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Response of duckweed to lead exposure: phytomining, bioindicators and bioremediation

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ABSTRACT

The ability of aquatic macrophytes to bioaccumulate toxic metals relative to the concentrations of these metals in wastewater has led to their use as phytoremediators. Lead (Pb) is among the most serious environmental contaminants. This study assesses the gibbous duckweed (*Lemna gibba* L.) as a bioaccumulator and bioindicator of Pb pollution. The plant recovery from a 12-d exposure period in terms of re-releases of Pb from its tissues, and recovery of pigmentation was monitored. Duckweed was exposed to Pb-contaminated water by adding $PbCO_3$ at concentrations from 10 to 100 mg/L. At 2-d intervals, bioaccumulation, contaminant removal efficiency, pigment content, and bleaching were assessed. The efficiency of Pb removal after 12 d reached nearly 50% at the lowest Pb treatment (10 mg/L), but decreased at higher levels of Pb up to 100 mg/L. The highest bioconcentration factors (BCF) were achieved at low Pb treatment of 10 mg/L, which increased from nearly 200 mg/L after 2 d, to 943 mg/L after 12 d of exposure. Recovery from bleaching was around 50% for all photosynthetic pigments in plants exposed to 10–40 mg/L concentrations of Pb. The response of duckweed to Pb treatment and recovery from stress suggest its possible use as biosensor or biomonitor of Pb pollution, considering that active uptake, rather than low concentration gradient, is driving the absorption of Pb from the water medium.

Keywords: *Lemna gibba*; Bioaccumulation; Removal efficiency; Photosynthetic pigments; Bleaching; Phytoremediation

1. Introduction

Heavy metals, here after "toxic metals", unlike organic pollutants, are elements and therefore not typically degraded by chemical or biological processes. They persist in the environment and pose a serious health hazard for living organisms including humans [1]. Lead (Pb) is one of the most abundant, ubiquitously distributed toxic metals that can cause major damage to biota [2]. Air, water, and food; dust; and soil can all be contaminated as a result of mining and smelting activities, solder, lead-containing paints, paper and pulp manufacturing, leaded gasoline, and explosives as well as from landfill leachate including old batteries and the disposal of municipal sewage sludge enriched with Pb [3]. The exposure to Pb is a serious health hazard that can result in damage to neurons; it is especially dangerous for babies, infants, and pregnant females. Many of the detrimental effects of lead are due to the tendency of Pb^{++} ions to substitute for Ca^{++} ions in biological systems. According to the World Health Organization [4], the maximum acceptable concentration (MAC) of Pb in drinking water, measured at the tap, is 0.010 mg/L (i.e., 10 parts per billion, or 10 mg/L). Traditional technologies such as chemical

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reduction, precipitation, and ion exchange to remove toxic metals from wastewater are often ineffective or very expensive and are significantly affected by changes in the pH and alkalinity of the water [1].

The ability of some plants to tolerate and even accumulate metals offers new strategies for the treatment of soils and wastewater through phytoremediation [5]. This offers an eco-friendly cost-effective technology not just for removal of toxic metals but also for removal of other contaminants. The advantages of phytoremediation over traditional treatments include lower cost, ease of monitoring plants, and the possibility of the recovery and re-use of valuable metals by "phytomining" [1,6]. Depending on whether they are floating, rooted, or submersed, aquatic plants may be able to accumulate contaminants either from the water medium, or from the sediments below or from both [7,8].

Generally, plants species are selected for phytoremediation based on their potential to accumulate and bioconcentrate metals, their growth and yield, and the depth of their rooting zone. Rhizosphere microorganisms in the rooting zone may play an important role in contaminant uptake, and in the case of organic contaminants, in the breakdown of these materials [9]. Plants have evolved a variety of defenses in response to toxic metals. In the case of Pb, once ions enter the plant cytosol, chemical chelators such as proteins (e.g., heat shock proteins), peptides (e.g., metallothioneins), ligands (e.g., glutathione), proline, and polyamines can bind to the metal and reduce its toxicity [10,11].

Earlier studies have shown the potential of aquatic plants in phytoremediation and monitoring of pollution levels in wastewater [8,12–16]. Duckweed has been used as an ecological and early warning indicator of the phytotoxicity of pollutants worldwide [17,18]. Some duckweed species, such as *Lemna gibba* L., *Lemna minor* L. and *Spirodela polyrhiza* L. (Lemnaceae), have received particular attention because of their rapid clonal growth and easy propagation, and demonstrated ability to remove certain pollutants, such as toxic metals and metalloids from water [15,19].

In wastewater treatment plants, episodes of intense exposure may be followed by periods of relatively low or no exposure to contaminants, allowing exposed organisms to recover from toxic injury [20]. Recently, it has been suggested that the concept of eco-toxicological risk assessment should be modified towards greater attention to "vulnerability analysis", combining susceptibility to exposure, sensitivity to stressor(s), and ability to recover from that exposure [21]. Later, the US Environmental Protection Agency (US EPA) guidelines [22] for ecological risk assessment proposed that not only the nature and intensity of potential effects and their spatial and temporal scales be considered but also the potential for system recovery should be incorporated in the assessment. In most cases, information on the dynamics of toxicity and the recovery potential or organisms following metal exposure is not available. The intent of the present study of *L. gibba* was to: (a) determine the ability of *L. gibba* to accumulate lead from water under laboratory conditions; (b) characterize the physiological response of plants to Pb accumulation; (c) track the process of recovery; and (d) assess the potential of *L. gibba* as an indicator of levels of Pb contamination.

2. Materials and methods

2.1. Study species

The gibbous, or "swollen" duckweed, *L. gibba*, is found in Mediterranean and warm temperate climates, and tropical mountain zones on all continents except Australia. It is a small freshwater floating macrophyte in the Araceae associated with ponds and lentic (slow-moving) water, becoming abundant, largely through vegetative propagation in nutrient-rich or eutrophic conditions. Individual plants are tiny and consist of just two leaf-like structures (fronds) connected to a fine rootlet [22]. The species is commonly used for wastewater treatment in the Mediterranean climate [24] and is an attractive candidate for phytoremediation of contaminated water due to its tolerance of a wide range of temperature, pH, and nutrient levels [25]. Duckweed also has a low fibre (5%) and high protein (10%–40%) content, which makes it a valuable food source for fish and other animals. Due to the surface cover they provide duckweed-based treatment systems typically evaporate some 20% less water than other open treatment systems do, such as waste stabilization ponds [25].

2.2. Experimental procedure

Duckweed and water were collected from the lake of Ghadir El-Banat dam, Taif, Saudi Arabia. The Pb level in the lake water was 9.3 μg/L, which is below WHO limit for drinking water (10 μg/L). Duckweed plants were collected by mesh and placed in open containers containing water taken from the lake. The containers were transferred to the laboratory for the experimentation. The Canadian phycological culture collection (CPCC), formerly known as University of Toronto culture collection (UTCC) formulation of Hoagland's solution plus ethylenediamine tetraacetic acid (EDTA)-chelated iron was used as nutrient medium. Three replicate experimental containers were set up with six levels of added dissolved lead, as Pb carbonate ($PbCO_3$) at 10, 20, 40, 60, 80, and 100 mg/L (corresponding to 10, 20, 40, 60, 80, and 100 ppm, respectively). The initial values of Pb in the water and plants, and the bioconcentration factor (BCF), which is calculated as the concentration of Pb in plant tissues divided by the concentration of Pb remaining in the water column) were determined at 2-d intervals over a period of 12 d. Light was provided by four fluorescent tubes (50 cm length) hanged 30 cm above the growth containers. Light intensity at the water surface was 6,300 Lux, which was provided for 12-h day length and turned off at night. The pH of the growth medium ranged between 5.9 ± 0.3 and 6.2 ± 0.2 (pH ± SE) during the experiment duration.

Recovery was assessed by taking plants that had been exposed to contaminated water at various lead concentrations, for 12 d, and placing them in distilled water for 12 more days to wash out any bound Pb to the cell surface. This was intended to simulate the episodic exposure of plants to Pb that might occur in a water treatment system. Percent recovery was calculated as a relative measure of release of absorbed Pb after 12 d in distilled water, that is, as (Pb concentration in plants after 12 more days in clean water)/(Pb concentration following 12 d of exposure to the treatment – original concentration of Pb in the plant tissues) × 100. In each case (at each contaminant concentration), five replicate plants were used to determine recovery.

The physiological response of plants can be assessed in terms of such variables as stunted growth, blackening of the root systems, and loss of chlorophyll (bleaching). In the present study, plant health was assessed in terms of plant colour, measured as the total concentration in mg/g of chlorophyll-a, chlorophyll-b, and the other accessory pigments, grouped as carotenoids. Again that was done for plants in the six levels of added Pb, ranging from 10 to 100 mg/L, and at each of six, 2-d intervals from 2–12 d after exposure began. The restoration of pigment following 12 d in distilled water after the 12-d exposure period was also assessed in order to evaluate the ability of plants to recover from different levels of exposure to dissolved Pb.

2.3. Measurement of Pb and pigments

The Pb concentrations in water and in acid digested plant tissues were measured using the Varian inductively coupled plasma atomic emission spectroscopy [15]. The BCF was calculated as the ratio of [Pb] in plant tissue compared with that in water. Pigments (chlorophylls a and b and carotenes) were extracted in 80% acetone and measured with a spectrophotometer at λ = 470, 646.6, and 663.6 nm, respectively [26]. Pb concentrations were measured by atomic absorption spectrophotometry expressed in mg/L in the case of water samples, and mg/kg in plant tissues [15]. Concentrations of pigments were expressed in terms of mg/g dry mass. Data were calculated and processed using statistical package for social sciences (SPSS) for windows.

3. Results

3.1. Bioaccumulation and BCF

The lowest level of Pb added (at 10 mg/L) to the natural water used in the untreated control was over 1,000 times this value. The additional contribution of the natural water used in the study was negligible relative to the treatments, which ranged from 1,000 to 10,000 times background. The concentration of Pb remaining in the water in each treatment tank over the 12-d study is shown in Fig. 1(a). Accumulation of Pb in *L. gibba* is shown in Fig. 1(b). In general, Pb accumulation in *L. gibba* increased with increasing levels of Pb in the water. After only 2 d of exposure, tissues of *L. gibba* biomass had accumulated 1.82, 3.71, and 3.91 mg/kg in media dosed with 10, 20, and 40 mg/L of Pb, respectively. The Pb uptake levelled off at the higher levels of exposure (60, 80, and 100 mg/L). As a result the BCF ranged from 195 to 943 at lower levels of exposure (10 and 20 mg/L).

Tracking the process of uptake over time provided a useful measure of how long the plants would take to accumulate and remove the metal from water. At all levels of exposure, high rates of bioaccumulation occurred in the first 2–4 d (Fig. 1(c)). The efficiency of BCF attained high values at low levels of Pb at 10 mg/L treatment and decreased with the increased levels of exposure. The high concentrations of Pb in plant tissues reached 5.22 mg/kg that were found in plants exposed to 40 mg/L of Pb for 12 d.

3.2. Photosynthetic pigments

In comparison with plants that were not exposed to increased Pb treatment, pigment concentrations in *L. gibba* declined with the increasing levels and duration of exposure (Fig. 2). The most severe effect was seen after 12 d of exposure, in the most concentrated Pb treatment (100 mg/L). The values measured for chlorophyll-a, chlorophyll-b, and carotenoids were 6.38, 7.31, and 7.51 mg/g fresh weight, respectively.

3.3. Recovery after exposure

The recovery potential of *L. gibba* after 12 d of exposure to different Pb concentrations was assessed by transferring the exposed plants to untreated water for a further 12 d. The recovery potential of *L. gibba* from Pb exposure, in terms of re-release of lead from their tissues, reached at 57.78%, in plants exposed to 10 mg/L Pb (Fig. 3). The level of re-release decreased in plants exposed to high Pb concentrations, reaching a recovery rate of 40.76% for plants exposed to 100 mg/L of Pb.

During the 12-d recovery period in untreated water, the pigment content of *L. gibba* fronds was determined to assess their ability to recover from bleaching (Fig. 4). Plants exposed to low levels of Pb was recovered in terms of pigment content, with those experiencing 20 mg/L recovering 59.06%, 78.67%, and 53.63% of their original levels of chlorophyll-a; chlorophyll-b, and carotenoids, respectively. When plants exposed to 100 mg/L Pb concentrations, their recovery was 4.7% for chlorophylls a and b, and 2.25% for carotenoids.

4. Discussion

4.1. Bioaccumulation and BCF

Duckweed was proved to be effective bioconcentrator of Pb from the water medium. At all levels of added Pb, early uptake is rapid, reaching removal of, on average, 4.25 mg/L in all treatments up to 100 mg/L after 12-d duration. This represents almost half of the Pb in solution at the lowest treatment (10 mg/L) but only 3.4% of the Pb in solution at the highest Pb treatment (100 mg/L). This pattern suggests that *L. gibba* is the most efficient as a bioremediator when exposure levels are lower (10 mg/L or less), and that these plants can only remove a maximum of about 4.25 mg/L of Pb independent of the concentration of Pb in the surrounding water. This may reflect saturation of active sites where Pb can be immobilized [27]. In a similar study it was found that uptake of zinc by *L. gibba* was most efficient when zinc concentrations in water were low, but the efficiency of removal decreased somewhat at higher concentrations [28]. In the present study the duration of exposure affected the rate of absorption in that the concentration of Pb in *L. gibba* tissues increased with time for plants exposed to 10, 20, 40, and 60 mg/L, but declined after 6 d at 80 mg/L, and after 2 d at 100 mg/L. Similarly, the rate of accumulation of silver and gold ions in *L. gibba* showed a significant decrease after 6 d of exposure because the metals reached saturation levels in the plant tissues [29]. Earlier studies of Pb uptake in *L. gibba* suggest saturation is achieved after 5 d of exposure [30]. The process of Pb uptake in *L. gibba* growing in mine effluent seems to involve mass flow into the plant tissues until they are saturated with Pb absorbed from the water column; this may occur either via apoplastic or symplastic pathways and involve either active or passive diffusion [19]. Fig. 1(b) showing Pb concentration in plant tissues suggests that final uptake is high and more rapid at

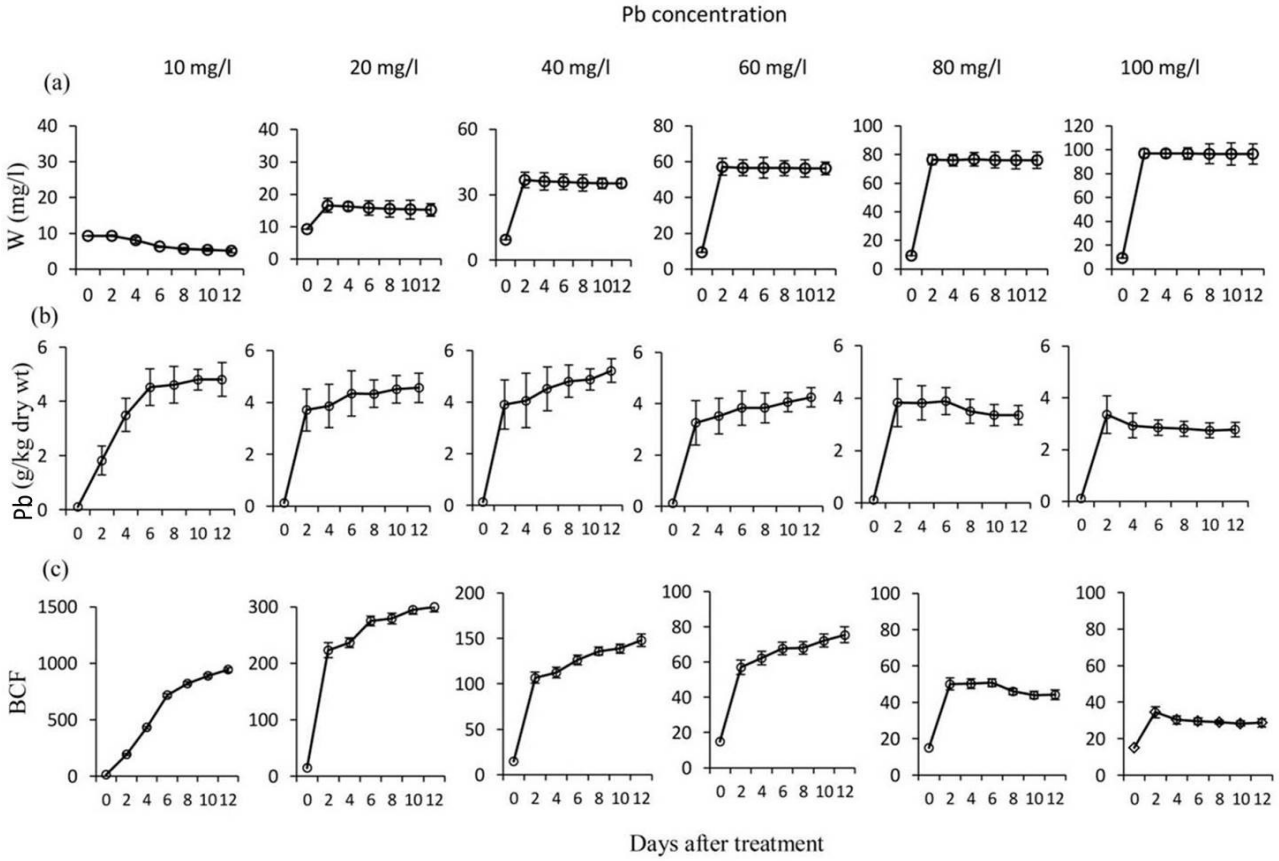

Fig. 1. (a) Changes in lead concentration in water (W) over 12 d of exposure; (b) lead concentration in plants (Pb); and (c) bioconcentration factor (BCF) under contrasting lead treatments. 0 = baseline value. Error bars represent the standard error of the mean (±SE).

low concentrations of Pb treatments. This suggests that active uptake, rather than a low concentration gradient, is driving the absorption of Pb from the water medium.

The BCF can be used as an indicator of the efficiency of contaminant removal in *L. gibba.* This decreased with increasing Pb exposure (Fig. 1(c)). Other studies have reported similar results for both lead and other toxic metals [19,31,32]. The BCF value of the related species, *L. minor*, decreased significantly with exposure to increasing concentrations of chromium [1]. In the present study the BCF increased over time when plants were exposed to 10, 20, 40, and 60 mg/L of lead, but when plants were exposed to 80 and 100 mg/L, the BCF decreased after 2 d of exposure, suggesting damage to the plants. The BCF is a more useful metric of the effectiveness of plants in removing contaminants since it provides an index of the ability of the plants to accumulate the particular contaminant relative to its concentration in the surrounding water [33]. For a plant to be regarded as an effective bioaccumulator and good candidate for phytoremediation, it needs to show a BCF >1,000 [33]. In the present study, *L. gibba* achieved that level of Pb accumulation after 12-d exposure at 10 mg/L of Pb treatment. However, it became progressively less effective at high levels of Pb pollution. In comparison with other studies, the levels of Pb found in Pb/zinc mine wastes in Ireland reached 0.15 mg/L [34]; the most severe levels of Pb detected in homes in Flint, Michigan, reached 12 mg/L [35], that is, in the order of the lowest Pb concentration applied in this study. In an earlier study of industrial wastewater in Sadat city, Egypt, we found Pb concentrations of 20.4 mg/L (0.0204 mg/L), which is about twice the WHO standard for maximum acceptable concentration (MAC) of 0.01 mg/L [4,15]. Based on our results we conclude that *L. gibba* is an effective and useful bioaccumulator of Pb having significant potential for phytoremediation where Pb is present in concentrations up to 10 mg/L, which is about 1,000 times the WHO standard for drinking water, and may be accepted for the range normally encountered in industrial and mine wastewater.

4.2. Photosynthetic pigments

The change in levels of photosynthetic pigments is likely to negatively affect the entire metabolism of the plant [36]. In the present study, increased exposure to Pb over time resulted in negative effects on pigment concentrations in *L. gibba* tissues. Pigment breakdown was responsible for chlorosis in the aquatic plants, *Ceratophyllum demersum* and *Myriophyllum spicatum* [27]. Toxic metal exposure is often associated with degradation of chlorophyll and evidence of disrupted chloroplast ultrastructure resulting in reduced chlorophyll content and a lower photosynthetic rate [15]. Many authors report that Pb exposure inhibits

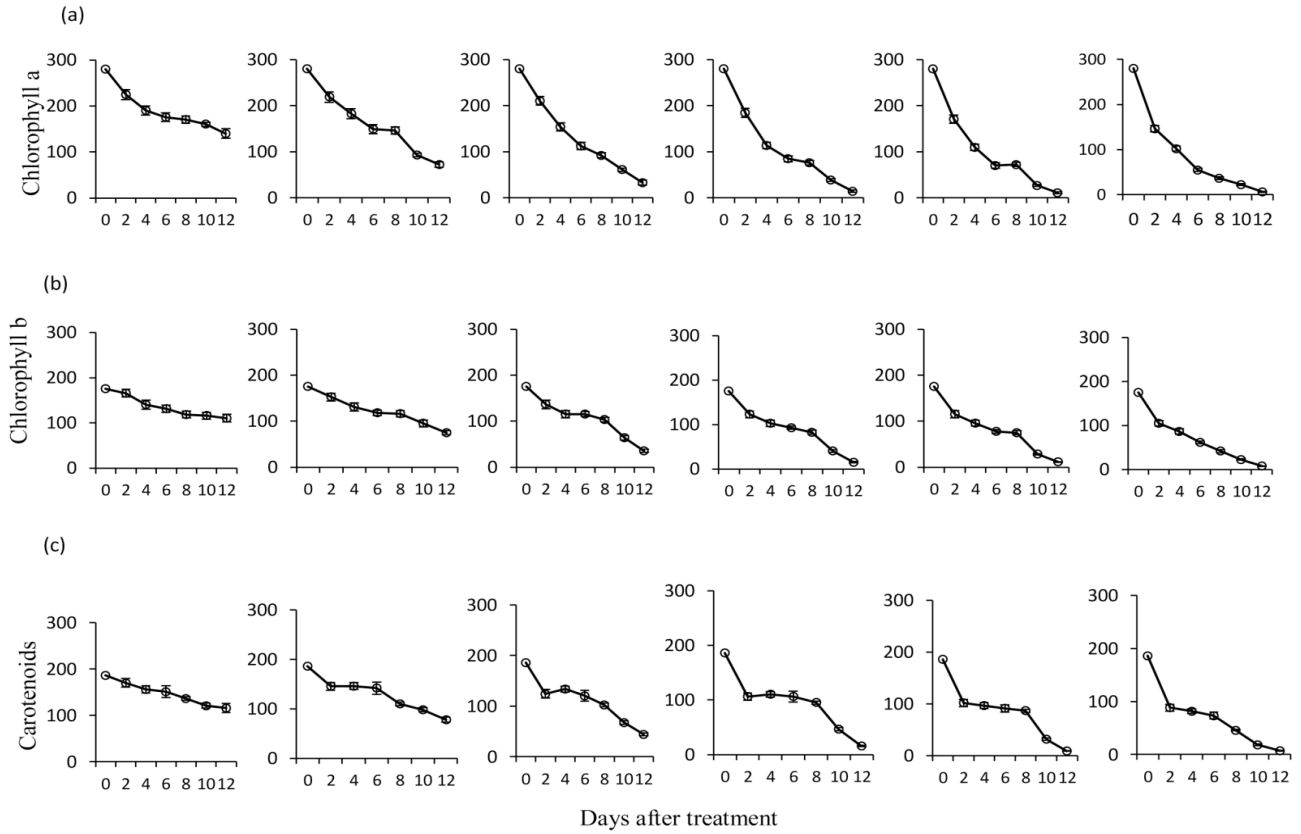

Fig. 2. Changes in pigment content (mg/g fresh mass) of plants exposed to varying levels of lead, tracked over 12 d of exposure. Pigments are shown, in sequence, as: (a) chlorophyll-a; (b) chlorophyll-b, and (c) carotenoids (mg/g fresh mass). Error bars represent the standard error of the mean (±SE).

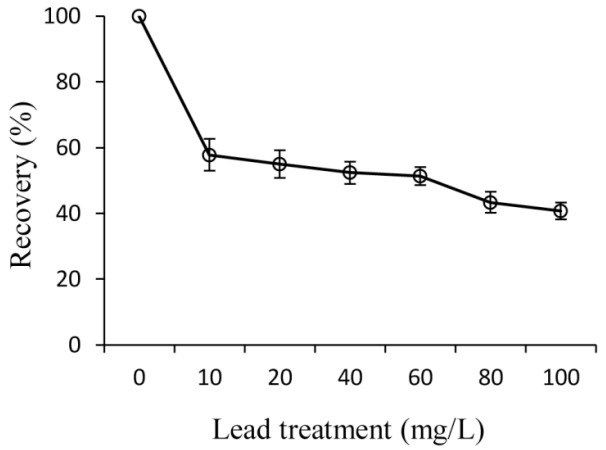

Fig. 3. Relative recovery from lead exposure (re-release of absorbed lead after 12 d of exposure, followed by 12 d in clean water). The first point, at 100%, represents the baseline level of lead in plants growing in distilled water; contrasting (prior) lead treatment levels are shown on the *X*-axis. Error bars represent the standard error of the mean (±SE).

chlorophyll biosynthesis and disrupts its structure, leading to bleaching of photosynthetic pigments [15,27,37,38]. Clearly, changes in chlorophyll concentrations, evident as bleaching following exposure to Pb, may provide a simple, visible biomarker of exposure in a biomonitoring program. In particular, fluorescence of chlorophyll-a and chlorophyll-b was identified as a sensitive biomarker of plant exposure to toxic metals [39,40].

A similar response related to the decrease in carotenoid concentrations was observed in *Ceratophyllum demersum* growing under toxic metal stress [10,27]. Toxic metals cause a reduction in chlorophyll, and this is accompanied by an increase in peroxidase activity and the depletion of other antioxidants such as carotenoids [41]. It is known that carotenoids protect photosystems from oxidative stress as, in addition to being accessory pigments for photosynthesis, they act as reactive oxygen species scavengers (ROS). Under intense toxic metal stress, the imbalance between carotenoid production and carotenoid oxidation, due to ROS activity, results in a decreased carotenoid content. Overall, the destruction of photosynthetic pigments by toxic metals may be due to interference with the electron transport chain, whereby mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecules are displaced, and reactive oxygen species cause inhibition of important enzymes associated with chlorophyll biosynthesis or peroxidation processes in the lipids of the chloroplast membrane [42].

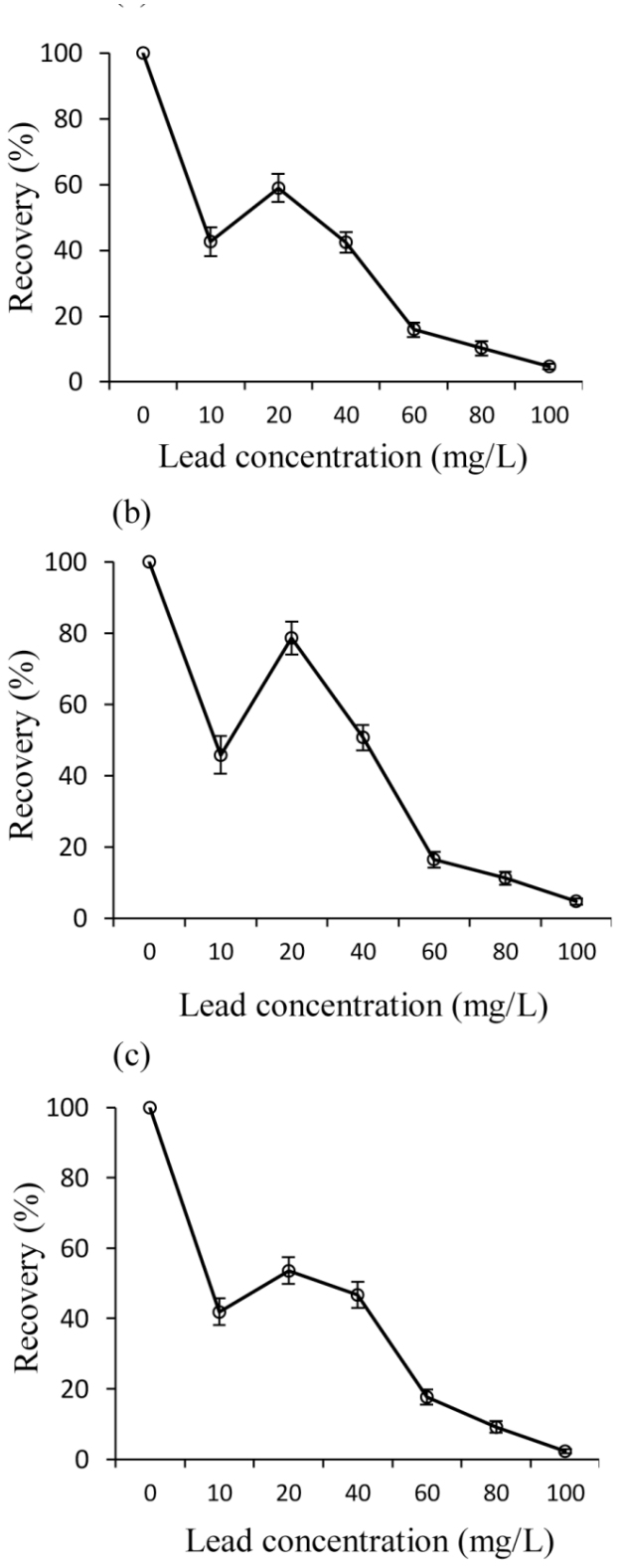

Fig. 4. Relative recovery of pigment intensity following lead exposure (after 12 d of exposure, followed by 12 d in distilled water). The first point, at 100%, represents full pigmentation; contrasting (prior) lead treatment levels are shown on the X-axis. Error bars represent the standard error of the mean (±SE): (a) changes in chlorophyll-a; (b) chlorophyll-b, and (c) carotenoids.

4.3. Recovery pattern

Because contaminants often enter a watercourse intermittently, rather than steadily, at a single concentration, it is useful while investigating plants as bioremediators or bioindicators, to assess their ability to survive, and recover from periods of exposure. In the present study, following 12 d of exposure to a given concentration of Pb, *L. gibba* plants placed in distilled water for a further 12 d were able to re-release about half of the previously absorbed Pb. Rather, they can be re-released to the environment. Similar effects were reported for many toxic metals during the recovery phase [20]. This suggests that the absorbed Pb from the water during the exposure period is not irreversibly bound to any cell structure such as cell walls as suggested elsewhere [20]. In principle, two processes may be responsible for the decrease in tissue concentrations of toxic metals: (a) active or passive excretion of the toxic metal from the plant and (b) the effective "dilution" of tissue concentrations of Pb due to the increase in total biomass as plants adjust to growth in distilled water [20].

During recovery, pigment concentrations was higher in plants exposed to low Pb concentration up to 20 mg/L than in the plants exposed to higher concentrations. The plants exposed to high levels of Pb of 60, 80, and 100 mg/L showed lower levels of pigment recovery. This suggests that some irreversible damage may have occurred to chloroplasts or to enzymes involved in chlorophyll and carotene biosynthesis at the high Pb concentrations. These findings suggest that phytotoxicity induced by exposure to lower Pb concentrations is reversible, but damage from higher Pb exposure is not reversible.

In other studies, when *L. gibba* was treated with the herbicide atrazine, the bleaching of photosynthetic pigments was reversible [43]. Similarly, bleaching in the algal species *Chlorella fusca* was shown to be a temporary effect of nitrogen starvation [15]. The ability of *L. gibba* to respond to short-term variation and pulses of exposure to Pb can be an asset for phytoremediation and harvest of contaminants from wastewater, or used to reduce the extremes of variability in Pb levels in water discharged from the industrial systems.

4.4. Potential use of duckweed as bioindicator

Bioindicator species can provide information on the quality of the environment when they are calibrated against known levels of contamination [19,44]. In the present study, *L. gibba* accumulated high amounts of Pb and showed potential as a phytotoremediator of Pb in aquatic bodies. The observed physiological changes in duckweed are reflected in the simple characteristic of colour, such as bleaching in response to Pb exposure. It could therefore be calibrated to provide a fairly effective indicator of the level of Pb contamination of mine or industrial waste streams, urban wastes, or other contaminated aquatic systems. This biological approach offers a simple, readily applied measure of pollution that can be used for routine monitoring, without the high costs of chemical analyses [19,23]. It is still valuable to verify the accuracy of bioindicators from time to time, and to calibrate plant colour or growth against measured contaminant concentrations, as plants may adapt to persistent contaminant exposure.

A significant attraction of this method, however, is the fact that local residents can readily be trained to monitor contaminants, and draw the attention of water protection agencies and local government staff to any unusual observations. Some additional biomarkers besides the colour of fronds of *L. gibba* may be useful indicators of contaminated waters. For example, levels of antioxidative enzymes such as peroxidase, chlorophyll, and soluble protein content could be assessed to provide a more complete eco-toxicological risk assessment, in addition to other potential ecological indicator species.

5. Conclusions

Our results show that *L. gibba* is an effective accumulator of Pb in the water medium, and that this species is able to recover from that exposure in terms of re-release of previously accumulated Pb, and recovery of photosynthetic pigments provided exposure levels do not exceed 40 mg/L. It therefore can readily be used as a bioaccumulator and sensitive bioindicator for Pb. The phenomenon of pigment bleaching can readily be calibrated against a standardized colour chart such as the Munsell Plant Tissue Colour charts (http://www.pantone.com/munsell-plant-tissue-color-charts), and used in the field with the appropriate training. Pigment concentrations can also be measured using more precise measurements in the laboratory.

As pointed out by [45,46], the process of phytoaccumulation requires metal absorption followed by translocation and accumulation in plant tissues. This bioremoval process includes biosorption, which is fast, reversible and a metal-binding process, and bioaccumulation, which is slow, reversible, and ion-sequestration step. Tolerance and accumulation of pollutants are important plant properties for an efficient phytoremediation, which are determined by uptake, translocation, intracellular sequestration, chemical modification, and degradation, which may result in stress resistance of the plant to various contaminants [47,48].

If *L. gibba* is used in the treatment of contaminated wastewater, when plants reach their maximum saturation level at about 4–5 mg/kg dry mass, they can be harvested and replaced with a fresh population of fronds. An interesting application of this work has *L. gibba* "biomining" a polluted aquatic environment, where plant tissues are harvested in order to recover metal resources. Recycling and recovery of Pb from waste materials are contributing a growing fraction of the Pb used in industry (~50% worldwide and over 90% in the United States, http://www.essentialchemicalindustry.org/metals/lead.html). According to a 2006 estimate, reserves of Pb were thought to be sufficient only for the next 42 years, but this was before increases in the level of recycling, and improvements in the design of fuel cells [49]. It is quite expensive and challenging to separate Pb ore from zinc ore; the concentration of Pb in ores is typically from 3% to 8% Pb, whereas the exposed plant materials in the present study had already concentrated the Pb to about 5 g/kg, or 0.5% by mass. It would be much easier to extract the metal from dried plant tissues than from complex ores.

Additional benefits of using *L. gibba* for bioremediation of water contaminated with toxic metals would be the reduction of evaporative losses from treatment lagoons, and the ability of this species to tolerate relatively high concentrations of Pb. The present study also clearly demonstrates the value of including recovery studies as well as exposure treatments to provide a more complete assessment of an ecological hazard, in this case, high levels of Pb in water.

References

[1] M. Thayaparan, S.S. Iqbal, M.C.M. Iqbal, Phytoremediation potential of *Lemna minor* for removal of Cr(VI) in aqueous solution at the optimum nutrient strength, OUSL J., 9 (2015) 97–111.

[2] L.S. Zeng, M. Liao, C.L. Chen, C.Y. Huang, Effects of lead contamination on soil microbial activity and rice physiological indices in soil–Pb–rice (*Oryza sativa* L.) system, Chemosphere, 65 (2006) 567–574.

[3] P. Sharma, R.S. Dubey, Lead toxicity in plants, Braz. J. Plant Physiol., 17 (2005) 35–52.

[4] World Health Organization (WHO), Lead in Drinking-water Background Document for Development of WHO Guidelines for Drinking-water Quality, WHO/SDE/WSH/03.04/09/Rev/1, 2011.

[5] K. Bouchama, R. Rouabhi, M.R. Djebar, Behavior of *Phragmites australis (CAV.) Trin. Ex Steud* used in phytoremediation of wastewater contaminated by cadmium, Desal. Wat. Treat., 57 (2016) 5325–5330.

[6] V. Sheorana, A.S. Sheoran, P. Pooniaa, Phytomining: a review, Miner. Eng., 22 (2009) 1007–1019.

[7] J. Lovett-Doust, M. Schmidt, L. Lovett-Doust, Biological assessment of aquatic pollution: a review, with emphasis on plants as biomonitors, Biol. Rev. Camb. Philos. Soc., 69 (1994) 147–186.

[8] J. Lovett-Doust, L. Lovett-Doust, M. Biernacki, T.K. Mal, R. Lazar, Organic contaminants in submersed macrophytes drifting in the Detroit River, Can. J. Fish. Aquat. Sci., 54 (1997) 2417–2427.

[9] H. Moubasher, A.K. Hegazy, N. Mohamed, Y. Moustafa, H. Kabiel, A. Hamad, Phytoremediation of soils polluted with crude petroleum oil using *Bassia scoparia* and its associated rhizosphere microorganisms, Int. Biodeterior. Biodegrad., 98 (2015) 113–120.

[10] S. Mishra, S. Srivastava, R. Tripathi, R. Kumar, C. Seth, D. Gupta, Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation, Chemosphere, 65 (2006) 1027–1039.

[11] E. Islam, D. Liu, T. Li, X. Yang, X. Jin, Q. Mahmood, S. Tian, J. Li, Effect of Pb toxicity on leaf growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*, J. Hazard. Mater., 154 (2008) 914–926.

[12] M. Biernacki, J. Lovett-Doust, L. Lovett-Doust, *Vallisneria americana* as a biomonitor of aquatic ecosystems: leaf-to-root surface area ratios and organic contamination in the Huron-Erie Corridor, J. Great Lakes Res., 22 (1996) 289–303.

[13] M. Biernacki, J. Lovett-Doust, L. Lovett-Doust, Laboratory assay of sediment phytotoxicity using the submersed macrophyte *Vallisneria americana*, Environ. Toxicol. Chem., 16 (1997) 472–478.

[14] K. Potter, L. Lovett-Doust, Site parameters and the efficacy of *Vallisneria americana* as a biomonitor of stressed aquatic ecosystems: implications for widespread biomonitoring, Ecol. Appl., 11 (2001) 215–225.

[15] A.K. Hegazy, H.F. Kabiel, M. Fawzy, Duckweed as heavy metal accumulator and pollution indicator in industrial wastewater ponds, Desal. Wat. Treat., 12 (2009) 400–406.

[16] S. Radic, D. Stipanicev, P. Cvjetko, S. Sırac, B. Pevalek-Kozlina, M. Pavlica, Duckweed *Lemna minor* as a tool for testing toxicity and genotoxicity of surface waters, Ecotoxicol. Environ. Saf., 74 (2011) 182–187.

[17] K.J. Appenroth, K. Krech, Á. Keresztes, W. Fischer, H. Koloczek, Effects of nickel on the chloroplasts of the duckweeds Spirodela polyrhiza and Lemna minor and their possible use in biomonitoring and phytoremediation, Chemosphere, 78 (2010) 216–223.

[18] R. Henke, M. Eberiusb, K.J. Appenroth, Induction of frond abscission by metals and other toxic compounds in *Lemna minor*, Aquat. Toxicol., 101 (2011) 261–265.
[19] H. Böcük, A. Yakar, O.C. Türker, Assessment of *Lemna gibba* L. (duckweed) as a potential ecological indicator for contaminated aquatic ecosystem by boron mine effluent, Ecol. Indic., 29 (2013) 538–548.
[20] W. Drost, M. Matzke, T. Backhaus, Heavy metal toxicity to Lemna minor: studies on the time dependence of growth inhibition and the recovery after exposure, Chemosphere, 67 (2007) 36–43.
[21] I. Teodorović, V. Knežević, T. Tunić, M. Čučak, J.N. Lečić, A. Leovac, I.I. Tumbas, *Myriophyllum aquaticum* versus *Lemna minor*: sensitivity and recovery potential after exposure to atrazine, Environ. Toxicol. Chem., 31 (2012) 417–426.
[22] US Environmental Protection Agency, Guidelines for Ecological Risk Assessment, EPA/630/R-95/002F, Washington, D.C., USA, 1998.
[23] M.M. Gausman, A Comparison of Duckweed and Standard Algal Phytotoxicity Tests as Indicators of Aquatic Toxicology, MSc Thesis, Institute of Environmental Sciences, Miami University, Oxford, Ohio, 2006.
[24] M. Ater, N.A. Ali, H. Kasmi, Tolerance and accumulation of copper and chromium in two duckweed species: *Lemna minor* L. and *Lemna gibba* L., Rev. Sci. l'Eau. (Water Sci. Rev.), 19 (2006) 57–67.
[25] A. Allam, A. Tawfik, A. El-Saadi, A. Negm, Potentials of using duckweed (*Lemna gibba*) for treatment of drainage water for reuse in irrigation purposes, Desal. Wat. Treat., 57 (2014) 459–467.
[26] H.K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods Enzymol., 148 (1987) 350–382.
[27] A.A. El-Khatib, A.K. Hegazy, A.M. Abo-El-Kassem, Bioaccumulation potential and physiological responses of aquatic macrophytes to Pb pollution, Int. J. Phytorem., 16 (2014) 29–45.
[28] N. Khellaf, M. Zerdaoui, Phytoaccumulation of zinc by the aquatic plant, *Lemna gibba* L., Bioresour. Technol., 100 (2009) 6137–6140.
[29] A. Sasmaz, E. Obek, The accumulation of silver and gold in *Lemna gibba* L. exposed to secondary effluents, Chem. Erde, 72 (2012) 149–15.
[30] E. Obek, Bioaccumulation of heavy metals from the secondary treated municipal wastewater by *Lemna gibba*, Fresen. Environ. Bull., 18 (2009) 2159–2164.
[31] N. Khellaf, M. Zerdaou, Growth response of the duckweed *Lemna gibba* L. to copper and nickel phytoaccumulation, Ecotoxicology, 19 (2010) 1363–1368.
[32] N. Dirilgen, Mercury and lead: assessing the toxic effects on growth and metal accumulation by *Lemna minor*, Ecotoxicol. Environ. Saf., 74 (2011) 48–54.
[33] A. Zayed, S. Gowthaman, N. Terry, Phytoaccumulation of trace elements by wetland plants: I. Duckweed, J. Environ. Qual., 27 (1998) 715–721.
[34] A.D. O'Sullivan, D.A. Murray, M.L. Otte, Removal of sulfate, zinc, and lead from alkaline mine wastewater using pilot-scale surface-flow wetlands at Tara Mines, Ireland, Mine Water Environ., 23 (2004) 58–65.
[35] Flint Water Study, Website Reporting Field Data Gathered by Virginia Tech Researchers. Available at: http://flintwaterstudy.org/ (Accessed July 21, 2016).
[36] S. Mishra, S.B. Agrawal, Interactive effects between supplemental ultraviolet-B radiation and heavy metals on the growth and biochemical characteristics of *Spinacia oleracea* L., Braz. J. Plant Physiol., 18 (2006) 307–314.
[37] Z. Leblebici, A. Aksoy, Growth and lead accumulation capacity of *Lemna minor* and *Spirodela polyrhiza* (Lemnaceae): interactions with nutrient enrichment, Water Air Soil Pollut., 214 (2011) 175–184.
[38] K. Uruç Parlak, D. Demirezen Yilmaz, Response of antioxidant defences to Zn stress in three duckweed species, Ecotoxicol. Environ. Saf., 85 (2012) 52–58.
[39] R. Popovic, D. Dewez, P. Juneau, Application of Chlorophyll a Fluorescence Parameters in Ecotoxicological Studies of Pollutants: Heavy Metals, Herbicides and Air Pollutants, P. Toivonen, J. DeEll, Eds., Practical Applications of Chlorophyll Fluorescence in Plant Biology, Kluwer, Dordrecht, 2003, p. 152.
[40] C. Bragato, H. Brix, M. Malagoli, Accumulation of nutrients and heavy metals in *Phragmites australis* (Cav.) Trin. ex Steudel and *Bolboschoenus maritimus* (L.) Palla in a constructed wetland of the Venice lagoon watershed, Environ. Pollut., 144 (2006) 967–975.
[41] K.J. Dietz, M. Baier, U. Kramer, Free Radicals and Reactive Oxygen Species as Mediators of Heavy Metal Toxicity in Plants, M.N.V. Prasad, J. Hagemayer, Eds., Heavy Metal Stress in Plants: From Molecules to Ecosystems, Springer, Berlin, 1999, p. 73.
[42] K. Uruç Parlak, D. Demirezen Yilmaz, Ecophysiological tolerance of *Lemna gibba* L. exposed to cadmium, Ecotoxicol. Environ. Saf., 91 (2013) 79–85.
[43] R.A. Brain, A.J. Hosmer, D. Desjardins, T.Z. Kendall, H.O. Krueger, S.B. Wall, Recovery of duckweed from time varying exposure to atrazine, Environ. Toxicol. Chem., 31 (2012) 1121–1128.
[44] G. Pérèsa, F. Vandenbulcke, M. Guernion, M. Hedde, T. Beguiristain, F. Douay, S. Houot, D. Piron, A. Richard, A. Bispo, C. Grand, L. Galsomies, D. Cluzeau, Earthworm indicators as tools for soil monitoring, characterization and risk assessment. An example from the national Bioindicator programme (France), Pedobiologia, 54 (2011) 77–87.
[45] S. Demim, N. Drouiche, A. Aouabed, S. Semsari, CCD study on the ecophysiological effects of heavy metals on *Lemna gibba*, Ecol. Eng., 57 (2013) 302–313.
[46] S. Demim, N. Drouiche, A. Aouabed, T. Benayad, O. Dendene-Badache, S. Semsari, Cadmium and nickel: assessment of the physiological effects and heavy metal removal using a response surface approach by *L. gibba*, Ecol. Eng., 61 (2013) 426–435.
[47] N. Fedala, H. Lounici, N. Drouiche, N. Mameri, M. Drouiche, Physical parameters affecting coagulation of turbid water with Opuntia ficus-indica cactus, Ecol. Eng., 77 (2015) 33–36.
[48] S. Demim, N. Drouiche, A. Aouabed, T. Benayad, M. Couderchet, S. Semsari, Study of heavy metal removal from heavy metal mixture using the CCD method, J. Ind. Eng. Chem., 20 (2014) 512–520.
[49] M. Reilly, How long will it last? New Sci., 194 (2007) 38–39.