Removal Efficiency, Accumulation and Biochemical Response of Lemna minor L. Exposed to Some Heavy Metals

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Abstract. Both terrestrial and aquatic plants can be used for removing various pollutants such as hydrocarbons, heavy metals, etc via phytotechnology. This study applied different concentrations of Cd, Pb, and Cr (1, 4, and 8) mg/L to evaluate heavy metal removal and toxicological effects on *Lemna minor* L. in laboratory experiments for 12 days. At the beginning and the end of experiments, the parameters were measured, including fresh weight, relative growth rate (RGR), total chlorophyll, protein, and proline content, as well as other parameters measuring at the end of experiments such as metal remaining in water, removal efficiency, and bioconcentration factor(BCF) in plant. The results demonstrated a decrease in metal concentrations in aqueous solution at all treatments with different values. The highest removal efficiencies were Pb >Cr >Cd, respectively. It was 88.8 % at the 1 mg/L of lead and 22.22 % at 8 mg/L of Cd. The toxicological effects on plants as a response to selected metals were increased with increasing concentrations and periods of exposure. The reduction in fresh, dry weight, t. chlorophyll, and protein content were more in Cd>Cr>Pb respectively compared with increased in control treatments. The highest reduction at 8 mg/L of Cd were 0.329 µg/gram, 18.656 % for total chlorophyll and protein content respectively. The result showed the proline content increased with increasing concentration and the value was highest in Cd>Cr>Pb. The results suggest that *Lemna minor* L. is a good candidate for treating polluted water with heavy metals.

Keywords. Aquatic plant, Phytoremediation, Heavy elements, *Lemna minor* , Toxicological effect, Tolerance , Removal effeminacy , Toxicity , BCF , Biochemical .

1. Introduction

Environmental pollution by organic and inorganic pollutants can be treated via phytoremediation technology [1]. Heavy metals pollutants are particularly difficult to remediate from the soil, water and air because it cannot break down into harmless molecules, persistence in the environment and can enter the food chain and accumulated causing dangerous effect for both humans and environment [2]. Metals and metalloid groups with an atomic density greater than 5 g/cm3 and an atomic number greater than 20 are referred to as heavy metals [3]. It can be classified as essential and non-essential elements according their physiological and metabolic role for living organisms. Fe, Mo, and Mn are essential micronutrients; Zn, Ni, Cu, Co, V, W, and Cr are trace elements that are hazardous at high concentrations. Other metals such as As, Hg, Ag, Sb, Cd, Pd, and U have no biological role and appear to be harmful to microbes and plants [4]. It can derive from both natural and anthropogenic sources. Heavy metals are increasing discharged into the environment, due to the rapid development of

industries such as metal plating facilities, mining operations, fertilizer industries, tanneries, batteries, paper industries, and pesticides, and others [5]. Macrophytes are plants that grow near or in water. They are classified as free-floating, merged, or submerged. Because of their rapid growth, high biomass production, and relative greater ability to accumulate heavy metals. [6]. Several aquatic plant using for removal heavy metals by phytotechnology. Plants respond to heavy metal exposure in a variety of ways, including chelation, compartmentalization, immobilization, exclusion and production of stress response mechanisms [7]. *Lemna minor* L. is a monocotyledonous aquatic plant in the Landoltia, Spirodela, Lemna, Wolffia, and Wolffiella genera. It can be found in tropical and temperate region, and it thrives in slow-moving water in freshwater, marshes, and nutrient-rich water, where it develops a mat on the water's surface that can reach a depth of several feet [8]. A simple Semi-rootlets plant without a stem or leaves, has only of a thallus, a frond, and a few cells that contain the aerenchyma that helps the plant float [9, 10]. The optimium temperature for plant growth is 20 - 30 °C. otherwise growth is slowed at temperatures below 10 °C and beyond 40 °C. [2] studied the toxicity of lead on *Eichhornia crassipes* that the result showed that the Pb inhibition of growth was 50 % at 1000 mg/L as a response of lead stress and the accumulation in root is more than in shoot. The result also revealed that the increased of lead concentration reduce the chlorophyll in plant compare with control treatment. [11] demonstrated the ability of *Lemna minor* L. exposed to different concentrations of zinc and lead in laboratory experiment for 4 days. The result showed that the toxicity was more in high concentrations of metals and causing toxic effects on fresh weight, dry weight, growth and photosynthesis pigment. The aim of this study is to determine the ability of duckweed *Lemna minor* L. to grow in different metal concentrations of Cd, Pb and Cr and to study the toxic effect of these metals on growth and some biochemical parameters

2. Methodology

2.1. Plant Collection and Acclimatization

The plant *Lemna minor* L. was collected in a labeled plastic bag from the Shatt Al-Arab river, Basrah, Iraq. It was properly washed in the laboratory with tap water to removed solid particles before being rinsed with de-ionized water. Hoagland nutrient solution (1%) was used to grow the plants in plastic aquarium containing tap water. The plant was kept for one week to allow it to grow and acclimate.

2.2. Metal Concentration Preparation and Experimental Setup

This experiments carried out at the control environment in the laboratory. The average temperture 20-25 °C and 12/12 hours day/night. Standard salts of lead acetate Pb $(CH_3COO)_2$, Cadium sulphate $CdSO_4$ and potassium chromate K_2CrO_4 were used to prepare desire concentration of selected metals (pb, Cd and Cr). The concentration was (1, 4, 8 mg/L) of each metal separately prepared. While plant without metal treatment were used as a control. For each treatment, triplicates were maintained. Plastic aquarium capacity 1.6 L were used that filled with 1 L of selected concentrations. After one-week acclimatization, 4 gram of healthy plant were selected for each treatment. 1% of Hoagland nutrient solution was added to provide the desire nutrients for plant at the period of experiments. At the end of experiments after 12 days the sampling were taken to study, any changing occur on plant. The plant were thoroughly harvested and washed with tap and distilled water before being analyzed for plant analysis. In addition, heavy metal remaining in water and removal efficiencies were calculated.

2.3. Water Analysis

2.3.1. Metal Remaining in Water

The heavy metals in water were measured using the [12] method. 100 ml of water sample was obtained. It was heated on a hot plate after adding 5 ml of concentrated nitric acid (near dryness, then added another 5 ml of concentrated nitric acid and returned to the hot plate near drying) to make sure the sample was completely digested. it was allowed to cool before being placed in a volumetric flask and completely to 100 ml with distilled water. The samples were analyzed with a Flame Atomic

Absorption Spectrometer (spectro absorption atomic flame) (FAAS) and the result is expressed in mg/L).

2.3.2. Removal Efficiency %

The removal efficiency calculated using equation mentioned in [13]

$$\text{Removal efficiency \%} = \frac{Intial\ Concentration\left(\frac{mg}{L}\right) - final\ Concentration\left(\frac{mg}{L}\right)}{Intial\ concentration\ \left(\frac{mg}{L}\right)} \times 100$$

2.4. Plant Analysis (biochemical)

2.4.1. Fresh Weight and Relative Growth Rate

Harvested plant were rinsed with tap water and distilled water. Put it on filter paper to remove excess water. Then weighed of plant with satorious balance (four digits) as fresh weight in gram. The relative growth rate was calculated at the end of experiments by using the equation mentioned in [14]

$$\text{Relative growth rate} = \frac{final\ fresh\ weight}{intial\ fresh\ weight}$$

2.4.2. Heavy Metals Inside Plant Tissue and Bio Concentration Factor (BCF)

The concentration of the metal in the plant was determined using the method described in [15] by transferring 0.5 g of dry , crushed plant to the digestive tubes and adding 10 ml of an acidic liquid. After that, the contents of the tube were transferred into a 100 mL beaker. The tube was rinsed three times with distilled water before being placed to the plastic beaker and heated on the hot plate. 5 ml concentrated nitric acid was added and transferred to a 25 ml volumetric flask at a temperature of 70-80 C near dryness, then rinsed three times, added to volumetric flask, and volume to 25 ml was completed. It was transferred to a plastic cap with a capacity of 30 ml after being diluted with 5% nitric acid until it was measured. The sampling were measuring using an atomic absorption spectrophotometer (FAAS) and the result expressed in mg/gram. The BCF was calculated using equation mention in [16].

$$\text{BCF} = \frac{metal\ concentrations\ in\ plant\ tissue\ mg/gram}{intilal\ metal\ concentration\ in\ water}$$

2.4.3. Total Chlorophyll Content (µg/g fresh weight)

The chlorophyll content was determined using the [17] . It is involved extracting the chlorophyll using 0.2 g of fresh weight in 20 ml of 80 % acetone, centrifuging at 5000 rpm for 5 minutes to remove any remaining particles, and measuring the absorbance of the extracted solution at 645 and 663 nm with a spectrophotometer to calculate the total chlorophyll content in µg/gram.

$$\text{Total chlorophyll} = (12.7 * OD\ 663) + (16.8 * OD\ 645)$$

2.4.4. Protein Content

The protein in the plant was estimation according to [18] as a percentage by calculating the total nitrogen in it and multiplying it by a factor of 6.25

2.4.5. Proline Content mg/g

The determination of proline content using the method described in [19] . 0.2 gram of dry, powdered plant was mixed with 5 ml of 95 % ethanol for an hour before centrifugation at 5000 circle/min for five minute. The transparent part of the extract was evaporated near dryness. 2mL distilled water was added to the mixture. 1 mL extract solution was analyzed using spectroscopy at 520 nm, and the proline content was calculated in units using the proline standard curve.

2.5. Statistical Analysis

The statistical program spss model 23 was used. Under the significance level of 0.05, descriptive analysis and one-way variance (ANOVA) were applied between concentrations of metals and control treatments.

3. Results

The result showed that the reduction in metal concentrations in all treatments decreased at the end of experiments as shown in figure 1 . The decreased was in Pb > Cr> Cd. with a significant difference between each metal concentration.

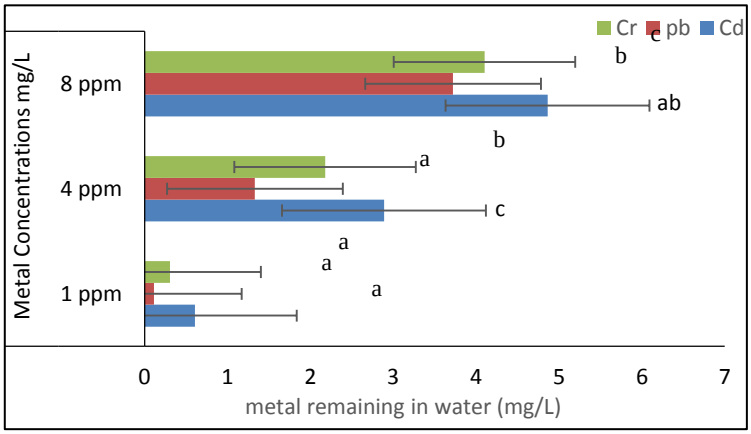

Figure 1. The average reduction of metal in aqueous solution after 12 days of exposure to different concentrations of cadmium, lead and chromium metals.

The result indicated that the removal efficiency increased with low metal concentration more than the high concentrations and with different values between metals. The removal efficiency was more in Pb> Cr > Cd. Table 1 .

Table 1. The average removal efficiency of metal after 12 days of exposure to different concentrations of cadmium, lead and chromium metals.

Metals	Removal efficiency %		
	1 ppm	4 ppm	8 ppm
Cd	39.4 ± 21.589 [a]	27.8 ± 15.226 [a]	22.225 ± 7.791 [a]
Pb	88.8 ± 48.637 [a]	66.75 ± 3.565 [a]	53.4875 ± 9.293 [b]
Cr	69.3 ± 7.957 [a]	45.575 ± 4.960 [a]	48.7375 ± 6.691 [b]

*Different letter for each columns of metal concentration refer to significant difference between each metal concentrations at a probability level of 0.05 ($p \leq 0.05$) according to ANOVA test.

When compared to control treatment, the result revealed that the average fresh weight and relative growth rate of the *Lemna minor* L. plant was affected as a response of heavy metal stress, and the effect was directly affected by the increasing in the time period of exposure and the increase in concentration, also the plant was most affected by Cd> Cr > Pb with asignificatant difference at 0.05 between each metal concentrations and control. Figure 2 .

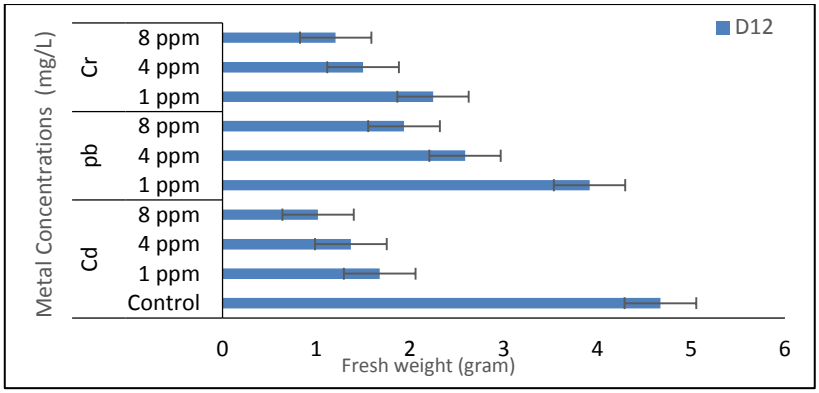

Figure 2. The average change of fresh weight after 12 days of exposure to different concentrations of cadmium, lead and chromium metals.

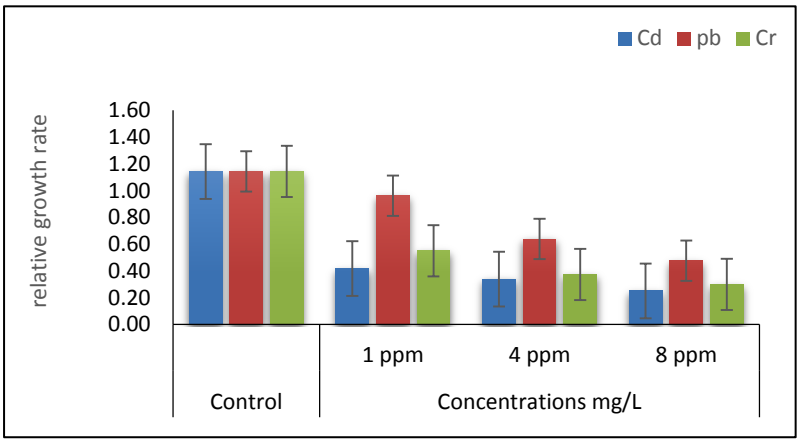

Figure 3. The average change in the relative growth rate after 12 days of exposure to different concentrations of cadmium, lead and chromium metals.

The BCF value was increased in all treatments at the end of experiments with different values with a significant difference between treatments. The biggest value was in 1 ppm Pb and the smallest value was at 8 ppm Cd. Figure

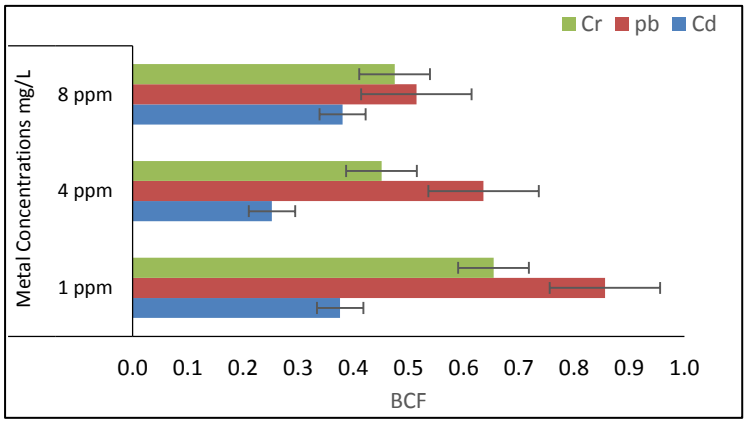

Figure 4. The average BCF in plant after 12 days of exposure to different concentrations of cadmium, lead and chromium metals.

The intial concentration of total chlorophyll , protein and proline content were 4.547 µg/g , 33.76 % and 23.55 mg/gram respectively. Table 2 indicated that the reduction in total chlorophyll and protein content increased with increasing metal concentrations and period of exposure. The reduction was

greater in Cd , Cr , Pb respectively compared with increasing in control treatments. The clorosis and necrosis as a response to Cd began to appeared at 3rd days of exposure in all treatments of Cd.

Table 2. Toxicological effects of Cd, Pb and Cr concentrations after 12 days of exposure.

Metals and control treatments	Concentrations mg/L	Total Chlorophyll µg/g	Protein content %	Proline content mg/gram
Control	0	4.972 ± 0.236^{b}	40.332 ± 3.650^{b}	26.54 ± 1.760^{a}
	1	4.061 ± 0.969^{a}	22.545 ± 6.143^{a}	354.876 ± 181.487^{a}
Cd	4	1.9142 ± 1.440^{a}	20.454 ± 7.342^{a}	552.3 ± 289.663^{a}
	8	0.329 ± 2.310^{a}	18.656 ± 8.273^{a}	755.75 ± 401.042^{b}
Control	0	4.6252 ± 0.230^{a}	40.332 ± 3.650^{a}	26.54 ± 1.760^{a}
	1	3.702 ± 0.462^{a}	32.434 ± 1.165^{a}	123.54 ± 54.766^{a}
Pb	4	2.529 ± 1.108^{a}	29.612 ± 2.272^{ab}	205.54 ± 99.680^{a}
	8	2.12 ± 1.333^{b}	26.671 ± 3.828^{c}	288.65 ± 145.201^{b}
Control	0	4.8542 ± 0.268^{b}	40.332 ± 3.650^{a}	26.54 ± 1.760^{a}
	1	2.542 ± 1.098^{ab}	28.213 ± 3.038^{ac}	303.87 ± 15.548^{ac}
Cr	4	1.851 ± 1.476^{ac}	23.334 ± 2.664^{ab}	450.7 ± 22.959^{ab}
	8	1.629 ± 1.598^{a}	21.545 ± 6.692^{c}	501.03 ± 26.532^{c}

*Different letter for each columns of metal concentration refer to significant difference between each metal concentrations at a probability level of 0.05 ($p \leq 0.05$) according to ANOVA test

4. Discussion

Rhizofilteration mechanism by aquatic plants is useful for eliminating pollutants from both aqueous solutions and polluted water . *Lemna minor* L. plant has semi roots that have demonstrated their ability to absorb the chosen metals [20 , 21]. The reduction of metal concentrations and removal effciency as shown in table 1 revealed that the effciency of plant to eleminate low concentrations of metals was greater than that in high concentrations; the reson could be attributed to the less toxicological effects on plant growth, whereas high concentrations of cadmium and chromium inhibited the fresh weight and relative growth, reducing its ability to absorb the element from its aqueous solution. This results agreed with [22] which investigated *Hydrilla verticillata*'s ability to remove chromium (Cr) and Cadmium (Cd) from aqueous solution at low concentrations, while high concentrations inhibit plant growth, resulting in a reduction in metal absorbed by the plant and reduced removal effciencies .

Plants' ability to absorb and accumulate metals is affected by their health and growth. As a result, as concentrations increased, the metals inside the plant decreased. The high metal concentrations may be causing poisoning and saturation of metal in the plant as well as the decrease in mitotic index reported in the heavy metals exposure could be linked to growth inhibition , resulting in a loss in the its ability to absorb metals and accumulation inside plant tissue especially in vacuoles.[23]. This result agreed with [24].

Reduced chlorophyll content can be used to monitor heavy-metal-induced damage in plant fronds as a visible symptom. Based on the existing evidence, it's possible that metals accumulation restrict chlorophyll production by impairing the intake of key components for photosynthetic pigments like magnesium, potassium, calcium, and iron. Furthermore, enhanced chlorophyllase activity causes an increase in chlorophyll degradation in plants growing in the presence of metal ions [25]. Peroxidation of chloroplast membranes due to increased rate of ROS production could also be a cause of photosynthetic pigment accumulation inhibition in response to heavy metal stress. This finding is consistent with the enhanced rate of H_2O_2 and lipid peroxide generation in W.arrhiza treated to Cd and other metals. Cd caused DNA damage, slowed DNA synthesis, and stopped cell division in synchronized soybean (Glycine max) cell suspension culture, according to experiments [26]. This result agreed with [27].

Plants' protein content, which is a key indicator of both reversible and irreversible metabolic alterations, has been shown to respond to a wide range of stresses [28]. At all concentrations, the protein content of *lemna minor* L. fronds decreasing as heavy-metal concentrations increased, with the largest decline occurring in response to 8 mg/L Cd. The failure of plant fronds to accumulate proteins after exposure to heavy metals such as Cd and Pb and Cr could be due to acute oxidative stress generated by heavy metal overload in plant cells. Many aquatic plants, including *Lemna minor*, L. and the free-floating freshwater macrophyte *Ceratophyllum demersum*, have been reported to degrade protein as a result of metal exposure [29].
The accumulation of soluble proline, proteins, and higher MDA content, will enhance osmotic balance for the adaptive response and allow plants to tolerate stress [30]. In higher plants, proline serves as a stress marker, and it accumulates in large amounts in response to environmental stressors [31]. Proline is important for protein protection, osmoregulation, and the prevention of oxidative damage, as well as the stabilization of cellular membranes [32]. Proline buildup has been seen in HMs-stressed plants of diverse species [33]. Similarly, the increased level of proline in this investigation indicated its antioxidant capacity in detoxifying HMs buildup.this result agreed with [34]

Conclusion

From the result, the *Lemna minor* L. showed tolerance to selected metal used with different value. The removal efficiencies at low concentrations of metals was more than in high concentrations. the removal and lowest toxicological effect was Pb < Cr< Cd . The toxicological effect of Cd is more than Pb and Cr in all concentrations. The present study suggest that Lemna minor could tolerate heavy metals and can be candidate for phytoremediation of polluted water with low and moderate concentration of heavy metals.

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