

Comparative Studies of the Phytoextraction Capacity of Five Aquatic Plants in Heavy Metal Contaminated Water

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Abstract

The uptake capacity of the aquatic plants (*Salvinia natans* Kunth., *Eichhornia crassipes* Mart., *Lemna minor* L., *Elodea canadensis* Michx., *Pistia stratiotes* L.) was analyzed in phytoextraction of Cu²⁺, Zn²⁺, and Cd²⁺. It was attend to study the plants capacity comparatively using mono and multimetallic systems. In particular, the chlorophyll, protein and carotenoids contents were studied during heavy metals uptake, in order to observe the stress effect on plants. The results obtained for the monometallic system showed that *Salvinia natans* Kunth. accumulated the highest quantity of Cu²⁺ (4.72 mg/g), Zn²⁺ (2.23 mg/g) and Cd²⁺ (1.90 mg/g). The leaves of *Lemna minor* L. accumulated the highest concentration of Cu²⁺ (10.80 mg/g) and Cd²⁺ (2.78 mg/g) in multimetallic system. The water lettuce (*Pistia stratiotes* L.) translocated the highest quantity of Zn²⁺ in its roots (4.80 mg/g). The chlorophyll and the carotenoids levels decreased under the stress of heavy metals in both systems, while protein content increased under the influence of Cu²⁺ and Cd²⁺, but decreased for Zn²⁺. The studied hydrophytes proved to be useful in the uptake of heavy metals in monometallic system and much more effective in the multimetallic system and showed great potential for further applications in the industrial and commercial wastewater treatments.

Keywords: hydrophytes, photosynthetic pigments, phytoaccumulation, pollution, proteins

Abbreviations: DW, dry wt – dry weight; chl – chlorophyll; chl *a* – chlorophyll *a*; chl *b* – chlorophyll *b*; total chl – total chlorophyll; car – carotenoids; FW – fresh weight; DW – dry weight; HM – heavy metals; NPK – nitrogen, phosphorus, potassium.

Introduction

The environmental contamination with toxic elements is a major issue on a global scale. It can damage or alter the biosphere reducing the agricultural productivity and can affect both animal and human bodies (Buszewski *et al.*, 2000; Cvjetko *et al.*, 2010; Kamal *et al.*, 2004; Kaur *et al.*, 2010; Lu *et al.*, 2004; Skinner *et al.*, 2007).

The remediation of pollutants in different environmental conditions can be achieved through plants (phytoremediation), being considered an alternative technology to clean up contaminated waters. This method was also used in the 18th century to treat wastewater, leading to extensive researches that allowed the study of metal phytoextraction processes. Moreover, the understanding of the physiological mechanisms of metal absorption, transportation and assimilation became possible (Mudgal *et al.*, 2010).

Phytoremediation includes: phytoextraction, phytostabilization, rhizofiltration, phytodegradation and phytovolatilization. Through

phytoextraction, plants remove the pollutants from a contaminated site (Maczulak, 2009). Hydrophytes can translocate and concentrate many kinds of contaminants including heavy metals, pesticides, explosives, oils (Hasan *et al.*, 2007; Kamal *et al.*, 2004) and nutrients (nitrate, ammonium, phosphate).

The hyperaccumulator plants can gather high concentrations of heavy metals (Kachenko *et al.*, 2009) from contaminated sites, at a minimum percentage, which can vary depending on the pollutant involved (more than 1000 mg/kg of dry weight for copper, cobalt, nickel, chromium and lead; or more than 10,000 mg/kg for magnesium and zinc – Baker and Brooks, 1989). These plants are able to absorb heavy metals such as Pb, Hg, Zn, Co, Cd, and Cu via roots, stems and leaves, and deposit them in their organism. Some of these elements are essential for the metabolic function for a large class of organisms, e.g. Zn, Fe, Cu, Cr and Co (Hogan, 2010), while others are very toxic (Cd, Hg, Pb).

In the case of a high heavy metal concentration, the uptake inhibition and transportation can take place for essential metals as well (Fe, Zn and Mn), through

antagonistic effects (Naaz and Pandey, 2010). Other authors analyzed the distribution, accumulation and selection of heavy metals on selective plants in the presence of chelating compounds (Csog *et al.*, 2012; Mihucz *et al.*, 2012).

The main source of these heavy metals usually find their path in the aquatic ecosystems, e.g. ponds, rivers and lakes (Kumar, 2010), and consist mostly of industrial residues, mining, military operations (Liang *et al.*, 2009), fertilizers and pesticide applications, fuel production and urban wastes. Recently, the use of aquatic plants for treating wastewater has been reported as an economical device for the treatment of heavy metal pollution in contaminated wastewaters (Kumar, 2010). Several studies proved that the cost of phytoextraction is lower than other conventional engineering technologies. Phytoremediation is not just environmentally friendly, cost-effective and non-intrusive, but it also conserves the ecosystem efficiently (Kamal *et al.*, 2004).

Many researches have been conducted regarding the ability of aquatics to remove heavy metals from contaminated waters. For a diversity of purposes, a number of studies have been made on several hydrophytes: *Salvinia sp.* (Dhir, 2009; Espinoza-Quinones *et al.*, 2005), *Potamogeton crispus* (Aysel *et al.*, 2010), *Potamogeton pectinatus* and *Potamogeton malaianus* (Peng *et al.*, 2008). Recent studies highlight the effectiveness of *Eichhornia crassipes* (Hogan, 2010; Naaz and Pandey, 2010; Singh and Kalamdhad, 2013; Skinner *et al.*, 2007), *Typha angustifolia* (Dilek and Aksoy, 2004), *Myriophyllum heterophyllum* (Aysel *et al.*, 2010), *Lemna minor* (Alka and Tripathi, 2007; Rahman and Hasegawa, 2011; Yeşim *et al.*, 2003) and *Azolla caroliniana* (Bennicelli *et al.*, 2004). Some research studies demonstrate that *Nymphaea* varieties can accumulate heavy metals, consequently, they can be used as hyperaccumulators (Lavid *et al.*, 2000).

The purpose of the present paper is to compare the phytoextraction capacity of five aquatic plants and monitor the accumulation and distribution of heavy metals into their morphological parts (leaves and roots). In order to evaluate the markers for stress responses in plants under heavy metal stress treatment, biochemical parameters such as chlorophyll, proteins and carotenoids were investigated.

Materials and methods

Plant material and growing conditions. During the experiment the following five hydrophytes were used: floating fern (*Salvinia natans* Kunth.), water hyacinth (*Eichhornia crassipes* Mart.), duckweed (*Lemna minor* L.), waterweed (*Elodea canadensis* Michx.) and water lettuce (*Pistia stratiotes* L.). The above mentioned species were grown in an educational greenhouse, for a period of 30 days at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. The plants were kept separately in 5 plastic tanks, filled with tap water, in addition of 0.5% Complex III (NPK) fertilizer.

After the growing period, the plants were used for Cu^{2+} , Zn^{2+} and Cd^{2+} phytoremediation experiments. They were kept for acclimatization for a period of 3 days in modified Hoagland nutrient solution in laboratory conditions. The

Hoagland nutrient solution contained macro elements such as: 1.25 mM KNO_3 (Potassium nitrate), 1.25 mM $\text{Ca}(\text{NO}_3)_2$ (Calcium nitrate), 0.5 mM MgSO_4 (Magnesium sulfate), 0.25 mM KH_2PO_4 (Monopotassium sulphate) and micro-nutrients such as: 10 μM FeEDTA (Ethylenediaminetetraacetic acid) as iron source, 11.6 μM H_3BO_3 (Boric acid), 4.5 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Manganese chloride tetrahydrate), 0.19 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Magnesium sulphate heptahydrate), 0.12 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (Sodium molybdate), 0.08 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Copper II sulphate pentahydrate). The dimension of the aquarium was 21 cm x 38 cm x 28 cm. During the phytoremediation experiments the plants were exposed to electric light for 14 hours then to darkness for 10 hours at a temperature of 24-28 °C.

The heavy metal stock solutions were made of salts such as $\text{Cd}(\text{NO}_3)_2$ (Cadmium nitrate), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Zinc sulphate heptahydrate) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Copper II sulphate pentahydrate). For accuracy purposes, the initial concentrations were analyzed by the Atomic Absorbance Spectrophotometer and the Inductively Coupled Plasma Mass Spectrometry. The concentrations of Cu^{2+} , Zn^{2+} and Cd^{2+} , used in all the aquatics studied, were of 10 mg/L.

The heavy metal contamination was investigated in the monometallic and multimetallic system, but the control plants were left without heavy metal treatment. It is essential to specify that the monometallic system can be defined as a process of each plant's separate absorption of every heavy metal one-by-one. Moreover, the multimetallic system uses the treatment of the plants with the mixture of the mentioned heavy metals. In order to determine the phytoextraction capacity, the results of the treated plant samples were compared with the results of the untreated plant samples. The untreated and treated plant samples were dried at 70-80 °C for a period of 48 hours. After digestion with HNO_3 (digestion of 1g plant material with 10 ml nitric acid - 69% HNO_3 , left for 24 hours at 20 °C and for 8 hours at 100 °C) the Cu^{2+} , Zn^{2+} and Cd^{2+} concentrations were determined. Three replications were obtained of both systems.

In order to investigate the responses of the plants through the heavy metal treatments, the photosynthetic pigments (chlorophyll *a*-chl *a*; chlorophyll *b*-chl *b*, carotenoids-car) and the total content of protein were analyzed. The determination of chlorophyll in treated and untreated plant samples (250 mg) was achieved through the extraction of photosynthetic pigments with 96% chilled ethanol being kept in the dark (for 12 hours). After a ten-minute centrifugation, the supernatant absorbance (chl *a* A_{665} , chl *b* A_{649} , car A_{470}) was measured using the UV-Vis Spectrophotometer (GBC Cintra 202). The content of chl *a*, chl *b* and total chl and car was determined by the method of Arnon (1949) using the formula suggested by Lichtenthaler and Wellburn (1983) and Lichtenthaler (1987). The protein content was estimated following the method of Bradford (1976) using serum albumin as a standard protein and it was measured in both mono and multimetallic solutions.

In order to establish quantitative differences between values, statistical analyses were performed using Microsoft Office Excel. The data results were expressed as standard deviation (SD) from three replicates for each sample, consequently Duncan's multiple range test was undertaken.

Results and discussion

Based on the preliminary experiments and observations, the hydrophytes studied had a phytoremediation period of six days. In Romania, the permissible metal content in wastewaters in compliance with the law is: 0.1 mg/dm³ for Cu²⁺; 0.5 mg/dm³ for Zn²⁺ and 0.2 mg/dm³ for Cd²⁺ (<http://www.epurare.euriteh.ro/legislatie/NTPA001%202.8.02.2002.pdf>). The phytoextraction capacity of the plants was determined by measuring the heavy metal concentrations before and after the experiments undertaken from the plant samples.

Monometallic system

The uptake of the plants studied and the bioaccumulation depend on the plant species and on the metal ion characteristics. Two hydrophytes *E. crassipes* Mart. and *P. stratiotes* L. were selected in order to analyze the heavy metal accumulation and distribution in the parts of the plant (root and leaves) and the obtained results are presented in Tab. 1. The controlled plant samples contain lower quantities of Cu²⁺ and Zn²⁺ ions (Tab. 1). The heavy metal distribution in the different morphological parts of the smaller plants studied was not investigated in monometallic condition.

Tab. 1. Accumulation and distribution of heavy metals (mg/g) in different parts of *E. crassipes* Mart. and *P. stratiotes* L., in monometallic system

Monometallic system					
Species	Treatment	Concentration (mg/g)		Accumulated concentration (mg/g)	
		leaves	roots	leaves	roots
<i>E. crassipes</i> Mart.	Cu ²⁺	0.005	0.003	0.36±0.02 ^d	1.46±0.3 ^b
	Zn ²⁺	0.075	0.041	0.32±0.03 ^d	0.81±0.07 ^c
	Cd ²⁺	0	0	0.13±0.01 ^d	1.72±0.2 ^a
<i>P. stratiotes</i> L.	Cu ²⁺	0.009	0.004	2.49±0.6 ^a	1.33±0.2 ^b
	Zn ²⁺	0.033	0.013	0.95±0.01 ^b	1.11±0.4 ^b
	Cd ²⁺	0	0	0.27±0.04 ^f	1.42±0.3 ^b

Values represent ± SD (n=3), small letters represent the statistical significant difference at P<0.05.

The results obtained showed that the concentration of the three heavy metal ions was higher in the root and lower in the leaves. One exception was noticed in the case of Cu²⁺ treatment for *P. stratiotes* L. which accumulated a higher concentration of Cu²⁺ ion (2.49 mg/g) in the leaves. These results demonstrate that *P. stratiotes* L. is the most effective plant for the removal of this metal from aqueous solutions. In diversion of the *E. crassipes* Mart., Cu²⁺ accumulation was measured in a lower quantity in the leaves. As for the Zn²⁺ monometallic treatment the highest concentration was accumulated in *P. stratiotes* L. roots (1.11 mg/g). This means that the phytoextraction period was not enough for the translocation of the Zn²⁺ ions from the root to the leaves. The results demonstrated that the highest Cd²⁺ ion accumulation was very significant in the case of *E. crassipes* Mart. in the plant roots (1.72 mg/g). In the case of Zn²⁺ ions, the Cd²⁺ heavy metal ions have been translocated only in lower concentrations in the leaves during the phytoremediation period. These results

corresponded with Zhu *et al.* (1999) reports, regarding the phytoaccumulation and distribution process in the case of *E. crassipes* Mart.

The results concerning the accumulation of heavy metals in the entire plant of *El. canadensis* Michx., *S. natans* Kunth., *L. minor* L. are presented in the Tab. 2. The highest concentration of Cu²⁺ was registered in the leaves of *S. natans* Kunth. (4.71 mg/g), followed by *L. minor* L. (1.55 mg/g) with significant differences. Similar concentration in this element was removed by *El. canadensis* Michx. (1.34 mg/g). The highest amount of Zn²⁺ and Cd²⁺ ions was accumulated in *S. natans* Kunth. leaves (2.26 and 1.90 mg/g). The lowest concentration of Zn²⁺ (0.44 mg/g) was registered in *L. minor* L. The results showed that the greatest capacity to uptake and bind the heavy metals in monometallic systems was reached by *S. natans* Kunth. and *P. stratiotes* L. On the other hand, the ions distribution is representative only in the case of Cu²⁺ in the leaves of *P. stratiotes* L.

Tab. 2. The accumulated heavy metal concentration in leaves of different hydrophytes (*El. canadensis* Michx., *L. minor* L. and *S. natans* Kunth.), in monometallic system

Monometallic system			
Species	Treatment	Concentration in leaves (mg/g)	Accumulated concentration in leaves (mg/g)
<i>El. canadensis</i> Michx.	Cu ²⁺	0.044	1.34±0.1 ^a
	Zn ²⁺	0.086	0.58±0.02 ^b
	Cd ²⁺	0	0.44±0.01 ^b
<i>L. minor</i> L.	Cu ²⁺	0.003	1.55±0.6 ^c
	Zn ²⁺	0.044	0.44±0.01 ^c
<i>S. natans</i> Kunth.	Cd ²⁺	0	0.06±0.06 ^{NS}
	Cu ²⁺	0.007	4.71±0.5 ^a
	Zn ²⁺	0.003	2.23±0.6 ^c
	Cd ²⁺	0	1.90±0.2 ^c

Values represent ± SD (n=3), small letters represent the statistical significant difference at P<0.05.

Multimetallc system

An attempt was made to appraise the phytoextraction capacity of the plants, to present the competition between metals for a better understanding of metabolic pathways and to identify the phytoremediation mechanism of the plants.

Tab. 3 contains the results related to the accumulation and distribution of heavy metals in different morphological parts of *P. stratiotes* L. and *E. crassipes* Mart. The compared results showed that the presence of the three heavy metal ions has a major influence on the plants phytoextraction efficiency, and that the heavy metal bioaccumulation capacity has increased values. The heavy metal bioaccumulation of hydrophytes is not proportional in the plant body. A higher concentration of heavy metals was observed in the roots of the hydrophytes. An exception was noticed in the case of *P. stratiotes* L. where the highest quantity of Cu²⁺ ions was bioconcentrated in the leaves. The same effect was observed in the case of monometallic treatment. The highest quantity of Cu²⁺ was registered in the case of the *E. crassipes* Mart. The Cu²⁺ uptake of this plant increased; it was seven times higher in comparison

with the monometallic system. The result obtained in the multimetallic experiment confirmed that *P. stratiotes* L. has removed the highest concentrations of Zn^{2+} ions from the synthetic, water in a concentration of 8.07 mg/g plant biomass (4.89 mg/g in the root and 3.17 mg/g in leaves). The two hydrophytes Cd^{2+} ions uptake had increased. The highest removed quantity of cadmium can be noticed in *P. stratiotes* L. The Cd^{2+} ions concentrations is three time higher than in the case of the monometallic treatment. Generally, the heavy metals are accumulated in the root and may be due to the process of rhizofiltration, which is commonly observed in aquatic plants (Vesely *et al.*, 2011).

Tab. 3. Accumulation and distribution of heavy metals (mg/g) in different parts of *E. crassipes* Mart. and *P. stratiotes* L., in multimetallic solutions

Multimetallic system					
Species	Treatment	Concentration (mg/g)		Accumulated concentration (mg/g)	
		leaves	roots	leaves	roots
<i>E. crassipes</i> Mart.	Cu^{2+}	0.004	0.005	1.76 ± 0.6^c	5.84 ± 0.4^a
	Zn^{2+}	0.029	0.006	1.73 ± 0.8^c	3.03 ± 0.2^b
	Cd^{2+}	0	0	0.39 ± 0.3^d	3.58 ± 0.5^b
<i>P. stratiotes</i> L.	Cu^{2+}	0.003	0.007	3.64 ± 0.06^b	2.98 ± 0.7^b
	Zn^{2+}	0.013	0.047	3.17 ± 0.3^b	4.89 ± 1.1^a
	Cd^{2+}	0	0	0.88 ± 0.02^c	3.29 ± 0.4^b

Values represent \pm SD (n=3), small letters represent the statistical significant difference at $P < 0.05$.

Results regarding the accumulation of HM (without distribution) in the smaller studied hydrophytes (*El. canadensis* Michx., *L. minor* L. and *S. natans* Kunth.) are presented in Tab. 4. The heavy metal uptake capacity was increased with the presence of three heavy metal ions in all the three aquatic plants. The highest Cu^{2+} ions bioaccumulation was measured in *L. minor* L. This plant Cu^{2+} ions uptake capacity has grown nine times more than in the monometallic system. Moreover, the obtained data showed that *L. minor* L. was the most efficient also in the uptake of Zn^{2+} and Cd^{2+} ions from the multimetallic systems. *El. canadensis* Michx. proved to be a weak HM accumulator, in both the systems, even though it has been seen, that in the monometallic system this plant accumulates high amounts of Cu^{2+} (1.34 mg/g), meanwhile registering low values of Zn^{2+} and Cd^{2+} . In the multimetallic system, this species accumulated high amounts of Cd^{2+} (1.51 mg/g), but low Cu^{2+} and Zn^{2+} quantities. The *S. natans* Kunth. Zn^{2+} ions uptake has increased (4.47 mg/g) in the presence of the Cu^{2+} and Cd^{2+} ions. The obtained results suggested that the poisoned plant system needs more Zn^{2+} ions for protection. The Zn^{2+} ion is known to be involved in the chlorophyll biosynthesis. The all-presence of these metals usually raises the phytoextraction capacities, showing non-concurrence between the metals during absorption.

Plants Biochemical Response (chl and car content).

Heavy metal pollution causes certain physiological and biochemical changes in the plants. The most significant biochemical changes can be observed in the photosynthetic pigment concentrations and in the total

protein content (Dhir *et al.*, 2008). Heavy metals stress can inhibit or stimulate the formation of new proteins in the plant samples. The photosynthetic pigments are considered as indicators of environmental stress (Liechtenthaler and Miehe, 1997) and their degradation can be observed as a response to HM toxicity (Bunea *et al.*, 2012).

El. canadensis Michx. was selected to analyze the biochemical changes after the monometallic treatment. Fig. 1 contains the results of chl and car content from the treated plant samples. The results showed that after the Zn^{2+} monometallic exposure the plants were the most affected.

Tab. 4. The accumulated heavy metal concentration in leaves of *El. canadensis* Michx., *L. minor* L. and *S. natans* Kunth., in multimetallic system

Multimetallic system			
Species	Treatment	Concentration in leaves (mg/g)	Accumulated concentration in leaves (mg/g)
<i>El. canadensis</i> Michx.	Cu^{2+}	0.036	0.48 ± 0.01^b
	Zn^{2+}	0.089	0.59 ± 0.03^b
	Cd^{2+}	0.003	1.51 ± 0.2^a
<i>L. minor</i> L.	Cu^{2+}	0.003	10.81 ± 1.3^a
	Zn^{2+}	0.046	4.89 ± 1.2^b
	Cd^{2+}	0.000	2.78 ± 0.9^c
<i>S. natans</i> Kunth.	Cu^{2+}	0.006	3.56 ± 0.1^b
	Zn^{2+}	0.002	4.47 ± 0.3^b
	Cd^{2+}	0.000	1.09 ± 0.4^d

Values represent \pm SD (n=3), small letters represent the statistical significant difference at $P < 0.05$.

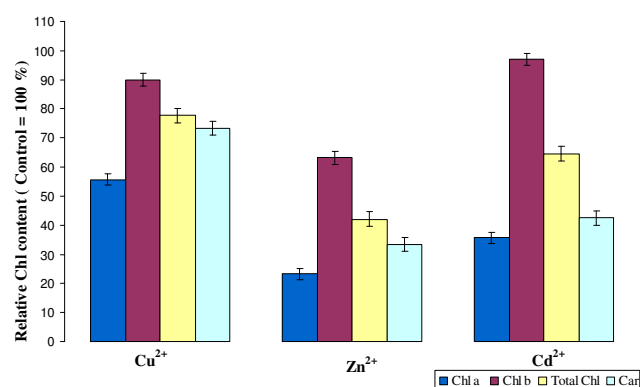


Fig. 1. The content of chl a, chl b, total chl and carotenoids of *El. canadensis* Michx. measured after the heavy metal treatment, in monometallic system

The multimetallic stress effects on the photosynthetic pigments content of the five studied aquatic plants are shown in Fig. 2 and 3. In this study, the chlorophyll concentrations decreased after six days of multimetallic exposure. A comparison of the relative sensitivity of the aquatic plants suggested that *S. natans* Kunth. is less sensitive to the three heavy metal ions than *L. minor* L., *P. stratiotes* L. and *E. crassipes* Mart. Hydrophytes showed the lowest chlorophyll content (Fig. 2) after the treatment. Moreover, the results confirmed that the three heavy metal ions had a major impact on the photosynthetic activity of the aquatic plants.

The carotenoids content in this experiment have shown a decreasing tendency in all the studied species. The lowest concentration was registered in the case of *El. canadensis* Michx. followed by *P. stratiotes* L. (Fig. 3). The obtained results are in agreement with previous literature reports about the monometallic systems. Naumann *et al.* (2007) showed that Cu^{2+} may inhibit the chl and car biosynthesis in case of *L. minor* L. The most sensitive parameters were observed on chl and car, followed by FW (fresh weight) and DW (dry weight). Hegazy *et al.* (2009) reported that chl and car content in *L. gibba* L. was altered by bioaccumulation of Zn^{2+} and Cu^{2+} , therefore the plants have shown a significant discoloration of leaves. In the same time, with the growth of concentration of HM, the content of chl *a* decreased, chl *b* increased and the car became higher than total chl. Singh *et al.* (2012) reported that the effect of combined heavy metal treatment on toxicity symptoms of aquatic plants depends on the HM concentration and also, on the exposure time.

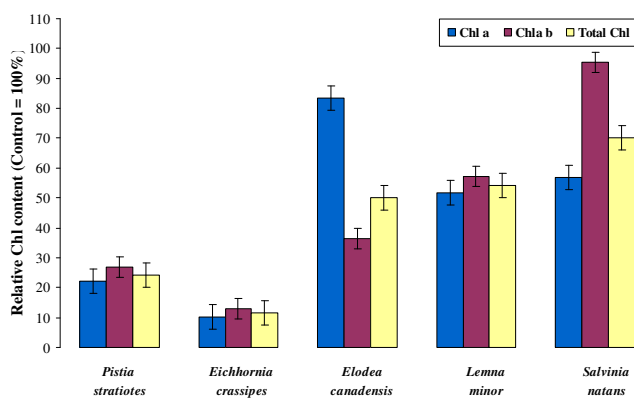


Fig. 2. The content of chl *a*, chl *b*, total chl of five aquatic plants measured after the multimetallic treatment

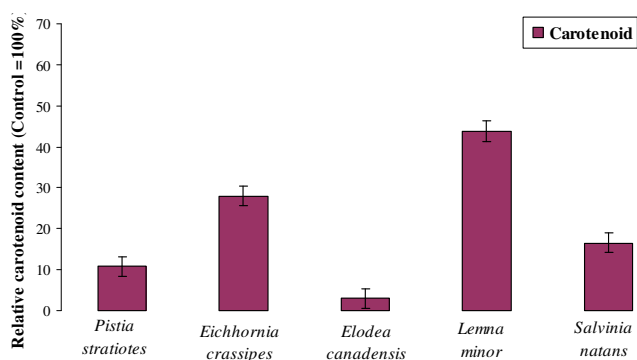


Fig. 3. The content of carotenoids of five aquatic plants measured after the multimetallic treatment

Protein Content

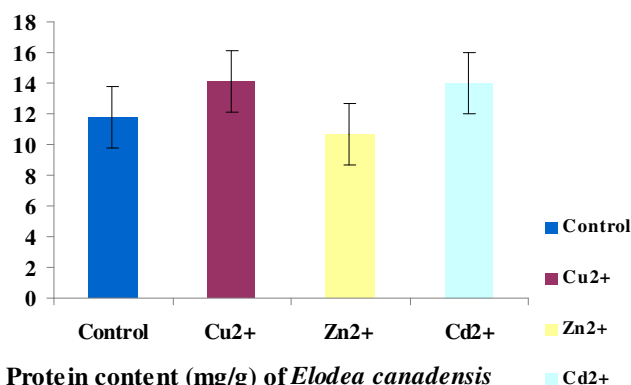
After the phytoremediation treatment with anorganic contaminants the plant metabolism is affected by lower or higher modifications in the protein content. The obtained results suggested that the correlation between removal rate and HM toxicity affected the protein content. The *El. canadensis* Michx. was selected for analysing the total protein content after the monometallic system. The results are presented in Fig. 4. Regarding these, the total protein

content had increased after the Cu^{2+} and Cd^{2+} treatments. The total protein level decreased in the case of the Zn^{2+} exposure, which is in agreement with the report of Mishra and Tripathi (2008).

In the case of multimetallic systems the total protein content was measured in all five aquatic species. The results are shown in Fig. 5 in comparisons with the control plants content. The presence of the three toxic heavy metals in the aquatic environment has a main effect on the protein content in the case of *P. stratiotes* L., *E. crassipes* Mart. and *El. canadensis* Michx. The three mentioned aquatic plants protein concentration has increased after the multimetallic exposure. The plants possess a defence system, therefore the increased amount of protein could indicate the induction of stress proteins.

In the studied species with smaller morphological parts such as *L. minor* L. and *S. natans* Kunth., protein content has decreased after the heavy metal treatments. These results are in agreement with Singh's *et al.* (2012) reports concerning the decrease of total protein content in multimetallic system, which can generate higher toxicity effects on plant protein concentration.

The consequence of this study showed that the protein level in different aquatic species is in accordance with the HM accumulation. Phytoextraction results showed that the increasing protein content is involved in HM stress



Protein content (mg/g) of *Elodea canadensis*

Fig. 4. The protein content of *El. canadensis* Michx. measured after the heavy metal treatment, in monometallic system (mg/g).

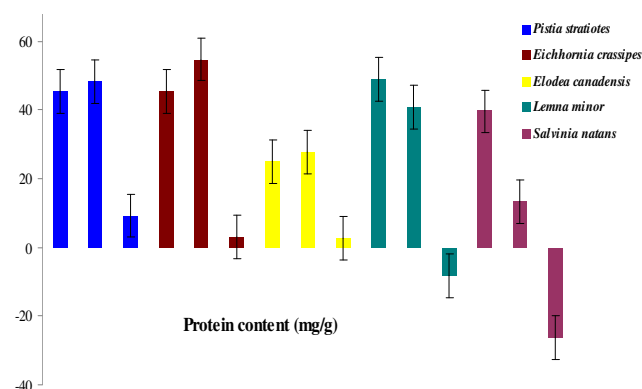


Fig. 5. Total protein content in control plants (mg/g), in treated plants and differences compared with control (untreated plants) and the studied species

response. It was also observed, that in case of *El. canadensis* Michx. the mixture of the three metal ions did not disturb the accumulation mechanism and the level of total proteins was higher than in the monometallic treatment.

Conclusions

The studied macrophytes proved to be useful in the uptake of heavy metals in monometallic system and much more effective in multimetallic system. The plant uptake and accumulation of toxic Cd^{2+} ions have been advantageously influenced by the presence of Cu^{2+} and Zn^{2+} ions. The responses of plants to heavy metal stress can vary depending on: the decrease in chl and car content, the inhibition and induction of soluble protein quantity. In the case of protein content two kinds of reactions were suggested: 1. the reduction of protein level as an effect of the metal ions toxicity, 2. the increase, from the production point of view, of new protein synthesis. The measurements of photosynthetic pigment concentration, revealed the highest heavy metal tolerance in the *L. minor* L. and *S. natans* Kunth species.

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