Research Square

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ETDA as a legacy soil chelatant; a comparative study to a more environmentally-sensitive alternative for metal removal by Pistia stratiotes L.

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Research Article

Keywords: Soil chelatants, DOC leaching, Metal speciation modelling, Rhizofiltration, Water lettuce

Posted Date: March 2nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2486631/v1

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Version of Record: A version of this preprint was published at Environmental Science and Pollution Research on May 19th, 2023. See the published version at https://doi.org/10.1007/s11356-023-27537-6.

Abstract

The accuracy of environmental risk assessment depends upon selecting appropriate matrices to extract the most risk-relevant portion of contaminant(s) from the soil. Here we applied the chelatants Na-EDTA and tartaric acid to extract a metal-contaminated soil. *Pistia stratiotes* was applied as an indicator plant to measure accumulation from the metal-laden bulk solutions generated, in a hydroponic experiment lasting 15 days. Speciation modelling was used to elucidate key geo-chemical mechanisms impacting matrix and metal-specific uptake revealed by experimental work. The highest concentrations of soil-borne metals were extracted from soil by Na-EDTA (7.4% for Cd), but their uptake and translocation to the plant were restricted due to the formation of stable metal complexes predominantly with DOC. Tartaric acid solubilized metals to a lesser extent (4.6% for Cd), but a higher proportion was plant-available due to its presence mainly in the form of bivalent metal cations. The water extraction showed the lowest metal extraction (e.g., 3.9% for Cd) but the metal species behaved similarly to those extracted by tartaric acid. This study demonstrates that not all extractions are equal and that metal-specific speciation will impact accurate risk assessment in soil (water)-plant systems. In the case of EDTA, a deleterious impact on DOC leaching is an obvious drawback. As such, further work should now determine soil, and not only metal, specific impacts of chelatants on the extraction of environmentally relevant portions of metal(loid)s.

1. Introduction

An environmentally-friendly and cost-effective solution to remediate soils or waters contaminated by metal(loid)s is to extract the pollutants using plants (often referred to as phytoextraction or rhizofiltration) (Awa and Hadibarata, 2020). The quantity of metal(loid)s that a plant will take up is not only dependent on the plant biomass and capacity to uptake and translocate metal(loid)s but also on the availability of the metal(loid)s in the soil, for which soil properties such as pH, organic matter content, and texture, are major influencing factors. To increase metal(loid) availability and thus potential uptake by plants, chelating solutions can be applied to the soil (Ali et al., 2020; Liu et al., 2022). Much research has focused on the use of synthetic chelating agents, especially EDTA (ethylenediaminetetraacetic acid), to augment plant metal(loid) removal (Hasan et al., 2019) under the pretext that applying EDTA can increase metal(loid) mobility and render an increased bioavailability. For example, increases in the net uptake of metal(loid)s of 20 to 110% have been reported (Ali et al., 2020; Liu et al., 2008; Shahid et al., 2011; Yuan et al., 2007). However, EDTA, like most synthetic chelatants, has several disadvantages: (i) it has a high cost, (ii) is poorly biodegradable, (iii) can lead to secondary pollution (Liu et al., 2022), and (iv) can have negative impacts on plant growth (Liu et al., 2008). Alternatives addressing some or all of these concerns are low molecular weight organic acids (LMWOA) (Lu et al., 2013), which are naturally secreted by plants and microorganisms. Organic acids also have the advantage of being cheaper and biodegradable (Lu et al., 2013; Tao et al., 2020; Veselý et al., 2012a) compared to synthetic chelatants. Several studies have evaluated the impact of exogenous application of LMWOA on metal(loid) mobility and accumulation in plants, demonstrating an increase in metal(loid) mobility and uptake. For instance, when diverse organic acids (oxalic acid, citric acid, vanillic acid, and gallic acid) were applied to a metal(loid) enriched soil, shoot and root Cd, Pb, Zn, Cu, and Ni concentrations in Indian mustard plants grown on that soil increased 1.4 to 10 times (do Nascimento et al., 2006). In another study by Veselý et al., (2012a) water lettuce removed 95% of Pb from a control spiked solution and 97% and 99% when citric acid and tartaric acid were added to the solution, respectively. The hyperaccumulator *Sedum alfredii* has also shown increased Cd accumulation through the exudation of tartaric acid (Tao et al., 2020). When known metal(loid) tolerant and accumulating species are tested, studies have demonstrated favourable outcomes. For example, the aquatic macrophyte *Pistia stratoties* L. (common name: water lettuce) showed tolerance and accumulation capacity towards various metal(loid)s being able to grow in the presence of up to 20 ppm Cd (Das et al., 2014). This plant can also accumulate high concentrations of Cr, Cu, Fe, Mn, Ni, Pb, and Zn (Lu et al., 2011). Moreover, rhizofiltration studies evaluated its removal potential to be up to 4 g $Cd.m^{-2}.y^{-1}$, 193 g $Pb.m^{-2}.y^{-1}$, 2.2 kg $Fe.m^{-2}.y^{-1}$, 426 g $Cr.m^{-2}.y^{-1}$, 129 g $Cu.m^{-2}.y^{-1}$, 77 g $Zn.m^{-2}.y^{-1}$ and 18 g $Ni.m^{-2}.y^{-1}$ (Galal et al., 2018; Veselý et al., 2011), making water lettuce an interesting phytoextractor plant.

This study follows a previous study published in 2012 (Veselý et al., 2012b), which focused on the secondary elements present in the soil, *i.e.*, Al, Fe, and Mn. In this paper, Cd, Cu, Pb, and Zn are the pollutants of focus because of their contrasting toxicities (Pb-Cd > Cu-Zn) and mobilities (Cd-Zn > Pb-Cu).

We hypothesized that the use of chelatants Na-EDTA and tartaric acid will (i) increase the solubilization of metals Cd, Cu, Pb, and Zn from the soil solution compared to deionized water, and (ii) modify metal speciation, which will (iii) affect the accumulation of those elements in *Pistia stratiotes*.

2. Material And Methods

2.1. Soil sampling and solution extraction

The contaminated soil was sampled from an alluvial plain in the vicinity of the former heavily industrialized city of Přibram (Czech Republic). This site bears a legacy of the historic atmospheric deposition of Pb via smelting activities and, latterly, the reprocessing of heavy metals from batteries. Previous studies at this site (for example; Michálková et al., 2016; Teodoro et al., 2020; Trakal et al., 2011) have resulted in a body of knowledge on the solubility of metals in soils, indicating that Pb and Zn in particular are present in pore waters at concentrations greater than would be expected, even from technogenic soils and substrates in the vicinity of mine areas (Moreno-Jiménez et al., 2011). As such this soil was chosen to be a suitable media from which to extract metal-laden solutions in bulk. Soil sampling was carried out in a W-formation in the field, obtaining and then bulking a representative sample of topsoil (0-30cm). After sampling, the soil was air dried and sieved to < 2 mm prior to characterization (Table 1a). To extract bulk solutions soil (1 kg) was mixed with an extraction solution (10 L) for 24 h, with an end-over shaking and under room temperature. The extraction solutions were: (A) deionized water, (B) deionized water with 10 mM kg^{-1} soil tartaric acid, and (C) deionized water with 3 mM kg^{-1} soil Na-EDTA. After the 24 h shaking, solutions were recovered through overnight decantation and filtration. Each condition (A, B, C) was replicated three times.

Table 1
Chemical properties of the initial soil (a) and of the initial solutions (b) after extraction with deionized water (DW), tartaric acid (TA), and Na-EDTA (EDTA); all c the soil leakages are in mg L^{-1}.

pH *	CEC *	TOC*	DOC*	Available content of nutrients* *(mg kg^{-1})*				N_{min}*	Total metal content * *(g kg^{-1})*			
(-)	*(mmol kg^{-1})*	*(%)*	*(mg kg^{-1})*	Ca	K	Mg	P	*(mg kg^{-1})*	Al	Fe	Mn	Pb
5.7	134 ± 3	3.72	146	995 ± 68	155 ± 4	125 ± 1	58.8 ± 1	80.5	9.0 ± 0.5	20.6 ± 0.1	3.4 ± 0.3	2.5 ± 0.1

* Analytical methods described in Vesely et al. (2012b)

	DOC	Ca	K	Mg	Na	NO_3-N	NH_4-N	Al	Fe	Mn	Pb	Zn
DW	5.1 ± 0.3	12.6 ± 0.1	2.14 ± 0.04	0.84 ± 0.01	1.57 ± 0.02	0.18	0.27	134 ± 2	102 ± 1	2.48 ± 0.07	7.6 ± 0.1	27.8
TA	5.1 ± 0.1	22.4 ± 0.3	2.41 ± 0.04	1.33 ± 0.01	1.42 ± 0.02	0.01	0.03	140 ± 1	109 ± 1	2.83 ± 0.02	8.1 ± 0.1	31.0
EDTA	24.7 ± 0.6	15.8 ± 0.1	2.56 ± 0.07	1.32 ± 0.02	8.76 ± 0.11	0.27	0.39	102 ± 2	76 ± 1	1.82 ± 0.01	7.6 ± 0.1	32.1

2.2. Hydroponic plant growth experiment

Aquatic macrophyte (*Pistia stratiotes* L.), obtained from the Botanical Garden of Charles University (Prague, Czech Republic), was selected as a suitable metal(loid) tolerant species (Veselý et al., 2012b, 2011).

Nine pots (5 L capacity) were prepared containing 1 L of each of the three solutions detailed above (total 27 combinations). Those nine pots corresponded to nine sampling times, *i.e.*, 0, 1, 2, 4, 8, 24, 72, 168, and 360 h. Each pot contained 40–50 g of plant fresh weight. Over the entire experiment time course, solution pH was maintained at pH 5 using hydrochloric acid, to ensure solubility of the metals and evaluate the effects of the modification of the speciation of the metals by the extractants on its uptake by plants.

At each sampling time, solutions and plants were collected, except for the first time (hour 0) which corresponded to the initiation of the experiment (plant introduced into the pots) and for which only the solution was collected. The solutions were analyzed for total metal risk concentrations (Cd, Cu, Pb, Zn), total nutrient concentrations (Ca, K, Mg, Na), using ICP-OES (optical emission spectrometry with inductively coupled plasma) (720 ES, Varian Inc., CA, USA), total dissolved organic carbon (DOC) content using total organic carbon analyzer TOC-L a (CPH / CPN, Shimadzu), TNM-L segment flow analyzer (Shimadzu) and anion (fluoride, chloride, sulfate, phosphate, nitrate, ammonium) and organic acids (acetate, malate, formate, lactate, oxalate) concentrations using ion chromatography (ICS 6000 and ICS 90, Thermo Scientific Dionex, USA). In addition, the speciation of Cd, Cu, Pb and Zn changes in time as well as according to the selected chelate were determined using Visual MINTEQ software. For this modeling, solution pH, metal and nutrient concentrations, organic acid concentration and the forms of all detected anions were used as an input data.

All the procedures ensuring laboratory QC and QA were done according to the standard laboratory procedures including blanks, fortified standards referential materials etc.

Plants were harvested and roots were cleaned with 5 mM $CaCl_2$ solution to remove the metals sorbed onto the root surface (Macfie and Welbourn, 2000; Veselý et al., 2011). Measurement of the metal concentration in the cleaning solution using ICP-OES allowed the determination of the concentration of metals sorbed on the root surface. After that, leaves and roots were separated and dried. The concentrations of metals into the plant tissue were determined by ICP-OES. Translocation factors (TF) and bioconcentration factors (BCF) were calculated using equations (1) and (2), respectively.

$$(1)\, TF = \frac{\text{Concentrationintheleaves}}{\text{Concentrationintheroots}}$$

$$(2)\, BCF = \frac{\text{Concentrationintheleaves (orroots)}}{\text{Concentrationinthesolution (initial)}}$$

2.3. Statistical analysis

The data were analyzed using R Software version 4.2. After testing normality (Shapiro test) and homoscedasticity (Bartlett test or Fligner test) of the data, means were compared using either the ANOVA test or the Kruskal-Wallis test, for parametric and non-parametric data, respectively, followed by a posthoc test. The difference was considered significant at $p < 0.05$.

3. Results

3.1. Solution composition over time

The elemental composition of the solutions after the extraction process are shown in Table 1b. The concentrations of metals measured in the solutions before plant introduction, i.e. $t = 0$ h, showed that, compared to deionized water, using 10 $mM.kg^{-1}$ tartaric acid extracted 18% more Cd, 11% more Zn and 7% more Pb, while using 3 $mM.kg^{-1}$ Na-EDTA extracted 88% more Cd and 15% more Zn. Except for Cu (for which the tartaric solution induced a higher extraction rate from the soil compared to the Na-EDTA solution (6%)), higher concentrations of metals were measured when Na-EDTA was used as the extractant, despite the Na-EDTA solution being less concentrated than the tartaric acid. The concentration of dissolved organic matter was higher in the Na-EDTA solution than the tartaric acid treatment and the deionized water. The solubilization of the nutrients (Ca, K, Mg, Na) by the different solutions was dependent on the extraction solution and the element. In more detail, calcium was measured in higher concentrations in the tartaric acid treatment and the Na-EDTA treatment compared to the deionized water, with 77% and 25% higher concentrations than in deionized water, respectively. Magnesium was found in similar concentrations in the treatments with tartaric acid and Na-EDTA, which had 60% higher concentrations of Mg compared to the deionized water treatment. The concentrations of potassium were highest in the Na-EDTA treatment, followed by tartaric acid and finally deionized water. Tartaric acid did not affect the concentrations of sodium while the solution of Na-EDTA had almost 6-times higher concentrations of Na relative to the deionized water solution. Moreover, when comparing the amount measured in the soil used for the extraction and the amount measured in the 10 L extracting solutions after extraction, it can be seen that tartaric acid extracted the highest proportion of Ca (22.5%), Mg (10.6%), Al (15.6%), Fe (5.3%), Pb (3.2%), and Zn (7.2%); while using Na-EDTA extracted the least Fe (3.7%) and Mn (0.5%) but the highest K (16.5%), Mg (10.6%), DOC (8.2%), Zn (7.5%) and Cd (7.4%). It can be noted that the use of Na-EDTA was particularly aggressive towards the soil and removed essential fertility elements, such as K, DOC and nitrogen, in the form of ammonium and nitrate.

3.2. Total metal concentrations

Over the 360 h of the experiment time course, concentrations of Cd in solution varied greatly, with the concentrations decreasing in the order Na-EDTA > Tartaric acid > Deionized water. For Pb, Cu and Zn, concentrations decreased from 0 to 24 h then slightly increased, with the highest concentrations measured in the Na-EDTA treatment (Fig. 1).

At the end of the experiment ($t = 360$ h), Cd was higher in the Na-EDTA solution (7-fold) than in the tartaric acid and the deionized water treatments. The concentrations of copper were similar in the Na-EDTA solution and the deionized water but lower in the tartaric acid. Concentrations of lead and zinc decreased in the order Na-EDTA > Deionized water > Tartaric acid. The comparison of the concentrations of metals between the initial ($t = 0$ h) and the final ($t = 360$ h) samplings showed that the growth of water lettuce in the deionized water removed 92%, 53%, 84% and 83% of Cd, Cu, Pb and Zn, respectively: In comparison, its growth in the tartaric acid solution removed 94%, 77%, 90% and 91% of Cd, Cu, Pb and Zn, respectively, and only 71%, 45%, 78% and 65% of Cd, Cu, Pb and Zn when plants grew in the Na-EDTA solution.

3.3. Metal speciation and DOM concentration in the solutions

The concentrations of dissolved organic matter were higher in the Na-EDTA solution than the tartaric acid treatment and the deionized water throughout the experiment time course (Table S1). At the end of the experiment ($t = 360$ h), compared to the deionized water, the concentration of DOM was on average 57% higher in the Na-EDTA and 30% lower in the tartaric acid treatment.

Using the chemical composition of the growing solutions, the Visual MINTEQ software was used to model the forms of the metals found in the solution. These forms were grouped into four categories: (i) free ions (Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}), (ii) the metals linked to fulvic and humic acids (FA/HA), (iii) the metals linked to LMWOA and (iv) the metals in “other” forms (Fig. 1).

Throughout the experiment time course, Cd was mainly found as free ion Cd^{2+}. The increase in Cd concentration in the solution with tartaric acid and especially Na-EDTA was mainly attributed to an increase in free Cd ion and, to a lesser extent, the “other” fraction (composed mainly of $CdCl^{+}$, $CdSO_4$, $CdNO_3$ and CdF^{+}). In addition, Na-EDTA greatly increased the concentration of Cd associated mainly with acids. Over the 360 h of the experiment time course, concentrations of free Cd^{2+} ions tended to decrease especially in the deionized water and tartaric acid treatments, while the concentration of Cd associated with fulvic and humic acids increased with time in the Na-EDTA treatment. This implies that Cd consumption by plants was mostly through the uptake of free Cd^{2+} ions.

At the initial time ($t = 0$ h), Cu was mostly present as free Cu^{2+} ions, followed by Cu associated with fulvic and humic acids, Cu associated with LMWOA and the “other” Cu forms (composed mainly of $CuSO_4$, $CuOH^{+}$, $CuNO_3^{+}$, $CuCl^{+}$ and CuF^{+}), in the deionized water treatment. The use of tartaric acid did not affect the relative concentrations of Cu present as different species, while Na-EDTA decreased concentrations of free Cu^{2+} ions and increased the fraction associated with fulvic and humic acids. Over the experiment time course, the decrease in the total concentrations of Cu were primarily due to a decrease in the concentrations of free Cu^{2+} ions, reflecting an uptake of Cu by plants via free Cu ion assimilation.

Although the concentration decreased over time, Pb was mostly present as free Pb^{2+} ions, in all three treatments. Such a decrease in the concentrations of free Pb^{2+} ions is resultant of the uptake of the Pb^{2+} ions by the plants. The second most encountered form, “other” (*i.e.*, $PbSO_4$, $PbCl^{+}$, $PbOH^{+}$, $PbNO_3^{+}$, PbF^{+}), also decreased with time, while the concentrations of Pb associated with fulvic and humic acids tended to increase. The main effect of the Na-EDTA treatment was the increase in the concentration of Pb associated with fulvic and humic acids.

Similar to the other elements, the main form of Zn in the solution was free Zn^{2+} ion, concentrations of which decreased over time in all three treatments, relative to plant uptake. The main effect of the treatment was a small increase in the concentration of Zn associated with humic and fulvic acids with Na-EDTA, which was more important with time.

3.4. Nutrient contents

Concentrations of nutrients concentrations tended to stay stable until hours t = 8 or 24, after which they decreased in all treatments. At the end of the experiment (t = 360 h), nutrient concentrations decreased in the order Na-EDTA > Tartaric acid > Deionized water. Compared to the concentrations measured in the solutions at the beginning of the experiment, after 360 h, plants consumed 86%, 74%, 97% and 96% Ca, Mg, K and Na, respectively in the control, 83%, 73%, 94% and 83% in the tartaric acid and 70%, 55%, 97% and 80% in the Na-EDTA treatment.

Regarding their ionic forms, the anion concentrations showed more variability through time (Table S1). At the end of the experiment, all anion concentrations decreased compared to t = 0, except for fluoride and sulphate. All treatments presented similar concentrations except for chloride (tartaric acid > Na-EDTA > deionized water) and sulphate (deionized water > Na-EDTA > tartaric acid).

Similar to the reported concentrations of anions, an important variability with time was seen in the case of organic acid (Table S1). On average, concentrations of lactate decreased in the order tartaric acid > Na-EDTA > deionized water; acetate decreased in the order Na-EDTA > deionized water > tartaric acid; formate in the order tartaric acid > deionized water > Na-EDTA; and malate and oxalate decreased in the order deionized water > tartaric acid > Na-EDTA.

This shows that there were higher concentrations of nutrients in the Na-EDTA and tartaric solutions than the deionized water, but the Na-EDTA treatment extracted the most elevated concentrations of Na which could induce salt stress in plants.

3.5. Metal accumulation in plants

The accumulation of Cd, Cu, Pb and Zn onto the roots and inside the leaves and the roots of *Pistia stratiotes* was monitored over the 360 hours of the experiment (Fig. 2). Most of the metals accumulated inside the roots, followed by an accumulation inside the leaves and onto the root surfaces.

The sorption of Cd onto root surfaces increased with time but generally decreased in the order Tartaric acid > Na-EDTA > Deionized water (Fig. 2). The highest levels of Cd adsorption to root surfaces were measured at 24 h for the control, 360 h for the solutions containing tartaric acid and at 72 h for the Na-EDTA. Copper sorption onto the roots showed greater variability over both time and treatments compared to Cd, although Cu sorption was generally higher with tartaric acid solution and Na-EDTA solution compared to deionized water and decreased with time, except at the final sampling. The highest concentrations were found at 2 h for the control, 360 h for the tartaric acid and 4 h for the Na-EDTA. Sorption of Pb onto the roots was low in all treatments at the beginning of the experiment and increased with time, with higher levels of sorption associated with the Na-EDTA and tartaric acid treatments compared to the deionized water treatment. The highest concentrations were measured at 360 h for the tartaric acid and Na-EDTA treatments and 72 h for the control. Finally, Zn sorption onto the roots increased with time and was higher when tartaric acid or Na-EDTA was used to obtain the growing solutions than deionized water solution. The concentration in the control did not evolve much while the highest concentrations in the tartaric acid and Na-EDTA treatments were measured at 360 h.

The different treatments used to obtain the growing solutions had little effects on the concentrations of Cd, Cu, Pb and Zn in the shoot tissues, while the concentrations in the root tissues were more affected (Fig. 2). Concentrations of Cd in plant tissues were elevated in the tartaric acid and Na-EDTA treatments relative to the deionized water treatment, while concentrations of Pb decreased with both treatments, Cu initially decreased then increased in the treatments with tartaric acid and Na-EDTA, and Zn was little affected by time or treatment. Except for Cu, for which concentrations in the roots decreased while concentrations in the shoots remained the same with time; the concentrations of Cd, Pb and Zn increased with time in both shoots and roots and for all three treatments. Maximum concentrations of Cd, Pb and Zn in shoot tissues were measured at 360 h in all treatments while maximum concentrations of Cu in shoots was measured at 360 h only in the case of Na-EDTA and at 24 h for the other two treatments. Maximum concentrations of Cd in root tissues were observed at 24 h for the control, 360 h for the tartaric acid and 168 h for the Na-EDTA treatment. Maximum concentrations of Cu in both plant tissues were measured in the initial phases of the experiment: 4 h for the control, 8 h for the tartaric acid and 2 h for the Na-EDTA. In the control, the maximum concentrations of Pb in root tissues occurred at 360 h while in the other two treatments it was earlier (72 h). Finally, in all cases, maximum concentrations of Zn in roots were measured at 360 h.

When looking at the repartition of the metals within the plants, it can be seen that the treatments “tartaric acid” and “Na-EDTA” had mainly an effect in the latter phases of the experiment (*i.e,.* after 24 h) (Fig. 2). More precisely, at the last sampling, the proportion of metals sorbed on the surface of the roots increased while the proportion in the leaves decreased, with a more pronounced effect observed in the case of tartaric acid.

The translocation factors were low in all treatments and for all elements at the beginning of the experiment (Table 2), except for Zn. They all increased over the time of the experiment but remained below 1. The treatment effect was mostly visible at the last sampling points, in which a higher TF was observed in the deionized water treatment relative to the other two treatments. The exception to this was Pb which showed the reverse. The translocation efficiency followed the mobility of the elements, rather than the essentiality, with higher TF values for Zn and Cd than Pb and Cu, regardless of the treatments. In accordance with TF, bioconcentration factors were higher in the roots than in the leaves (Table 2). Moreover, BCFs increased with time for all elements and both organs, except for Cu. On average, for both roots and leaves, the highest values of BCF were associated with the uptake of Cu, followed by Cd, Zn and Pb was the lowest.

Table 2
Translocation (a) and bioconcentration factors for leaves (b) and roots (c) in the different treatments and during the 360h experiment. Data are presented as mean ± sd (n = 3). DW = deionized water, TA = tartaric acid, EDTA = Na-EDTA.

Translocation factor		**1**	**2**	**4**	**8**	**24**	**72**	**168**	**360**
Cadmium	*DW*	0.039 ± 0.008	0.023 ± 0.005	0.022 ± 0.004	0.040 ± 0.004	0.067 ± 0.005	0.088 ± 0.004	0.204 ± 0.113	0.514 ± 0.230
	TA	0.021 ± 0.003	0.025 ± 0.001	0.018 ± 0.004	0.037 ± 0.023	0.051 ± 0.027	0.102 ± 0.006	0.244 ± 0.142	0.158 ± 0.004
	EDTA	0.057 ± 0.043	0.024 ± 0.003	0.024 ± 0.004	0.024 ± 0.006	0.029 ± 0.007	0.104 ± 0.034	0.147 ± 0.019	0.333 ± 0.140
Lead	*DW*	0.099 ± 0.010	0.060 ± 0.007	0.086 ± 0.009	0.104 ± 0.005	0.059 ± 0.012	0.023 ± 0.001	0.031 ± 0.008	0.069 ± 0.004
	TA	0.064 ± 0.012	0.070 ± 0.011	0.071 ± 0.014	0.117 ± 0.088	0.054 ± 0.016	0.042 ± 0.002	0.093 ± 0.011	0.088 ± 0.000
	EDTA	0.062 ± 0.009	0.077 ± 0.008	0.089 ± 0.010	0.038 ± 0.008	0.038 ± 0.015	0.057 ± 0.002	0.071 ± 0.002	0.140 ± 0.039
Copper	*DW*	0.037 ± 0.001	0.028 ± 0.005	0.033 ± 0.008	0.037 ± 0.001	0.050 ± 0.003	0.078 ± 0.002	0.080 ± 0.033	0.242 ± 0.050
	TA	0.037 ± 0.004	0.037 ± 0.002	0.039 ± 0.004	0.058 ± 0.027	0.075 ± 0.008	0.090 ± 0.001	0.078 ± 0.024	0.096 ± 0.006
	EDTA	0.037 ± 0.001	0.037 ± 0.004	0.058 ± 0.006	0.043 ± 0.001	0.035 ± 0.009	0.102 ± 0.008	0.075 ± 0.005	0.092 ± 0.003
Zinc	*DW*	0.155 ± 0.015	0.110 ± 0.010	0.125 ± 0.006	0.156 ± 0.005	0.114 ± 0.005	0.073 ± 0.002	0.112 ± 0.034	0.297 ± 0.009
	TA	0.171 ± 0.023	0.122 ± 0.015	0.117 ± 0.023	0.202 ± 0.158	0.129 ± 0.023	0.120 ± 0.011	0.220 ± 0.056	0.196 ± 0.011
	EDTA	0.164 ± 0.025	0.165 ± 0.008	0.201 ± 0.005	0.123 ± 0.011	0.090 ± 0.035	0.178 ± 0.011	0.173 ± 0.003	0.273 ± 0.026
Bioaccumulation Factor: Leaves		**1**	**2**	**4**	**8**	**24**	**72**	**168**	**360**
Cadmium	*DW*	8.43 ± 1.97	5.10 ± 1.07	8.28 ± 1.75	14.58 ± 1.68	25.38 ± 2.44	23.18 ± 1.25	52.20 ± 26.50	134.26 ± 64.17
	TA	4.46 ± 0.79	5.37 ± 0.24	6.32 ± 1.81	14.41 ± 8.93	15.80 ± 9.02	26.07 ± 0.99	64.53 ± 34.88	94.01 ± 0.15
	EDTA	6.32 ± 4.31	3.77 ± 0.31	4.73 ± 0.26	4.47 ± 1.18	6.28 ± 1.75	21.87 ± 4.40	39.77 ± 5.03	70.58 ± 31.03
Lead	*DW*	1.89 ± 0.27	1.97 ± 0.25	3.36 ± 0.33	6.72 ± 0.47	5.52 ± 0.95	3.58 ± 0.24	5.62 ± 1.66	14.72 ± 0.47
	TA	1.16 ± 0.02	2.04 ± 0.22	3.25 ± 0.76	6.16 ± 4.72	7.06 ± 2.79	6.35 ± 0.58	10.63 ± 0.65	11.28 ± 0.09
	EDTA	1.28 ± 0.19	1.57 ± 0.17	3.43 ± 0.18	2.82 ± 0.55	3.82 ± 1.58	7.78 ± 0.87	8.46 ± 0.39	17.94 ± 4.91
Copper	*DW*	65.91 ± 0.34	54.23 ± 8.04	67.98 ± 16.94	73.23 ± 6.10	92.95 ± 5.82	83.65 ± 5.18	90.31 ± 23.18	81.36 ± 12.62
	TA	65.48 ± 0.30	56.06 ± 3.94	65.11 ± 7.71	106.78 ± 46.26	108.88 ± 16.44	76.14 ± 0.29	85.25 ± 26.01	80.50 ± 4.57
	EDTA	65.54 ± 1.19	87.99 ± 7.48	82.28 ± 6.21	80.48 ± 1.27	75.03 ± 24.10	84.56 ± 4.90	87.02 ± 3.24	117.25 ± 10.21
Zinc	*DW*	10.98 ± 1.22	8.10 ± 0.61	11.19 ± 0.85	15.90 ± 0.88	15.77 ± 0.37	11.07 ± 0.29	17.82 ± 4.94	48.53 ± 3.61
	TA	10.24 ± 2.03	7.76 ± 1.29	11.68 ± 2.57	19.97 ± 14.93	17.75 ± 4.26	15.36 ± 1.50	26.47 ± 5.17	33.40 ± 2.76
	EDTA	8.28 ± 0.45	10.73 ± 0.94	12.06 ± 1.33	10.85 ± 1.03	9.87 ± 4.18	18.06 ± 0.29	20.56 ± 0.43	41.89 ± 4.73
Bioaccumulation Factor: Roots		**1**	**2**	**4**	**8**	**24**	**72**	**168**	**360**
Cadmium	*DW*	215.7 ± 7.5	222.0 ± 1.5	371.8 ± 4.4	367.2 ± 3.6	380.2 ± 6.2	262.2 ± 1.0	260.0 ± 14.5	259.2 ± 9.1
	TA	215.8 ± 7.8	214.7 ± 1.6	353.5 ± 12.5	387.6 ± 1.2	306.7 ± 16.9	255.7 ± 6.5	268.5 ± 13.1	595.8 ± 16.6
	EDTA	115.4 ± 12.0	157.7 ± 7.0	202.1 ± 24.8	185.1 ± 3.7	218.4 ± 6.9	215.1 ± 27.5	271.0 ± 1.0	210.9 ± 4.6

Translocation factor		1	2	4	8	24	72	168	360
Lead	*DW*	19.0 ± 0.7	32.8 ± 0.4	38.9 ± 0.0	64.6 ± 1.4	93.8 ± 2.3	155.6 ± 3.6	178.0 ± 7.8	212.4 ± 3.9
	TA	18.5 ± 3.7	29.2 ± 1.6	45.8 ± 1.7	52.1 ± 1.0	128.2 ± 13.6	152.1 ± 7.2	115.1 ± 7.3	127.7 ± 0.8
	EDTA	20.6 ± 0.0	20.4 ± 0.1	38.6 ± 2.4	74.1 ± 0.6	100.7 ± 3.1	136.1 ± 9.3	118.5 ± 8.1	128.2 ± 0.3
Copper	*DW*	1803.6 ± 62.4	1950.0 ± 50.4	2068.2 ± 8.8	1955.8 ± 108.7	1862.6 ± 23.1	1078.4 ± 36.8	1161.1 ± 187.0	338.1 ± 17.9
	TA	1773.8 ± 195.6	1514.1 ± 38.7	1670.2 ± 14.1	1864.3 ± 72.1	1439.4 ± 73.9	848.9 ± 13.4	1091.5 ± 9.1	835.2 ± 1.5
	EDTA	1753.4 ± 94.9	2350.6 ± 27.0	1429.7 ± 33.9	1885.3 ± 63.0	2112.2 ± 137.0	836.1 ± 114.5	1165.0 ± 34.2	1273.7 ± 74.7
Zinc	*DW*	71.0 ± 0.8	73.9 ± 1.0	89.7 ± 2.3	102.1 ± 2.3	138.3 ± 2.4	151.7 ± 0.3	159.3 ± 4.3	163.1 ± 7.0
	TA	59.6 ± 3.8	63.4 ± 2.9	99.8 ± 2.5	100.8 ± 4.7	136.9 ± 8.7	128.3 ± 0.7	121.2 ± 7.5	169.9 ± 4.9
	EDTA	50.8 ± 5.1	65.0 ± 2.4	59.9 ± 5.0	88.0 ± 0.5	109.4 ± 4.0	102.0 ± 7.9	118.7 ± 4.5	153.4 ± 2.7

4. Discussion

Taken together, these results show that although Na-EDTA solubilized higher concentrations of metals than tartaric acid and deionized water, at the end of the experiment, higher concentrations remained in this treatment, while tartaric acid solubilized higher metal concentrations than the control and metal removal by plants was the highest in this treatment.

4.1. Metal mobility as influenced by the extractants

In this study the use of synthetic and natural chelatants were compared for the extraction of metals, and their subsequent (bio)availability. It has already been well documented that chelatants are efficient for this purpose (Liu et al., 2008; Veselý et al., 2012a; Yuan et al., 2007) and the mechanisms have been identified to be the competition for sorption sites and the formation of soluble LMWOA- or EDTA-metal complexes (Liu et al., 2022; Tao et al., 2020; Yuan et al., 2007). In most cases tested here, apart from Cu, the synthetic chelatant Na-EDTA was more efficient than the natural one (tartaric acid) at increasing the mobility of metals. This is consistent with previous studies (do Nascimento et al., 2006; Evangelou et al., 2006; Liu et al., 2008), and is related to the high chelation stability constant of EDTA (de Santiago-Martín et al., 2013). In addition to this, chelating solutions increased not only the solubility of metals but also modified their speciation. For example, the use of Na-EDTA to extract metals induced a high concentration of metal associated with fulvic and humic acids, in addition to the high proportion of metal present as free divalent ions that was also observed for the other two chelatants/extractants. In previous studies, humic/fulvic acid complexes have been linked to the strong chelating capacity of EDTA (Yang et al., 2001; Zhang et al., 2010), which not only mobilize elements weakly bound to extant soil matrix constituents but can also extract soil organic matter (of which humic acids are a major component), releasing additional elements (bound to this fraction) into solution (Yang et al., 2001; Zhang et al., 2012, 2010). This is supported by the higher concentrations of DOM measured in the Na-EDTA treatment relative to the control and tartaric acid treatments. The different forms of metals present in the growing media will influence their toxicity and potential for uptake by plants and biota (Chen et al., 2010). For instance, the study of Ondrasek et al., (2018) showed that the application of humic acids to the soil decreased the accumulation of Cu, Zn, Cd and Mn in plants due to the formation of metal-humate complexes, whereas the application of EDTA increased the accumulation of Pb in *Vicia faba* plants due to the formation of metal-EDTA complexes (Shahid et al., 2014a). As speciation also evolved during the experiment time course in our study, it can be hypothesized that the presence of plants adjusted metal speciation through preferential uptake of different metal species, as well as the secretion of root exudates, which may have differed between treatments (Haoliang et al., 2007; Kidd et al., 2009). This was exemplified in our previous study, where concentrations of both total and singular amino acids in the leaves of *Pistia stratiotes* were modified over time by the use of tartaric acid and Na-EDTA (Veselý et al., 2012b). Although the measurements were made in the leaves in that study, it demonstrates that the metabolism of plants changed with both treatment and time. From this it is reasonable to infer that root exudation was also modified in a similar way, thus plant-mediated metal speciation differed between treatments and with time. This was also supported by the high variation of organic acid contents in the growing media with time in the different treatments (Table S1).

An elevated concentration of Na in the solutions obtained using the Na-EDTA treatment could also have affected the growth of plants as well as the uptake of metals (Haddad and Al-Jada, 2021).

4.2. Changes in speciation affected the plant uptake

The concentrations of metals measured in the shoots and the roots of *Pistia stratiotes* in this study were greater than those found in previous studies using the same plant species under higher soil/solution metal concentrations (Galal et al., 2018; Kumar et al., 2019; Lu et al., 2011; Veselý et al., 2012a; Xie et al., 2022). This demonstrates the efficiency of plants to uptake metals in solution particularly using the chelatants (tartaric acid and Na-EDTA). Tartaric acid enhanced the uptake of metals by the plants to the greatest extent. This can be explained by a higher root uptake, an increase of xylem transport and an enhanced biomass production (Lu et al., 2013). The results of the metal uptake by plants, and removal capacity, also revealed that although Na-EDTA increased metal solubility more than tartaric acid, it did not induce a higher removal of metals by plants. Such an effect can be attributed to speciation differences and selective uptake by plants. Previous studies showed that EDTA increased the uptake of metals by plant roots and their translocation towards the upper parts (Shahid et al., 2014a, 2014b; Shamshad et al., 2018). This effect is usually attributed to the high concentration of soluble EDTA-metal complexes, as well as the capacity of EDTA to damage the root physiological barrier (do Nascimento et al., 2006). However, in our case, metal contents in the

plant were minimally affected by the addition of Na-EDTA, despite elevated concentrations of metals in solution. This finding was in contrast to the work of Shamshad et al., (2018) and Bunluesin et al., (2007), but in accordance with the finding of Lambrechts et al., (2011); clearly there is not a settled consensus on this effect. When looking at the speciation of the elements in the solution, it can be seen that tartaric acid increased the concentrations of metals in solution primarily through the increase of free divalent ions. In contrast, and as described above, the Na-EDTA treatment also increased the concentrations of metals associated with humic and fulvic acids. Such metal species may not be available for plant uptake and translocation and thus the uptake of metals by plants has been shown to be mainly related to the concentration in free Me^+ forms in the growing media (Chen et al., 2010; Kumar et al., 2021; Shabbir et al., 2020; Wang et al., 2008). The application of humic acids has also been shown to decrease the accumulation of metals by plants (Bunluesin et al., 2007; Rong et al., 2020). The authors related such an observation to a decrease in the concentration of free metal ions in the growing solution and the formation of stable complex with metals. Such an explanation is supported by the evolution of the concentration of the metal forms through time; the concentrations of free metal ions decreased in all cases, but in the Na-EDTA treatment, the content of metal linked to humic and fulvic acids did not decrease, indicating that such complexes were not taken up by the plants. Furthermore, even if taken up by the roots, such complexes are large molecules with a reduced transport from the roots to the shoots (Chen et al., 2010; Wang et al., 2008). This could explain the lower translocation factor of Cd and Cu in the Na-EDTA treatment. Finally, the higher amount of Ca and Mg in the Na-EDTA treatment could have contributed to suppressing the removal of metals by the plants, as those elements are antagonists of cations for plant uptake (Kabata-Pendias, 2004). In summary, tartaric acid mobilized a small proportion of metals that were readily available for plant uptake (free ion form) while Na-EDTA solubilized a high quantity of metals that were less readily available for plant uptake (form complexed with fulvic and humic acids).

4.3. Risks and benefits associated with the application of chelatants

In most situations where phytoextraction of metals from soils or waters is employed, the plant material is ultimately removed and used for non-food-chain purposes such as feedstock for anaerobic digestion. The main considerations are therefore the impacts of the chelator on longer-term soil and water quality (the ability of the soil to support plant growth once metals have been removed), as well as any potential the chelator might have to impair growth of the selected accumulator crop. The fact that Na-EDTA solubilized DOM reduces its useful application to soils because fertility will be reduced, and soil washing with this agent has been shown to be detrimental for soil structure and microbial activity (Jelusic and Lestan, 2014). From the results of our study it can also be seen that Na-EDTA induced a higher stress to the plants, especially after 360 h, as plants contained higher concentrations of proline (stress marker) comparative to the other treatments (Veselý et al., 2012b), even though metal accumulation was similar, showing that toxicity stress came from other elements than just metals, Na for instance. This brings into question the use of Na-EDTA for soil remediation applications in the field, and our study indicates that the use of tartaric acid is a more appropriate option where soil quality needs to be preserved.

5. Conclusions

A hydroponic experiment was performed to extract metal (Cd, Cu, Pb and Zn) laden solutions from a contaminated soil with two chelating agents and test the accumulation of those metals in water lettuce, as compared to water. Both chelatants were more extractive than water, and Na-EDTA mobilized the greatest concentration of metals of from soil but their uptake and translocation to plants was restricted because stable complexes were formed. In contrast, metals solubilized by tartaric acid were present largely as free ions and more easily taken up by the plants, but its stability will need to be assess in subsequent experiments. Applied to remediation scenarios, soil washing using tartaric acid in combination with water lettuce growth could be efficient to reallocate soil metals to plants. The usage of Na-EDTA should be avoided due to its aggressive propensity to render metals soluble (leachable) but not bioavailable. The focus of future research in this area should be upon the considered usage of tartaric acid as an environmentally relevant chelatant in a variety of soils having been treated with remedial amendments.

Abbreviations

EDTA
ethylenediaminetetraacetic acid
LMWOA
low molecular weight organic acid
DOC; DOM
dissolved organic carbon; dissolved organic matter

Declarations

Funding: This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic (project CZ.02.1.01/0.0/0.0/16_026/0008403).

Competing Interests: The authors have no relevant financial or non-financial interests to disclose

Author Contributions: The corresponding author **Lukáš Trakal** together with **Luke Beesley** and **Hailong Wang** contributed to the study conception and design. Material preparation, data collection and analysis were performed by **Jiřina Száková, Ondřej Drábek, Václav Tejnecký, Manhattan Lebrun** and **Rupert Lloyd Hough**. The first draft of the manuscript was written by **Manhattan Lebrun** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethical approval: This manuscript is only submitted to this journal.

Consent to participate: Not applicable.

Consent to publish: All the authors consent to publish this work.

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Figures

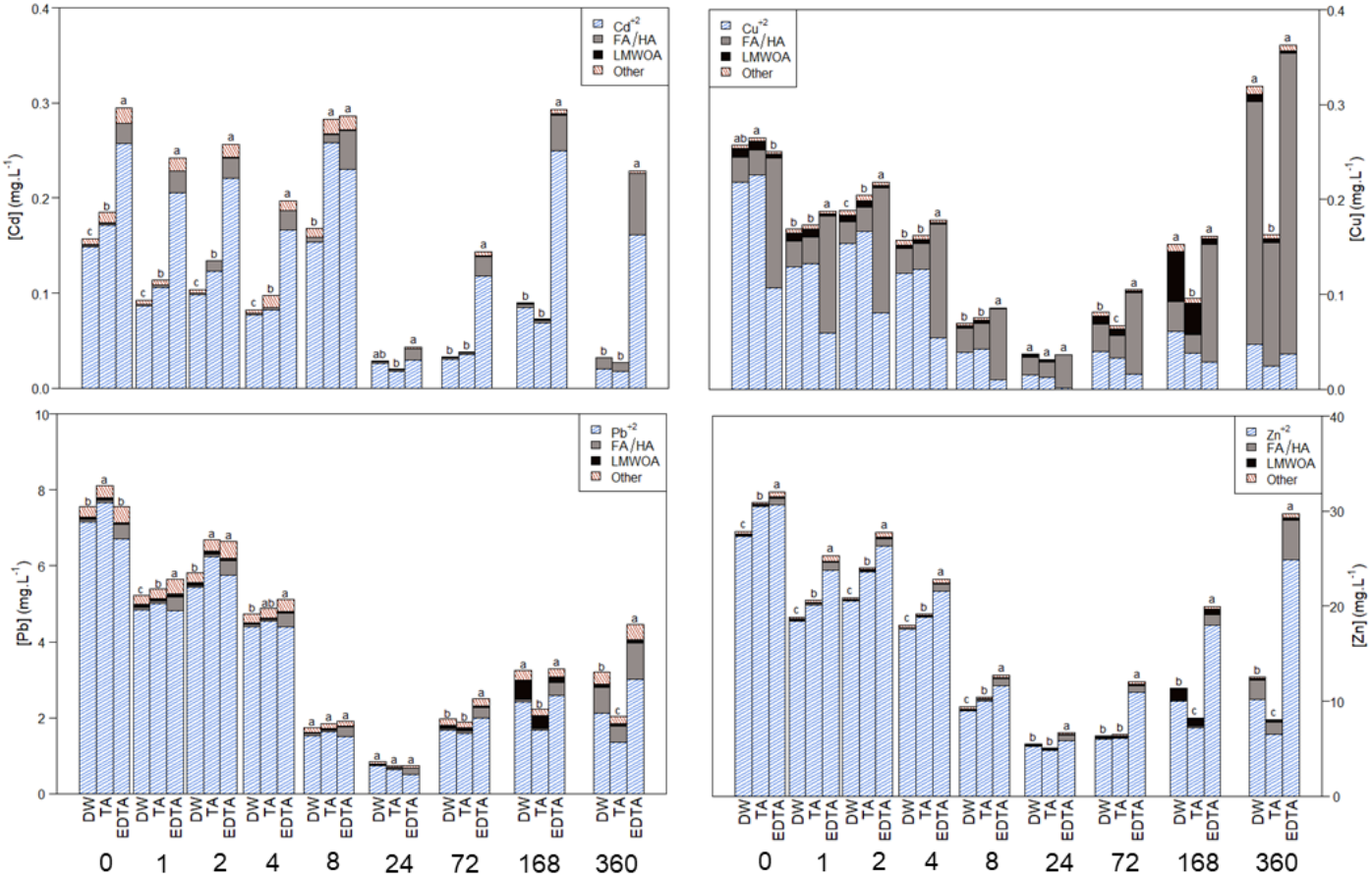

Figure 1

Concentrations of the different metal forms (assessed by visual MINTEQ model) in the different growing solutions over the 360 hours of the experiment. Letters indicate significant differences in total metal concentration between treatments at each sampling time (n = 3) ($p < 0.05$). DW = deionized water, TA = tartaric acid, EDTA = Na-EDTA

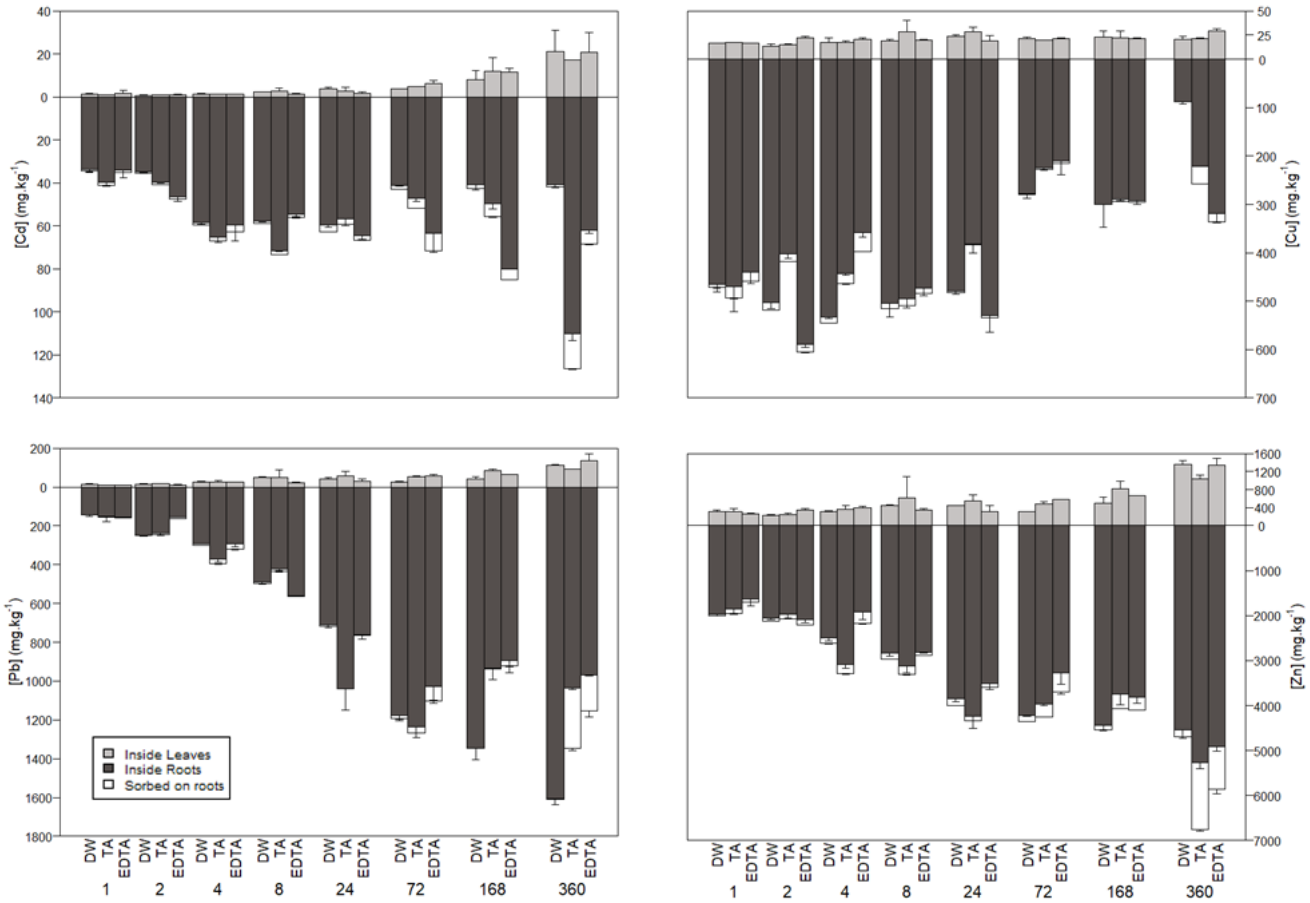

Figure 2

Metal concentration sorbed onto the roots and accumulated inside the leaves and inside the roots under the three treatments and over the 360 hours of the experiment. Data are presented as mean ± SD (n = 2-3). DW = deionized water, TA = tartaric acid, EDTA = Na-EDTA

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