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Removal of Al, Fe and Mn by *Pistia stratiotes* L. and its stress response

Research Article

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Abstract: The influence of different chelates applied in the soil primary on Al and secondary on Fe and Mn mobilization and their removal from solution was investigated. The work compared the efficiency of 10 mM tartaric acid and 3 mM EDTA in soil washing process and accumulation potential of *Pistia stratiotes* in rhizofiltration process. The plant response on the toxic element Al and other elements Fe and Mn was determined through the nitrogen and free amino acids content in plants. The efficiency of chelates decreased in order 10 mM tartaric acid > deionized water > 3 mM EDTA for all studied elements. *P. stratiotes* was able to remove up to 90% of elements during the 15 days period. Higher content of toxic element Al and potential toxic elements Fe and Mn were observed in the roots than in the leaves with the increased time. The trend of Al accumulation correlated with Fe accumulation (R^2=0.89). Toxicity impact of high level of Al was observed by increased free amino acids (AA) level. Proline, histidine, glutamic acid and glycine were the most synthesised free AA in leaves. Total AA content in leaves was significantly higher under chelates addition compared to control.

Keywords: *Water macrophyte • Accumulation • Toxic element • Toxicity • Enhanced rhizofiltration*

1. Introduction

Although the choice of suitable plants and cultivation techniques (extraction, filtration, restoration) is vitally important in phytoextraction, in most cases, the key to successful extraction is the right choice of chelators and their concentration [1,2]. Although EDTA is effective in the mobilization of metals in soils, EDTA and EDTA-metal complexes can be toxic to plants and persist in the environment [3]. The leaching of metal complexes through the soil profile could be prevented efficiently through the use of natural organic compounds such as low molecular weight organic acids (LMWOA) [4]. In addition to the leaching of metals, potentially toxic elements such as Al, Mn or Fe are also leached during mobilization of metals [5]. A factor that has not been sufficiently discussed in previous studies describing soil washing or soil leaching, but the pH changes due to leaching have a crucial role in toxic and potentially toxic element availability. The application of chelates decreases the pH, the most important aspect for Al mobilization and bioavailability. Other elements such as Fe and Mn are important for plant metabolism, but could be excluded from metabolism or replaced by other elements due

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to increased risk of their availability. Furthermore, the effect of the combined treatment of water polluted by toxic elements by rhizofiltration processing and chelate addition is poorly understood [6].

The current study has four objectives. The first objective was to compare the impact of the synthetic chelate (EDTA) and natural chelate (tartaric acid) on the amount of Al, Mn and Fe leaching from soil. Due to the findings of previous studies, we predict a higher impact of natural chelate on Al adsorption compared to EDTA, due to the natural release of organic acids by plants during the decrease of Al toxicity in rhizosphere [7]. The second objective was to investigate the bioaccumulation of Al, Fe and Mn by water macrophytes. The third objective was to evaluate the influence of Al in solution on nitrogen uptake and sequestration in leaves. Finally, the stress response of water macrophytes was determined measuring the free amino acids content in leaves.

2. Experimental Procedures

2.1. Experimental materials

Soil samples came from the Příbram area [8]. Soil was air dried, homogenized, and filtered through a 2 mm stainless steel sieve prior to analyses. Aquatic macrophyte *Pistia stratiotes* L. (Botany Garden of Charles University, Prague, Czech Republic) was used in the rhizofiltration experiment. Two different chelate agents were applied to the soil to provide the release of toxic metals into the solution. Disodium EDTA salt (Analytika, Prague, Czech Republic) with high toxicity to plants [9] and tartaric acid (Lach-Ner, Neratovice, Czech Republic) nontoxic for plants [10].

2.2.Solution preparation

Three soil samples (1 kg) were inserted separately into polyethylene bottles of volume 15 L. Experimental solutions A, B or C were added in the amount of 10 L. Solutions contained (A) deionized water, (B) deionized water with tartaric acid (10 mM kg^{-1} soil) (C) deionized water with Na-EDTA (3 mM kg^{-1} soil). Each treatment was prepared in three replicates. Bottles were shaken on the end-over-end shaker for 24 hours at room temperature and filtrated solutions (using sedimentation overnight and filtration paper) were used were used in the rhizofiltration experiment.

2.3.Rhizofiltration experiment

Experimental pots (volume, 5 L) were rinsed with 0.1 M hydrochloric acid and filled with 1 L of solutions A, B or C obtained from the previous step. Each replicate pf each treatment consisted of nine separate pots representing 9 time steps (0, 1, 2, 4, 8, 24, 72, 168 and 360 hours). The pH of the solutions was maintaind at pH 5.0 using soft hydrochloric acid. Finally, 40–50 g of fresh weight plants were used in each pot.

2.4.Analytical procedures

Soil pH was measured in the suspension using a 1:1.25 (w/v) ratio of soil and deionized water and 0.01 M $CaCl_2$. Samples for soil cation exchange capacity (CEC) were prepared in suspension using a 1:50 (w/v) ratio of soil and 0.1 M $BaCl_2$. Total Al, Mn and Fe content in the soil were determined with the use of a two-step procedure [11], and measured by optical emission spectrometry with inductively coupled plasma (ICP-OES). The content of mineral N (N_{min}) and dissolved organic carbon (DOC) in the soil were determined in 0.01 M $CaCl_2$ extracts [12] using a colorimeter on a SKALAR (San$^+$ System, Nederland) apparatus. Total organic carbon (TOC) was determined by wet oxidation with $K_2Cr_2O_7$ and measurement of the absorbance at 590 nm [13]. Determined soil parameters are summarized in Table 1. Dried leaves and roots were weighed, ground and total content of Al, Mn and Fe in the plant samples were determined in mineral extracts obtained by a dry decomposition procedure [14,15] and analyzed using ICP-OES. The free amino acids content was analyzed according to Neuberg *et al.* [16] using an EZ-faast amino acid analysis procedure (Phenomenex, U.S.A.) and determined by gas chromatography coupled with mass spectrometry detection using a HP 6890N/5975 instrument (Agilent Technologies, U.S.A.). Content of total extractable N (N_{H2O}), N-NO_3^- and N-NH_4^+ levels in leaves were determined by segmental flow-analysis using a colorimetric method on a SKALAR (San$^+$ System, Nederland).

pH	CEC	TOC	DOC	Toxic elements content (mg kg^{-1})			N_{min}
(-)	(mmol kg^{-1})	(%)	(mg kg^{-1})	Al	Fe	Mn	(mg kg^{-1})
5.70	134 ± 3	3.72	146	9039 ± 496	20550 ± 136	3396 ± 285	80.5

Table 1. The chemical parameters of studied Cambisol from Příbram area sampled in 2011.

2.5.Phytoremediation factors

Bio-concentration factor (BCF; 1) and translocation factor (TF; 2) were determined according to Abhilash *et al.* [17].

$$BCF = \frac{accumulated\ element\ amount\ (mg\ kg^{-1}\ DW)}{element\ concentration\ in\ the\ solution\ (mg\ L^{-1})} \quad (1)$$

$$TF = \frac{element\ concentration\ in\ leaves\ (mg\ kg^{-1}\ DW)}{element\ concentration\ in\ roots\ (mg\ kg^{-1}\ DW)} \quad (2)$$

2.6. 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 HSD test. One-way ANOVA was used to determine the significance of differences in the data set. Metal concentrations were expressed in mg L^{-1} in water and mg kg^{-1} in plant tissues. The amount of free amino acids was expressed in nmol g^{-1} fresh weight and total nitrogen content in leaves in mg N kg^{-1} dry weight. Standard deviations were calculated for the three replicates for every set of data. All analyses were performed by using software Statistica 8.0 (StatSoft, U.S.A.).

3. Results and Discussion

3.1.The toxicity levels of Al and Fe in solution after chelate application

Our results showed high mobilized amount of Al (approximately 4.3 mM); Fe (1.4 mM) and Mn (55 µM) into the solution. Umebese and Motajo [18] reported a toxic influence of Al at a concentration of 360 µM on hornwort plants. Giannakoula *et al.* [19] reported that Al at a concentration of 480 µM in solution is already toxic for plants. Rout *et al.* [20] observed Al toxicity already at a concentration of 20 µM. Additionally, Mn toxicity was observed at 50 µM for the Cowpea (*Vigna unguiculata* L.; [21,22]).

The results of our study showed concentrations of free Fe ion between 70-110 mg L^{-1}. Wheeler *et al.* [23] reported free Fe ion toxicity at level 50 mg L^{-1} and higher. Nowack [24] documented the influence of EDTA on Fe desorption from soil. The toxicity of chelated Fe is dependent on the stability of Fe(II)EDTA or Fe(III)EDTA complexes [25]. The Fe(II)EDTA complex is widely used form of micronutrient in mixed fertilizers. However, Fe(III)EDTA complex could be poorly accumulated by roots due to a lower availability of Fe(III). Furthermore, due to Fe(III)EDTA instability the free EDTA could cause a higher toxic effect than the free Fe ions.

3.2. Removal of Al, Fe and Mn from solution

The concentration of Al, Fe and Mn in solutions significantly decreased ($P<0.05$) with increased time. The significantly largest decrease was observed during the first 24 hours for all elements (Figure 1). Concentration of Al in solution at the end of the experiment decreased after extraction by solutions A, B and C by 13.9%, 11.3% and 11.5%, respectively. Similar results were observed for Fe. Concentration of Fe at the end of the experiment decreased by 9.9%, 8.0% and 8.1% after the application of solutions A, B and C, respectively. The concentration of Mn at the end of the experiment decreased by 8.0%, 6.3% and 6.1% after application of solutions A, B and C, respectively.

3.3.The accumulation of Al, Fe and Mn by water macrophytes

The accumulation of all elements was significantly ($P<0.05$) higher in the roots than in leaves (Figure 2). The highest concentration of Al in roots was 6146 mg kg^{-1} after three days growth in solution A. We observed in our results bio-concentration factors (BCF) for Al of 151 at 18.6 mg L^{-1}, 279 at 15.9 mg L^{-1} and 282 at 11.7 mg L^{-1}. Lower BCF values were presented by Nevertheless, in contrast with the lower values in our study, Umebese and Motajo [18] but only with free Al(III) ion in the solution. Umebese and Motajo [18] presented BCF values of 221 at 9 mg L^{-1} and 171 at 3 mg L^{-1}. Ma *et al.* [7] studied Al toxicity on root development of wheat and found that uptake of Al was dependent on the Al form that was in contact with the roots. In our study, there were no specific toxic symptoms of Al on root development observed. The reason was likely to be the use of mature plants with well-developed root systems containing a large amount of hairy roots. We observed only dropping of old inactive roots. However, the plants produced a low number of new roots immediately after dropping of old ones.

Chelated Al form with citrate or oxalate had lower negative impact than the $AlCl_3$ form. Ma *et al.* [7] confirmed the protection effect of Al-OA complexes on root apexes under free Al(III) ion toxicity. In our study, *P. stratiotes* also provided a higher BCF after chelate addition. Generally, the rapid accumulation of Al and Fe by roots during the first 24 hours could be explained by the primary fast free ion uptake and secondary chelated form uptake. These findings are in agreement with Veselý *et al.* [26]. We observed inhibition of risk element accumulation after the addition of chelates into the solution.

Accumulation of Fe was highly correlated with Al accumulation. The potential of *P. stratiotes* to accumulate Fe was previously documented by Mishra

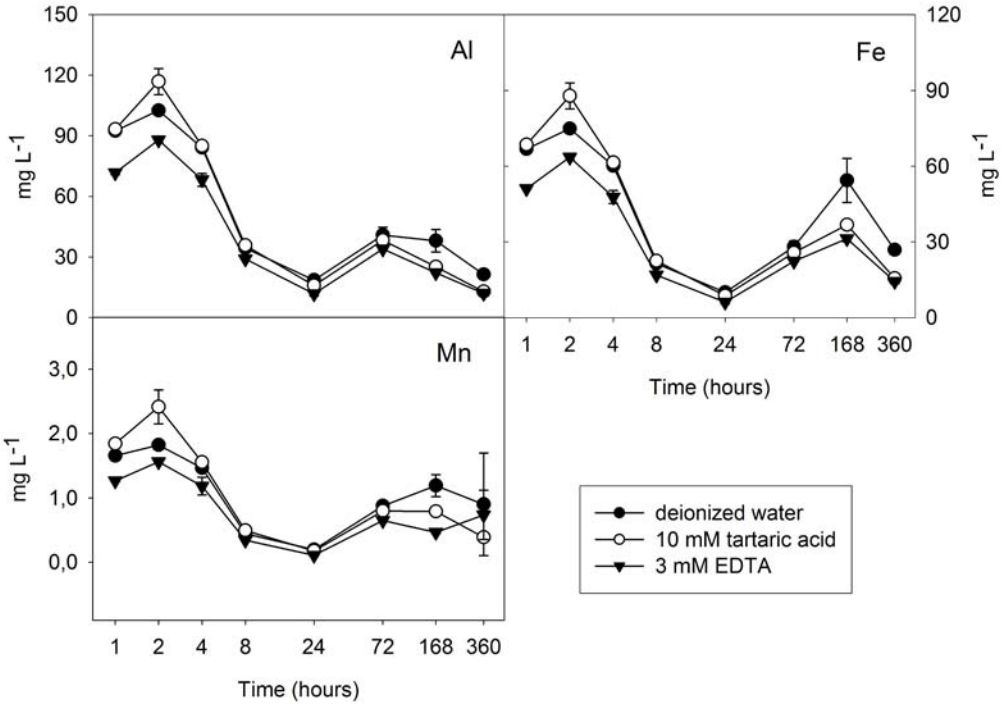

Figure 1. The concentration of Al, Fe and Mn in solution over time in the presence of water macrophyte *P. stratiotes* in the rhizofiltration experiment. Data are presented as mean ± SD (n=3).

and Tripathi [27]. In that study high removal of Fe from solution during 12 days was reported. In our study we observed the highest concentration of Fe in leaves after 15 days growth, 2297 mg kg^{-1}, 1853 mg kg^{-1} and 2937 mg kg^{-1} with solutions A, B and C, respectively. Little increase of the amount of toxic elements in solution after 168 hours was documented by Veselý *et al.* [6] and Abhilash *et al.* [17]. The effect could be caused by the negative impact of toxic elements on water balance in plants [28] or due to reversal desorption of adsorbed elements onto the roots surface.

3.4.The impact of Al on plant biochemical parameters

The results of the total content of free amino acid experiment showed three different phases (Table 2). The most interesting of these is the second phase, between 72 and 168 hours, when free amino acids content significantly increased by 85.2%, 223% and 105% under growth in solutions A, B and C, respectively. Increased proline concentration is the first response to toxicity [29]. Proline concentration rapidly increased between 72 and 168 hours. Similar results were observed in tobacco plants [30]. Sharma and Dubey [31] also reported proline accumulation in plants grown under Al stress. From the other determined free amino acids, focus was concentrated on the glycine, glutamic acid, asparagine and histidine [32]. The increased level of these amino acids in leaves was correlated with the increase in proline levels (Figure 3). Glutamic acid and glycine are directly involved in the synthesis of glutathione or in synthesis of phytochelatin, which plays a major role in metal binding and detoxification in plants [33]. The concentration of histidine increased with increased toxic metal concentration in solution and it allowed the accumulation of a higher amount of toxic elements [34].

3.5. Nitrogen uptake by Pistia stratiotes

Pista stratiotes proved to have a different strategy for the uptake of N-NO_3^- and N-NH_4^+ form (Figure 4). The N-NH_4^+ form was predominantly removed during the first 24 hours compared to N-NO_3^- form which was mainly removed between 8 and 72 hours. According to Nelson *et al.* [35], the nitrogen uptake rate by *P. stratiotes* was higher for N-NH_4^+ than for N-NO_3^-. In our study the nitrogen content in leaves rapidly increased during the first 2 to 8 hours for all treatments (Figure 5). After 8 hours, N-NO_3^- content in leaves constantly decreased. The rapid decrease of N-NO_3^- concentration in leaves could be explained by consumption for amino acid synthesis. Sharma and

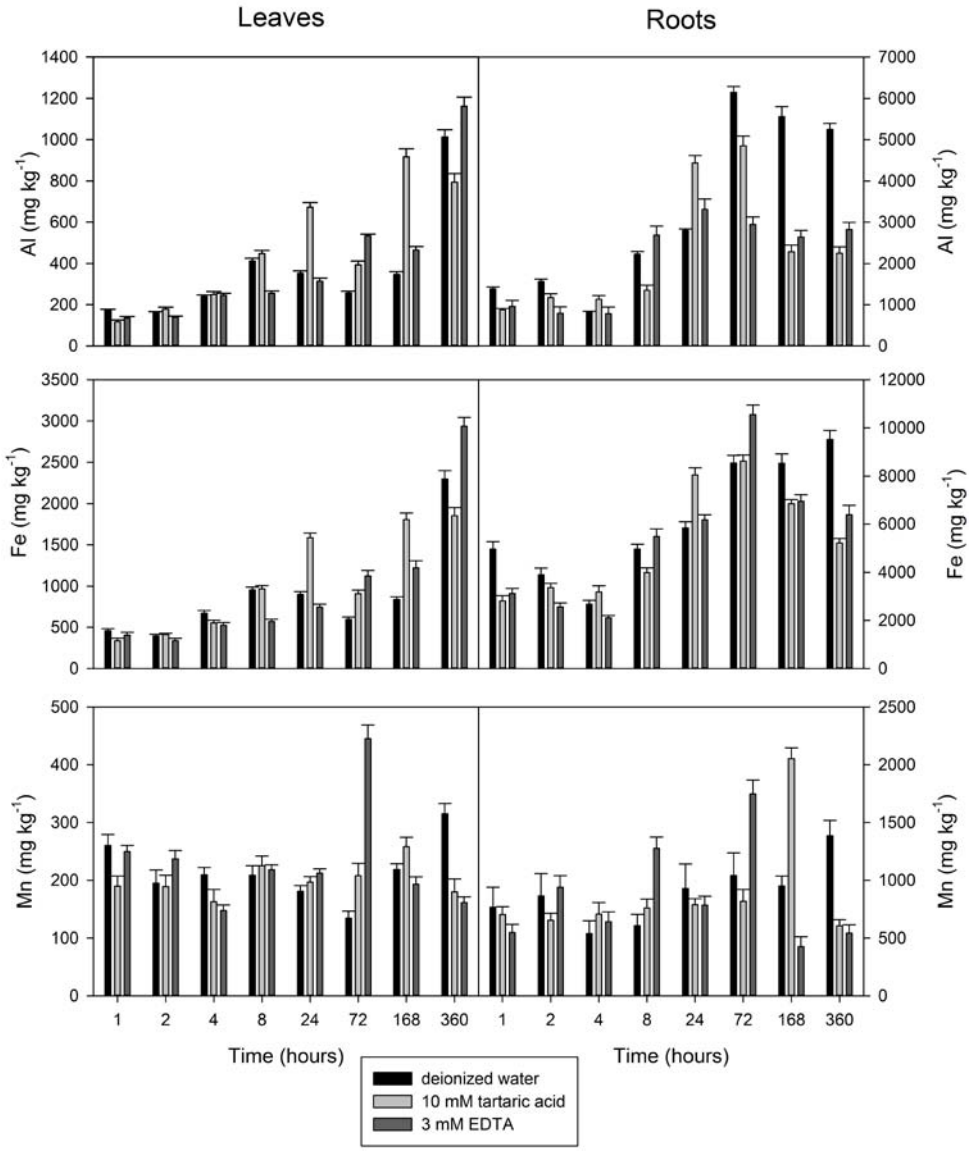

Figure 2. The Al, Fe and Mn concentrations in leaves and roots during the 15 day rhizofiltration experiment using *P. stratiotes* on solutions from flushing experiment. Data are presented as mean ± SD (n=3).

	Time (hours)			
	24	72	168	360
Total AA content	(nM mg^{-1} FW)			
deionized water	1.763 ^{Aa}	1.530 ^{Aa}	2.833 ^{Ab}	1.194 ^{Ac}
10 mM tartaric acid	1.893 ^{Aa}	1.387 ^{Aa}	4.492 ^{Bb}	2.236 ^{Ba}
3 mM EDTA	1.488 ^{Aa}	1.659 ^{Aa}	3.396 ^{Bb}	2.659 ^{Bc}

Table 2. The total free amino acid content in leaves of *P. stratiotes* grown in solution obtained from desorption experiment. Data are presented as mean (n=3).

Upper case subscript text explains the significant differences among the varieties and the lower case subscript text explains the significant differences among the time steps.

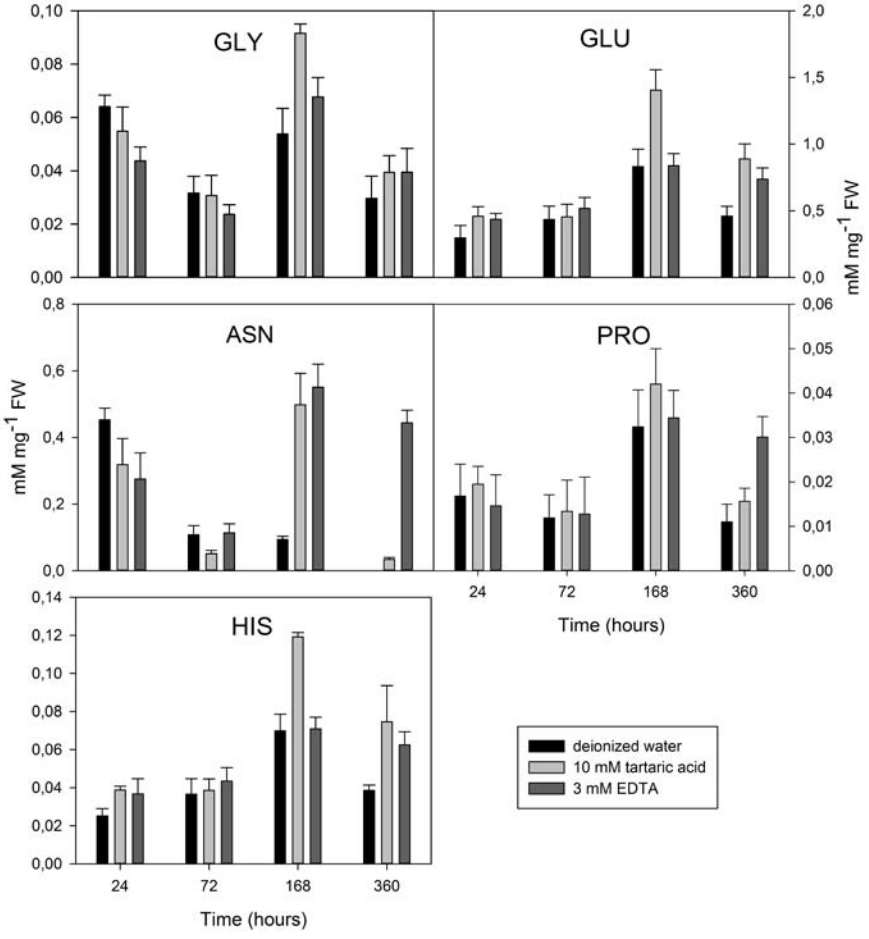

Figure 3. The content of free amino acids (Gly-glycine, Glu-glutamic acid, Pro-proline, His-histidine and Asn-asparagine) in *P. stratiotes* leaves most affected by potential toxic elements in solution. Data are presented as mean ± SD (n=3).

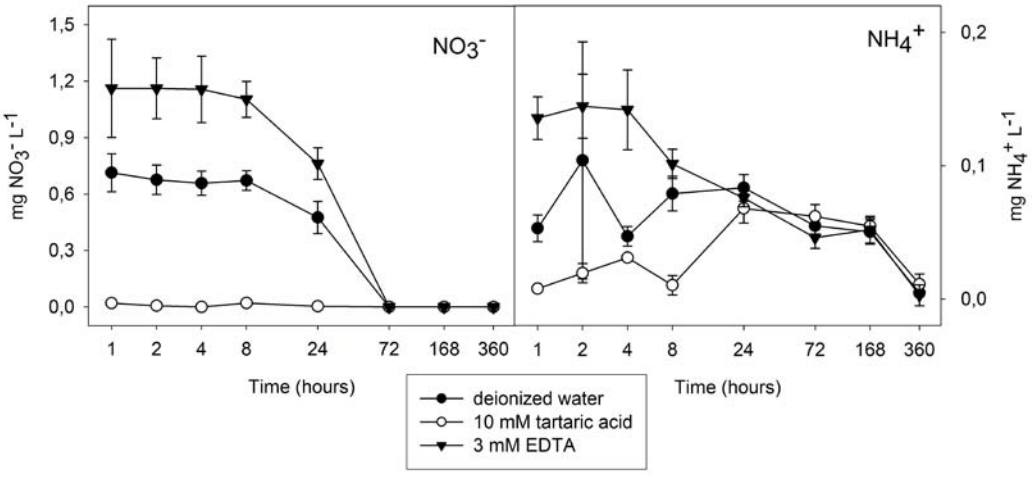

Figure 4. The efficiency of chelates on nitrate and ammonium N desorption from soil (1 hour) and uptake by water macrophyte *P. stratiotes* in rhizofiltration experiment. Data are presented as mean ± SD (n=3).

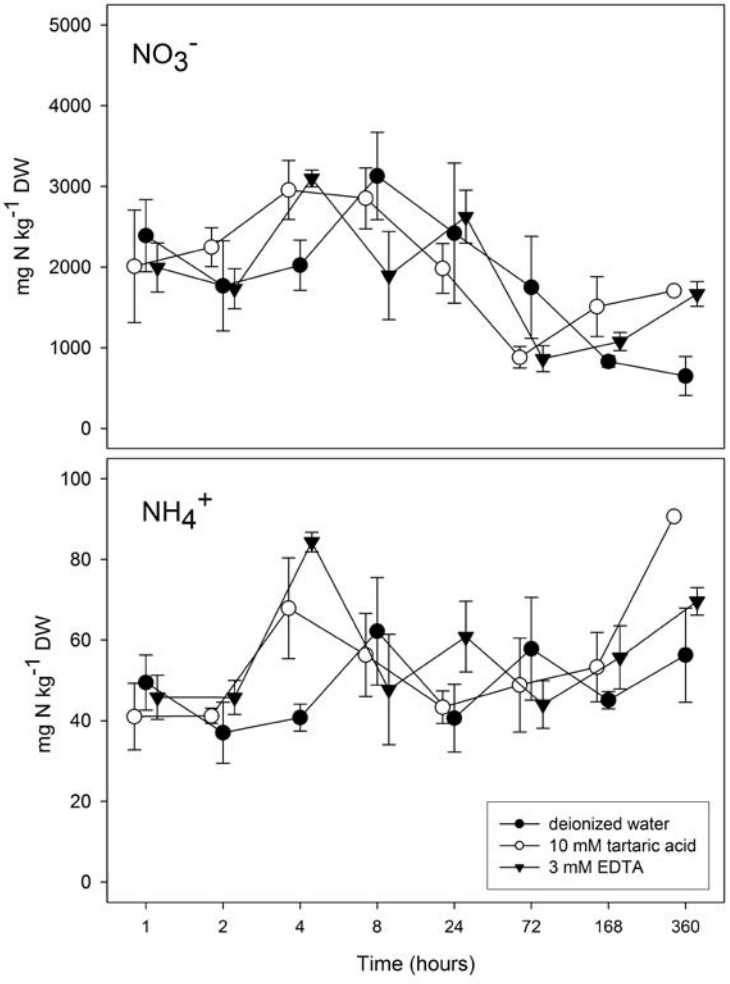

Figure 5. The content of different N form (ammonium and nitrate) in *P. stratiotes* leaves during the rhizofiltration experiment. Data are presented as mean ± SD (n=3).

Dietz [33] reported increased synthesis of diverse metabolites under toxic elements stress, which contained particularly free amino acids.

4. Conclusions

The major conclusions of this study are: (i) the efficiency of chelates decreased in the order of 10 mM tartaric acid > deionized water > 3 mM EDTA for all elements, (ii) *P. stratiotes* was able to remove up to 90% of Al, Fe and Mn during the 15 day period, (iii) higher content of Al, Fe and Mn were observed in the roots than in the leaves, (iv) toxicity impact of a high level of Al was observed by increased free amino acid (AA) level (mainly due to proline), (v) total AA content in leaves was significantly higher under chelate addition compared to control, and (vi) the N-NH_4^+ form was removed diversely compared to the N-NO_3^- form.

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