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COMPARATIVE STUDY OF THE TOXICITY AND PHYTO-EXTRACTION CAPACITY OF *L. MINOR* AND *L. GIBBA* IN POLLUTED WATER BY CADMIUM

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ABSTRACT: A comparative study on the potential of phytoremediation on *Lemna minor* and *Lemna gibba* for the removal of different concentrations of cadmium was carried out. The duckweed species were treated with a wide range of cadmium concentrations in the environment [10- 30 µg L^{-1}] for seven days. Plant growth was stimulated at 10 and 15 µg L^{-1}, while higher concentrations unfavourably affected plant growth. It was observed that the metal considerably impaired chloroplast ultra-structure and caused a significant reduction in pigment content and progressively increased from 20 to 30 µg L^{-1} compared to the control, indicating that cadmium induced considerable oxidative stress. Antioxidant enzymes were generally significantly increased in the presence of cadmium in a dose-dependent manner. Cadmium accumulations in duckweed species were increased with increasing cadmium concentration in growth medium. The maximum BCF values for root and shoot tissues were obtained for 15 µg L^{-1}cadmium, which indicated that both species were cadmium hyper accumulators. In addition, most of the cadmium accumulated in the roots, but some was also trans-located and accumulated in the fronds. However, L. minor was shown to have a slightly better performance in removing cadmium and more tolerant, when compared with *L. gibba*.
Key words: *Lemna* species, Cadmium, Metal efficiency, Antioxidant enzymes.

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INTRODCUTION

The presence of heavy metals and other strangers in aquatic environments are turning into a serious environmental problem, as most of them are toxic or carcinogenic in nature with potentially harmful consequences for the entire world. Cadmium was chosen in the current investigation since this metal ion is very widespread. It also has an extremely long biological half-life (30 years), large water solubility, and high mobility in soils, and among the worst pollution chemicals as it accumulates throughout the food chain posing a serious threat to human health [1].

In plants, cadmium causes disturbing to several physiological, biochemical and structural changes [2] like mineral nutrition, enzyme activities, and alterations in membrane permeability [3].However, several conventional metal removal ways are useful for detecting the presence and the concentration of metals in the environment, but these methods do not come with an eco-friendly technique and need high operational and maintenance cost. On the contrary, the use of aquatic plants in metal removal appeared as an effective alternative in the removal process of heavy metal excess from water, wastewater and soil [4]. The efficiency of phytoremediation differs between species, as different mechanisms of ion uptake are operative in each species, depended on their morphological, physiological and anatomical characteristics [5].

One of the most unique challenges is how to make the efficiency of phytoremediation better through increasing the accumulation of metals in both roots and shoots of plants or via improving key plant biological traits that should enhance metal uptake [6]. Macrophytes are among the most suitable plants for toxicity. The ability to accumulate heavy metals as most of uptakes are facilitated by dissolution in water, which make them interesting research area especially for the treatment of industrial effluents and wastewaters [7].

High tolerance and accumulation of heavy metals in their green parts, when producing high biomass, their photosynthetic rate and the availability of the species are the most characteristics to make plants ideal for phytoextraction process [8].

Different species of aquatic plants are used as a bio-filter to increase the quality of water and wastewater [9]. *Lemnace* family in particular floating one is among the common used aquatic plant families. *Lemna minor* and *Lemna gibba* are perennial and free floating aquatic plants; they are monocotyledonous aquatic macrophytes consisting of floating plant bodies (fronds) and submerged greenish roots[10].

They produce rapidly- expanding mat of foliage on water surface. They growth under a wide range of temperature (7 to 30 ^{0}C), as they and can be found worldwide on the surface of nutrient- rich fresh and brackish waters. They are excellent plants for molecular, biochemical and physiological studies as well as for phytoremediation of polluted waters [12].

These particular plants absorb metallic ions and deposit them in various parts of the plants body depending on their affinity towards a particular metal [7]. However, there is a scarce knowledge about the physiological and accumulation mechanisms of cadmium toxicity in these plants. Since those toxicological protocols agree for the utilization of a different species of *lemnaceae* in experimental studies, building the comparative sensitivity of different *Lemna spp*. It is really crucial to develop theses protocols.

In view of above, the present study was designed to demonstrate the efficiency and tolerance of two common free- floating aquatic plants exposed to cadmium. The influence was investigated with reference to relative growth rate of fresh weight (RGR FW), chlorophyll content, enzymatic activities, cadmium content in roots and fronds, residual cadmium and BCF and TF, which are the basic steps regarding metabolic adaptations of plants growing in heavy metal stress conditions.

MATERIALS AND METHODS

Plant material and growing Conditions

L.minor and *L. gibba* clones were kindly provided by the university of Jena-Germany. The stock cultures were kept separately in two transparences plastic tanks (35cm × 70cm) filled with modified Swedish Institute Standard medium (SIS) containing 85mg L^{-1}NaNO3, 13.4 mg L^{-1} KH_2PO_4, 17 mg L^{-1} $MgSO_4$. $7H_2O$, 36 mg L^{-1} $CaCl_2$. $2H_2O$, 20 mg $L^{-1}$$Na_2CO_3$, 1 mg L^{-1} H_3BO_3, 0.20 mg $L^{-1}$$MnCl_2$. $4H_2O$, 0.010 mg $L^{-1}$$Na_2MoO_4$. 2H2O, 0.050 mg $L^{-1}$$ZnSO_4$. $7H_2O$, 0.005 mg $L^{-1}$$CuSO_4$. 5H2O, 0.010 mg L^{-1} $Co(NO_3)2$. 6H2O, 0.84 mg L^{-1} $FeCl_3$. 6H2O.The pH value of the nutrient solution was adjusted to 6.0± 0.1 using a pH meter (Denver instrument company, USA). Plants were grown at 23–27/14–19 ^{0}C day/night temperature, and 65%–70% humidity under greenhouse conditions.

After the growing period, plants were transferred to the laboratory in small plastic tanks (15cm × 20cm) and washed with Mill-Q water several times to remove dirt, and disinfected by immersion in 1% (v/v) NaClO for one minute then re-washed with distilled water. The plants were set into culture containers with a capacity of 500 ml and filled with SIS. They were producing extra fresh materials in the culture solutions and allow acclimating to laboratory conditions for two weeks, before exposing to cadmium. The pH value of SIS medium was taken and maintained at 6.5± 0.1. All culture solutions were maintained in a growth chamber at 22 ^{0}C with 16:8 (light: dark) photoperiod at a photosynthetic photon density of 8.5 × 10 µmol m^{-2} S^{-1}[13].

Experimental design and statisitical analysies

Stock solution of cadmium was used from $CdCl_2$ (Sigma Aldrich) to prepare 10, 15, 20, and 30 µg L^{-1}. All solutions were prepared with high purity Milli-Q water. All plastic-ware and glassware used in the experiment were chemically sterilized (acid-washed) and cleaned. After the acclimatization period, light-green fronds of about 50 colonies with 3-4 fronds per colony and similar size were randomly chosen from the pre-culture as test specimens using a disposable plastic inoculation loop. All colonies were individual healthy, with no discolored areas, chlorosis, necrosis, and gibbosity [13]. The colonies of each species were exposed to four test concentrations of cadmium maintained in SIS medium in separate 500 ml plastic tanks. Test concentrations were verified by running some samples from culture solution on Inductively Couple Plasma Mass Spectrophotometer ICP-MS. Varian-Germany). Control plants were grown in the nutrient solution without cadmium amendment. The tanks were placed in a growth chamber under the aforementioned conditions for seven days.

Data were analyzed using one-way analysis of variance (ANOVA) and the Tukey test ($P<0.05$) to compare the means of the treatments with the control. Data presented here are expressed as the mean ± SD of three independent replicates.

GROWTH MONITORING

Relative growth rate of fresh weight (RGR FW)

Relative growth rate of duckweed species was calculated according to Hunt's equation:

$$\textbf{(1)}\ R = \ln W_2 - \ln W_1 / T_2 - T_1$$

Where R is the relative growth rate (g^{-1} day^{-1}), W_1 and W_2 are the initial and final fresh weights, respectively, and (T_2 - T_1) is the experimental period [13].

Photosynthetic Pigment

Pigments of the exposed and control plants were measured following the method described by [14] with some modifications. Fresh materials (5 gm) were homogenized in 10 ml cold acetone 80% (w/v) inindividual dark sterilize scintillation vials and incubated in the dark place for 24hours at 4 ^{0}C for homogenization. Samples were centrifuged at 5000 rpm for 10 min. The supernatant was read at 663, 646 and 470 nm to determine chlorophyll a, b and total carotenoid, respectively. The photosynthetic pigment contents (mg g^{-1}FW) were calculated according to Lichtenthaler [15].

Enzyme Extraction and soluble Protein

Fresh biomass (1 gm) was homogenized in 5 ml of 100 mM potassium phosphate buffer (pH: 7.8) containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone with a homogenizer. The homogenate was centrifuged at 15000 rpm for 10 min at 4^{0}C [16]. Total soluble protein was measured using serum albumin as a protein standard [17]. The supernatant was used for enzymatic assay.

Catalase activity CAT: The activity of catalase was measured spectrophotometric ally [18]. The reaction mixture contained 50 mM KPO_4 buffer (pH 7.0), 10mM H_2O_2 and enzyme extract. Activity was calculated using the extinction coefficient (0.04 mM^{-1} cm^{-1}) and mM H_2O_2decomposed g^{-1} FW min^{-1} was used as a unit of CAT.

Superoxide dismutase SOD: The activity of superoxide dismutase was evaluated spectrophotometric ally [19].

Cadmium content in Plants and in Growth medium

Cadmium content in plants and residual cadmium in growth culture were determined following the method described by [20]. Plant materials were collected after each period of times and rinsed with Mill-Q water three times and blotted on tissue papers for 3 minutes, and then separated into fronds and roots. Fresh materials were freeze dried for 48hr and dry materials were wet digested in 10ml analytical grade of HNO_3 (12 N; 5 ml) at 70^{0}C for 20 minutes in the Teflon vessels, which were returned to the microwave, then at 130^{0}C for another 5 minutes. After cooling, the samples were diluted with up to the total volume of 10 ml. Cadmium was analyzed by Inductively Couple Plasma Mass Spectroscopy ICP-MS. Samples from growth medium, 10 ml per replicate were taken by digital pipette and placed in small container with 1 ml concentrated HNO_3 to measure the cadmium concentration removed from the nutrient solution. The percentage metal efficiency was calculated according to [21].

$$\textbf{(1)}\ \%\ \text{Efficiency} = \frac{C0 - C1}{C0} \times 100$$

Bioaccumulation factor (BCF) was calculated to estimate the phyto-extraction ability of selected plants [22].

$$\textbf{(2)}\ \text{BCF} = \frac{\text{Cadmium in plant biomass (mg kg}-1)}{\text{Cadmium in soultion (mg L}-1)}$$

Cadmium translocation from frond to root was measured by TF, which is given below:

$$\textbf{(3)}\ \text{TF} = \frac{\text{Cadmium in fronds}}{\text{Cadmium in roots}}$$

The standard reference material (Rye Grass) was digested and analyzed in the same fashion as the plant samples. The results of the reference (0.119 mg Kg^{-1}) material showed that the recovery values fell in an acceptable range 0.110-0.125 mg Kg^{-1}.

RESULTS AND DISCUSSION

Comparative Growth Performance of Selected plants

The phytotoxicity of different contaminants in metals towards several different aquatic plant species has been determined [1]. The relative sensitivity of specific species of *Lemnaceae*, mostly *L. minor*, to a range of toxicants has also been investigated [23]. However, few researches have compared the sensitivity of individual duckweed species. This study presents evidence that two species of *Lemnaceae* differ in their relative sensitivities and accumulation capacity to cadmium.

Growth performance, which is indicative of biomass accumulation, is one of the essential symptoms for identification of prospective plants for successful cadmium phytoremediation application. The impacts of different concentrations on *L. minor* and *L. gibba* within certain exposure time were evaluated to study the cadmium induced growth behavior in forms of plant biomass. Throughout the experiment, all fronds appeared green and vigorous, and no signs of senescence were observed in the control cultures after exposure duration.

It has been shown that some toxic chemicals stimulate the growth of plants at lower doses and have a severe toxic effect at higher doses [24]. Similarly, the present study ascertained that a lower dose of cadmium (up to 15 µg L^{-1}) stimulates growth ($P>0.05$), but not significantly (Figure1). However, little is known of the possible stimulatory effect of metals at low concentrations, but this particular situation by the effect of toxicants at lower doses called hormesis effect [25]. Furthermore, in both species, root biomass increased, which could be due to hydroponics system that all metabolic activities are governed by root. In contrast, in the cultures treated with 20, and 30 µg L^{-1} of cadmium, *L. minor* began to reveal visual phytotoxicity symptoms (necrosis and chlorosis) even after 24hr short-term stress. Furthermore, toxicity symptoms were shown after 48 hours in case of *L. gibba* and, necrosis started from margin and tip of frond, and then spread to their central parts during further cultivation as well as shrinkage of fronds.

After researchingin the literature, it revealed that not all studies have shown such differences in toxin sensitivity among *Lemnaceae* species, for instance [26]. In contrast, [27] found *L. minor* to be more sensitive than *L. gibba* when exposed to copper and cadmium induced plants to release daughter fronds from the mother frond, resulting colony disintegration. Moreover, roots separated from the fronds (root abscission) became brownish and very fragile. Although these fronds usually contained roots, they were considerably shorter than those of the controls.

Biomass of duckweed species increased at lower doses. It was recorded that the RGRFW values may tend to decrease when a high dose of cadmium is applied based on the fact that the cell wall and the cell membrane may be destroyed, due to the reactive oxygen species formed as result of the stress depending on an increased concentration of exposure [27]. Under heavy metal stress, change in plants growth is the first symptoms of heavy metal toxicity [28].

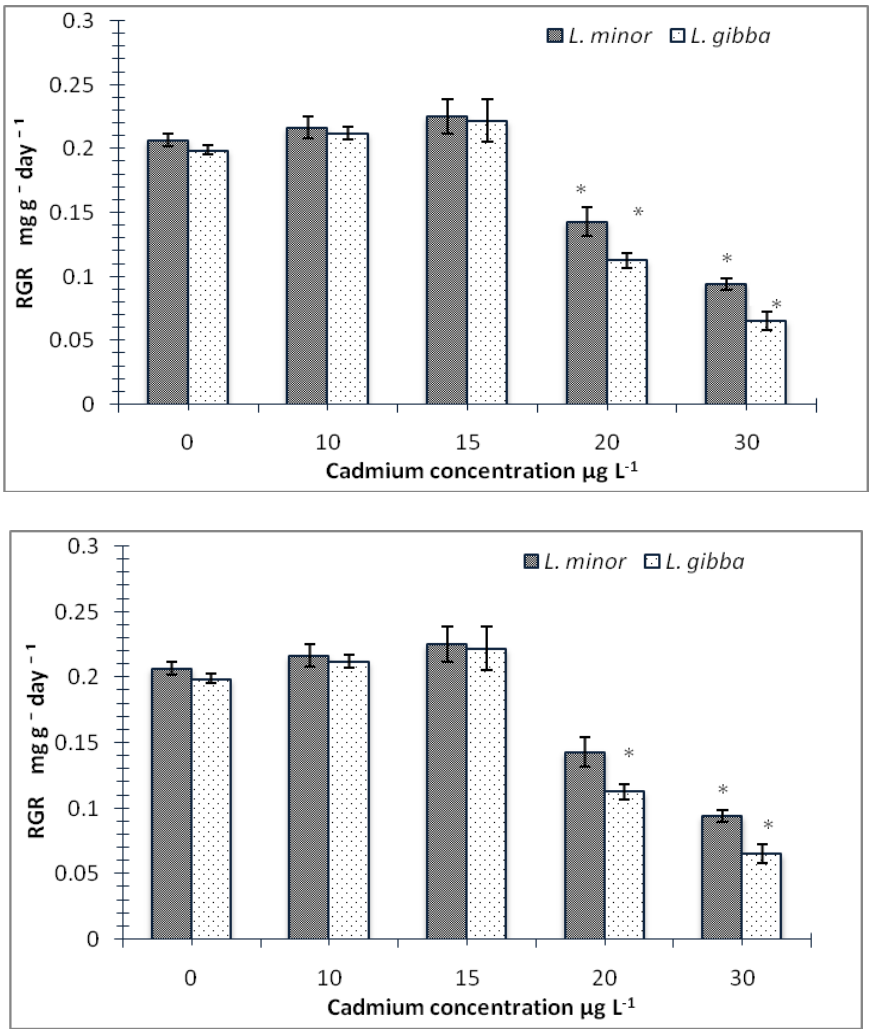


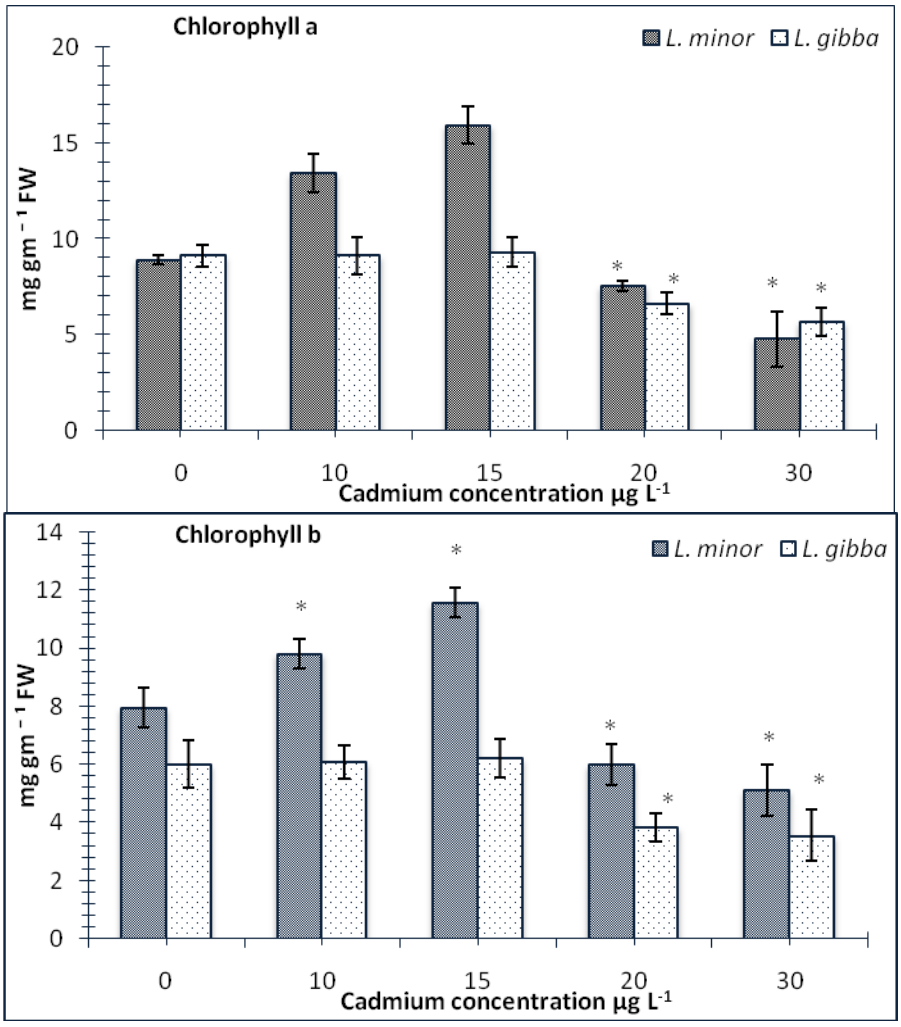
Fig-1: Effect of cadmium concentrations in SIS medium on relative growth rate based on fresh biomass of duckweed species after 7 days exposure. Error bars represent the SD of mean (n= 3).

Certain physiological and biochemical changes in the plants observed as a result of heavy metal pollution. The most significant biochemical changes can be seen in the photosynthetic pigment concentrations, which considered as indicators of environmental stress [29]. Compared to the control, *L. minor* and *L. gibba* showed increase in chlorophyll a, b and total carotenoid up to 10 and 15 µg L^{-1} cadmium after exposure duration. However, at 20, and 30 µg L^{-1} of cadmium, a significant decrease (p<0.005) in chlorophyll a, b and total carotenoid content was observed after seven days of exposure as compared to the control (Figure 2). This result suggests that pigment biosynthesis and/or pigment accumulation might be an important target of stress induced by low doses of cadmium in aquatic macrophytes [30].

Plant species showed necrosis symptoms and also reduction in biomass. The chlorosis symptoms are explained due to inhibited of Fe^{3+} reductase in the roots by cadmium. This scenario results in Fe|| deficiency and affects several photosynthesis processes. Decline in chlorophyll in response to cadmium stress in aquatic plants due to inhibition of important enzymes associated with chlorophyll biosynthesis of fronds, and thus growth of plants[31]Also, the inhibition of photosynthesis may also be due to inhibition of other photosynthesis-related factors, such as Rubisco and photochemical activities [32]. Research reported that heavy metals might impair the amount of chlorophyll in plants either directly or indirectly inhibition chlorophyll synthesis [33].

Carotenoids are a class of natural fat-soluble pigments found in plants, where they have a key role in the photosynthetic process as well as membrane associated antioxidant activity.

A dose dependent increase in carotenoid content was observed in cadmium treated plants of *L. minor* and *L. gibba* at highest cadmium concentration where carotenoid level decrease significantly after exposure duration. Maximum carotenoid content was found at 30 after 96hr of exposure. Plants have defense system, which work to nullify the toxic effect of free radicals generated during metal stress. Research figured out that many plants have quite large difference in the absolute level of carotenoid and synthesize zeaanthin in response to stress condition [34].



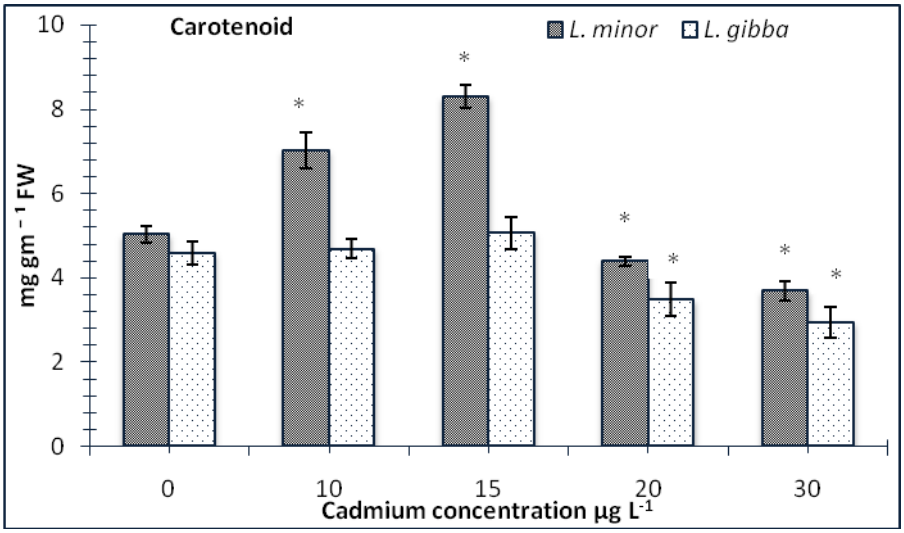


Fig-2: (A, B and C)Effect of cadmium concentrations in SIS medium on a chlorophyll a, chlorophyll b and carotenoid content of duckweed species after 7 days exposure. Error bars represent the SD of mean (n= 3).

It has been reported that pigment content in *L. gibba* was altered by bioaccumulation of cadmium; therefore the plants have shown a significant discoloration of leaves [35].Moreover, the results confirmed that the cadmium had a major impact on the photosynthetic activity of the aquatic plants. Research reported that at high concentrations of metal pollutants damage to cells occurs as a result of excessing ROS, which the cells unable to cope with the toxic situation [36]. Eventually, the selection of species for conducting toxicity and bioaccumulation tests should be made very precisely, with due consideration given to the most sensitive parameter for the species selected and the nature of the toxicant being tested.

Protein content and antioidant enyme Activities

To assess the biochemical responses of duckweed populations under cadmium exposure, the protein content was determined (Figure 3). Protein content was increased non-significantly (p>0.05) at low concentrations. The maximum increase in protein content was 1% and 4% in *L. minor* and 2% and 4% in *L. gibba* exposed to 10 and 15 µg L^{-1} of cadmium. However, exposure to higher concentrations resulted in a decrease in the level of protein. The decrease was 56% and 69% in *L. minor*, 17% and 39% in *L. gibba*, respectively at 20 and 30 µg L^{-1} of cadmium, as compared to the control (P<0.05).

In this work, a cadmium concentration-dependent decline of protein content was observed for the two populations of duckweed. [37] stated that under cadmium exposure, protein content decrease is an enhancement of the proteolytic activity. The oxidative variation of the proteins is the first indicating step leading to protein degradation [38]. Hence, an increase in the proteolysis rate could be linked to the oxidizing properties of cadmium.

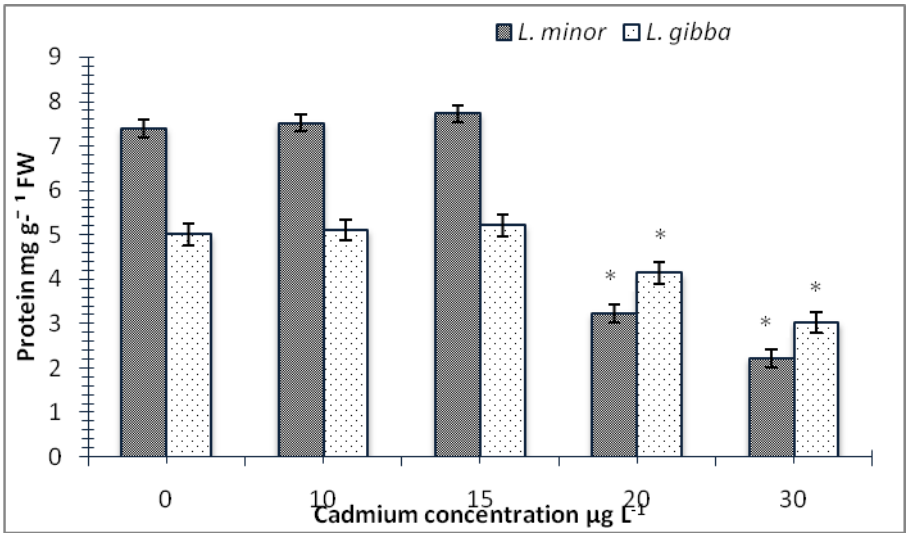


Fig-3: Effect of cadmium concentrations in SIS medium on soluble proteins content in duckweed species after 7 days exposure. Error bars represent the SD of mean (n= 3).

Parallel to the decrease in protein content, our results revealed a concentration-dependent increase of cadmium in the activity of SOD for both species (Figure 4 A and B). SOD is considered as a primary defense against ROS as it acts on superoxide radicals, which are pronounced in different compartments of the cell [39]. The increase in the SOD activity was %117 and %223 in *L. minor* and %92, %188 in *L. gibba*, respectively at 20 and 30 µg L^{-1} of cadmium, as compared to the control (p<0.05). As for protein content, the impact of cadmium on SOD activity was observed to be higher in*L. Minor* than *L. gibba*. The increase of SOD activity is the consequence of an increased production of ROS (Devi and Prasad 1998) and it may happen through the activation of latent SOD [40]or the increased expression of genes encoding SOD [41]. Many species were already shown an increase in the activity of SOD after an exposure to cadmium [42]; [43]; [44].

SOD blocks O2- radicals; the enzyme does not supply proper protection to the cell since H_2O_2 emerges as a product of its functioning [45]. H_2O_2, a precursor of the highly reactive hydroxyl radical is be destroyed by CAT.

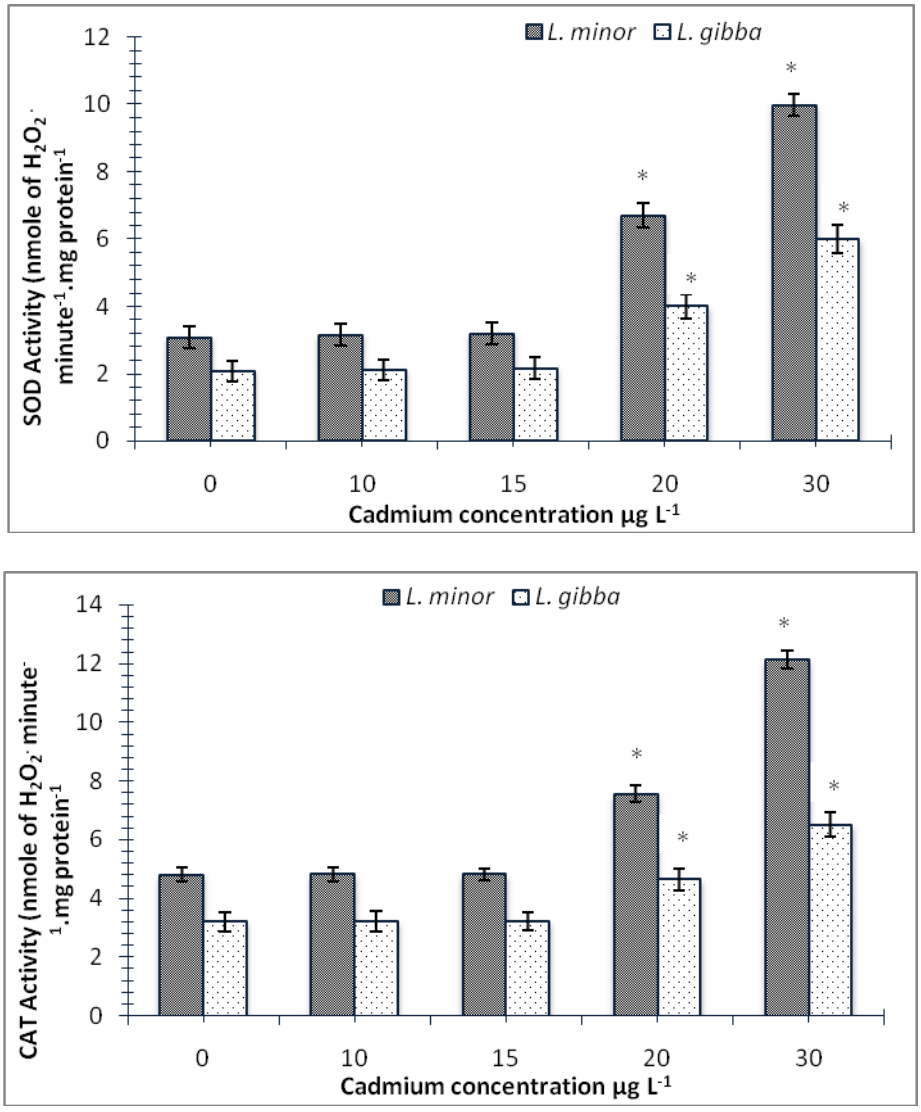


Fig-4: Effect of cadmium concentrations in SIS medium on (A) superoxide dismutase (SOD) and (B) catalase (CAT) in duckweed species after 7 days exposure. Error bars represent the SD of mean (n= 3).

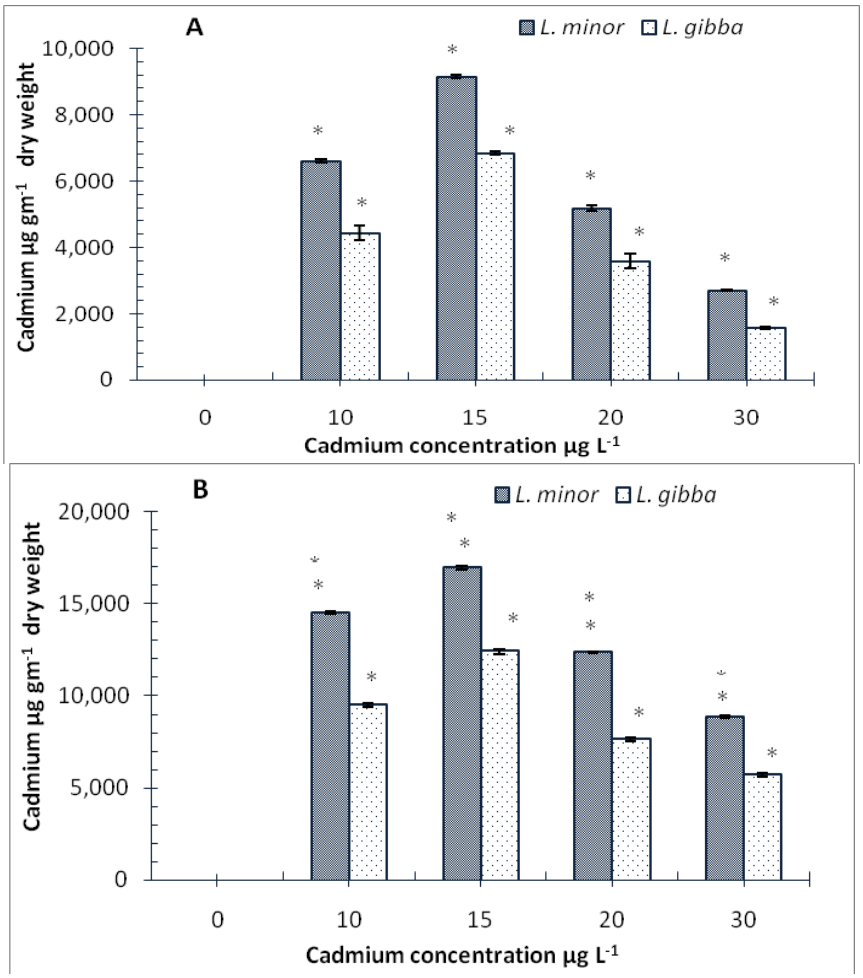


Fig-5: Cadmium accumulation: (A) in fronds of *L. minor* and *L. gibba*(B) in roots of duckweed species exposed to cadmium for seven days at the indicated concentrations. Error bars represent the SD of mean (n= 3).

It has an important role in adjusting the level of hydrogen peroxide and several stress factors were reported to stimulate CAT enzyme [46].CAT activity displayed increasing trends with increasing concentration of cadmium. It can be seen in figure 4B,when the concentration of cadmium was 20 and 30 µg L^{-1}, CAT activity increased statistically (p<0.05) by %56 and 151 in *L. minor*,%44 and %102 in *L. gibba*, respectively in comparison to the control. It is well established that cadmium toxicity results in enhanced ROS generation [47]. The level of ROS in duckweeds are controlled as usual by a complex antioxidant system that consists of enzymes, scavenger enzymes such as glutathione S-transferase (GST)- and non enzymatic low molecular mass antioxidants such as glutathione (GSH), ascorbate and proline [47].The increasing activity of CAT can be explained by substrate induction to maintain low levels of H_2O_2 as an adaptive mechanism[48].

Cadmium accumulation in roots and fronds

Aquatic plants have the potential to accumulate metals in their tissue. The tissue metal concentration depends on the concentration of the metal in the ambient water and time of exposure. Our results clearly showed two different trends of accumulation capacity between *L. minor* and *L. gibba* from the growth medium. Cadmium accumulation in tested plant species were significantly increased (P<0.05) in both fronds and roots with increasing cadmium concentration up to 15 µg L^{-1}, while there was a significant decline in cadmium accumulation in fronds and roots at 20 and 30 µg L^{-1} in duckweed species, compared with the control in growth medium. An overall higher accumulation was observed in roots rather than fronds for all treatments (Figure5 A and B).

In control plant species had low cadmium contents that reflected its uptake from the atmosphere. The highest cadmium accumulation was seen at a dose of 15 µg L^{-1}. Low concentration of heavy metals might stimulate growth of some plant species. Measurements of cadmium concentrations showed that both species accumulated one and a half-fold more cadmium in their roots than in their fronds (Figure 3 A and B).

Cadmium ions are retained in the roots, and only very small amounts are transported to the shoots. Cadmium easily enters the root via the cortical tissue and is trans-located to the aboveground tissues and as soon as they enter the roots, they reach the xylem through an apoplastic or symplastic pathway [49].

This difference in concentrations between roots to fronds reveals a great restriction of the internal transport of cadmium from root towards fronds via xylem sap flow, resulting in higher root concentrations rather than translocation to leaves. For an ideal hyper accumulator, TF values should be higher than 1 [50]. But in the present research, maximum TF was 0.541 and 0.553 in *L. minor* and *L. gibba*, respectively. Such lower values of TF are most likely related to an exclusion strategy [51].

Tissue concentrations of the pollutants and the total biomass produced should be taken to the consideration when plants select for phytoremediation. Therefore, the only critical factor in assessing the efficiency of a given plant species for the phytoremediation of wastewater is the amount of metal accumulated by the harvested parts [52].

In order to measure the potential of tested species to accumulate cadmium, the BCFwere calculated (Table 1).

From these data, it would appear that there was a gradual decrease in cadmium uptake potential in both species with an increase in cadmium concentration of the nutrient solutions. The value of the metal concentration factor for *L. minor* in each group is higher than the value for *L. gibba*, indicating that the uptake rate of cadmium by *L. minor* is higher than that for *L. gibba*. Aquatic macrophytes take up metals from the water, producing an internal concentration several fold greater than their surroundings [53].

Results after seven days of exposure to concentrated cadmium solutions indicate that, *L. minor* achieves high cadmium removal efficiencies, followed by *L. gibba* (Table 1). Metal concentrations in growth medium decreased with time in all the experiments. Results from other research were stated that metal removal from the medium was, due to accumulation in/on plants since *L. minor* was able to remove between 63% and 94% of cadmium [54].

A greater understanding of our findings, *L. minor* was considered to be the most suitable target for further studies, due to effective performance in the cadmium removal studies, easy to deal with in the laboratory in particular during the harvesting, high wet content, and its low environmental impact in case of accidental release into surrounding water bodies.

Table 1. Percentage cadmium efficiency for different initial concentrations. Bio-concentration factor (BCF), Translocation factor (TF) of cadmium uptake in L. minor and L. gibba.

Species	Initial concentrations µg L^{-1}		Final concentration µg L^{-1}	Removal %
	Selected	Measured		
L. minor	10	10.002 ± 0.094	0.135 ± 0.051	98.650
	15	14.855 ± 1.048	0.225 ± 0.063	98.485
	20	20.044 ± 1.952	1.992 ± 0.105	90.062
	30	30.183 ± 1.403	3.991 ± 0.066	86.775
L. gibba	10	9.984 ± 0.843	0.758 ± 0.078	92.408
	15	14.827 ± 1.025	1.257 ± 0.092	91.522
	20	20.102 ± 1.027	2.882 ± 0.717	85.663
	30	30.075 ± 0.765	4.834 ± 0.544	83.927

Cadmium concentrations µg L^{-1}	*L. minor*			*L. gibba*		
	BCF Root	BCF Frond	TF	BCF Root	BCF Frond	TF
10	1452.846	662.930	0.456	948.945	444.902	0.469
15	1128.719	610.446	0.541	825.928	456.368	0.553
20	616.520	259.209	0.420	381.838	179.703	0.471
30	295.155	90.398	0.306	189.235	52.400	0.277

CONCLUSIONS

This study was carried out on two *L. minor* and *L. gibba*, with the target to determine the capacity for accumulation of cadmium that is quite important for bio-indication, bioremediation and bio- monitoring of aquatic ecosystems. With regards to metal (growth biomass, chlorophyll, carotenoid contents, and protein as well as antioxidant enzymes) indicators, cadmium was found to be toxic to both species in the higher observed concentration ranges. *L. minor* revealed the highest accumulation of cadmium, followed by *L. gibba* as well as showed related phytotoxicity. Bio-concentration factor decreased as a function of increased initial cadmium concentrations. Results showed that all species proved to be hyper-accumulator of cadmium.

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