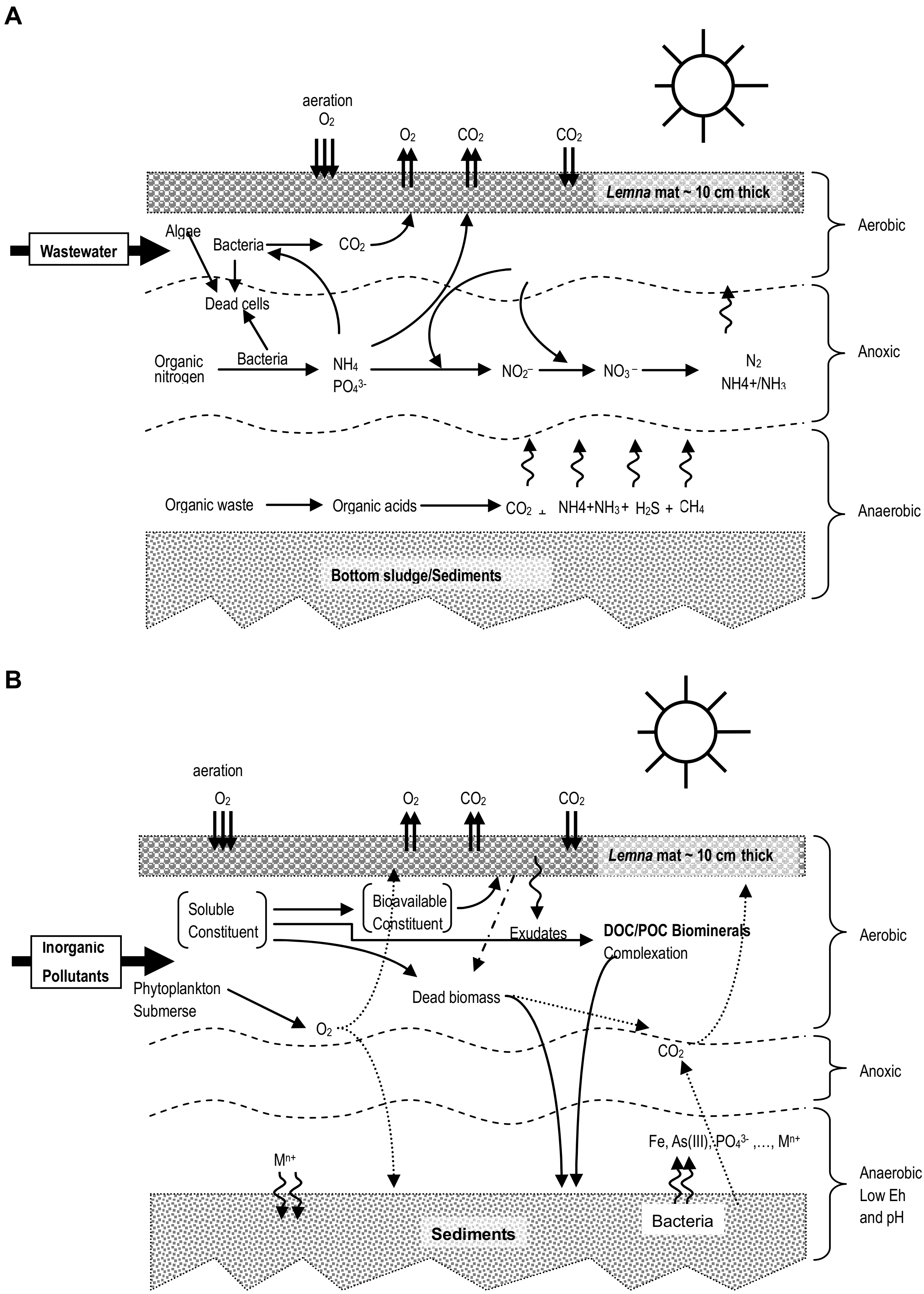

Fig. 3 Biological processes in *Lemna*-based aquatic phytoremediation system for: (A) wastewater as modified from Smith and Moelyowati (2001) and (B) inorganic pollutants especially metal(loid)s as modified from Mkandawire (2005).

Table 5 Selected bioaccumulation and transfer factors of some heavy metals in some *Lemna* spp. as reported in the literature (Charpentier *et al.* 1987; Steveninck *et al.* 1992; Miranda and llangovan 1996; Dirilgen 2001; Mkandawire *et al.* 2004c; Ater *et al.* 2006).

Species	Metal	Bioaccumulation (mg kg^{-1} dry biomass)	Bioaccumulation coefficient*
L. gibba	Pb	-	>500
	As	1000-1500	~10000
	Cu	745-1050	~3000
	U	850-1100	~7000
	Cr	900-1710	~1000
L. minor	Zn	212.5-1010	>1000
	Cd	14200	~20000
	Cu	200-800	>500
	Cd	14200	~12000
	Co	200-2000	>1500
	Pb	>750	>500
	Cr	13.48	~250
	Ba	226.1	~800
	Al	1700-4560	>4000
L. trisulca	Cd	130-1200	>3500
	Al	19238.09	~18000
	Cr	1555.30	~2000
	Cu	217.06	~300
	Ba	107.69	>500
	Zn	1308.56	~700
	Pb	233.38	>500

* **Bioaccumulation coefficients** were estimated from values from the same literature source.

Furthermore, the growth rates combined to this metal bioaccumulation potential and harvest necessity make *Lemna* a very good candidate for phytoremediation.

Are *Lemna* really hyperaccumulator plants?

Plant species accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the media are classified as hyperaccumulator. As a rule of thumb, plant species should accumulate above 1000 mg kg^{-1} of dry-biomass (Wang *et al.* 2002; Cobbett 2003). About a thousand plant species are known as metal hyperaccumulators. The dominating species are from the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae families. According to the literature, whose data is partially summarised in **Table 5**, most *Lemna* species can formally be classified in the hyperaccumulator category. However, other hyperaccumulators listed above have ontogenetic and productivity characteristics not typically of *Lemna* spp. Normally, hyperaccumulator plants have coevolved in a metal-rich environment like bed rock (Baker and Walker 1990). Hyperaccumulation in *Lemna* spp. is related to the direct contact between the plant and the contaminants, which are controlled by the water chemical conditions (i.e. anaerobic and micro-anaerobic) below the *Lemna* canopy. These conditions are triggered by *Lemna*'s organic matter production, and they can be especially induced and regulated by changing the physicochemical conditions, nutrient regime and contaminant loading in the *milieu* (Dudel *et al.* 2002; Mkandawire *et al.* 2004a, 2005c).

Biotransformation of organics

In relation to the degradation of complex synthetic organics (xenobiotics) and natural complex organics there are some reports on complete mineralisation of hazardous and toxic substances to CO_2 or to lower toxic decay products in *Lemna* communities with associated microbes. However, the role and share of the associated bacteria and fungi in this degradation process is not clear. Most *Lemna* plants take up and transform organic pollutants of which some are well known persistent organic pollutant (POP). For instance, Goa *et al.* (2000) investigated the uptake and phytotransformation of pesticide dichlordiphenyltrichlorethane (DDT) using three axenically cultivated *Spirodela oligorrhiza* and a few other aquatic macrophytes including *Lemna* spp. During the 6-day incubation period, almost all of the DDT was removed from the medium, and most of it accumulated in or was transformed by the plants. *Spirodela oligorrhiza* demonstrated the greatest potential to transform both DDT isomers; 50-66% was degraded or bound in a non-extractable manner with the plant material. Dichlorodiphenyldichloroethane (DDD) were the major metabolites and small amounts of dichlorodiphenylethane (DDE) were also found. Apparently, reduction of the aliphatic chlorine atoms of DDT is the major pathway for this transformation. *L. minor* and *L. gibba* can metabolise phenol and a series of chlorinated phenols (i.e. 4-chlorophenol to pentachlorophenol) producing β-glucoside conjugate metabolites 2,4-dichlorophenol and 2,4,5-trichlorophenol (Ensley *et al.* 1996). It seems that chlorophenols are incorporated into the vacuoles and cell walls of *L. minor* as either 2,4-dichlorophenyl-β-D-glucopyranoside (DCPG), 2,4-dichlorophenyl-β-D-(6-O-malonyl)-glucopyranoside (DCPMG) or 2,4-dichlorophenyl-β-D-glucopyranosyl-(6, 1)-β-D-apiofuranoside (DCPAG). Further, the plants are able to progressively dechlorinate the phenols (Ensley *et al.* 1996). Chlorophenols are an important class of xenobiotics used in a variety of biocides and have been shown to be resistant to microbial degradation. *L. minor* can also metabolise and transform metolachlor (MET)-contaminated waters (Fairchild *et al.* 1998). However, it is principally a poor sequester for the herbicide atrazine (ATR) in the surface water (Fairchild *et al.* 1997, 1998; Crum *et al.* 1999). Nonetheless, the presence of *Lemna* accelerates extracellular degradation of metolachlor and atrazine, which significantly reduces herbicides content from the water pathway, even more than the sequestering of the herbicides into the plant.

Datko and Mudd (1985) investigated the capacity and preferential uptake of organic compounds by *Lemna paucicostata* from their *milieu*. *L. paucicostata* had preferential uptake for neutral L-α-amino acids, basic amino acids, purine bases, choline, ethanolamine, tyramine, urea, and aldohexoses. They found that the neutral amino acid system neither transports basic amino acids nor is inhibited by these compounds in *Lemna* spp. The basic amino acid system does not transport neutral amino acids but is strongly inhibited by some, but not all, of these compounds. Therefore, the maintenance of these active, specific, and discrete systems in *Lemna* suggests they play important roles permitting *Lemna* to remove organic compounds occurring naturally in its environment.

Survival strategies in extreme *milieu* conditions

Sequestration and compartmentalisation of contaminants

Most *Lemna* plants are capable of withstanding an extreme concentration of contaminants by sequestrating and compartmentalising them into cell organelles (**Fig. 4**). The principle was first illustrated by Pilon-Smits (2005) for plants, but we tested and proved that the principle functions in the same way but with minor difference in *L. gibba* (Mkandawire 2005; Mkandawire and Dudel 2005a). Steveninck *et al.* (1992) showed that a Zn-tolerant clone of *L. minor* exposed to a high level of Zn had cellular deposits of Zn, Mg, K and P or Zn, K and P (Zn phytate). The same clone had globular deposits consisting of Cd, K and P in mature fronds, but the immature cells of the enclosed daughter fronds contained relatively large deposits with Cd and S as the main components which were presumed Cd-phytochelatin. Ex-cess Ca^{2+} in *L. minor* and *L. gibba* is deposited in the cells as Ca oxalate (de Kock *et al.* 1973; Cohan and Tirimzi 1997; Volk *et al.* 2004).

In *L. gibba*, arsenic was deposited in the cell wall as arsenite species as observed in a few other plant species (Zhang *et al.* 2002; Quaghebeur and Rengel 2004;

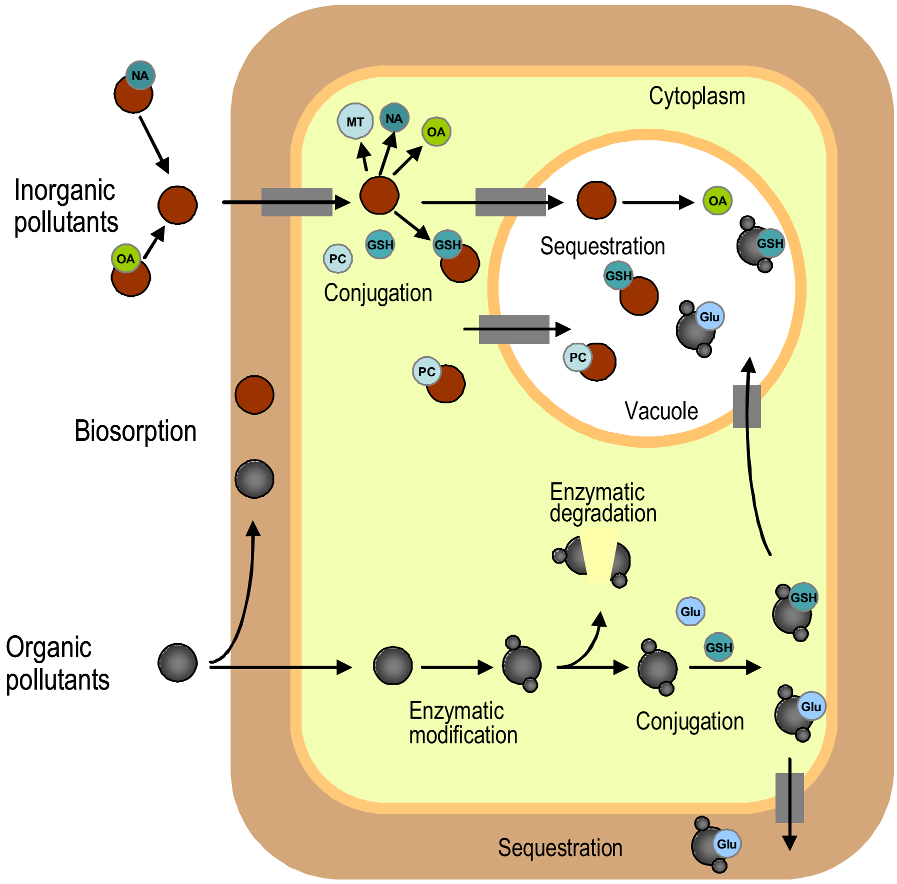

Fig. 4 Tolerance mechanism for inorganic and organic pollutants in *Lemna* cells. Detoxification involves in active sequestration in parts that can do least harm. The chelators involved are: glutathione (GSH); glucose (Glu); Metallothioneins (MT); nicotiamines (NA); organic acids (OA); and ploychelatines. This is the reason *Lemna* species accumulate high concentration of contaminants. Modified from Pilon-Smits (2005).

Mkandawire 2005). Uranium and arsenic were detected in the vacuoles of *L. gibba* cells (Mkandawire 2005; Mkandawire *et al*. 2005b). *Lemna* spp. transforms organic substances into less toxic compounds and either volatilisation (e.g. methyl arsenic species) or probably the contaminants are sequestrated and deposited in the different cell compartments, mainly the vacuole and cell wall material (Sachs and Michael 1971; Nissen and Benson 1982; Cullen *et al*. 1994; Guerin *et al*. 2000; Abedin *et al*. 2002; Miguens-Rodriguez *et al*. 2002). Thus, *Lemna* and other species manage to survive extreme concentrations through this strategy.

Strategy for synthesizing organic compounds

A few low molecular weight organic acids have been studied in *Lemna* species, especial *L. gibba*. For instance, Slovin and Cohen (1988) and later Rapparini *et al*. (2002) reported the occurrence of indole-3-acetic acid (IAA) during the growth of *L. gibba* G-3. Mkandawire *et al*. (2002) reported the occurrence of oxalic acids in *L. gibba*, while a few authors have reported the presence of oxalates in *L. minor* and *Lemna* spp. in general (de Kock *et al*. 1973; Franceschi 1987; Kostman *et al*. 2001; Volk *et al*. 2002; Hudgins *et al*. 2003; Mazen *et al*. 2004; Volk *et al*. 2004). Jasmonic acid was extracted and determined in *L. minor* (Kristl *et al*. 2005). Glutathione and organic acid metabolism plays a key role in metal tolerance in plants, including *Lemna* spp., where detoxification of arsenic, cadmium and uranium have been attributed to these organic compounds (Schmöger *et al*. 2000; Geoffrey *et al*. 2002; Schäffner *et al*. 2002; Schutzendubel and Polle 2002; Yin *et al*. 2002; Zhao *et al*. 2003). Formation of arsenosugars in *Lemna* spp. exposed to different arsenic species have also been widely reported in the literature (Gomez-Ariza *et al*. 2000; Miguens-Rodriguez *et al*. 2002; Sanchez-Rodas *et al*. 2002).

In extensive physiological studies by Datko's research group between 1977 and 1990, a number of organic compounds in *Lemna* spp. were identified and quantified. These include sulphur complex-forming organic compounds to enzymes that catalyze *S*-adenosylmethionine-dependent N-methylations of phosphatidylmethylethanolamine, phosphatidyldimethylethanolamine, and phosphatidylcholine syntheses (e.g. inorganic sulphate, glutathione, homocysteine, cysteine, methionine, *S*-methylmethionine sulphonium, *S*-adenosylmethionine, *S*-adenosylhomocysteine, cystathionine, sulpholipid, protein cysteine, and protein methionine) (Datko *et al*. 1978a, 1978b; Datko and Mudd 1982, 1985; Giovanelli *et al*. 1986; Datko and Mudd 1988). Thus, organic compounds play a major role in metal tolerance in *Lemna* spp., specifically where organic acids directly conjugate the metals or organic pollutants, to initiate extra-cellular degradation or fixation. Indirectly, some of the compounds as well as heavy metals induce survival strategies like flowering (Satish and Subhashni 1967; Tanaka *et al*. 1982; Yamaguchi *et al*. 2001) to facilitate propagation and dispersion of *Lemna* plants. This survival strategy may seem more important in ecotoxicology, it is equally important in phytoremediation because it enables survival of *Lemna* spp. even in adverse conditions.

Morphological plasticity

A few *Lemna* species have been confirmed to demonstrate phenotypic plasticity. The plasticity in most *Lemna* spp. has been observed at morphological level such as frond production rate, change of shoot to root ratio, and yield as well enzymatic protein production at molecular level like in terrestrial vascular plants (Vasseur and Aarssen 1992; Crawford *et al*. 2001). In a study involving different genotypes of *L. minor* from four continents and grown in different environmental treatments, Vasseur and Aarssen (1992) and later Scheiner (1993) described that the rate of frond multiplication and production biomass vary significantly among genotypes likewise among environmental conditions. Mkandawire *et al*. (2005b) and Mkandawire (2006) demonstrated that root length and leaflet size of *L. gibba* can be regulated by varying phosphorus supply and uranium loading in microcosms. Under phosphorus and nitrogen deficiency, and high iron, uranium or arsenic, and high redox potential environments, *L. gibba* develops long roots and a relatively smaller than average normal frond (Dudel *et al*. 2002; Mkandawire 2005; Mkandawire *et al*. 2005c). The pattern and level of plasticity are also influenced by initial treatments. Since some genotypes may be more affected than others by environmental conditions, the origin of the effect may accentuate the interaction and therefore, modify the pattern and amount of plasticity (Vasseur and Aarssen 1992; Scheiner 1993). The traits related to fitness, such as frond production rate, phenotypic variation of adaptive advantage makes *Lemna* sp. apt to survive under a wide range of environmental conditions. Thus, the ability of most *Lemna* spp. to withstand high levels of contamination in the environment directly benefits from this property.

Hormesis

L. gibba and *L. minor* multiply quickly when exposed to low dosages of arsenic, uranium, and metamitron as well as under relatively nutrient deficient environment, especially phosphorus (Engelen *et al*. 1998; Brock *et al*. 2004; Mkandawire *et al*. 2004b, 2006a). *L. gibba* culture exposed to doses of arsenic exhibited a "U"-shaped dose-response curve, commonly observed with essential elements, particularly PO_4^{3-} (Lockhart *et al*. 1989; Mkandawire *et al*. 2004b). This is a hormetic response, which is an adaptive response characterised by biphasic dose-responses that are either directly induced by the quantity of stimulant or the result of compensatory biological processes following an initial disruption in homeostasis (Calabrese and Baldwin 2003). Essentially, hormesis has been the subject of controversy due to its challenge of basic understandings of the dose-response relationship and implications for phytoremediation and ecotoxicology (Mkandawire *et al*. 2004b). Some studies argued that hormesis in *Lemna* sp. is an adaptive response with distinguishing dose-response characteristics that is induced by either direct acting or overcompensation-induced stimulatory processes at low doses. Thus, it is *Lemna*'s strategy to optimising resource allocation to maintain homeostasis (Calabrese and Baldwin 2003; Mkandawire 2005). Others suggest that it is an adaptive response that operates within normal maintenance functions that allows for metabolic excursions at extremely low concentrations (Meyer *et al*. 1998). Nonetheless, it is a steady-state adaptive response that modulates physiological dynamics, and plays an important role in *Lemna* spp. capacity to survive extreme conditions in the environment; subsequently, this is advantageous for the use of *Lemna* spp. as phytoremediation agents.

Rare properties and behaviour advantageous for remediation

Resource trade-off and homeostatic-induced decontamination

Mkandawire and Dudel (2002) and later Mkandawire *et al*. (2005) reported DOC accumulated in the *Lemna*-test culture in correspondence to nutrient limitation and uranium stress. They attributed to *L. gibba* an ability to exude low molecular weight organic acids such as IAA (Slovin and Cohen 1988; Rapparini *et al*. 2002) and oxalic acids (Dudel *et al*. 2002; Mkandawire *et al*. 2005b). The exudation was induced by the disruption of homeostasis due to interaction between nutrients, metabolites and contaminants which results in changes of chemical speciation in the *milieu*. The speciation changes render some essential nutrients including toxic metals non-bioavailable. Phosphates may form complexes with some metals like uranium which is not taken up by *L. gibba*. Consequently, *L. gibba* exudes low molecular weight organic compounds to improve the status of the growth-limiting element P whose deficiency is induced by speciation. Therefore, the organic acids form complexes with metals which may lead to precipitation or initiate redox processes which eventually render the contaminant less toxic. They may also conjugate organic compounds and facilitate their degradation. The speciation changes can also be caused by dynamics of O_2 and CO_2 concentration below the *Lemna* mat and consumption of nutrients and uptake of contaminants, as well as the accumulation of plant excreta (Mkandawire *et al*. 2004b, 2005a) (recapped in **Fig. 2**). Further studies are required to ascertain if some surface microflora of *Lemna* are capable of using electrons from some metals as a source of energy as anoxic conditions partially develops. For organic contacts, the microflora may induce degradation and transformation (e.g. fermentation).

Allozymic variation

The ability of *Lemna* species to withstand extreme environmental conditions is also related to allozymic variation. There is high level of allozymic variation within species in the general *Lemnaceae*, despite being a predominantly clonal plant. Despite very seldom flowering (sexual reproducetion), the genome of *Lemna* is as complex and diverse as in other vascular plants. They are commonly composed of several genetically different clones (Amado *et al*. 1980; Landolt 1980, 1982; Crawford *et al*. 2001; Les *et al*. 2002). Vasseur *et al*. (1991) reported that allozymic similarity among clones of *L. minor* is usually neither related to morphemetric similarity nor the degree of geographic separation nor climatic similarity of their sites of origin. Thus, the variations are largely neutral and not a consequence of differenttial selection. Most of the studies in the literature, however, have focused on a relatively small geographical area. Few studies have investigated the genetic structure among clones that are representative of the overall distribution of a species. Therefore, this is an area requiring more investigation to give more insight not only the genomics and the role of allozymism as a survival benefit for *Lemna* spp., but also the overall contribution to phytoremediation and ecotoxicology.

Benefits from associated surface microflora

Since the beginning of 1960s, several studies on the association between *Lemna* and microflora have been conducted. They mostly focused on microscopic observations and enumeration of bacteria on plant surfaces as well as several culture-dependent studies (Hosselland and Baker 1979; Körner *et al*. 1998; Szabo *et al*. 1999; El-Alawi *et al*. 2002; Falabi *et al*. 2002; Stout and Nusslein 2005; Vogel *et al*. 2006). Other areas widely studied and reported in the literature are the removal of excess nutrients – especially nitrogen and phosphorus – from waste water using the *Lemna*-microbial association; and N_2 fixation by N_2-fixing heterotrophic bacteria and cyanobacteria were associated with the duckweed mats (Zuberer 1982, 1984). Definitely, inoculation of some micro-organisms in *Lemna* culture would yield positive effect because, Chang *et al*. (2006) report that inoculation of bacteria to a floating macrophytes *Eichhornia crassipes* like wise a submerge *Elodea nuttallii* increases significantly the capacity of improving water quality in eutrophic water bodies compared to the un-inoculated treatments. Earlier, Körner and Vermaat (1998) reported that *L. gibba* was itself directly responsible for 30% and up to 52% of the total N- and P-loss, respectively. The indirect contribution of *L. gibba* to the total nutrient removal was through algae and bacteria in biofilm on the plant surface which accounted for 35 and 32 % of the total N- and P-loss, respectively.

Despite the increased interest in *Lemna* spp. for aquatic phytoremediation, there have been limited studies of microbial communities associated with the plant, especially in relation to heavy metal uptake or immobilisation (Stout and Nusslein 2005). So far in the literature, only Stout and Nusslein (2005) and Vogel *et al*. (2006) have direct addressed microbial communities associated with *Lemna* spp. in the presence of heavy metals and metalloids. The former studied the influence of indigenous rhizospheric bacterial communities of *L. minor* on Cd uptake and immobilisation from water while the latter investigated and isolated arsenic-resistant surface microflora associated with *L. gibba* and *L. minor* in abandoned uranium mines. The isolated microflora was found to belong mainly to six physiological groups, namely: oligotrophs, nitrogen fixing, phosphate solubilising, ammonifying, nitrifying and denitrifying bacteria. Most resistant bacterial to arsenic were the pseudomonas and reactive to gram negative. Otherwise, this is an important area not very exploited and published despite the fact that plant-associated microbial communities have previously been shown to be involved in controlling plant uptake of metals and metalloids from their surrounding environment (Sizova *et al*. 2002; Evans *et al*. 2005; Lyubun *et al*. 2006).

Last but not least, it is worth to mention that culture-dependent studies have been criticised for two reason: (1) they are mostly conducted in batch culture mode with extremely high test and nutrient concentrations compared to natural water bodies (Asad *et al*. 1997; Mkandawire *et al*. 2005a). As a result the validity of results obtained from laboratory tests performed batch-wise are often questioned because of dissimilarities to natural systems in respect to physical characters as well as process conditions such as high nutrient and test substance concentrations (Asad *et al*. 1997; Mkandawire *et al*. 2005a); and (2) most *Lemna* spp. are nutrient media sensitive that their performance get affected when a wrong media is used. Several studies have reported stress resulting from the medium content which reduced the life span of the plants by inducing early flowering (Satish and Subhashni 1967; Posner 1973; Tanaka *et al*. 1982; Cleland and Tanaka 1986; Yamaguchi *et al*. 2001). Normally, *Lemna* spp. rarely flower and reproduce mainly vegetative. To overcome the problems associated with high concentrations of nutrient and test chemicals, semicontinuous culture system should adopted except where the studies are intended to simulate highly contaminated stagnant ponds. Generally, it is advisable to avoid media that induce flowering of *Lemna*.

Nucleation of biomineralisation

There are circumstantial evidence that metals sequestrated in *Lemna* biomass may result into biominerals. Some studies have reported that some *Lemna* spp. assimilate metals in sugars, peptides, proteins and some low-molecular-weight organic acids (de Kock *et al*. 1973; Mazen and El-Maghraby, 1998; Bovet *et al*. 2000; Weiss *et al*. 2000; Abbas *et al*. 2001; Prasad *et al*. 2001; Yin *et al*. 2002; Mazen *et al*. 2004), which have been reported elsewhere as the nucleus of biomineralisation (Mann *et al*. 1989; Mkandawire *et al*. 2005b). Mkandawire *et al*. (2005b) investigated the durability of uranium fixation in *L. gibba* biomass, and found that about 40-50% of uranium is easily eluted from dead biomass within a month of contact with water, weak acids and EDTA. However, further elution did not take place and there was no release of uranium from the decaying biomass after four months. Using microscopy and Energy Dispersive X-ray (EDX) technology, we discovered possible crystals of uranium oxalate in *L. gibba* fronds. Others have also reported the presence of calcium oxalate crystals and other organometalic compounds in *L. minor*, *L. gibba* and *L. polyrhiza* (de Kock *et al*. 1973; Franceschi 1987; Prychid and Rudall 1999; Kostman *et al*. 2001; Hudgins *et al*. 2003; Mazen *et al*. 2004). Thus the evidence is so far circumstantial and requires further studies. If *Lemna* spp. really initiates biomineralisation through sequestration of metals in their biomass, this would be a big breakthrough in the application of *Lemna*-based phytoremediation systems.

Other environmental benefits

Lemna species have more environmental benefits than mere phytoremediation potential. Initially, a great deal of work has been done on the nutritional value of species of the *Lemnaceae* in aquaculture and livestock production (Abdulayef 1969; Trewavas 1972, 1973; Culley *et al*. 1981; Skillicorn *et al*. 1993; FAO 2001). Even the World Bank and FAO have been promoting the use of *Lemna* spp. as a source of feed for fish, poultry and cattle production because of its protein content (Culley *et al*. 1981; Skillicorn *et al*. 1993; FAO 2001). **Table 6** shows the chemical fraction in *Lemna* biomass, while **Table 7** shows the protein content in *L. gibba* as an example for *Lemna* species. Generally, the amino acid composition is more comparable to animal protein than plant protein because it has a high lysine and methionine content, two amino acids normally deficient in plant products (Dewanji *et al*. 1997; Landesman 2000). Mkandawire and Dudel (2005b) reported allelopathic behaviour of *L. gibba* where it inhibited growth of some unicellular algae and blue-green algae through exudation. Others have claimed that *L. minor* produces anti-mosquito-larval compounds that can be exploited for public health benefit and can have commercial significance (Culley *et al*. 1981; FAO 2001). The compounds exuded by *L. minor* interfere with egg oviposition by *Culex pipiens pipiens* L., and is lethal to *Culex p. pipiens* larvae and *Aedes aegypti* L. (FAO 2001; Heidrich *et al*. 2004). These mosquitoes are a known vector of deadly human diseases such as malaria and yellow fever.

It is estimated that wastewater treatment systems coved by *Lemna* have up to 20% less evaporation compared to open wastewater systems because of the development of the 10 cm thick *Lemna* mat on the water body (Oron *et al*. 1986; Landesman 2000). The reduced evaporation in *Lemna*-covered wastewater treatment is an asset in arid climates. The *Lemna* mat also has ecological significance in the aquatic system. It can be used in biological control of algae bloom through reducing light penetration and nutrient competition (Körner and Vermaat 1998; Szabo *et al*. 1999; Parr *et al*. 2002). The outer margins of *Lemna* spp. fronds (phyllosphere) support dense populations of diatoms, green algae, rotifers, and bacteria (Coler and Gunner 1969; Zuberer 1982, 1984). Associated with this epiphytic community of *Lemna* spp. are an assortment of insects, including beetles, flies,

Table 6 Chemical fractions in *Lemna* sp. biomass. Modified from Landesman (2000) and FAO (2001).

	Chemical Composition (%age total dry biomass)
Dry matter	3.5
Crude protein	20-45
Crude fat	4.4
Fiber (cellulose)	8-10
Non-fibre carbohydrate	17.6
Ash	16.0

Table 7 Amino acid fractions in dried *Lemna* species. Modified from Landesman (2000) and FAO (2001).

	Content in dry biomass ($g\ kg^{-1}$)
Alanine	23.0
Arginine	21.4
Aspartic Acid	35.1
Cysteine	4.4
Glutamic Acid	36.7
Glycine	19.3
Histidine	7.3
Isoleucine	16.6
Leucine*	28.9
Lysine*	18.5
Methionine*	6.4
Ornithine	0.5
Phenylalanine	17.5
Proline	14.2
Serine	13.9
Taurine	0.3
Threonine	16.8
Tryptophan	4.0
Tyrosine	12.7
Valine*	21.2

* Essential amino acid

weevils, aphids, and water striders (Scotland 1940). The presence of *Lemna* spp. contributes dissolved organic matter into a water body in form of exudates and excrete like low organic acids (Baker and Farr 1987; Mkandawire and Dudel 2002; Mkandawire 2005), amino-acids and enzymes, and recalcitrant organic matter (Baker and Farr 1987; Thomas and Eaton 1996) and humic substances through humification of dead biomass which provide nutrients to other organisms such as bacteria, epiphytic algae, invertebrates and other microdetrivores (Baker and Farr 1987; Thomas and Eaton 1996). In addition cyanobacteria residing in the phyllosphere of *Lemna* spp. fronds and fix atmospheric nitrogen, providing nitrogen input in oligotrophic environments (Tran and Tiedje 1985; Cheng *et al*. 2002a, 2002b; Landesman *et al*. 2005).

A system with flexibility to manipulate

Significant progress has been made in engineering phytoremediation systems for higher capacity, efficiency and durability of fixation. The knowledge generated is being efficiently exploited in *Lemna*-based phytoremediation system that it is possible to engineer the system to enhance natural attenuation (Stahl and Swindoll 1999; Dudel *et al*. 2002, 2004; Mkandawire *et al*. 2004a). For instance, the mechanisms and processes involved in uptake and accumulation in *Lemna* are well described and a number of manipulations are currently possible. One way of manipulating the system is the facilitation of contaminant bioavailability to *Lemna* plants through the application of various acidifying agents, fertilizer salts and chelating materials. Reports in the literature show that the general phytoextraction of most metals is enhanced when acidifying agents are added to the media thereby increasing the bioavailability to *Lemna* (Kumar *et al*. 1995; Salt *et al*. 1995; Kayser *et al*. 2000; Lasat 2002; Watt *et al*. 2002). The retention of metals to sediment organic matter is also weaker at a low pH, resulting in more available metal in the water system for uptake. Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn (Maywald and Weigel 1997; Fargasova 2001; Abollinoa *et al*. 2002; Buykx *et al*. 2002). Polar and Kucukcezzar (1986) demonstrated that the amendment of culture medium with some metal chelators – ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N,N'-bis-(*o*-hydroxyphenylacetic acid) (EDDHA) or salicylic acid – significantly increased the uptake and accumulation of cadmium in *L. gibba*. When applied to soils, chelates accelerate the leaching of metals into groundwater. However, it may be an acceptable strategy in stagnant and slow flowing shallow surface water with a dense sediment layer because chelate would lenders metals bound in deeper water and sediments bioavailable to *Lemn*a plants in the up layers of water.

In metabolically active uptake, *L. minor* had reduces the elimination of Zn^{2+} by continuously increasing the degree of Fe^{3+} uptake (Cecal *et al*. 2002; Popa *et al*. 2006). Therefore, a system targeting Zn elimination can be manipulated by reducing content of Fe^{3+}. Further, *L. gibba* responds homeositically to chemical speciation changes in the media (Mkandawire and Dudel 2002; Mkandawire 2005). Hence, any substance that may influence the speciation of the media without significantly affecting the growth of *Lemna* can be used to manipulate uptake, accumulation, immobilisation and even degradation of the contaminants.

A *Lemna*-phytoremediation system can be regulated through resource stoichiometry. We also manipulated uranium and arsenic uptake and accumulation in *L. gibba* with specific resources – PO_4^{3-} and NH^{4+} – amended under field and laboratory conditions. An increase in the supply of PO_4^{3-} increased the accumulation of uranium in *L. gibba* but had the opposite effect on arsenic bioaccumulation. NH^{4+} increased bioaccumulation of both uranium and arsenic in *L. gibba* but lower than the effect of PO_4^{3-} (Mkandawire *et al*. 2004b, 2005c; Mkandawire 2005; Mkandawire and Dudel 2005c). In a related study we managed to regulate the accumulation of DOC and exudation of low-molecular weight organic acids by *L. gibba* with varying PO_4^{3-} supply. The organic acids were later found to be responsible for chemical speciation, which was crucial in determining uranium bioavailability, and consequently ecotoxicity effects. In a field study, we found that arsenic and uranium accumulation in *L. gibba* was highly influenced by water quality like water hardness, pH, presence of other metal cations and the amount of sulphates in the aquatic system. For instance, uptake of uranium was greatly reduced in high Mg^{2+}, Ca^{2+} and CO_3^{2-} , PO_4^{3-}, like high conductivity of the water while presence of SO_4^{2-} and lower pH favoured uptake of uranium (Mkandawire *et al*. 2004b, 2006, 2007). For arsenic, the higher the content of PO_4^{3-}, the lower the uptake and accumulation of arsenic (Mkandawire and Dudel 2005a). The reason behind this is ion competition, complexation of the contaminant and on top of all chemical speciation which result in contaminants being non-bioavailable. Hence, these factors can all be used to manipulate the *Lemna*-phytoremediation system to enhance natural attenuation.

The *Lemna* biomass can also be manipulated to increase biosorption capacity by protonation (i.e. application of acidifying agents that donate H^+ to the biomass). Biosorption processes are very important because they are the initial contact between the plant material and the contaminants in question. The biosorption of Cd, Cu, Ni, Pb, and Zn by *L. minor* biomass can increase by almost 20% with the application of 0.1 N HNO_3 (Palit *et al*. 1994; Cecal *et al*. 1999; Singh *et al*. 2000; Mkandawire *et al*. 2003). Protonated biomass is a more effective sorbent signifying that the complexation process contributes to the uptake mechanisms in *L. minor* while the presence of Mg^{2+} and Ca^{2+} reduces the biosorption capacity of uranium by *L. gibba* biomass (Mkandawire *et al*. 2003; Mkandawire 2005). Similarly, alkalinity reduces uranium biosorption. Phosphates reduce biosorption of arsenate in *L. gibba* biomass while increasing the uptake of uranium (Mkandawire *et al*. 2004b; Mkandawire 2005).

LIMITATIONS OF *LEMNA* SPP. AS A PHYTOREMEDIATION AGENT

Flexibility to external *milieu* influence

Most *Lemna* species are relatively insensitive to environmental changes like pH, temperature and availability of resources including photosynthetic active radiation and nutriaents in comparison with other higher plants. Despite the optima growth condition for Lemna spp as presented in **Table 8**, most *Lemna* spp. have been observed to stop growing in temperature below 6°C and above 30°C, and below pH 3 (Landolt 1980, 1986; Mkandawire 2005). However, they are sensitive to most organic contaminants especially herbicides and heavy metals under certain environmental conditions. Changes in most physicochemical conditions result in reduced growth due to adaptation through reallocation of resources (e.g. developing longer roots, and small leaflet in hard water and high conductivity conditions) (Mkandawire and Dudel 2005b; Mkandawire *et al*. 2005c, 2007). The uptake and accumulation of contaminants in a few *Lemna* spp. depends on the milieu's conditions. For instance, we found that the accumulation of uranium and arsenic in *L. gibba* were reduced significantly with increase of water hardness vis-à-vis concentration of Ca^{2+}, Mg^{2+} and CO_3^{2-}, and acidity

Table 1 A generalized set of conditions for culturing *L. gibba* and *L. minor*. Adopted from Mkandawire *et al*. (2005a).

	Optimal condition	Information source
Temperature (°C)	18-24 °C	ISO/WD 20079
Salinity ($g\ l^{-1}$)	20-24 $g\ l^{-1}$	ISO/WD 20079
Light intensity (lux)	4200 and 6700 lux	EPA712-C-96-156
	85-125 $\mu E\ m^{-2}\ s^{-1}$	OPPTS 850.4400
	or 400-700 nm	ISO/WD 20079
Photoperiod	14-16 hr d^{-1}	Mkandawire (2005)
pH	5.6-7.5	Mkandawire *et al*. (2002)

in the form of Fe ions (Mkandawire *et al*. 2004a; Mkandawire 2005). Further, SO_3^{2-}, PO_4^{3-} and NH_4^+/NO_3^- influenced both growth and bioaccumulation of both uranium and arsenic. Increasing electric conductivity and redox potential equally the amount of dissolved oxygen reduced biosorption potential and accumulation of uranium in *L. gibba* (Mkandawire *et al*. 2004a). In summary, the physicochemical conditions influence the interaction among the chemical constituents of the media, likewise the interaction with the biotic constituents. The interactions lead to chemical speciation and influence the bioavailability of the contaminants. They may also result in ion competition for uptake. Other works investigated the factors influencing the response of to Cd and its interaction with *L. minor* photosynthesis At molecular level, a few studies have shown that sulphate uptake and reduction are essential to detoxify Cd in *L. minor* (Charpentier *et al*. 1987; Steveninck *et al*. 1992; Saygideger 2000). The Cd interferes with photosynthesis processed in *L. minor*. Consequently, *L. minor* responses by increasing sulphate metabolism, which is also energetically very expensive and thus, requires an efficient and active photosynthesis. Yet sulphates are found in large amounts in most wastewater and natural water bodies, which may present problem to implement an efficient *Lemna*-based phytoremediation system, particularly in large water bodies where: (1) *Lemna* spp. are only used to enhance natural attenuation, and (2) manipulation of the milieu's condition for optimal performance may be impossible due to the size of the water body requiring remediation, or it may be very costly.

Anatomical and physiological restrictions

One of the limitations of *Lemna*-based treatment systems is related to the size and existence as free-floating macrophytes (Landolt 1980, 1986; Landolt and Kandeler 1987). Even though *Lemna* form mats of about 10 cm above the water, their direct influence on contaminants like uptake, transformation or immobilisation with exudates or transformation only at the surface may be limited to a few centimetres deep (Amado *et al*. 1980; Boniardi *et al*. 1994; Lemon and Posluszny 2000; Les *et al*. 2002; Mazen *et al*. 2004). Hence, the influence in a few metres deep stagnant water ponds devoid of mixing should be very low. However, there may be the question of fibre contribution to sedimentation and humification of dying *Lemna* biomass to the deeper waters, discussed in detail below. Their small size results also in a high water volume to plant biomass ratio (Mkandawire 2005). This entails that the high accumulation of metals in the *Lemna* plant may not really have significance in removal or immobilisation of contaminants. Anatomically, *Lemna* are reduced diffuse plants without a vascular system (vessels in roots and shoots) (Lemon and Posluszny 2000; Les *et al*. 2002). Therefore, the plants are less advantaged in utilisation of solar-driven apoplastic flow of water into the plant together with contamination, but contaminants left by evapotranspiration accumulated in the plants is missing in the *Lemna* plant system. Therefore, most of the accumulation may be metabolically driven with energy consumption.

The biomass yield is considerably reduced with is own plant density. There is a minimum biomass at which yield decreases and an upper biomass where yield is limited by crowding. The influence of *Lemna* species in shallow ponds may require large areas that may not be available near urban areas. Thus harvesting of excess biomass is a must, yet the water volume to *Lemna* biomass ratio should be reduced in order to optimise its phytoextraction potential. Further, in temperate climates *Lemna* spp. grows slowly in the winter. This may restrict the use of such treatment systems in cooler climates particularly when applied in large constructed wetland. Water temperatures of about 11°C were recorded in winter in a *Lemna*-covered wetland tailing pond of a former uranium mine in eastern Germany while the atmospheric temperatures were below zero (Dudel *et al*. 2004; Mkandawire *et al*. 2004c). The observation suggests that in small constructed wetland or wastewater treatment, the *Lemna* mat covering small ponds function as an insulator and keep the water temperatures high. Nonetheless, *Lemna*-based treatment systems may be limited to treating secondary effluents from small communities where land costs are low (Cheng *et al*. 2002a).

Wet to dry biomass ratio

Several studies have shown that most *Lemna* species retain less than 3% of their weight biomass after drying (Landesman 2000; Mkandawire 2005). This means that the highest percentage of *Lemna* content is water just like many other aquatic emersed and even submersed macrophytes and algae. Therefore, the high bioaccumulation of metals and metalloids reported in the literature is attributed to the loss of water which leaves the contaminant to *Lemna* biomass ratio very high. That means, considering the wet biomass of *Lemna*, that high accumulations are very legible. Consequently, using *Lemna* for phytoremediation will require excessive high dry biomass production. Further, removal of water from *Lemna* in order to concentrate-up the contaminants requires much energy and technical equipment which may render the system less economic and limit the using *Lemna* as a phytoremediation agent.

Carbon sequestration into sediments

The fate of contaminants, especially metals and metalloid complexes on or accumulated in organic matter in the course of litter deposition and sedimentation, is decisive for plant-mediated elimination of contaminants from the water pathway. Dead and dying *Lemna* spp. fronds (Laube and Wohler 1973), specifically *L. gibba* (Szabo *et al*. 2000) fall to the bottom of the water column where their decay contributes organic matter, nitrogen, phosphorus, and other minerals to the benthos. Logically, the organic matter should later be part of recalcitrant organic carbon (e.g. humic matter after humification), thereby containing most contaminants, especially metals, bound to the organic matter fraction of decaying *Lemna* biomass. This is very important when the aim of using *Lemna* spp. is to stabilise or enhance natural attenuation, and not extraction of the contaminants.

In a study of two separate ponds in an abandoned uranium mine, we found that ponds covered by a thick *Lemna* mat had significantly organic carbon accumulation but low humic substance in the sediments compared to ponds dominated by halophyte communities with *Typhus latifolia* and *Phragmites australis*. The obvious reason was the size of the *Lemna* spp., crowned by the big ratio between dry and weight biomass. *Lemna* spp. are blown or swept away easily from the water surface by strong winds or flowing water, respectively. In a wastewater treatment pond, floating mats of fronds are held in place by partitions and baffles that prevent or reduce wind from blowing fronds off the water surface. These partitions and baffles are usually made of polyethylene in industrialised countries but may be made of bamboo or other materials in developing countries which may be costly, on one hand, and on the other, difficult to implement in natural systems.

This explains our finding that showed lower uranium deposition in the organic carbon-rich fraction of sediment cores from *Lemna*-covered rather than communities of halophyte-dominated ponds by Typhus latifolia in the same site. Generally, the layers of sediments rich in organic matter accumulated the highest uranium (Dudel *et al*. 2004). Neither we nor others in the literature have managed to establish the fraction of heavy metals (e.g. uranium, radium, etc.) that sediment together with recalcitrant organic carbon (e.g. lingocelluloses). Similarly, quantification of fixed heavy metals in the course of *Lemna* litter decay or originated in the course of microbial carbon mineralisation of dead *Lemna* biomass has never been tackled in literature. Thus, this sets limits in durability and permanent sink of metal contaminants in a *Lemna*-based phytoremediation system which defeats the reasons for using phytoremediation as an alternative conventional remediation technology.

Szabo *et al*. (1999, 2000) demonstrated that the nutrient flux from decomposing *Lemna* litter is mainly a microbial-mediated process. They found that organic matter in *L. gibba* litter lost about half its weight more rapidly in the presence of micro-organisms than in axenic vessels. In the case of axenic conditions, the C and K concentration of the remaining Lemna litter decreased; while the N, Ca, Fe and B concentration increased. The concentration of total N, P, K, Mg, and Mo increased in the receiving water. Mass balances of nutrients in the vessels and flux of these nutrients between *Lemna* litter, water and sediment compartments showed leaching of organic potassium and magnesium during the first term of incubation and then slowed down. Under biotic decomposition, the elemental content of the litter decreased in rates of K> Mo> C> Mg> S> P> Na> N> B in the course of the four month experiment. Calcium and iron immobilised in the litter. Most of the released N, S, P, K, Mg and Mo remained in the water, but B and Mn settled into the sediment. Therefore, application of *Lemna*-based phytoremediation requires removal or harvesting of biomass before death. This limits the use of *Lemna* in wastewater treatment plants only because harvesting or removal would require high amount of resources to be applied in a natural eutrophicated water body.

NEW PERSPECTIVE FOR PHYTOREMEDIATION STUDIES WITH *LEMNA* SPP.

Despite the limitations, the potency of *Lemna* spp. make this macrophyte an attractive phytoremediation agent worth further studies and application trials, mainly in the enhancement of natural attenuation and phytoextraction. Therefore, it is imperative to study *Lemna* spp. further in the context of phytoremediation. The studies should focus on the mechanisms and processes that enable the macrophytes to tolerate and detoxify multiple aquatic contaminants beyond what is currently known. This would require identification of metabolic pathways and genes involved in both toxicity tolerance mechanism and remediation processes. For instance, *Lemna* has been applied in decontamination of xenobiotic herbicides, applied against vascular plants. What makes *Lemna* exceptional to withstand herbicides is not clear, hence this needs further investigations. Recent advances in knowledge derived from the "omics" need to be considered high in *Lemna* phytoremediation studies because, there is considerable potential in developing this green technology using genomics and proteomics. However, strategies to produce genetically altered *Lemna* spp. to remove, destroy or sequester toxic substances and the long-term implications have either not been investigated thoroughly or not reported much. There is also a need for better knowledge of the processes that affect pollutant availability to *Lemna* and how the plants regulate the processes. This should include investigations on rhizospheric processes, pollutant uptake mechanisms, translocation, chelation, degradation, and volatilization. Currently, the influence of the *Lemna*-microflora consortium on pollutant detoxification, decay of organic polymers, or change of chemical speciation and removal of non-degradable contaminants like heavy metals from aquatic systems has been less exploited. Influence of microbial inoculation in *Lemna* culture on the phytoremediation potential requires more insight. Last but not least, selection of clones or strains of *Lemna* for phytoremediation is also an area less reported. It requires more insight.

CONCLUDING REMARKS

With respect to tolerance, bioaccumulation and biotransformation potential and biomass productivity, *Lemna* species have importance in the treatment of domestic and industrial wastewater and effluents as well as in the restoration of decommissioned mining sites. They have most of the properties of an ideal phytoremediation species. Hence, they can be part of constructed wetland systems, either as a component of a wetland receiving wastewater or as plants that polish nutrients from wetland-treated water. The potential of *Lemna* spp. as a phytoremediation agent are strengthened further by the ability to be applied to multiple pollutants and possession of other environmental benefits. The literature has clearly documented the use of *Lemna* spp. to major pollutants from domestic wastewater (e.g. DOC, and excess nutrients). They are confirmed to be good accumulators and potential hyperaccumulators for many metals including the widely reported Cu, Cr, Cd, Ni, Pb, U, As and Zn as well as ^{137}Cs, and ^{90}Sr. Some *Lemna* species, particularly *L. gibba* and *L. minor* have shown the ability to phytotransform some persistent organic pollutants (POP) (e.g., chlorophenols used in a variety of biocides, dichlordiphenyltrichlorethan DDT, organophosphorus (OP) pesticides including Malathion, demeton-*S*-methyl, and crufomate). Further, *Lemna* species have more environmental benefits than mere phytoremediation like reduction of evaporation of *Lemna*-covered surfaces in wastewater treatment, which is an asset in arid climates. *Lemna* cover can control growth of algae, and breeding of mosquitoes which is of public health signifycance and may provide a source of mosquito anti-larval compounds that could have commercial significance. *Lemna* spp. are already known and widely used as a protein source in aquaculture and livestock production. However, they are very limited in application because of their nature as small and free floating plants. The water volume to *Lemna* biomass ratio is always small. Therefore, *Lemna* spp. are confined to application for special remediation purposes and conditions only. The use of a *Lemna*-based phytoremediation system should be carefully tested before application.

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