Organic salts enhanced soil risk elements leaching and bioaccumulation in *Pistia stratiotes*

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ABSTRACT

It is well known that organic chelates have a positive influence on micronutrients mobilisation. The objective of the present study was to investigate the efficiency of organic acid salts to enhance mobility of Cd, Pb, and Zn in soil with subsequent bioaccumulation in water plant (*P. stratiotes*). Column flushing and pot rhizofiltration experiment were conducted. Statistical analyses ANOVA with Tukey's HSD test were used for results analysis. Results revealed statistically different efficiency of four tested organic acid salts ($P < 0.05$). Ammonium citrate was the most effective for release of all three risk elements. Cd, Pb, and Zn were mobilised by 45%, 321%, and 116% more than under 0.11 mol/L acetic acid (exchangeable form), respectively. Ammonium oxalate and acetate proved lower efficiency ($P < 0.05$). Young plants showed higher bioconcentration factor (BCF) than old ones in leaves as well in roots. Old plants proved lower residual metal concentration in solution. Mobilisation efficiency and metals accumulation in biomass were the highest under citrate and tartrate treatments.

Keywords: chelate; efficiency; cadmium; lead; mobility

The use of biochelators, such as natural low molecular weight organic acids (NLMWOA) or their salts, that are exudated by plants into the soil has positive effects on the phytoextraction of risk metals from soil (Evangelou et al. 2004). It is known that exudation of organic compounds by roots have the influence on the solubility of essential and toxic ions through their effects on microbial activity, rhizosphere physical properties, root growth dynamics, acidification, chelation and oxidation-reduction reactions in the rhizosphere (Nigam et al. 2000). Methods using living plants to remove metals from water (rhizofiltration or phytofiltration) are some of the phytoremediation processes (Espinoza-Quinones et al. 2008). Plants of a high metal bioaccumulation capacity and a good tolerance to high metal concentration over long periods of time are useful (Garbisu and Alkorta 2001). Among the great variety of plants, macrophytes are known to assimilate great amount of pollutants by surface adsorption and/or absorption and to incorporate them directly into their tissues (Hadad et al. 2006, Espinoza-Quinones et al. 2009).

The new approaches of the present study come from the use of natural organic chelates with low toxicity and short term application to provide high efficiency of chelates before their natural biodegradation through soil microorganisms. Questions are if the presence of organic salts in soil (10 mmol/kg) can enhance risk element mobility into soil solution in sufficient amount and if the presence of organo-metal complexes in water influences their accumulation in macrophytes biomass.

MATERIALS AND METHODS

Soil sampling and sample preparation. The Příbram area belongs to the most polluted areas in the Czech Republic due to atmospheric deposition of potentially toxic elements from the Pb smelter (Šichorová et al. 2004). Soil samples were taken from the arable layer (0–25 cm) of an agriculturally used soil. Samples used for soil characteristics determination, total cadmium, lead and zinc

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contents and fractionation and the experiment were air-dried, homogenised and sieved through a 2-mm stainless sieve prior to analyses.

Physico-chemical soil characteristics. The used soil was classified as Gleyic Cambisol developed on Proterozoic volcano-sedimentary rock complex belonging to the Příbram ore district (Komárek et al. 2006). Determined chemical parameters: the pH was determined using deionised water or 0.01 mol $CaCl_2$/L (w/v = 1:2.5). Cation exchange capacity was determined in 0.1 mol $BaCl_2$/L (w/v = 1:20) according to (Gao and Chang 1996). Available content of macronutrients was determined by the Mehlich III (Zbíral 2000) using the flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-300, Mulgrave, Australia). Reference Material ISE 989 Riverclay (Wageningen Agricultural University, The Netherlands) was used for quality assurance of analytical data.

Total risk elements content. Total trace and risk elements content in the soil was determined by the method of (Száková et al. 1999). Soil samples were decomposed by dry ashing in the Apion Dry Mode Mineraliser (Tessek, Prague, Czech Republic) in a mixture of oxidising gases ($O_2 + O_3 + NO_x$) at 400°C for 10 h and the ash was then decomposed in a mixture of HNO_3 + HF and evaporated to dryness on a hot plate at 160°C and dissolved in diluted aqua regia. Total metals content (Cd, Pb, and Zn) in digests were analysed using the ICP-OES. Reference Material 7003 Silty clay loam (Analytika Ltd., Czech Republic) was used for quality assurance of analytical data. The levels of studied metal in RM were determined as follows: Cd 0.74 ± 0.12, Pb 31.9 ± 4.7 and Zn 79.2 ± 8.2 mg/kg.

Cadmium, lead and zinc soil fractionation. Cadmium, lead and zinc fractionation in the soil were performed using the sequential extraction by Rauret et al. (1999). Exchangeable and acid extractable fraction (A) in 0.11 mol CH_3COOH/L; (ii) reducible fraction (B) in 0.1 mol $NH_2OH{\cdot}HCl$/L; (iii) oxidisable fraction (C) in 8.8 mol/L H_2O_2/ 1 mol/L CH_3COONH_4; and (iv) residual fraction, computed as the sum of fractions A, B, and C subtracted from the total content. Risk elements content in digests was analysed using ICP-OES (Vista Pro, Varian, Mulgrave, Australia).

Batch experiment. The device was consisted of two 250 ml volume PE bottles. The upper bottle performed as soil storage reservoir and lower bottle as solution collector. Total of 10 g of soil samples were homogenized and applied into the upper bottle. Solutions were prepared from acetate, tartrate, citrate and oxalate ammonium salts at concentration 10 mmol/kg (Wang et al. 2007) and applied in the volume of 100 ml to the soil samples. Each treatment was replicated 3 fold. The collected solutions were applied on soil again. Altogether 5 cycles were repeated. After the last cycle, collected solutions were analyzed on total risk elements content by ICP-OES (Vista Pro, Varian, Mulgrave, Australia).

Soil flushing experiment. The soil columns of 10 cm in internal diameter and 50 cm in height were packed with 200 g of air dried and 2.0 mm sieved soil. Columns were mounted on a framework and polyethylene collection pots were placed under the bottom of each column. Pots were 22.0 cm long, 14.0 cm wide and 10.0 cm high with volume approximately 2 L. Two out of three treatments contained solution with organic acid salts and solution of deionised water was used as control. Each column in three replicates was connected to 2.0 L reservoir tank that contained experimental solution (10 mmol/kg). Total time of 1 flushing cycle was 1.5 h. Collected solution was reused to next flushing cycle. Altogether 5 flushing cycles in total time of 8 h were applied. The collected solutions were filled to 2.0 L volume with deionised water and used in rhizofiltration process.

Rhizofiltration experiment. Free floating aquatic macrophyte *Pistia stratiotes* L. was used for subsequent rhizofiltration experiment. During the experiment, the solution volume in pots was kept constant at 2.0 L by adding deionised water to compensate the losses by evaporation and transpiration. Experiment was conducted with two different plant sizes (i) big and old (ii) small and young. Every plant was washed with tap and deionised water to remove the residual soil particles, dead parts of roots and leaves and bugs before the use in experiment.

Plant analyses. Plant samples were divided into leaves and roots. Leaves were kept in the oven at 60°C to constant weight. Roots were placed into 80 ml centrifuge tube to provide the desorption procedure. The 50 ml of soft and cold 5 mmol solution of $CaCl_2$ was used as desorption agent. After desorption, roots were dried in the oven at 60°C to constant weight. Total contents of elements in plant samples were determined after dry decomposition (Street et al. 2006). The contents of Cd, Pb and Zn were determined using ICP-OES. Certified Reference Material CTA-OTL-1 Oriental Tobacco Leaves (Polish Academy of Sciences and Institute of Nuclear Chemistry and Technology, Warsaw, Poland) was used for quality assurance of analytical data.

Bioconcentration and translocation factor. The translocation factor (TF) and bioconcentration factor (BCF) was calculated according to (Abhilash et al. 2009). Two bioconcentration factors were computed for the above (leaves) and submerged (roots) plant parts as;

$$TF = C_{leaves}/C_{roots} \quad (1)$$

$$BCF_{leaves} = C_{leaves}/C_{water} \quad (2)$$

$$BCF_{roots} = C_{roots}/C_{water} \quad (3)$$

Statistical analysis. All statistical analyses were performed using one-way analyses of variance (ANOVA) at a 95% ($P < 0.05$) significance level with a subsequent Tukey's HSD test. Metal concentrations were expressed in µg/L in water and mg/kg in plant tissues. Standard deviation was performed from three replicates for every set of data. All analyses were performed by using the Statistica 8.0 software (StatSoft, USA).

Table 1. Fractionation of 3 studied risk elements in soil

Cd (mg/kg) (*n* = 3)		(%)
Exchangeable and acid-extractable	1.927 ± 0.58	34
Reducible	0.685 ± 0.08	12
Oxidisable	0.142 ± 0.01	2
Residual	2.937 ± 0.49	52
Pb (mg/kg) (*n* = 3)		
Exchangeable and acid-extractable	18.42 ± 6.28	2
Reducible	339.6 ± 32.6	42
Oxidisable	34.91 ± 4.1	4
Residual	428.6 ± 30.3	52
Zn (mg/kg) (*n* = 3)		
Exchangeable and acid-extractable	27.36 ± 7.83	10
Reducible	21.68 ± 1.7	8
Oxidisable	19.52 ± 1.09	7
Residual	198.1 ± 5.35	75

Data are presented as mean ± SD

RESULTS AND DISCUSSION

Soil properties and total and fraction risk elements content. The pH_{H_2O} was 5.83 and pH_{CaCl_2} was 5.52. Cation exchange capacity, determined as sum of extractable Ca, Mg, K, Fe, Mn, and Al was 62.4 mmol/kg. Available macronutrient amounts were 2011 mg/kg, 65.7 mg/kg, and 61.5 mg/kg for Ca, Mg, and K, respectively. The total content of studied risk elements were (in mg/kg): Cd (5.68 ± 0.1), Pb (822 ± 14) and Zn (267 ± 36). Risk element fractions in the studied soil are summarised in Table 1.

Effectiveness of organic acid salts on risk elements content in soil leachate. The addition of organic acid salts in the concentration 10 mmol/kg soil had a significantly positive effect at the level of $P < 0.05$ on enhanced mobilisation all of three risk elements except oxalate and acetate (Table 2). The greatest positive influence on Cd mobilisation was that of citrate and tartrate ($P < 0.05$). Melo et al. (2008) presented different efficiency of single and three-application treatment of organic chelates on Cd mobility. Single treatment had by 32% lower effect than three-application treatment. This is clearly due to the high biodegrability of natural organic acids in soil by microorganisms (Meers et al. 2005). The significantly lower positive effect of oxalate could be affected by the great adsorption of oxalate-metal complexes onto soil particles. Formation and time-depending mineralization of organic-metal complexes with Pb is well dependent on organic compounds (Hees et al. 2003). The citrate was the most effective acid salt influencing Pb extraction into soil solution. Yang et al. (2006) reported an increase of Pb^{2+} desorption with an increase of organic treatment concentration. Nevertheless the other authors such as (Evangelou et al. 2006, Nascimento 2006) demonstrated the low ability of LMWOA and their derivates to extract less soluble metals (such as Pb) into soil solution. Citrate was the most effec-

Table 2. The efficiency of 4 tested low molecular weight organic acids on enhanced mobility of 3 risk elements (Cd, Pb and Zn) in batch experimen

Treatment	Cadmium	Lead	Zinc
		(mg/kg)	
Control	0.015 ± 0.018^a	0.148 ± 0.032^a	0.287 ± 0.009^a
Acetate	0.047 ± 0.002^a	0.053 ± 0.046^a	0.935 ± 0.070^a
Tartarate	0.667 ± 0.016^b	18.639 ± 0.502^b	26.916 ± 1.199^b
Citrate	0.862 ± 0.023^c	59.149 ± 1.720^c	31.861 ± 0.815^b
Oxalate	0.035 ± 0.010^a	3.869 ± 1.100^d	4.610 ± 0.068^c

Statistical differences are based on Thukey HSD test. All data are presented as mean ± SD (*n* = 3) tive solubilisation agent also for Zn. Our results corresponded with the work of Nascimento et al. (2006). They reported a substantial amount of desorbed Zn under 20 mmol/kg citrate and oxalate dose. Nevertheless Kos and Lestan (2004) supposed little impressive effects of organic chelates on metal solubilization due to relatively low doses tested in most works (5 to 10 mmol/kg).

Bioaccumulation of risk elements in biomass. Analytical results revealed that small plants of *P. stratiotes* were more efficient bioaccumulator than big plants ($P < 0.05$) for all of three risk elements (Figure 1). Results of the present study revealed a decreasing trend of Cd accumulation in leaves with the increasing length of experiment for all treatments. Upadhyay et al. (2007) reported an opposite accumulation trend. Accumulation of Cd in roots in our work had an increasing trend in time for all treatments except control. Upadhyay et al. (2007) reported bioaccumulation of Cd in *P. stratiotes* biomass at the level of 290 mg/kg at initial Cd concentration 0.09 mg/L. The accumula-

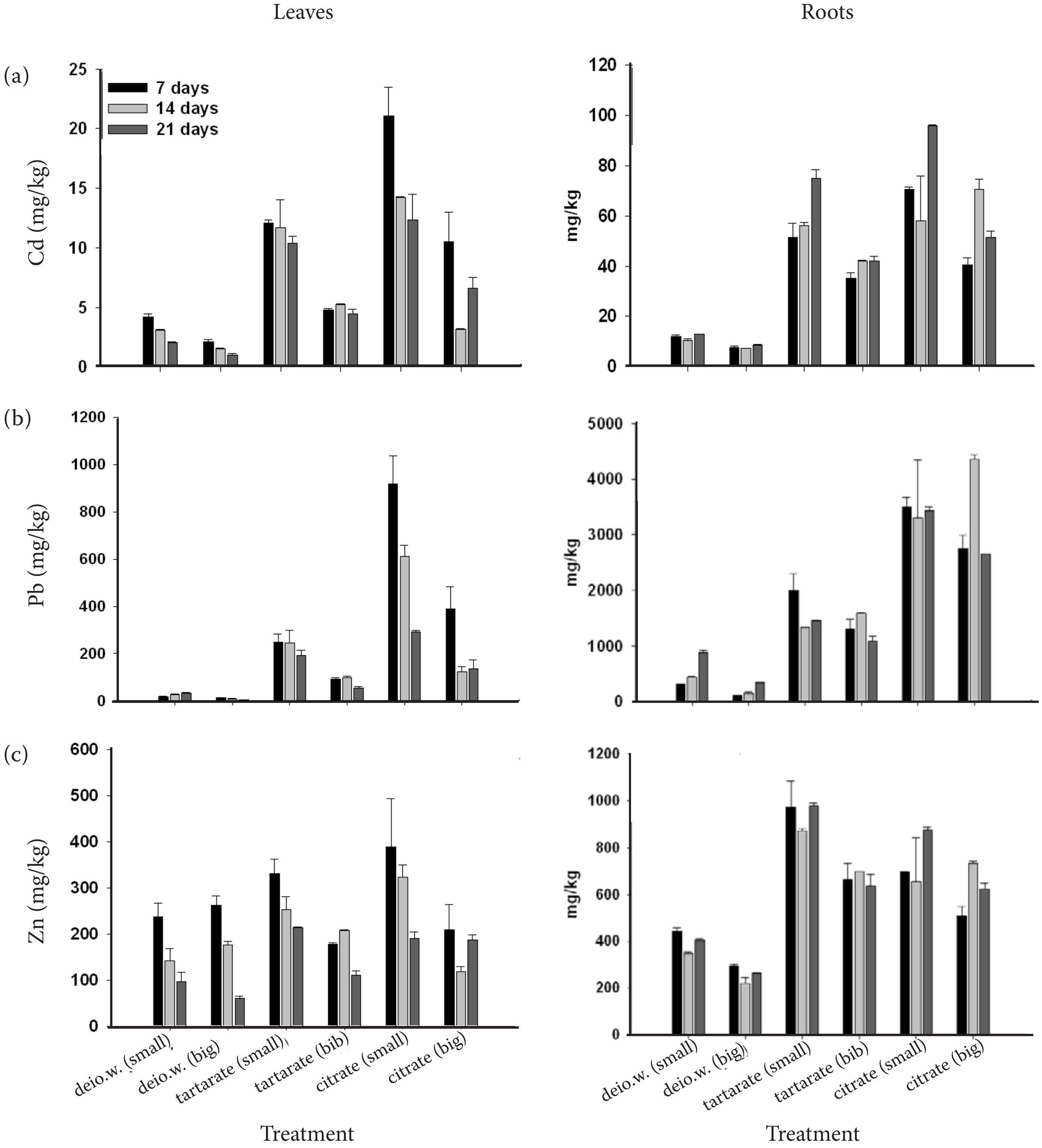

Figure 1. Accumulation of (a) Cd; (b) Pb and (c) Zn in leaves and roots of *P. stratiotes*. Characters 'small' and 'big' represent different experimental plant sizes (different variety). Each triplicate column represent 3 different sampling times (7, 14 and 21 days). Data are presented as mean ± SD ($n = 3$)

Table 3. Bioconcentration factor (BCF) values for Cd, Pb and Zn of *P. stratiotes* leaves and roots at 3 different sampling times, 7th, 14th and 21st day growth

Risk element	Plant part	Day	Treatment					
			control		tartarate		citrate	
			small	big	small	big	small	big
Cd	leaves	7	1847	422	202	42	274	96
		14	1973	639	224	60	266	108
		21	1400	386	248	67	328	76
	roots	7	7195	2775	1409	2956	2860	868
		14	16481	5127	1036	1049	989	877
		21	10907	3559	1281	979	1816	666
Pb	leaves	7	1261	384	182	32	261	64
		14	939	455	151	24	267	97
		21	1596	402	121	46	257	36
	roots	7	32666	2908	1343	1973	2768	671
		14	23403	14259	1271	3746	232	1848
		21	16475	41833	1088	1200	1494	633
Zn	leaves	7	30267	7726	235	50	189	107
		14	32826	8324	231	51	273	147
		21	30321	6781	283	69	256	94
	roots	7	122865	25847	862	529	1137	487
		14	149736	54108	1032	1712	129	1685
		21	103453	72046	1228	509	948	667

tion of Pb into the leaves had a significantly ($P < 0.05$) decreasing character in time for all treatments and plant sizes. Roots Pb accumulation had also a significantly ($P < 0.05$) decreasing trend in time for tartrate treatment. Uptake of metal ion is largely influenced by the bioavailability of the concrete metal and plant (Mishra and Tripathi 2009). The different role plays Zn as an essential element to higher plants because it involves certain metabolic processes. Zinc is toxic in high concentration and induces an increase of root membranes permeability (Paschke et al. 2000), which will cause nutrients to leak from the roots. Zinc was more accumulated in roots than in leaves due to its low mobility in plants. Young plants proved a higher accumulated concentration than old ones for both acid salt treatments. Phytotoxic symptoms as low photosynthetic activity were not observed.

Table 4. Translocation factor (TF) values

	Day	Treatment					
		control		tartarate		citrate	
		small	big	small	big	small	big
Cd	7	0.35	0.27	0.24	0.14	0.30	0.26
	14	0.29	0.21	0.21	0.12	0.24	0.04
	21	0.16	0.12	0.14	0.11	0.13	0.13
Pb	7	0.06	0.12	0.12	0.07	0.26	0.14
	14	0.06	0.06	0.18	0.06	0.18	0.03
	21	0.04	0.01	0.13	0.05	0.08	0.05
Zn	7	0.53	0.89	0.34	0.27	0.56	0.41
	14	0.41	0.80	0.29	0.30	0.49	0.16
	21	0.24	0.23	0.22	0.17	0.22	0.30

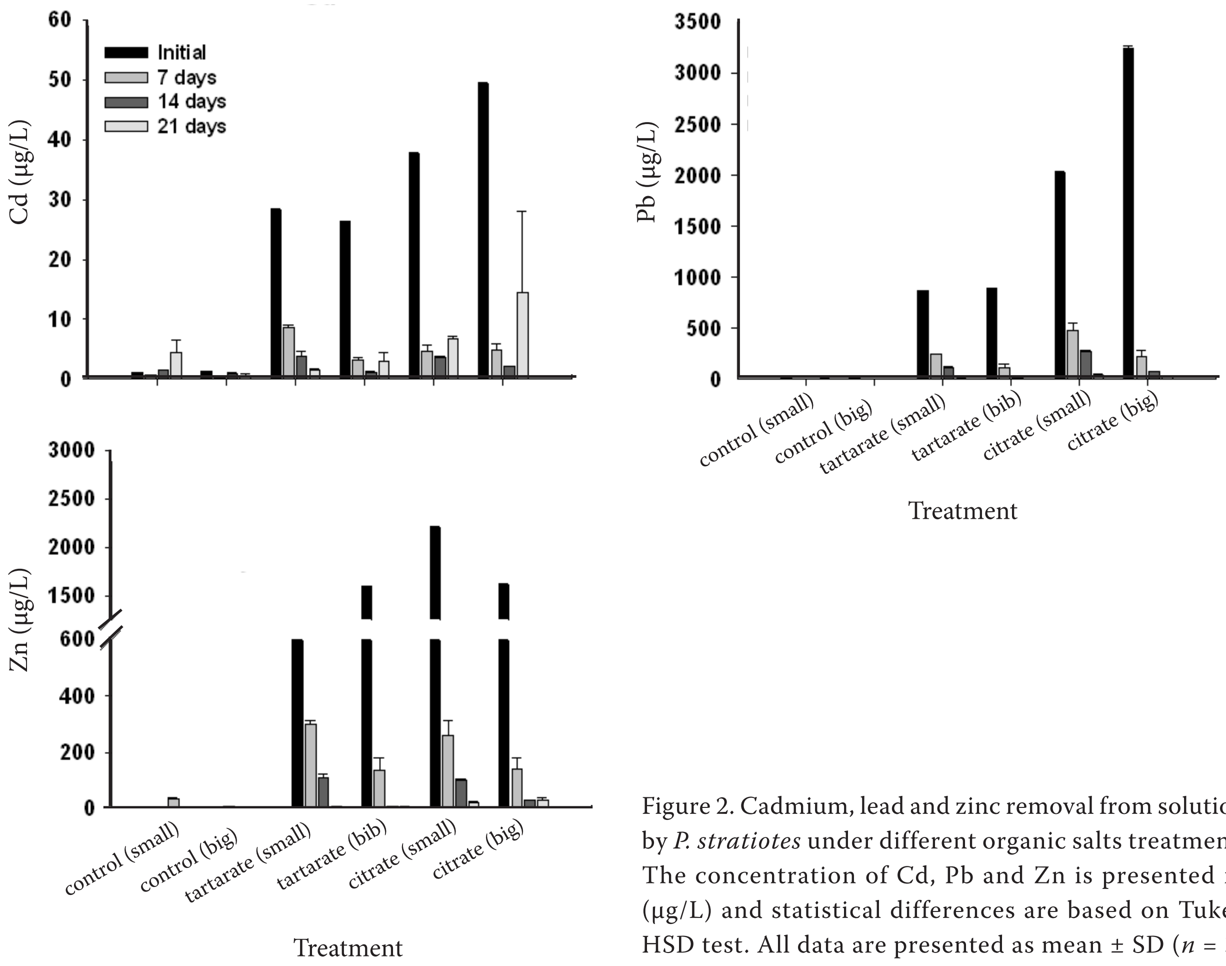

Figure 2. Cadmium, lead and zinc removal from solution by *P. stratiotes* under different organic salts treatment. The concentration of Cd, Pb and Zn is presented in (µg/L) and statistical differences are based on Tukey HSD test. All data are presented as mean ± SD ($n = 3$)

Bioconcentration and translocation factor of *P. stratiotes*. The BCF for leaves is more than 10 times lower as for roots. BCF was higher for young plants than old plants ($P < 0.05$) but not for all treatments (Table 3). Our results corresponded to work of Kara and Zeytunluoglu (2007). A good metal accumulator should meet the following criteria: (i) ability to accumulate high level of the metal in aboveground parts (ii) fast growth (iii) well-developed root system. Based on these criteria, *P. stratiotes* could be regarded as a good accumulator for Cd, Pb, and Zn (Lu et al. 2004); *P. stratiotes* is thus a potential and suitable candidate for the removal of Cd, Pb, and Zn. The TF values of various organic acid salt treatments are depicted in Table 4. Young plants proved higher potential to move risk elements from roots to aerial parts except control treatments.

Metal removal from solution. Experimental plants significantly decreased metals concentration in the solution ($P < 0.05$) with increased time (Figure 2). Mishra et al. (2008) confirmed a high removal efficiency of Cd for water lettuce during 12 days of an incubation experiment (70% at 5 mg/L and 82% at 2 mg/L). Lead removal from solution confirmed higher efficiency of big plants than small plants for all treatments. Espinoza-Quinones et al. (2009) achieved a 50% reduction in Pb concentration in the liquid phase after the first 24 h. Zinc removal had similar a trend as lead removal. Lu et al. (2004) reported Zn solution depletion from initial 5; 10; 20, and 40 mg/L on 0.82; 2.42; 5.06, and 6.29 mg/L after 12-day exposure.

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