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Comparative Study of Cd, Pb, and Ni removal potential by *Salvinia natans* (L.) All. and *Lemna minor* L.: Interactions with Growth Parameters

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Abstract

The aim of this study was to identify the biological responses and phytoremediation capability of two aquatic macropyhtes: Salvinia natans and Lemna minor. Lemna and Salvinia species were exposed to different concentrations of Pb, Cd, and Ni for 7 days. The bioconcentration factors (BCF) of heavy metals from water to these two aquatic macropyhtes were estimated and the removal potential of heavy metals was assessed. After 7 days, some biological parameters were measured, including metal accumulations in leaves, photosynthetic pigmentations, Lipid peroxidation activity, and growth rates. For S. natans, the highest Pb accumulation was found in 50 mg l^{-1} concentration. The amount of chlorophyll a (chl a) in L. minor was reduced to a minimum value of 0.120 mg g^{-1} with the 8 mg l^{-1} Cd concentration. The Relative Growth Rate (RGR) values of both plants were negatively associated with metal treatment. The levels of Malondialdehyde (MDA) in S. natans increased to a maximum value of 7.174 nmol/g with 50 mg l^{-1} at Pb concentration-dependent and time-depended action. S. natans was a more effective Pb and Ni accumulator than L. minor, but L. minor was a more effective Cd accumulator than S. natans. Our findings might be useful for the phytoremediation of water polluted with heavy metals.

Keywords: Heavy metal, Bioconcentration factor, *Salvinia natans*, *Lemna minor*, lipid peroxidation.

1. Introduction

There are inadequate fresh water resources throughout the world. Fresh water reservoirs and wetlands are under a heavy metal pollution threat from municipal, industrial waste, agricultural, mining, and urban activity. The presence of heavy metals in fresh water resources causes several serious health disorders in plants, animals, and people [1]. Because of water shortages, scientists have focused on the management of fresh water waste, water purification, and re-use [2].

There are several methods for removing heavy metals from water, including chemical precipitation, membrane filtration, electrolysis, reverses osmosis, and adsorption. As the most versatile method, reverse osmosis is most commonly used, but these methods demonstrate different levels of efficiency to different metal removal. Moreover, if the metal contaminated water volume is high, the process of decontamination becomes a very expensive operation. The primary processes by which heavy metals are removed from aquatic environments are physical, biological, and biochemical, and the removed metals will be held in water, biota, and suspended solids. The domination of one of them will depend on the composition of the system, pH, redox condition, and pollutant nature [3].

Phytoremediation is the preferred method for cleaning up contaminated areas because of its high efficiency and low pricing. Despite these advantages, few plant species are known to have properties that qualify them to be good phytoremediation species for terrestrial and aquatic environments [4,5]. Among aquatic species, genus *Lemna* has an important role because of its extensive use in phytoremediation studies [6–8]

In stress conditions, the free radical species (forms of active oxygen) may be increased, which will enhance the activities of these detoxifying enzymes. The activities of free radical species are induced in plant species by heavy metals [9–11]. At the cellular level, toxic metals lead to the inhibition of cellular function, which can be fatal. MDA is a cytotoxic by-product of resulting lipid peroxidation and it indicates the amount of tissue damage caused by the production of free radicals. RGR is significant parameter in assessing the physiological effects of toxic chemicals on plants. Stress conditions may increase protective processes such as the accumulation of compatible solutes [12].

Salvinia natans (water fern) is an annual floating aquatic fern that can appear superficially similar to moss [13]. *Lemna minor* (Duckweed) belongs to the *Lemnaceae* family and can be found throughout the world where there is plentiful standing fresh water and free-floating tiny macrophytes. In practice, duckweed can eliminate heavy metals and nutrients from polluted water and decrease algal abundance in waters, making it useful for waste water treatment [14]. Scientists have focused on studying the *Lemna* species because of its metal accumulation, providing exciting and significant responses to experimental model systems [2,15].

The first aim of the present study was to identify and compare the accumulation properties of Cd, Pb, and Ni on *Salvinia natans* and *Lemna minor*. This study also intended to show the biological responses of *Lemna* and *Salvinia* species to Cd, Pb, and Ni in different concentrations.

The findings of the research may help clarify the impacts of Cd, Pb, and Ni on aquatic plants, and accordingly, the biological reflexes of these plants.

2. Materials and Methods

2.1. Plant material and treatment conditions

Lemna minor were obtained from Soysalli-Kayseri and *Salvinia natans* were obtained from Adana, Turkey. At the beginning of the experiment, plants were washed in distilled water and acclimatized for 3 days in a climate chamber with a water temperature of 23°C, a relative humidity of 70%, and a 16:8 h light:dark cycle. In this study, cadmium chloride ($CdCl_2$), lead chloride ($PbCl_2$), and nickel chloride ($NiCl_2 6H_2O$) were used for experimental treatments. Different concentrations of Cd (1- 2- 4-8 mg l^{-1}), Pb (5, 10, 25, 50 mg l^{-1}), and Ni (1- 5- 10- 20 mg l^{-1}) were maintained in 10% Hoagland's solution in separate 400 mL conical beakers under the aforementioned conditions for 7 days [16]. Beakers without metals grown for each set of experimental groups acted as controls. The beakers that comprised the plant and heavy metal concentrates were placed in a climate chamber under the aforementioned conditions for 7 days [12]. All treatments were performed in triplicate.

2.2. Cd, Pb, and Ni determination

Plants were washed thoroughly with double deionized water, blotted, and oven dried at 80°C. Each sample was digested with 10 ml pure HNO_3, using a CEM-MARS 5 microwave digestion system. After digestion, the volume of each sample was set to 25 ml using double deionized water. Determinations of Pb, Ni, and Cd in plant samples were performed by inductively coupled plasma optical emission spectroscopy (Varian-Liberty II, ICP-OES). Reagent blanks were also prepared to determine any potential contamination during the digestion and analytical procedure [17]. Peach leaves (NIST, SRM- 1547) were used as the reference material for all of the performed analytical procedures. Recoveries of heavy metals from NIST proposed SRM-1547 (Cd: 0.005±0.01 μg l^{-1}; Ni: 0.03±0.01 μg l^{-1}; and Pb: 0.81±0.01 μg l^{-1}), and certified value of heavy metals of NIST proposed SRM 1547 (Cd: 0.007±0.01 μg l^{-1}; Ni: 0.04±0.01 μg l^{-1}; and Pb: 0.89± 0.02 μg l^{-1}) analyses were determined by ICP-OES. The detection limits of Cd^{2+}, Ni^{2+}, and Pb^{2+}, were 0.3×10^{-3}, 0.8×10^{-3}, and 2×10^{-3} mg/kg, respectively. All treatments were performed in triplicate. All chemicals used in this study were analytical reagent grade (Merck, Darmstadt, Germany) [12].

The bioconcentration factor (BCF) was calculated as follows [18]

$$BCF = \text{Pb in plant biomass (mg kg}^{-1}) / \text{Pb in solution (mg l}^{-1})$$

2.3. Relative Growth Rates and photosynthetic pigment contents

Relative Growth Rates of duckweed species were calculated in each group according to Hunt's equation:

$$R=\ln W_2-\ln W_1 / T_2-T_1$$

Where R is the relative growth rate (gg^{-1} d^{-1}), W_1 and W_2 are the initial and final dry weights, respectively, and (T_2-T_1) is the experimental period [19].

Plants were placed on blotting paper and allowed to drain for 5 min before weighing. Plant biomass was measured on the basis of fresh weight for photosynthetic pigments. Photosynthetic pigments (chlorophyll a and carotenoid) of treated and untreated plants (100 mg) were extracted in 80% chilled acetone in the dark. After centrifugation at 10,000 × g for 10 min, absorbance was taken at 450, 645 and 663 nm. The content of chlorophyll a, chlorophyll b and carotenoid were estimated as previously described [20].

2.4. Lipid peroxidation

To Determine of lipid peroxidation, leaf material (500 mg) was homogenized with 3 mL of 0.5% TBA in 20% TCA (w/v). The homogenate was incubated at 95°C for 30 min, and ice was used to stop the reaction. The samples were centrifuged at 10,000 × g for 10 min, and the absorbance of the resulting supernatant was recorded at 532 nm and 600 nm. The amount of MDA (extinction coefficient of 155 mM^{-1} cm^{-1}) was calculated by subtracting the non-specific absorbance at 600 nm from the absorbance at 532 nm [21].

2.5. Statistical analysis

The data was expressed as mean values with standard errors (SE). Two-way analysis (ANOVA) was done with all the data to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments. We used p=0.05 as the statistical significance threshold. All statistical analyses were made with the SPSS 17.0 software package.

3. Results and discussion

3.1. Accumulation and toxicity of heavy metals

The bioaccumulation of Cd was measured in both plants. As shown in Fig. 1, the maximum Cd accumulation was observed at a dose of 8 mg l^{-1} in *S. natans* (23550 μg g^{-1}), after 7 days. Cd accumulation and Cd concentrations were significantly positively correlated with each other in *S. natans* fronds (R=0.945, *P≤0.01*). Also shown in Fig. 1, the maximum Pb accumulation was observed at a dose of 50 mg l^{-1} in *S. natans* (9570 µg g^{-1}), after 7 days. Pb accumulation and Pb concentrations were positively correlated with each other in *L. minor* fronds (R=0.921, *P≤0.01*). Accumulation of Ni was determined in plant fronds. Fig. 1 also shows that the maximum Ni accumulation was seen at a dose of 20 mg l^{-1} in *S. natans* (42363 µg g^{-1}), after 7 days. Ni accumulation and Ni concentrations were positively correlated with each other in *S. natans* fronds (R=0.879, *P≤0.01*). Fig. 1 clearly shows that the bioaccumulation of metals in plants rises with the metal concentration as well as over time. In this study, the high accumulation of heavy metal was determined in plants over a seven-day period. As shown in Fig. 6, *S. natans* was more effective in accumulating Pb and Ni than *L. minor*, while *L. minor* was a more effective Cd accumulator than *S. natans*.

The potential of *Salvinia* for heavy metal removal has been investigated in many studies [22,23]. Researchers have proven the occurrence of a high concentration-dependent accumulation of heavy metal in aquatic plants [24–26]. As shown in Fig. 1, our study showed a high concentration of Cd, Pb, and Ni in *S. natans* and *L. minor* depending on concentration for 7 days.

The initial metal concentration is an important parameter for the accumulated metal concentration during exposure [27]. Maine et al. (2001) found that the higher the initial concentration of Cd, the greater the amount of Cd that the plants removed [28]. In the present study, the highest Cd accumulation was observed at the highest Cd concentration in *L. minor* and *S. natans*, as shown in Fig. 2.

A study conducted by Mishra and Tripathi (2008) with other aquatic macrophtes (*Spirodella polyrhiza*, *Pistia stratiotes,* and *Eichhornia crassipes*) determined that the removal of Cd was slower than that of Cu and Zn, and they explained that Cu and Zn are micronutrients for the plants while Cd is not an essential metal [29]. Similarly, the present study found that Cd was the most toxic metal for these plant species, followed by Ni and finally Pb.

Cowgill et al. (1991) compared the sensitivity of *L. minor* and *L. gibba* to eight different chemicals. They found that the *Lemna* species have the same sensitivity to all chemicals except diethanolamine, where *L. minor* had an EC50 value twice that of *L. gibba*, based on plant and frond numbers [30]. Metal concentrations in water decreased with time in all the experiments. In another study, *L. minor* and *S. polyrhiza* exposed to copper sulphate for 72 h were found to have similar levels of tolerance [5]. Contrary to that finding, the present study compared Ni sensitivity between two plants and found that *S. natans* were more tolerant to Ni than *L. minor*, as shown in Fig. 2.

According to Zayed et al. (1998), a plant that is considered a good accumulator must have a BCF over 1,000 [31]. As shown in Fig. 2, our results confirmed that *L. minor* and *S. natans* were good accumulators of Cd, Pb, and Ni and have potential for the remediation of heavy metal polluted water.

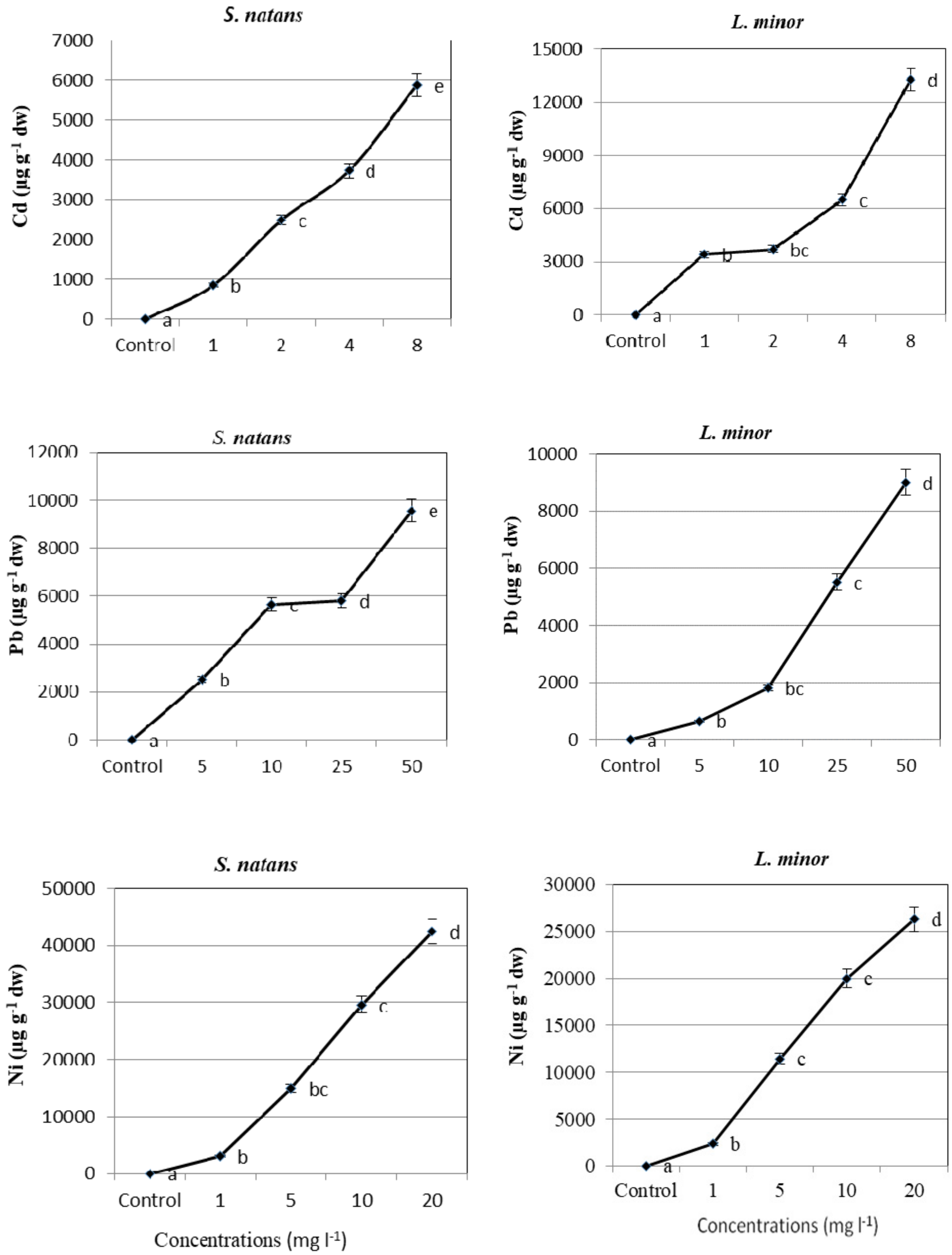

Fig. 1 Accumulation of Cd (1-2-4-8 mg l^{-1}), Pb (5-10-25-50 mg l^{-1}) and Ni (1-5-10-20 mg l^{-1}) by *Salvinia natans* and *Lemna minor*, exposed to different concentrations over various periods of time. All values are means of triplicates ± S.D. ANOVA significance was set at $p \leq 0.05$.

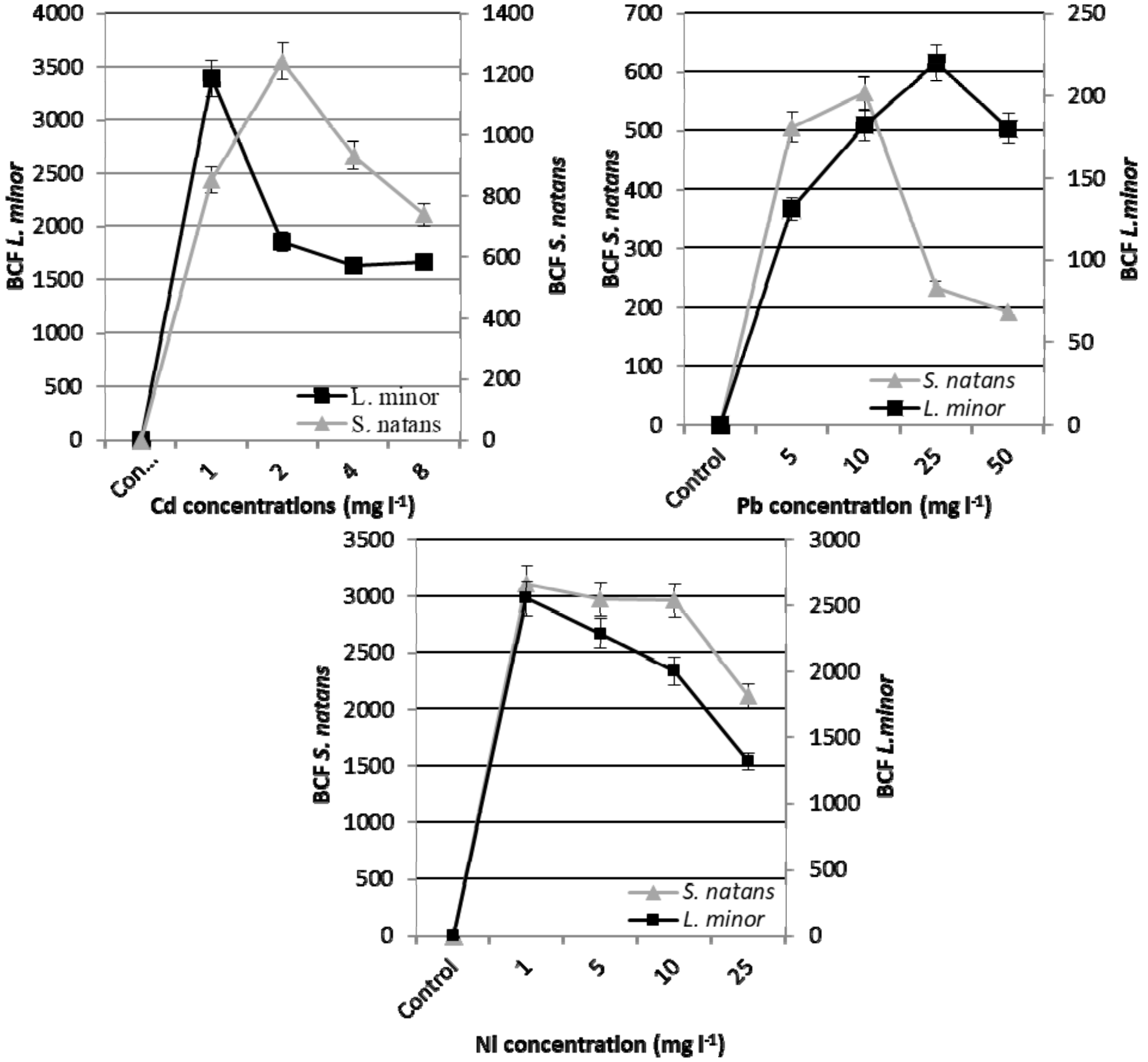

Fig. 2 Bioconcentration factor (BCF) values of three heavy metals in *Salvinia natans* and *Lemna minor*. Plants were exposed to heavy metals for 7 days. Vertical bars denote SD, n=3

3.2. Effects of heavy metals on growth of *S. natans* and *L. minor*

As shown in Fig. 3, the relative growth rates of plant decreased with the presence of heavy metal concentration and heavy metals produced a darker color on the leaves. Also shown in Fig. 3, the highest decline of RGR was found at 50 mg l^{-1} Pb exposure in *L. minor* after 7 days. The plants exposed to 25 and 50 mg l^{-1} of Pb revealed a significant effect on their growth parameters as is evident by the visual changes, such as, chlorosis of leaves. A significant negative correlation was determined between RGR values and concentrations of Pb for *L. minor* (R= -0.921, $P \leq 0.01$).

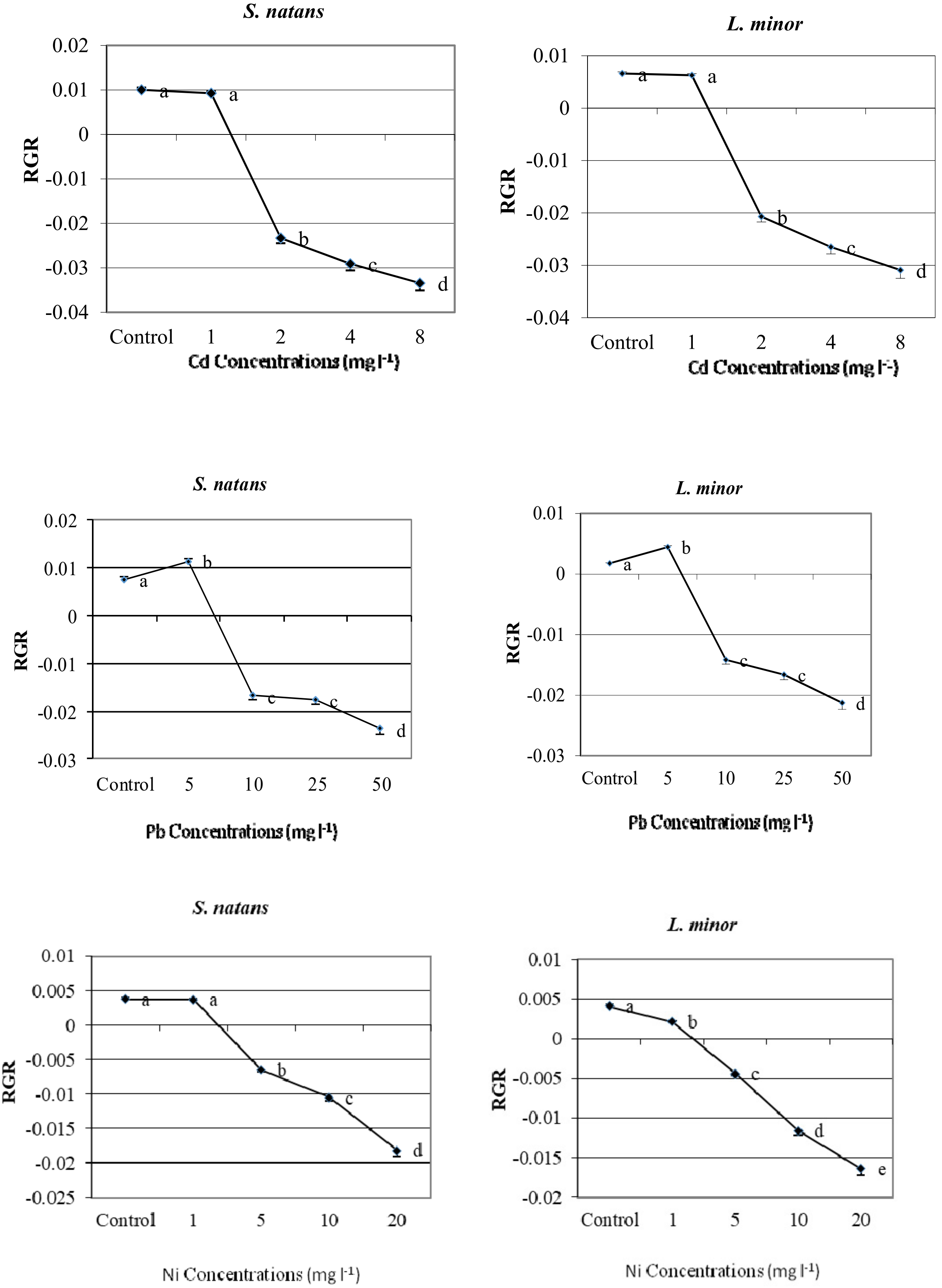

Fig. 3 Relative growth rates of Cd Pb and Ni treat by *Salvinia natans* and *Lemna minor*. All values are means of triplicates ± S.D. ANOVA significance was set at $p \leq 0.05$.

This study demonstrated that lower doses of the toxic metals Cd and Cr stimulated the growth of *S. natans* and *L. minor*. In higher doses, however, these toxic metals have disruptive effects on the growth of these plants. Similar to our findings, Cedergreen (2008) found that toxic chemicals accelerate growth at lower doses but have a toxic effect at higher doses [32]. It is well known that, depending on the concentration of exposure, plants under toxic metal stress produce reactive oxygen species (ROS) that destroy the cell wall and cell membrane. Because of ROS, RGR values may decrease in higher exposure concentrations [33]. Our findings completely support these findings.

3.3. Effect of metals on photosynthetic pigments

As shown in Fig. 4 and Fig. 5, chlorophyll concentrations in *S. natans* and *L. minor* were negatively correlated with heavy metal exposures. Fig. 5 shows that levels of chl *a* decreased in a Cd concentration-dependent and time-depended manner, with a minimum value of 0.120 mg g^{-1} in the 8 mg l^{-1} on *L. minor*, after 7 days (R= -0.829, $P \leq 0.01$). Fig. 5 also shows that levels of carotenoid decreased in a Pb concentration-dependent and time-depended manner, with a minimum value of 0.011 mg g^{-1} in the 50 mg l^{-1} on *L. minor* after 7 days (R= -0.916, $P \leq 0.01$). As shown in Fig. 4, levels of chl *a* decreased in a Pb concentration-dependent and time-depended manner, with a minimum value of 0.14 mg g^{-1} in the 50 mg l^{-1} on *S. natans* after 7 days (R= -0.718, $P \leq 0.01$). Fig. 4 and Fig. 5 show that chlorophyll concentrations in *S. natans* and *L. minor* were negatively correlated with Ni exposures. When *L. minor* fronds were exposed to Ni concentrations of 1 mg l^{-1} or higher, a dose-dependent decrease of chlorophyll pigments was also observed, with a minimum chl *a* value of 0.77 mg g^{-1} fresh weight on day 7 at 20 mg l^{-1} compared to 1.57 mg g^{-1} in controls (R= -0.917, $P \leq 0.01$), as shown in Fig. 5. Fig. 4 shows that the levels of carotenoid decreased in a Ni concentration-dependent and time-depended manner, with a minimum value of 0.014 mg g^{-1} in the 20 mg l^{-1} on *S. natans* (R= -0.912, $P \leq 0.01$). Under all exposure conditions, the photosynthetic pigment contents of the plants exposed to heavy metals revealed lower values than the control sample, as shown in Fig. 4 and Fig. 5.

In a study with aquatic plants in a heavy metal polluted wetland, Clijsters and Van Assche (1985) detected a significant reduction in the amount of aquatic plant chlorophyll, which they attributed to three different reasons: (I) metal accumulation that may reduce the effectiveness of the enzymes that synthesize chlorophyll, (II) reduced iron uptake, and (iii) the formation of metal substituted chlorophylls [34]. When exposing *Salvinia* species to heavy metals in a laboratory environment, Hadad et al. (2007) detected a decrease in photosynthetic pigment. Findings of the present study support this, as we determined that the chlorophyll content of both *S.natans* and *L.minor* decreased in increased concentrations of metal exposure [35].

Photosynthetic pigments (chlorophyll and carotenoids) are a central part of the photosynthesis system in green plants. A significant change in the amount of pigment causes a marked effect on the entire metabolism of the plant, including the peroxidative breakdown of pigments and chloroplast membrane lipids by the reactive oxygen species, an impaired uptake of nutrients (such as, Mn, Cu, Fe, and P), or the degradation of chlorophyll through an increase in chlorophylls activity [36]

Likewise, in this study, plants threatened with non-essential and toxic metals like Cd, Pb, and Ni saw a decrease in pigment molecules, subsequently interfering with the plant's ability to effectively photosynthesize and inhibiting plant growth and development.

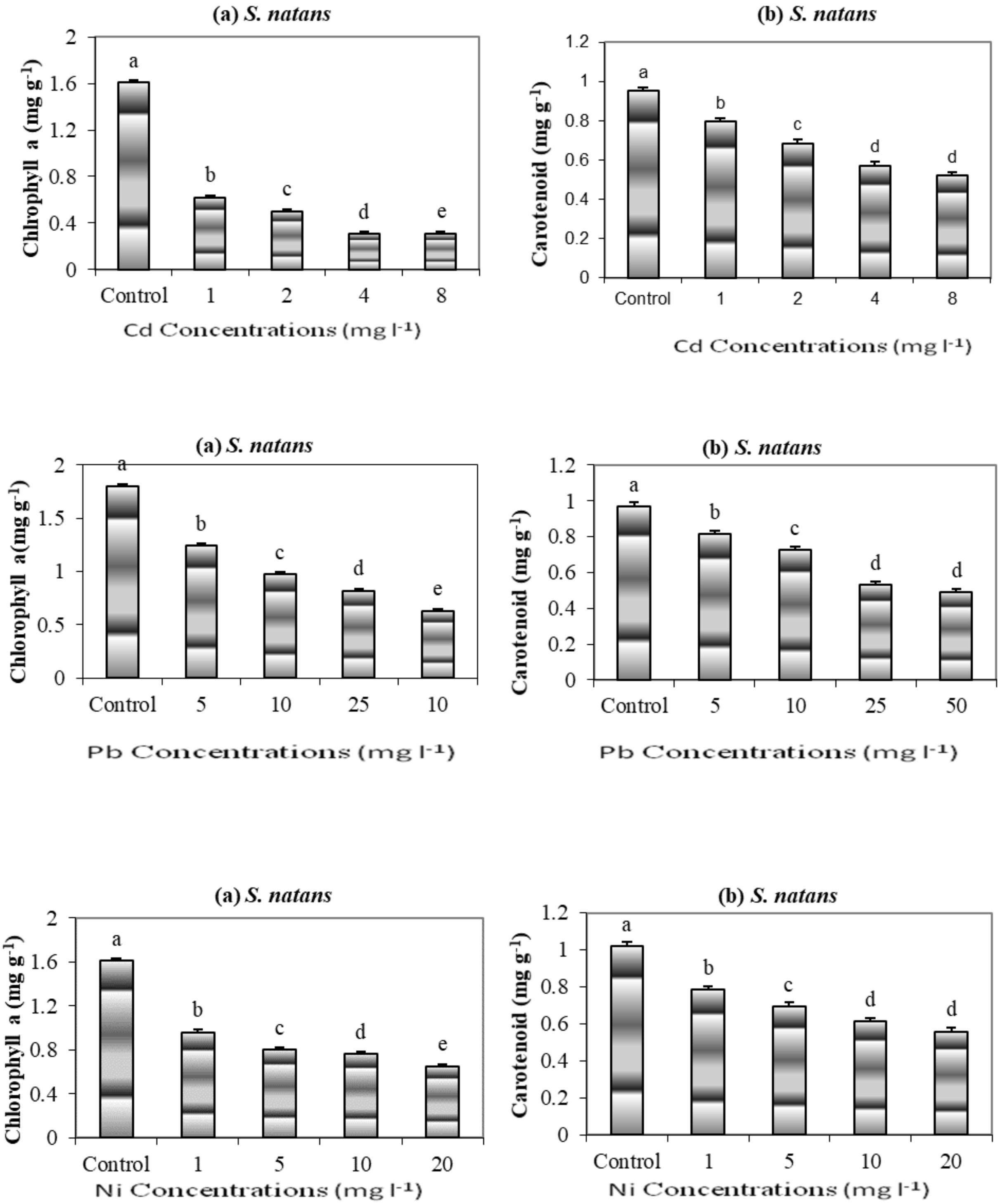

Fig. 4 Effects of Cd, Pb and Ni on chlorophyll *a* (a) and carotenoid (b) contents of *Salvinia natans*. All values are means of triplicates ± S.D. ANOVA significance was set at $p \leq 0.05$.

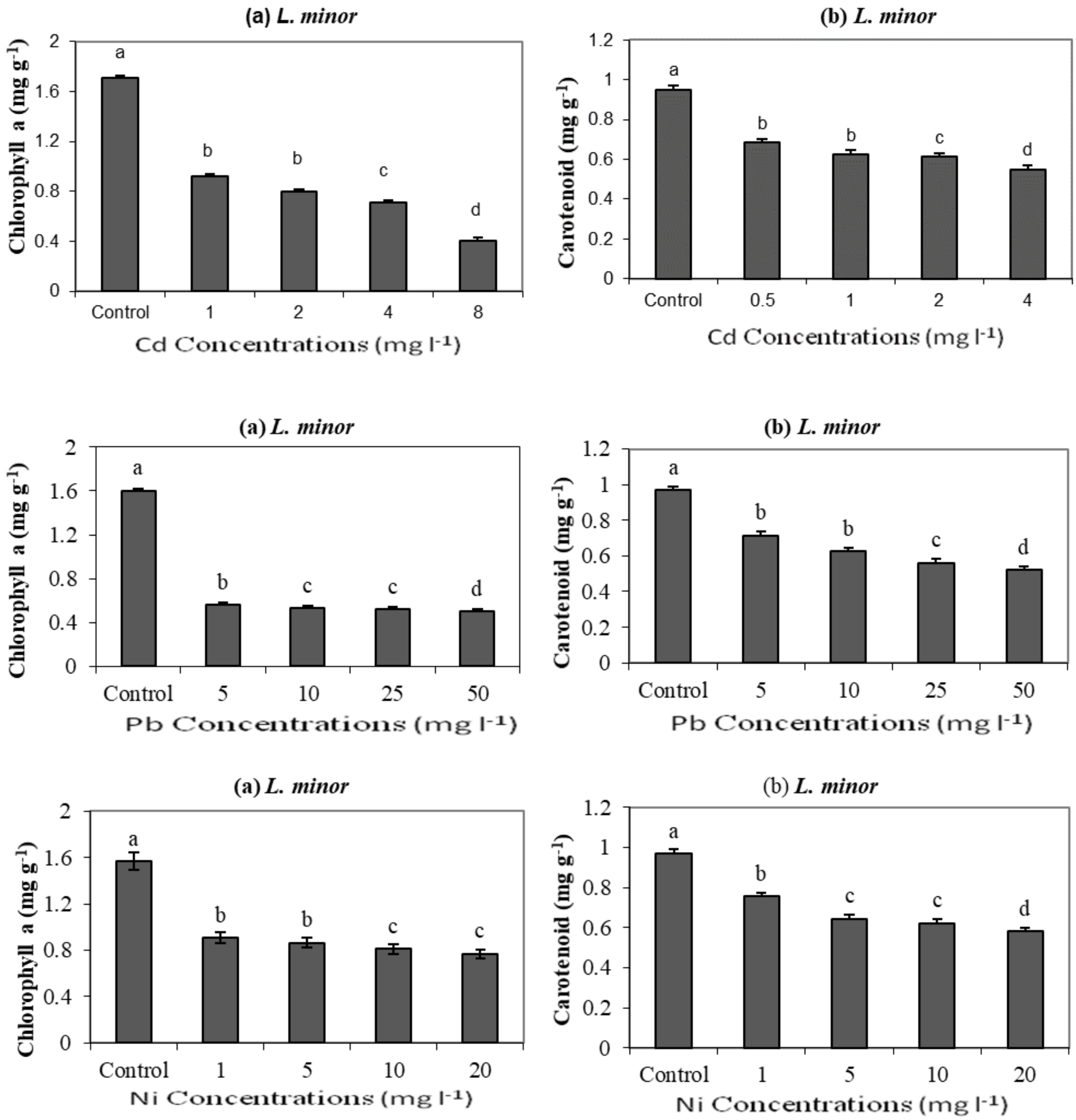

Fig. 5 Effects of Cd, Pb and Ni on chlorophyll *a* (a) and carotenoid (b) contents of *Lemna minor*. All values are means of triplicates ± S.D. ANOVA significance was set at $p \leq 0.05$.

3.4. Effect of metals on the level of MDA

As shown in Fig. 6, the MDA contents in *S. natans* and *L. minor* were positively correlated with heavy metal exposure. Furthermore, at a Pb application of 5 mg l^{-1}, the MDA content was not found to be significantly high when compared to the control. However, at a Pb application of 5–50 mg l^{-1}, the MDA content increased with an in increase in the Pb concentration up to 50 mg l^{-1}. Beyond that, the MDA content revealed a tendency to decrease in value, even though it never reached a level that was significantly lower than that of the control (P≤ 0.05), as shown in Fig. 6. The levels of MDA increased in both a Pb concentration-dependent and a time-depended manner, with a maximum value of 7.174 nmol/g in the 50 mg l^{-1} in *S. natans* after 7 days (R= 0.729, $P \leq 0.01$), as shown in Fig. 6.

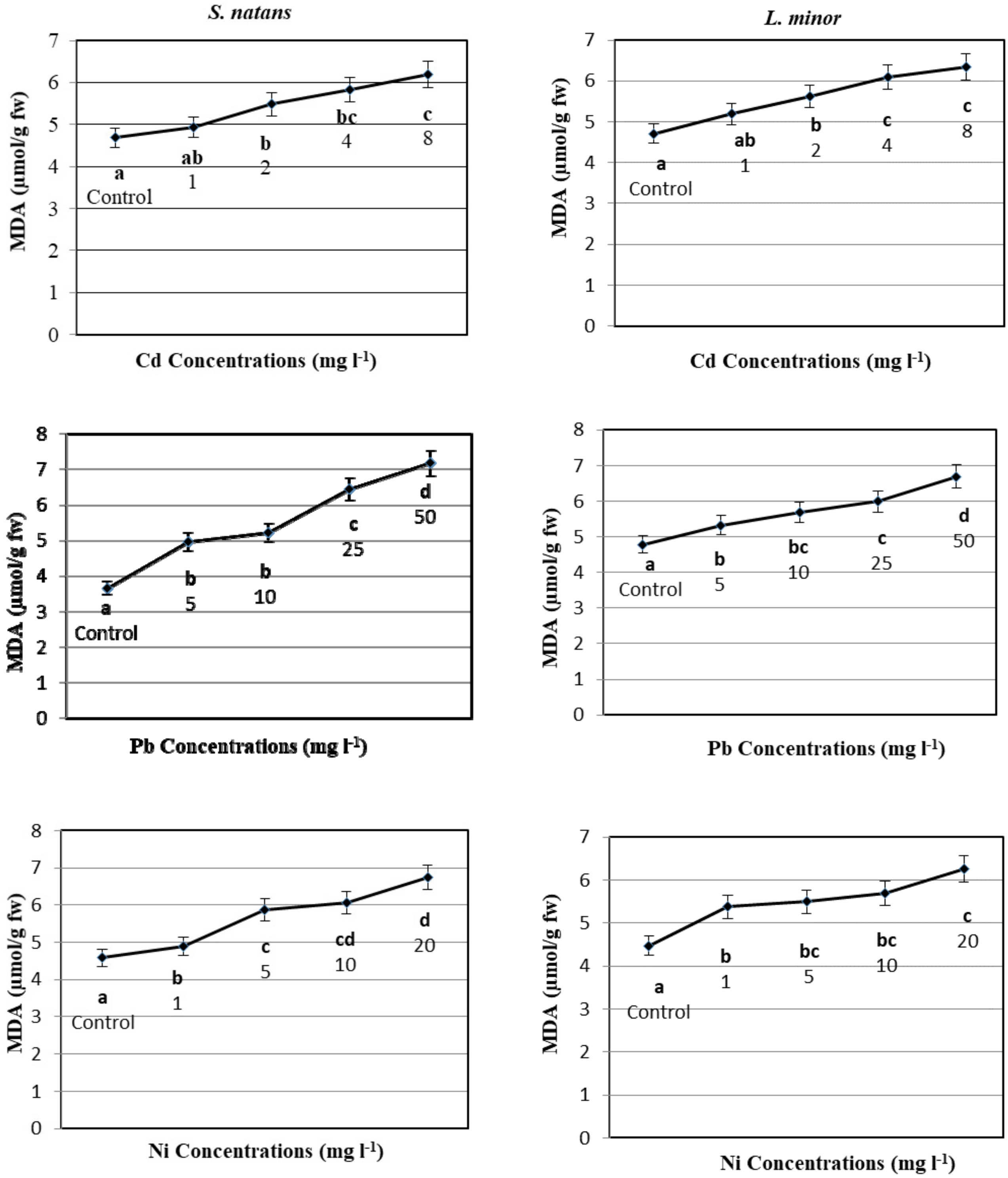

Fig. 6 Effects of Cd, Pb and Ni on lipid peroxidation of *Salvinia natans* and *Lemna minor*.
All values are means of triplicates ± S.D. ANOVA significance was set at $p \leq 0.05$.

MDA content provides an idea of a plant's antioxidant defense mechanism. According to the literature, plants with a high MDA content are under serious stress [37]. An increase in the accumulation of heavy metals increases the length of metal exposure, altering heavy metal enzyme activity, affecting the membrane permeability, creating ion leakage and damaging the plant [22]. This study supports the findings in the literature that the concentration end exposure duration increases MDA levels.

4. Conclusions

In conclusion, an efficient adaptation to hydroponics and the valuable Cd, Pb, and Ni accumulation observed for *S. natans* and *L. minor*, especially at higher doses of heavy metals, shows the great potential of this species for the decontamination of pollutants in water-based systems.

The release of heavy metals like Cd, Pb, and Ni into the environment has increased the decomposition of living and non-living organisms. Water is the potion of life and a universal solvent that carries nutrients and wastes to and from our cells. In recent years, water pollution has become a great problem.

Phytoremediation technology aims to eliminate the contamination of the metal uptake of heavy metals by plants. This technology has quite advantageous aspects compared to conventional methods already in use. To be successful with this method, one of the most important requirements is to select the appropriate plants for metal accumulation. Because of this, an investigation of the phytoremediation abilities of plants is a critical task for solving this environmental issue.

S. natans and *L. minor* are important in the treatment of domestic and industrial wastewater as well as in the restoration of decommissioned mining sites. They demonstrate most of the properties of an ideal plant species for phytoremediation.

It has been shown that *S. natans* was a more effective Pb and Ni accumulator than *L. minor,* but *L. minor* was a more effective Cd accumulator than *S. natans*. The results of the present study confirm the accumulation capacities of Cd, Pb, and Ni by these species and their great potential for phytoremediation.

5. Acknowledgements

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