Effects of chelates (EDTA, EDDS, NTA) on Phytoavailability of HMs (As, Cd, Cu, Pb, Zn) using Ryegrass (*Lolium multiflorum*)

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Abstract

This paper summarises a study of the application of the synthetic chelate ethylenediaminetetraacetic acid (EDTA), and the natural chelates ethylenediamine-N,N-disuccinic acid (EDDS) and nitrilotriacetate (NTA) to enhance ryegrass uptake of the heavy metal(oid)s (HMs) (As, Cd, Cu, Pb and Zn) from contaminated soils in mining sites. The study compares the effects of these chelates (EDTA, EDDS and NTA) on the phytoavailability of HMs (As, Cd, Cu, Pb, Zn) using ryegrass (*Lolium multiflorum*) through the single addition and sequential addition methods. The results show that application of EDTA, EDDS and NTA significantly increases ryegrass's (*Lolium multiflorum*) shoot uptake of some HMs when compared with no EDTA, EDDS or NTA application, particularly through sequential chelate treatment (EDTA 0.5:1 + 0.5:1; NTA 0.5:1 + 0.5:1; EDDS 0.5:1 + 0.5:1). EDTA 0.5:1 + 0.5:1 was more effective at increasing the concentration of Pb in shoots than were the other chelates (EDDS and NTA) and controls. Moreover, the concentrations of Zn in the shoots of ryegrass in LH significantly increased with the application of split dose (0.5:1 + 0.5:1). The plants displayed symptoms of toxicity including yellow and necrotic leaves at the end of the experiment. The selected chelates (EDTA, EDDS and NTA) led to a significant decrease in plant biomass (yield) 28 days after transfer with a maximum decrease in EDTA treatment (0.5:1 + 0.5:1) soils. This decrease was 3.43-fold in HT, 3-fold in LH and 1.59-fold, respectively, relative to the control. HM concentration and dissolved organic carbon (DOC) in pore water, provided an explanation for why fresh weight was significantly reduced with application of chelates in sequential dose (EDTA 0.5:1 + 0.5:1 and NTA 0.5:1 + 0.5:1).

1. Introduction

Heavy metal (HM) and metalloid pollution has become one of the most pressing threats worldwide to environmental soil, ecosystems and to human health by way of the food chain (Niinae et al., 2008, Sun et al., 2009, Petelka et al., 2019, Luo et al., 2005). Phytoremediation can potentially be used for the remediation of areas polluted by metal. The plant-based phytoextraction technique is a low-cost, cost-effective, environmentally friendly and sustainable method that has often been described as having the potential to remediate HMs in contaminated agricultural land (Garbisu and Alkorta, 2001, Evangelou et al., 2007b, Sun et al., 2009, Salt et al., 1998). Increased research attention has recently been devoted to phytoextraction, the use of plants to remove HMs from polluted soils (Bolan et al., 2014, Salt et al., 1998). Successful application of phytoextraction requires the ability to cultivate plants that generate a large biomass capable of accumulating high concentrations of toxic HMs in the shoots of plants from contaminated soil (Luo et al., 2006).

Enhancing the accumulation of metal(loid)s in existing high-yield crop plants without diminishing their yield is a viable strategy for improving the efficiency of phytoremediation (Evangelou et al., 2007a). Research has recommended adding chelating agents to increase metal(loid)s' accumulation capacity and the uptake speed of nonhyperaccumulator plants (Michael et al., 2007, Shen et al., 2002, Evangelou et al., 2007b). In contrast with conventional remediation methods, HMs obtained from polluted soil can be translocated to above-ground plant parts, and then metal-rich plant materials can be easily harvested

and cleared from sites without significant digging, disposal costs or destruction of topsoil (Blaylock, 2000, Blaylock et al., 1997, Kos and Leštan, 2003, Lee and Sung, 2014). Positive outcomes have been achieved through applying chelating agents to enhance the solubility of HMs in polluted soil and consequently enhance phytoextraction.

Many studies have applied various chelating agents (Evangelou et al., 2007b, Ramamurthy and Memarian, 2014, Suthar et al., 2014, Yu et al., 2020, Fang et al., 2017). Particularly, synthetic chelants such as ethylenediaminetetraacetic acid (EDTA), and natural chelants such as ethylenediamine-N,N-disuccinic acid (EDDS) (EDDS) and nitrilotriacetate (NTA) have enhanced the solubility of metal(loid)s in soils and their subsequent uptake and translocation in plant shoots (Blaylock et al., 1997, Shen et al., 2002, Ramamurthy and Memarian, 2014, Luo et al., 2005, Yu et al., 2020). Synthetic agents such as NTA, EDDS and EDTA are applied in phytoremediation with assisted chelates to enhance the phytoextraction of HMs in polluted soil by plants. Through the formation of metal-ligand complexes, the presence of a ligand affects the biouptake of HMs and changes the potential under the root zone to leach metal(loid)s (Seuntjens et al., 2004).

The effectiveness of various chelating agents differs according to the plants and HMs used (Evangelou et al., 2007b). Key considerations in selecting chelates include their fate, potential toxicity to plants and soil microorganisms, and degradation following application. The longevity of chelates as a stable compound in the soil is critical both for assisting metal release and hence phytoavailability for phytoextraction, as well as for the potential leaching of released metals (Van Ginneken et al., 2007). Persistent aminopolycarboxylic acids (APCAs) such as EDTA have been applied in many phytoextraction experiments to examine their potential for remediation. However, EDTA and the formed-EDTA complexes can create serious problems because of their low biodegradability in soils (Zhao et al., 2018, Luo et al., 2005, Grčman et al., 2003), and their presence in soils may result in greater risk of groundwater contamination through metal leaching (Luo et al., 2005, Quartacci et al., 2007, Zhao et al., 2016).

In the past decade, biodegradable chelant APCAs such as EDDS and NTA have been recommended as alternatives to EDTA nonbiodegradable APCAs and other persistent APCAs. Because of the need to avoid the problems caused by non-biodegradable chelants such as EDDS and NTA have the potential to enhance HM phytoextraction from contaminated soils (Lan et al., 2013, Evangelou et al., 2007b). Studies have found that compared with EDTA, EDDS and NTA cause much lower potential leaching (Luo et al., 2006); have faster biodegradability; stronger chelating ability; and are less toxic to microorganisms, ecosystems and plants (Grčman et al., 2003, Quartacci et al., 2007, Wenger et al., 2008). However, these studies do not provide underpinning mechanisms of metal(loid) dynamics in the presence of chelates. Thus, understanding their application in remediating high concentrations of multiple HMs in contaminated soils at different mining sites is important. It has been found that the concentration and daily addition of chelants affects the accumulation of metals in plant tissues (Grčman et al., 2003, Luo et al., 2006, Cao et al., 2007). Therefore, to improve HM phytoextraction in highly contaminated soils, the dosage of chelants and time for adding them require further study. To achieve this research aim, we

conducted a comparative study of the effect of three different chelates on the mobilisation of toxic metals present in contaminated mining soils in Vietnam. In particular, we aim to achieve the following:

(a) evaluate the effects of chelates (EDTA, EDDS and NTA) on the phytoavailability of the HMs (As, Cd, Cu, Pb, Zn) using ryegrass (*Lolium multiflorum*) through the single addition and sequential addition methods in contaminated soils in three different mining sites in Vietnam

(b) evaluate the important factors relating to the application of chelates (EDTA, EDDS and NTA) by the single addition and sequential addition methods in the phytoavailability of HMs (As, Cd, Cu, Pb and Zn) of ryegrass

2. Materials And Methods

2.1. Soil characterisation

The contaminated soils were collected from the following three selected mining sites in Thai Nguyen province (north-eastern mountainous region of Vietnam): Ha Thuong tin mine (HT); Hich Village lead and zinc mine (LH); Trai Cau iron mine (TC). Six contaminated soil samples with contrasting soil properties were taken from each selected mining site, but only one contaminated soil sample was selected to represent each mining site for the experiments. All soil samples were taken from the top 20 cm using stainless steel trowels; the soil samples were air dried and sieved through 4 mm sieves initially, and later through 2 mm sieves.

The general physicochemical properties of the soil samples were determined using standard procedures. Specifically, pH and conductivity were measured with Milli-Q water and 0.01 M $CaCl_2$ in 1:5(w/v) soil water suspensions (Rayment and Higginson, 1992). Soil textures were categorised according to United States Department of Agriculture (USDA, 1987). The hydrometer method was used to determine the soil texture in relation to the percentage of clay, sand and silt in the soil samples (Gee and Bauder, 1986). EC was measured according to a standard protocol using a pH/EC meter (smartCHEM-LAB - TPS, Australia) (Rayment and Higginson, 1992, Sposito, 2008). Dissolved organic carbon (DOC) was determined using a total carbon analyser (Shimadzu: TOC-LCSH, Japan). Soil organic carbon (OC) was determined by the high-temperature loss-on-ignition method after addition of HCl to remove carbonates (Leco TruMac CNS analyser, United States of America [USA]). Oxalate extractable, or ‘amorphous’ Fe, Al and Mn oxide content of sample, was measured using the acid oxalate method (pH = 3) (Rayment and Higginson, 1992). Soil OC including TC, TN and TS were determined using a LECO TOC analyser (TruMac CNS). CEC_B was determined by the modified compulsive exchange method (Gillman and Sumpter, 1986).

Air-dried soil (0.5 g, < 2 mm) was weighted directly into a Teflon digestion vessel for total metal analysis and added to 5 ml of concentrated HNO_3 aqua regia. The soil suspension was digested in accordance with Method 3051A (USEPA, 1997) in a microwave assisted acid digestion oven (MARS5, CEM, USA). A standard reference material (Montana Soil SRM2711, NIST, USA) approved and a blank for validating the

digestion operation were included in each microwave digestion batch. Using ICP-MS (Agilent 7900), the metal content in the digest was measured. Recoveries were on average from 80 to 120% for the selected HMs (As, Cd, Cu, Pb and Zn). The DOC concentration was determined by extracting soil with Milli-Q water (1:5) for 16 h in an end-over-end shaker. The suspensions were then purified by 0.45 µm membrane filters and analysed for DOC using an automatic total organic carbon (TOC) analyser (Model 1010, O.I. Analytical, USA) (Li et al., 2007).

2.2. Plant sample analyses

Plant samples (0.1–0.5 g) were digested in concentrated HNO_3 (5 ml) acid following the USEPA Method 3015a (Agazzi and Pirola, 2000). Testing the selected HM (As, Cd, Cu, Pb and Zn) content in plant tissue after exposure was conducted by digesting dried samples in 5 ml of concentrated high-purity nitric acid overnight and then heating each sample under programmed heating to 140°C to evacuate the acid to approximately 1 ml. The remaining acid was diluted to 20 ml using Milli-Q water. The solution was filtered using 0.45 µm membrane filters before analysis using ICP-MS Agilent 7900.

The roots and shoots of the harvested plants were separated, washed with Milli-Q water, and the dry weights were then recorded. The samples were then dried to a constant weight at 60°C by using a forced-air oven for 72 h and ground to a fine powder for metal analysis. The ground plant material (0.1–0.5 g) was weighed directly into a 75 ml digestion tube, 5 ml of concentrated nitric acid was added and left to cold digest in a fume cupboard overnight (Zarcinas et al., 1987). The tubes were heated using a temperature-controlled digestion block (AI Scientific Block Digestion System AIM 500, Australia) programmed to slowly increase the temperature to 140°C until approximately 1 ml of digest remained in the tube. The digests were diluted with Milli-Q water and analysed for the selected HMs using ICP-MS.

Plant standard reference material spinach leaves (SRM 2511A / SRM 1570A, NIST, USA) were used to check the consistency and accuracy of the digestion of plant samples. The level of precision can be manifested by the standard deviation (SD) of these data and comparison with certified or published data helps to evaluate the accuracy or consistency of the measurements. Quality control (QC) included blank controls and continuing calibration verification during analysis was monitored. QC during analysis was monitored by adding 10 µg/L check samples and blanks every 15–20 samples. All aqueous samples were analysed by ICP-MS after appropriate dilutions and matrix matching in standards. Triplicate samples were analysed. Recovery of check samples was always between 85 and 115%.

2.3. Pore water analysis

The pore water samples were extracted using rhizon samplers and analysed for pH, EC, cation and anion particulates, DOC, and total HM content. To measure DOC, the samples were filtered using 0.45 µm membrane filters and examined using the TOC analyser (Shimadzu: TOC-LCSH, Japan) (Choi et al., 2009). Total HM content was examined using ICP-MS 7900. Pore water samples were collected using the rhizon samplers and 10 ml syringes. These samples were taken at 1, 14 and 28 days after the ryegrass pots had been transferred to the chelate-treated soils. They were subsequently analysed for HM

concentration using ICP-MS Agilent 7900 and ICP-OES Perkin Elmer Optima 5300V; DOC was analysed using the TOC analyser; and pH using a pH meter.

2.4. Soil incubation

First, chelate solutions (EDTA, EDDS and NTA) with two ratio concentrations (1:1, 0.5:1 + 0.5:1) were prepared using Milli-Q water. The two selected ratio treatments were chosen from the results of the incubation experiments (4 g soil samples) using the chelates. The experiment was conducted with 63 sample pots, which included 2 selected chelate ratio treatments, 3 selected soil samples (200 g), 4 treatments (Control, EDTA, EDDS, NTA) and 3 replicates. The mine soils with chelates applied were then incubated at 60% water holding capacity (WHC).

Metal concentrations in pore water were monitored over three periods of time (after 1 day, 2 weeks and 4 weeks) following the addition of predetermined/calculated chelate solutions in the concentration. Residual metals including the effects of chelate on mobility of metals in different soils (chelate types, soil types and dose) were also investigated. The study compared two application methods (sequential and single). Following this study, ryegrass plants, which had been growing in synthesised soil were transferred into the chelate-treated mine soils to determine chelate-enhanced phytoextraction. This aspect of the experiment is detailed in the following subsection.

2.5. Plant growth experiment using chelates

For plant growth experiments, the approach introduced by (Stanford and DeMent, 1957) originally measured available nutrients such as phosphorus in the soil. However, this method has also been applied for other elements. This method makes it possible to measure metal absorption by plants to examine the bioavailability of HM in contaminated soil impacted by chelates. The method is particularly effective in conditions of root–soil contact over relatively brief periods.

Transparent plastic pots (capacity of 650 ml; 10 cm diameter) and removed bottoms were nested in similar containers that had intact bottoms and were filled with approximately 300 g of synthesis sand medium, including clean sand, coconut pit, and perlite ratio 1:1:1. Approximately 50 seeds (± 2) of ryegrass (*Lolium multiflorum*) were sown directly into the sand at a depth of 5 mm, then the pots were loosely with wet tissue paper for 5 days to maintain the moisture content (60% WHC) and achieve germination. In a temperature-controlled greenhouse environment (25 ± 3°C; 16 h light), each treatment was conducted in triplicate and the plant growth experiment was conducted. During the germination period, the pots were watered with deionised (DI) water every day for the first 7 days. After this time, to maintain the target moisture content (60% WHC), a modified Hoagland nutrient solution (HNS) was applied to each pot once per day.

The compositions of HNS including the macronutrients (μM/L) were as follows: 100 μM KH_2PO_4, 500 μM KNO_3, 500 μM $Ca(NO_3)_2$, 100μM NH_4HPO_4, and 200 μM $MgSO_4$. The micronutrients (μM/L) were as follows: 0.05 μM KCL, 0.1 μM $Cu(NO_3)_2$, 0.5 μM H_3BO_3, 0.5 μM $ZnSO_4$, 0.01 μM MoO_3, 0.04 μM $Co(NO_3)_2$,

1 μM $MnSO_4$, and 5 μM Fe-EDTA. The nutrient solution was adjusted to pH 6.0 before use and replaced weekly. The pH was adjusted to 6.0 using 0.1 M KOH or 0.1 M HCl solution.

The ryegrass (*Lolium multiflorum*) seeds were germinated in pots containing 300 g of clean synthesised sand, coconut pit and perlite; 12 weeks were set aside for adequate growth and biomass. The plant growth cycle began in May 2019 and continued until early July 2019. Because the study was conducted in winter, ryegrass growth was considerably slow compared with what would have been the growth rate in summer. After 12 weeks, at which time, the roots proliferated to the bottom of the pots, the plastic containers containing the plants were nested in the second plastic container holding 200 g of treated soil for an incubation experiment using selected chelate ratios, thus placing the dense mat of roots in intimate contact with the treated soil.

To have sufficient moisture in the sand, the roots were placed in contact with the soil. The combined pots were weighed, and this reference weight was recorded. The pots were irrigated every day or twice per day with Hoagland nutrient solution (containing Cu and Zn elements) to attain the reference weight. Rhizon samplers (one per pot, Rhizosphere Research Products, Wageningen, Netherlands) were placed horizontally at 1 cm from the bottom of the pot (or making a hole at a height of 2–3 cm from the bottom of the pots to connect to the rhizon samplers) so that sampling of the soil pore water at various periods during the experiment was possible. Moisture losses were replenished by DI/periodic water and nutrient solution additions. Plants were harvested at 16 weeks after sowing and this included the experiment time, which was 4 weeks long.

Pore water samples were obtained using the rhizon samplers at routine intervals after the transfer of seedlings to the contaminated soils and analysed for HMs. Pots without plants were retained as monitors, but pore water was obtained at specific intervals. In the sixteenth week (4 weeks after being moved to infected soil), plants were harvested. Top regrowth (the shoot part) of the ryegrass from all pots of the experiment was harvested at a 2 cm stubble when there was approximately 1 g of dry matter growth. At harvest time, the bottom of the pots in the three repetitions of each treatment experimental unit were dismantled and the roots of the ryegrass were retrieved by hand washing in DI water 3 times, and then in Milli-Q water 3 times to remove synthesised sand. The shoots and roots were separated after harvesting plants, washed with deionised water, oven dried, and their fresh weight and dry weight (dry biomass) were recorded. Then, the dried roots and shoot plants were ground and analysed for HMs content.

2.6. Statistical analysis

All experiments were conducted with three replicates. The statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 26.0 (SPSS Inc., Chicago, IL) and Excel. One-way analysis of variance (ANOVA) was conducted with SPSS 26.0 to determine the significance of the differences between treatments. Then treatment means were compared after applying *post hoc* Tukey's test at $p > 0.05$ or Dunnett's test at $p < 0.05$ to check the homogeneity of variances. The means of treatments were considered significantly different when a significant F-value ($p > 0.05$) was

obtained. The Duncan multiple range test was used to compare treatment means, data variability was expressed as the SD, and $p < 0.05$ was considered statistically significant. Where linear regressions were performed, the significance was also defined by SPSS 26.0 as $p < 0.05$. Minitab Express 1.5.1 determined the different letters and significance was $p > 0.05$ when compared with other treatments. Values were in the order a > b > c).

3. Results And Discussion

3.1. Physicochemical parameters of soils

The physicochemical properties of the selected soils are presented in Table 1 and these varied considerably with the pH ranging from 5.28 for HT soil to 8.58 for LH soil. As with the soil pH, soil texture also differed considerably. Given that the soils were from metal mining areas, the toxic metal content of the three soils was quite high. The widely different pH, textural content and metal concentration suggest potential differences in the phytoavailability of different metals.

Table 1
Soil properties of the three selected soils collected from the three selected mining sites (mean ± SD, n = 3)

Soil location	Ha Thuong tin mine (HT)	Hich Village Lead-zine mine (LH)	Trai Cau iron mine (TC)
GPS location	(21°40'N 105°50'E)	(21°43'30"N 105°51'23"E)	(21°35'39' 'N 105°57'06"E)
Selected sample	HT5.1	LH1.1	TC1.1
pH	5.28	8.58	6.29
EC µs/cm	28.40	205.3	113.7
CEC_B cmol/kg	2.21	1.2	11.18
Sand (%) > 0.05 mm	50.00	72.50	15.00
Silt (%) 0.05–0.002 mm	30.00	20.00	37.50
Clay (%) < 0.002 mm	20.00	7.50	47.50
USDA texture	Loam	Sandy Loam	Clay
WHC (%)	47.25	20.40	62.24
Moisture (%)	29.17	38.74	41.15
TC (%)	0.24	8.66	0.28
TOC (%)	0.21	0.12	0.49
TN (%)	0.02	0.02	0.03
TS (%)	0.01	0.54	0.21
TC (mg/L)	2.26	27.00	3.16
DOC (mg/L)	1.74	18.44	2.57
IC (mg/L)	0.52	8.66	0.52
As (mg/kg)	2104	34.61	242.8
Cd (mg/kg)	8.21	28.54	5.26
Cu (mg/kg)	328.8	29.72	177.0

Comparison of soil environmental quality standards for HMs of different countries (Chen et al., 2018) and compared with the Vietnam's environment standard for agricultural and industrial soil type (QCVN03-MT: 2015-BTNMT); Cu (100–300 mg/kg), Zn (200–300 mg/kg); As (15–25 mg/kg); Cd (1.5–10 mg/kg); and Pb (70–300 mg/kg), three selected samples including LH1.1, HT5.1 and TC1.1 contained high HMs concentrations (As, Cd, Cu, Pb and Zn) in contaminated soils. These were all affected by tailings in the three mining sites.

Soil location	Ha Thuong tin mine (HT)	Hich Village Lead-zine mine (LH)	Trai Cau iron mine (TC)
Pb (mg/kg)	1047	2898	2092
Zn (mg/kg)	2583	4287	2181
Fe (g/kg)	263.21	7.19	205.14
Al (g/kg)	14.27	1.49	21.25
Cr (mg/kg)	141.33	3.82	190.39
Mn (g/kg)	55.93	0.76	12.82
Fe oxide (g/kg)	2946	2048	1836
Al oxide (g/kg)	1546	131.1	862.6
Mn oxide (g/kg)	7823	1366	1836

Comparison of soil environmental quality standards for HMs of different countries (Chen et al., 2018) and compared with the Vietnam's environment standard for agricultural and industrial soil type (QCVN03-MT: 2015-BTNMT); Cu (100–300 mg/kg), Zn (200–300 mg/kg); As (15–25 mg/kg); Cd (1.5–10 mg/kg); and Pb (70–300 mg/kg), three selected samples including LH1.1, HT5.1 and TC1.1 contained high HMs concentrations (As, Cd, Cu, Pb and Zn) in contaminated soils. These were all affected by tailings in the three mining sites.

3.2. Effects of EDTA, EDDS and NTA in fresh shoot biomass in two selected doses on plant growth

Figure 1 below depicts plants growing in chelate-amended soils. In the absence of chelates, the plants displayed natural growth in control samples without visual signs of metal toxicity.

Plant growth was significantly affected in soils treated with chelates. As shown in Fig. 1, irrespective of which chelate (i.e. EDTA, EDDS or NTA), the amendment of soils led to a significant decrease in plant growth (Fig. 1 and Fig. 2). At the end of the experiment (28 days after the application of the chelates), the plants showed symptoms of toxicity, including yellow and necrotic leaves (Appendix 1), and root growth was seriously affected. Plant biomass is often used in experiments as an indicator of plants' ability to grow in chelate-amended soil and of the phytotoxicity of metals (Lee and Sung, 2014, Evangelou et al., 2007a, Luo et al., 2006).

The highest fresh weight of ryegrass (*Lolium multiflorum*) was found in the controls (Fig. 2). Results showed that selected chelates (EDTA, EDDS and NTA) led to a significant decrease in plant biomass (yield) 28 days after transfer. In particular, when the well-established ryegrass in sand medium with exposed roots was transferred to the chelate-incubated soils, the fresh weight of plant growth decreased, with the maximum decrease in the EDTA treatment (0.5:1 + 0.5:1) soils. This decrease was 3.43-fold in HT, 3.00-fold in LH, and 1.59-fold in TC, relative to the control (Fig. 2).

At the final harvest, EDTA 0.5:1 + 0.5:1 had the greatest effect on decrease of both fresh and dry weight production of the ryegrass compared with the control (Fig. 2 and Fig. 1 presented in Supporting information) in all soils, particularly in HT and LH, which had larger HM concentrations than did TC. However, there was no significant difference in the dry biomass of roots when the chelate was added (Fig. 2 presented in Supporting information). For the LH soil, ryegrass plants treated with the chelates (EDTA, EDDS and NTA) in split dose (0.5:1 + 0.5:1) treatments attained smaller plant fresh weight production in mg/kg than the single dose (1:1) treatments (Fig. 2). The lower biomass could be one reason for the higher HM (As, Cd, Cu, Pb and Zn) concentrations in the shoot and pore water of the split dose (0.5:1 + 0.5:1) treatments than those in single dose.

Decreased fresh plant biomass was strongly related to decreased chlorophyll content, which was caused by increased metal concentrations in the plant shoots (Lee and Sung, 2014). Moreover, it is considered that a more efficient chelate may have higher toxicity (Mühlbachová, 2011, Lee and Sung, 2014). As presented Table 1, Cd an Zn concentrations in the TC soil were lower than those in HT and LH soils. As plants accumulate more HMs, photosynthesis and biomass production can be reduced (Clijsters and Assche, 1985). This suggests a much lower decrease in plant shoot biomass in LH, particularly in the EDTA treatments, than was found in the HT and TC soils, and, there is less decrease in plant biomass shoot in the TC soil compared with the control soils.

Chelate effects on plant growth can differ with HM concentrations in soil and can be more significant when HM concentrations are low. When HM concentrations are high, plant growth is affected more by HMs than by chelates (Jiang et al., 2003). Plant growth could be affected by chelate toxicity and by the toxicity caused from increasing of HM concentrations in the soil solution and in the plant (Clijsters and Assche, 1985, Lee and Sung, 2014).

3.3. Effects of EDTA, EDDS and NTA on pore water metal concentration

3.3.1. Effects of EDTA, EDDS and NTA on pore water metal concentration by sites

The effects of chelate on pore water metal concentrations were investigated over 28 days. It is apparent from the pore water data (Tables 1, 2 and 3 presented in Supporting information), that except in the control, HM concentrations generally decreased as time passed, with the exception of Zn in the TC soil, which increased in the pore water over time. However, in the control soil, Cu and Cd in pore water increase over time in the three mining sites, as does Zn in LH. These differences in pore water metal concentrations could be connected to plant uptake of metals, which is likely to vary depending on the nature of the metals. EDTA 1:1 was the most effective for the mobilisation of metals in pore water.

3.3.2. Effects of EDTA, EDDS and NTA on DOC

It is apparent from the DOC data (Table 4 presented in Supporting information), DOC enhanced metal solubility and transport in the soil. Adding chelate treatments (EDTA, NTA and EDDS) significantly increased the amount of DOC in the soil compared with the control, and the DOC of the treatments decreased significantly after 28 days after applications in almost all HMs.

The transport and bioavailability of HMs can be strongly influenced by forming soluble and insoluble complexes with DOC. Such interactions can alter the chemical speciation of the HMs, modifying their affinity for sorptive surfaces in the soil matrix or their uptake, accumulation and eventual toxicity to organisms (Vulkan et al., 2000, Boyd et al., 2005, Arnold et al., 2010). Several studies have documented a positive correlation between DOC and HM concentrations in leachates, indicating DOC can act as a vehicle for moving HMs (Zhao et al., 2007, Bhatt and Gardner, 2009). The concentration of HMs in pore water in the soil was correlated with DOC.

3.4. Effects of EDTA, EDDS and NTA on root and shoot metal concentrations and phytoextraction

Control, EDTA 1:1, EDTA 0.5:1 + 0.5:1, NTA 1:1 and EDDS 0.5:1 + 0.5:1 chelate treatments significantly affected the fresh weight and dry biomass of shoot and Zn shoot concentration ($p < 0.01$ to $p < 0.05$) (Table 2).

Sequential chelate addition (0.5:1 + 0.5:1) for EDTA, NTA and EDDS significantly affected fresh weight of shoot (Table 3). Sequential addition (0.5:1 + 0.5:1) of EDTA also significantly increased uptake of Cd, Pb and Zn, while for NTA, As uptake was significantly increased, and for EDDS, Pb uptake was significantly increased with sequential addition. These results correspond to increasing HMs (Pb, Zn) in soil solution (pore water) and caused serious toxic symptoms in the ryegrass after the second addition of remaining sequential chelate dose.

The results for the TC soil showed that fresh weight of shoot was significantly affected by sequential chelate treatments of EDTA 0.5:1 + 0.5:1 and EDDS 0.5:1 + 0.5:1, at $p < 0.01$ and $p < 0.05$, respectively (Table 4). Cu concentration was significantly affected in controls, EDTA 1:1, EDTA 0.5:1 + 0.5:1 and EDDS 0.5:1 + 0.5:1, at $p < 0.01$. Pb concentration was significantly affected in controls, EDTA 1:1, at $p < 0.05$, while Pb concentration only was affected significantly differently by NTA 1:1, at $p < 0.01$.

Table 2
Dry biomass yields (g/pot) and concentrations of As, Cd, Cu, Pb and Zn (mg/kg DW) in the shoots of ryegrass 28 days after application of EDTA, EDDS and NTA in HT

Chelate ratios	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	7.43 ± 1.68	1.41 ± 0.31	0.82 ± 0.23	0.04 ± 0.00	26.00 ± 1.38	0.62 ± 0.13	61.71 ± 7.65
EDTA 1:1	3.99 ± 0.67	0.86 ± 0.15	0.29 ± 0.76	0.05 ± 0.01	17.79 ± 2.79	13.93 ± 4.52	123.00 ± 15.93
EDTA 0.5:1 + 0.5:1	2.44 ± 0.34	0.69 ± 0.20	0.65 ± 0.55	0.06 ± 0.04	20.05 ± 6.78	15.49 ± 14.16	97.82 ± 29.75
NTA 1:1	3.88 ± 0.57	0.71 ± 0.16	0.47 ± 0.10	0.17 ± 0.02	29.96 ± 5.17	1.90 ± 0.11	150.37 ± 7.77
NTA 0.5:1 + 0.5:1	5.93 ± 0.77	1.3 ± 0.14	0.50 ± 0.03	0.08 ± 0.02	22.78 ± 2.01	1.09 ± 0.18	101.91 ± 9.71
EDDS 1:1	7.05 ± 0.93	1.44 ± 0.16	0.52 ± 0.02	0.17 ± 0.04	25.37 ± 5.89	1.45 ± 0.34	71.38 ± 9.09
EDDS 0.5:1 + 0.5:1	3.75 ± 0.13	0.81 ± 0.1	0.72 ± 0.13	0.10 ± 0.04	17.68 ± 0.67	1.81 ± 0.49	58.87 ± 2.48
ANOVA	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	**	*	NS	NS	NS	NS	**
EDTA 1:1	**	*	NS	NS	NS	NS	**
EDTA 0.5:1 + 0.5:1	**	**	NS	NS	NS	NS	NS
NTA 1:1	**	**	NS	NS	NS	NS	**
NTA 0.5:+0.5:1	NS	NS	NS	NS	NS	NS	*
EDDS 1:1	NS	NS	NS	NS	NS	NS	NS
EDDS 0.5:1 + 0.5:1	**	*	NS	NS	NS	NS	NS

Values are means ± SD (n = 3); ** = $p < 0.01$; * = $p < 0.05$, NS = not significant.

Table 3
Dry biomass yields (g/pot) and concentrations of As, Cd, Cu, Pb and Zn (mg/kg DW) in the shoots of ryegrass 28 days after application of EDTA, EDDS and NTA in LH

Chelate ratios	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	6.20 ± 0.44	1.38 ± 0.18	0.22 ± 0.12	0.06 ± 0.02	24.05 ± 4.22	4.97 ± 1.35	63.43 ± 0.80
EDTA 1:1	4.44 ± 1.42	0.91 ± 0.34	0.58 ± 0.09	0.28 ± 0.95	17.80 ± 1.51	184.00 ± 22.50	136.43 ± 11.39
EDTA 0.5:1 + 0.5:1	2.07 ± 0.38	1.19 ± 0.05	0.90 ± 0.10	2.53 ± 0.78	48.59 ± 23.22	1339.38 ± 117.71	567.73 ± 51.53
NTA 1:1	6.35 ± 0.81	1.33 ± 0.11	0.43 ± 0.13	0.05 ± 0.02	21.89 ± 1.54	26.94 ± 10.15	143.41 ± 21.06
NTA 0.5:1 + 0.5:1	3.39 ± 0.19	0.75 ± 0.15	2.07 ± 1.24	0.23 ± 0.14	31.58 ± 18.88	347.95 ± 267.17	488.68 ± 107.67
EDDS 1:1	5.79 ± 0.47	1.16 ± 0.11	0.55 ± 0.09	0.12 ± 0.02	18.97 ± 1.52	306.26 ± 1.38	154.21 ± 31.71
EDDS 0.5:1 + 0.5:1	3.15 ± 0.62	0.68 ± 0.19	0.51 ± 0.14	0.57 ± 0.27	15.55 ± 1.18	434.59 ± 64.43	236.29 ± 24.30
ANOVA	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	NS	NS	NS	NS	NS	NS	NS
EDTA 1:1	NS	NS	NS	NS	NS	NS	NS
EDTA 0.5:1 + 0.5:1	**	NS	NS	**	NS	**	**
NTA 1:1	NS	NS	NS	NS	NS	NS	NS
NTA 0.5:1 + 0.5:1	**	*	**	NS	NS	NS	*
EDDS 1:1	NS	NS	NS	NS	NS	NS	NS
EDDS 0.5:1 + 0.5:1	**	**	NS	NS	NS	*	NS
Values are means ± SD (n = 3); ** = $p < 0.01$; * = $p < 0.05$, NS = not significant.							

Table 4
Dry biomass yields (g/pot) and concentrations of As, Cd, Cu, Pb and Zn (mg/kg DW) in the shoots of ryegrass 28 days after application of EDTA, EDDS and NTA in TC

Chelate ratios	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	7.08 ± 0.14	1.49 ± 0.11	0.17 ± 0.09	0.04 ± 0.01	24.03 ± 2.54	0.42 ± 0.15	68.43 ± 6.79
EDTA 1:1	5.81 ± 0.75	1.32 ± 0.19	0.07 ± 0.03	0.05 ± 0.03	14.82 ± 0.76	13.44 ± 9.93	66.88 ± 12.73
EDTA 0.5:1 + 0.5:1	4.46 ± 0.38	1.05 ± 0.11	0.07 ± 0.03	0.03 ± 0.01	13.95 ± 0.25	5.83 ± 4.55	46.49 ± 2.12
NTA 1:1	7.18 ± 0.60	1.47 ± 0.07	0.19 ± 0.07	0.15 ± 0.09	25.17 ± 0.68	8.85 ± 3.68	116.94 ± 10.45
NTA 0.5:1 + 0.5:1	6.88 ± 0.70	1.5 ± 0.15	0.26 ± 0.21	1.09 ± 0.99	28.80 ± 6.74	5.82 ± 1.64	88.82 ± 13.55
EDDS 1:1	6.00 ± 0.49	1.36 ± 0.19	0.17 ± 0.12	0.06 ± 0.02	20.10 ± 2.45	2.62 ± 0.27	69.35 ± 7.97
EDDS 0.5:1 + 0.5:1	5.53 ± 0.29	1.36 ± 0.11	0.10 ± 0.01	0.05 ± 0.00	13.57 ± 1.91	1.09 ± 0.56	62.64 ± 5.21
ANOVA	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	NS	NS	NS	NS	*	*	NS
EDTA 1:1	NS	NS	NS	NS	*	*	NS
EDTA 0.5:1 + 0.5:1	**	*	NS	NS	*	NS	NS
NTA 1:1	NS	NS	NS	NS	NS	NS	**
NTA 0.5:1 + 0.5:1	NS	NS	NS	NS	NS	NS	NS
EDDS 1:1	NS	NS	NS	NS	NS	NS	NS
EDDS 0.5:1 + 0.5:1	*	NS	NS	NS	*	NS	NS

Values are means ± SD (n = 3); ** = $p < 0.01$; * = $p < 0.05$, NS = not significant.

Table 5
One-way ANOVA analysis of difference between chelate treatments in the shoot of ryegrass 28 days after application of EDTA, EDDS and NTA in three mining sites

ANOVA	Fresh weight	Dry biomass	As	Cd	Cu	Pb	Zn
Control	*	NS	NS	NS	NS	NS	NS
EDTA 1:1	*	NS	NS	NS	NS	NS	NS
EDTA 0.5:1 + 0.5:1	*	*	NS	*	NS	*	NS
NTA 1:1	**	NS	NS	NS	NS	NS	NS
NTA 0.5:1 + 0.5:1	NS	NS	NS	NS	NS	NS	NS
EDDS 1:1	NS	NS	NS	NS	NS	NS	NS
EDDS 0.5:1 + 0.5:1	**	*	NS	NS	NS	NS	NS
Values are means ± SD (n = 3); ** = $p < 0.01$; * = $p < 0.05$, NS = not significant.							

When examining the effect of chelate treatment across the three soil specimens from the three mining sites, the results showed that fresh weight of shoot was significantly affected in controls, EDTA 1:1 and EDTA 0.5:1 + 0.5:1, at $p < 0.05$, and in NTA 1:1 and EDDS 0.5:1 + 0.5:1, at $p < 0.01$ (Table 5). Dry biomass of shoot was significantly affected by EDTA 0.5:1 + 0.5:1 and EDDS 0.5:1 + 0.5:1, at $p < 0.05$. Uptake of Cd and Pb was significantly affected by the EDTA 0.5:1 + 0.5:1 treatment, at $p < 0.05$.

Pearson's analysis was conducted to assess the correlations among fresh weight and dry biomass on the shoots of the ryegrass and the selected HMs' (As, Pb, Cu and Zn) concentration in the shoot of the ryegrass 28 days after application of EDTA, EDDS and NTA (Table 6). Cd concentration is so low (range from 0.02 to 0.3), not accurate detection, thus not signifficant for the analysis.

As expected, fresh weight of shoot of the ryegrass had a strong positive relationship with dry biomass (Pearson correlation = 0.85, $p < 0.01$), and a negative correlation with Pb (Pearson correlation = − 0.54, $p < 0.05$) and Zn (Pearson correlation = − 0.55, $p < 0.01$) concentration in the shoot. There was a strong positive correlation of As and Pb concentration with Zn concentration in the shoot since Pearson correlation = 0.71 and 0.88 at 1% significance respectively. This result indicated an association between increased uptake of As-Zn and Pb-Zn after chelate application. Combined with HM concentration and DOC results in pore water, this result provided an explanation for why fresh weight was significantly reduced with application of chelates in sequential dose (EDTA 0.5:1 + 0.5:1 and NTA 0.5:1 + 0.5:1). In the LH and HT soil samples, the ryegrass showed serious toxicity symptoms, including yellow leaves (Fig. 1).

Table 6
Pearson correlation between plant biomass and plant shoot uptake of metals

Correlations	Fresh Weight	Dry Biomass	As	Pb	Cu	Zn
Fresh_Weight	1	0.852**	−0.379	−0.535*	0.192	−0.553**
Dry_Biomass	0.852**	1	−0.428	−0.177	0.379	−0.287
As	−0.379	−0.428	1	0.367	0.139	0.709**
Pb	−0.535*	−0.177	0.367	1	0.396	0.873**
Cu	0.192	0.379	0.139	0.396	1	0.387
Zn	−0.553**	−0.287	0.709**	0.873**	0.387	1

** Correlation is significant at the 0.01 level (two-tailed). * = correlation is significant at the 0.05 level (two-tailed).

3.5. Effects of EDTA, EDDS and NTA on the uptake of HMs by ryegrass

Total metal phytoextraction by the shoots of ryegrass (*Lolium multiflorum*) is presented in Fig. 3, Fig. 4, Fig. 5, Fig. 6. The application of EDTA, EDDS and NTA to three selected soils from the three sites significantly increased the concentrations of As, Cd, Cu, Pb and Zn in the shoots of rye grass.

Compared with the control group, the addition of all three selected chelates (EDTA, NTA and EDDS) after 28 days treatment did not generally increase the concentrations of the HMs (As, Cd, Cu, Zn and Pb) in the root of ryegrass. The exceptions were NTA 1:1 for Cu in the LH soil (Fig. 3); EDDS 1:1 for Pb in the LH soil (Fig. 4); and NTA 1:1 for Pb in the HT and TC soil (Fig. 4).

EDTA 0.5:1 + 0.5:1 was more effective at increasing the concentration of Pb in shoots than were the other chelates (NTA, EDDS) and controls. On day 28 after the application of chelate treatments (EDTA 1:1 and EDTA 0.5:1 + 0.5:1), the concentrations of Pb in the shoots of the ryegrass reached 184 and 1339.38 mg/kg DW, respectively, which were 37-fold and 269.49-fold larger, respectively, than that of the controls (4.97 mg/kg DW without the application of chelates) (Fig. 4). The concentrations of Zn in the shoots of ryegrass (*Lolium multiflorum*) in LH significantly increased with the application of split dose (0.5:1 + 0.5:1) of the chelate treatments applied to the soil and was higher than those of control and the application of single dose (1:1). The application of split dose (0.5:1 + 0.5:1) of the chelate treatments was very comparable with the single dose (1:1) and the controls in LH (Fig. 6).

The selected chelates clearly affect Pb concentration in the shoots of ryegrass when compared with the controls. Particularly, EDTA 0.5:1 + 0.5:1 resulted in the highest accumulation of Pb in the ryegrass shoots. According to recent studies, Pb uptake by plant roots is greatly limited by Pb availability in soils (Ullah et al., 2015, Kushwaha et al., 2018). The availability of Pb in soils is closely connected to chemical forms. Usually, Pb may be present as a free iron or organic ligand or as a complex with inorganic

constituents (e.g. Cl-, SO_4=, CO_3^{2-} and HCO^{3-}) in soil solutions, or alternatively, Pb may be adsorbed onto particle surfaces, for example, onto organic matter, Fe-oxides and clay particles (Kushwaha et al., 2018, Tessier et al., 1979). Because of the strong binding with organic matter and colloids in soils, low bioavailability of Pb is evident for plant uptake (Kushwaha et al., 2018, Komárek et al., 2007).

Of the two selected ratios of chelates tested, split dose (0.5:1 + 0.5:1) was much better at phytoextraction than single dose (1:1), particularly for As, Pb and Zn in LH soil. As expected, the maximum phytoextraction of Zn, Pb and Cu occurred in the EDTA split dose (0.5:1 + 0.5:1) treatment, which increased 6.15-fold in Zn, 269.5-fold in Pb, and 1.49-fold in Cu compared with the control. However, the maximum phytoextraction of As was evident in the NTA split dose (0.5:1 + 0.5:1) treatment in the LH shoot, increasing 18.47-fold in As compared with the control (Fig. 5).

There was a statistically significant ($p < 0.01$ and $p < 0.05$) difference between the application of split dose (0.5:1 + 0.5:1) chelate treatments and controls; however, there was no significant difference between application of single dose (1:1) chelate treatments and controls. In addition, the application of chelate dose treatments of EDTA, EDDS and NTA in HT soils only slightly the concentrations of Zn in the ryegrass shoots over controls (significance at $p < 0.05$). Moreover, the application of chelate treatment (single dose 1:1) was more effective at increasing the concentration of Zn in shoots than when the chelate treatments (split dose 0.5:1 + 0.5:1) and controls in HT soils were applied. The total amount of Zn accumulated in the shoots and roots of ryegrass (*Lolium multiflorum*) following the application of EDTA, NTA and EDDS treatments to soil is presented in Fig. 6. A higher level of Zn phytoextraction was observed in the split dose (0.5:1 + 0.5:1) of EDTA and NTA in LH soils, in which the phytoextraction of Zn was 5.87-fold and 2.87-fold, respectively, larger than that of the control.

The application of EDTA, NTA and EDDS in single dose (1:1) of EDTA and EDDS in the TC soil had no significant effect on the total Zn concentration in the shoots of the ryegrass (*Lolium multiflorum*). However, in the application of single dose (1:1) NTA, NTA was much more effective than EDTA and EDDS at increasing the uptake of Zn by the ryegrass. Thus, the analysis demonstrates the effectiveness of chelates—particularly the split dose (0.5:1 + 0.5:1) over the single dose (1:1) treatments and the controls—in significantly increasing the concentrations of Zn in the shoots of the ryegrass in the LH soil. The LH soil had higher HM concentrations and particularly higher Pb and Zn concentrations than the other contaminated mining soils (Table 1). Further, the concentration of Zn in the shoots was higher in the plants treated with EDDS than in those treated with EDTA, except in the EDTA split dose 0.5:1 + 0.5:1 in LH soil, where higher Pb and Zn concentrations were found (Fig. 6).

4. Conclusions

Chemically enhanced phytoextraction has been suggested as a successful method for extracting HMs from contaminated soil by high biomass plants. This study presented the results of the application of EDTA, EDDS and NTA on the uptake of As, Cd, Cu, Pb and Zn by ryegrass using pot experiments.

The study found that when compared with no EDTA, EDDS or NTA application, the use of EDTA, EDDS and NTA significantly increase the uptake of some HMs by ryegrass. The study compared the effects of these chelates (EDTA, EDDS and NTA) on the phytoavailability of HMs (As, Cd, Cu, Pb, Zn) using ryegrass (*Lolium multiflorum*) employing the single addition and sequential addition methods.

The application of EDTA, EDDS and NTA to the three selected soil samples from the three mining sites in Vietnam significantly increased the concentrations of As, Cd, Cu, Pb and Zn in the shoots of the ryegrass. The EDTA split dose (0.5:1 + 0.5:1) was more effective than the other chelates (NTA and EDDS) and controls in increasing the concentration of Pb in shoots. On day 28 following the application of chelate treatments EDTA (1:1 and 0.5:1 + 0.5:1), the concentrations of Pb in the shoots of the ryegrass reached 184 and 1339.38 mg/kg DW, respectively, which were 37-fold and 269.49-fold larger than that of the control (4.97 mg/kg DW without the application of chelates). Ryegrass roots could grow in soils only after transferring the pot in all the TC soil samples and the control samples. This means that the plant could not grow in the treated soil samples when the chelate treatments were applied to the HT and LH soil samples.

The purpose of the chelating experiments was to mobilise the HMs, thereby enhancing the phytoaccumulation of mobilised HMs and their subsequent recovery/removal from soil. Ryegrass maintained growth only after transfer to the contaminated soils in all TC soil samples and control samples. The plants showed evidence of toxicity in the chelate-treated HT and LH soils because the chelate mobilised the high solution concentration of metals. When adding chelating agents before transferring the plants, the mobilised HMs including Al/Mn (which are highly phytotoxic) become toxic to plants and this results in their death (Zhang, 1993, Polák et al., 2018). Adding chelating agents while the plants were actively growing meant that the mobilised HMs were taken by the actively growing plant roots, and this resulted in the phytoaccumulation of HMs, leading to the death of the plants because of HM toxicity. Thus, in effect, dead plants were harvested and true phytoremediation meant recovery/removal of the HMs from soil.

We used the Stanford and DeMent test to investigate the nutrient bioavailability in plants. The current study shows that for toxic metals, transferring the actively growing plants to pots containing HMs that had already been mobilised using chelating agents may be the correct approach for assessing metal bioavailability. However, it must be considered that because of the, elevated levels of mobilised metals, the roots were exposed suddenly to high toxic concentrations of HMs. In essence, chelate-induced mobilisation of the HMs may kill the plants before the mobilised metals can be fully accumulated. In LH soil, concentrations of Pb in the shoots were also much higher in the plants treated with EDTA 0.5:1 + 0.5:1 than in those treated with EDTA 1:1, and with EDDS and NTA in both doses (1:1 and 0.5:1 + 0.5:1).

The paper is focused on a scientific approach to the pressing environmental issue of management of contaminated land. Specifically this paper addresses an enhanced phytoremediation approach using Ryegrass (*Lolium multiflorum*) and application of chelates (EDTA, EDDS, NTA) by single and sequential addition for remediation of metal contaminants in mine sites in Vietnam. A promising technique for

extracting HMs from soils using plants is chemically enhanced phytoextraction chelating agents have been applied in order to mobilise and enhance HM accumulation, especially Pb in soils with high toxic concentrations of HMs.

This research assessed independently the HMs (As, Cd, Cu, Pb and Zn) concentration in soils in 3 representative mining sites in Thai Nguyen province and the effects of the chelates on phytoavailability of the HMs by the two different addtion methods. This is the first public research using contaminated soils from mining sites in Vietnam about the effects of chelates (EDTA, EDDS, NTA) on phytoavailability of HMs (As, Cd, Cu, Pb, Zn) using Ryegrass (*Lolium multiflorum*) by single addition and sequential addition methods. Multivariate statistics was used to identify important factors for heavy metal accumulation in plants including associations between metals and soil properties. This studies focus on the effects of multiple chelates and dosing methods for heavy metal extraction from three soils with different properties makes a novel contribution to the field.

The different chelate treatments especially sequential addition are considered to have potential for enhancing phytoextraction of specific metals depending on soil properties of the contaminated soils. While a laboratory-based study demonstrates potential for phytoremediation of the metal contaminated mined soils, further research is needed in the field under controlled conditions. In particular, the extension of chelate-mobilised mining of metals by plants needs detailed analysis both in the laboratory and in the field. In conclusion, this research suggests more future work is required on phytoremediation of HMs, especially phytoextraction by assisted chelate moblisation at mining sites. Chelate-assisted mobilisation of HMs using the selected native plants in the mining sites, namely native plant (Lau plant (*Erianthus arundinaceus (Retz.*) and Reed plant (*Phragmites australis (Cav.)*) and exotic plants using the single or split application is quite promising for HMs accumulation as well as phytoextraction of the HMs.

Declarations

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Contributions

Dr. Nguyen Ngoc Son Hai carried out the research, data analysis, visualization and wrote the original draft. Other authors performed supervision and revisions of the manuscript as follows.

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Availability of data and materials

The datasets generated and analysed during the current study are not publicly available due the thesis in a period of university embargo but are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

We declare that we do not have human participants, human data or human tissue involved in the study.

Consent to publish

All authors consent to the publication of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

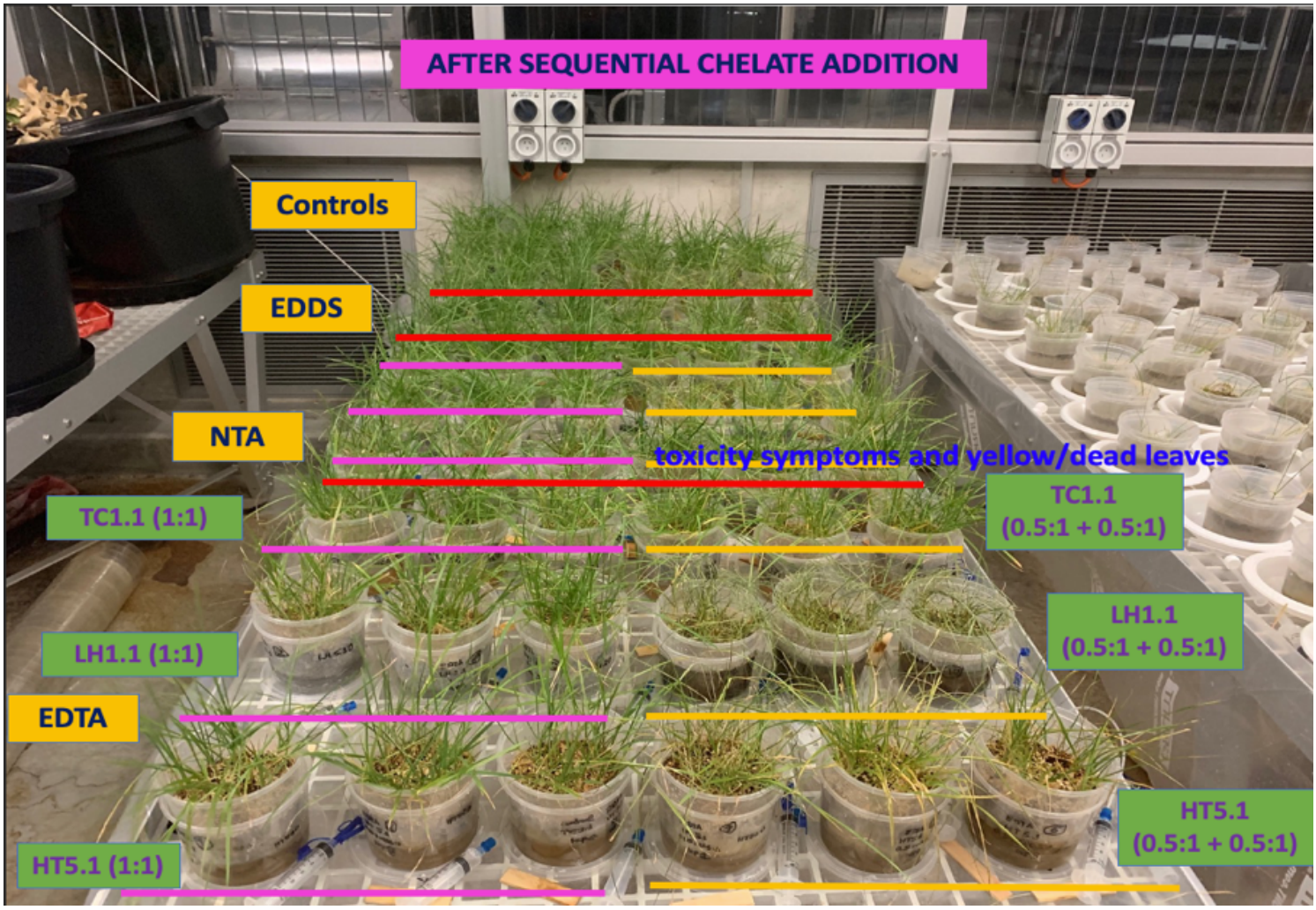

Figure 1

Plant experiments using chelates and plant toxicity symptoms

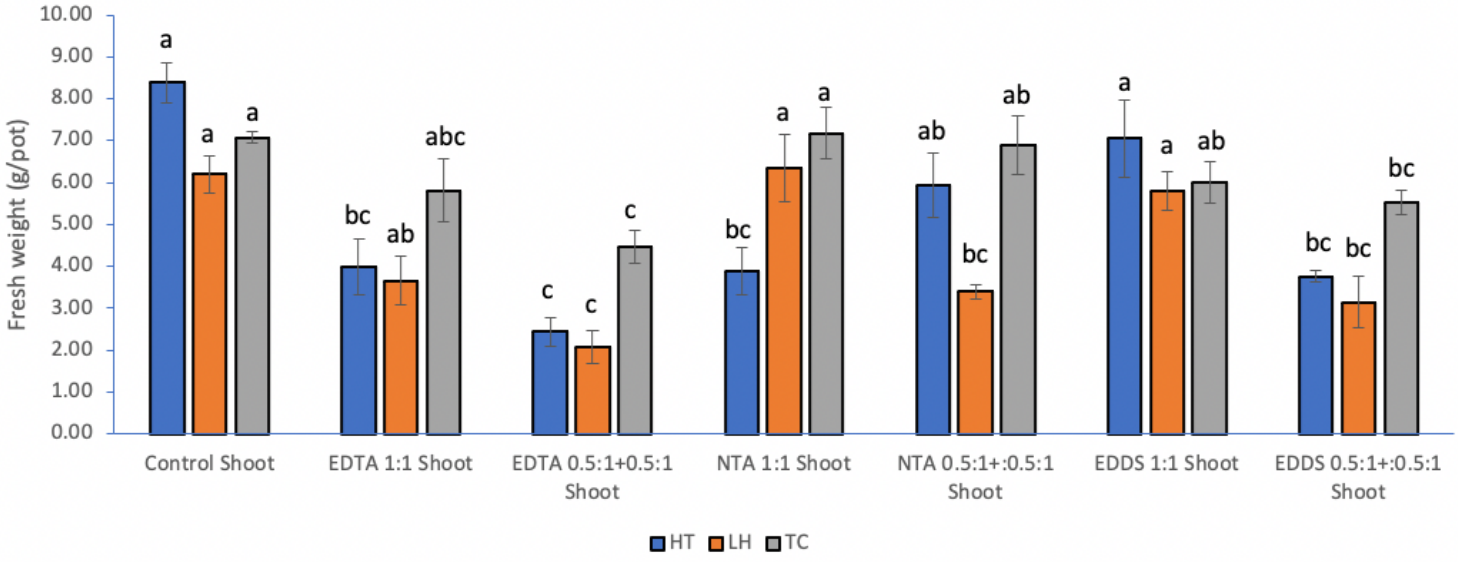

Figure 2

Effects of the application of chelates on the fresh weight of ryegrass (Lolium multiflorum) in three selected mining sites (HT, LH, TC). Lower-case letters represent significant difference between treatments

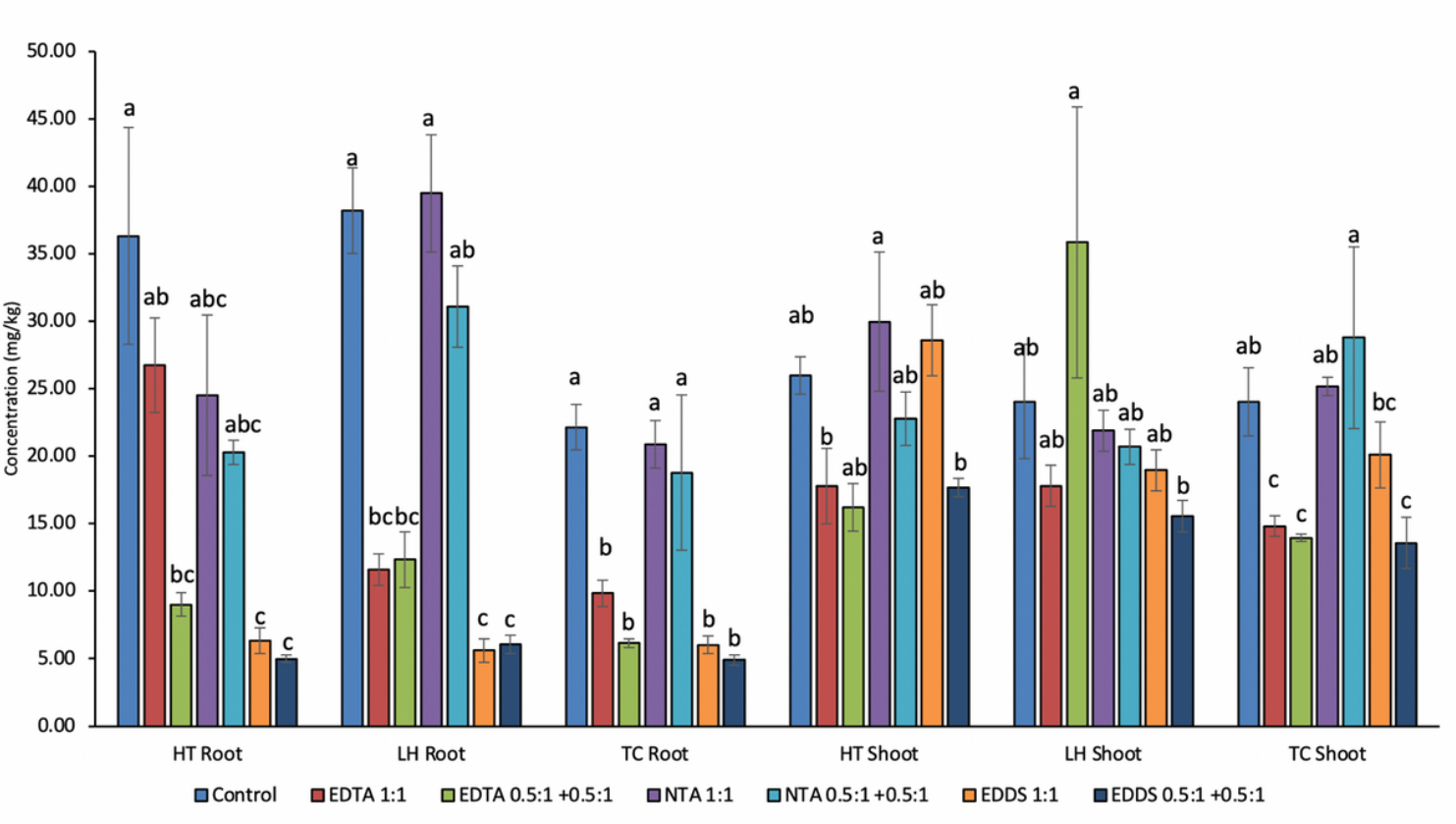

Figure 3

Effects of the application of chelates on the uptake of Cu in the roots and shoots of the ryegrass. Values are means ±SD (n = 3). Lower-case letters represent significant difference between treatments for shoots and roots at each soil sample; values are in the order a > b > c

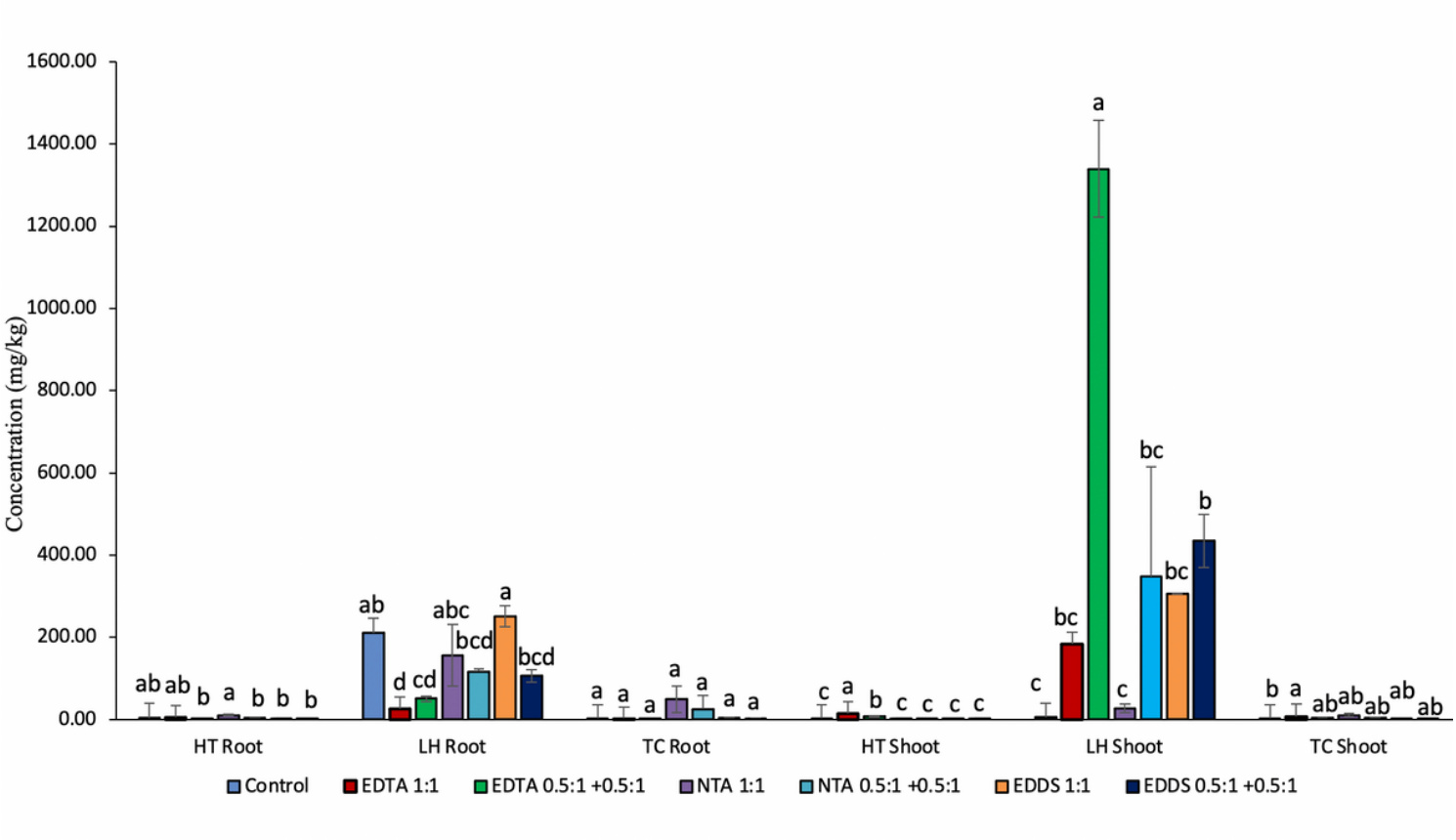

Figure 4

Effects of the application of chelates on the uptake of Pb in the roots and shoots of the ryegrass. Values are means ±SD (n = 3). Different letters indicate significant ($p < 0.05$) difference from other treatments; values are in the order a > b > c

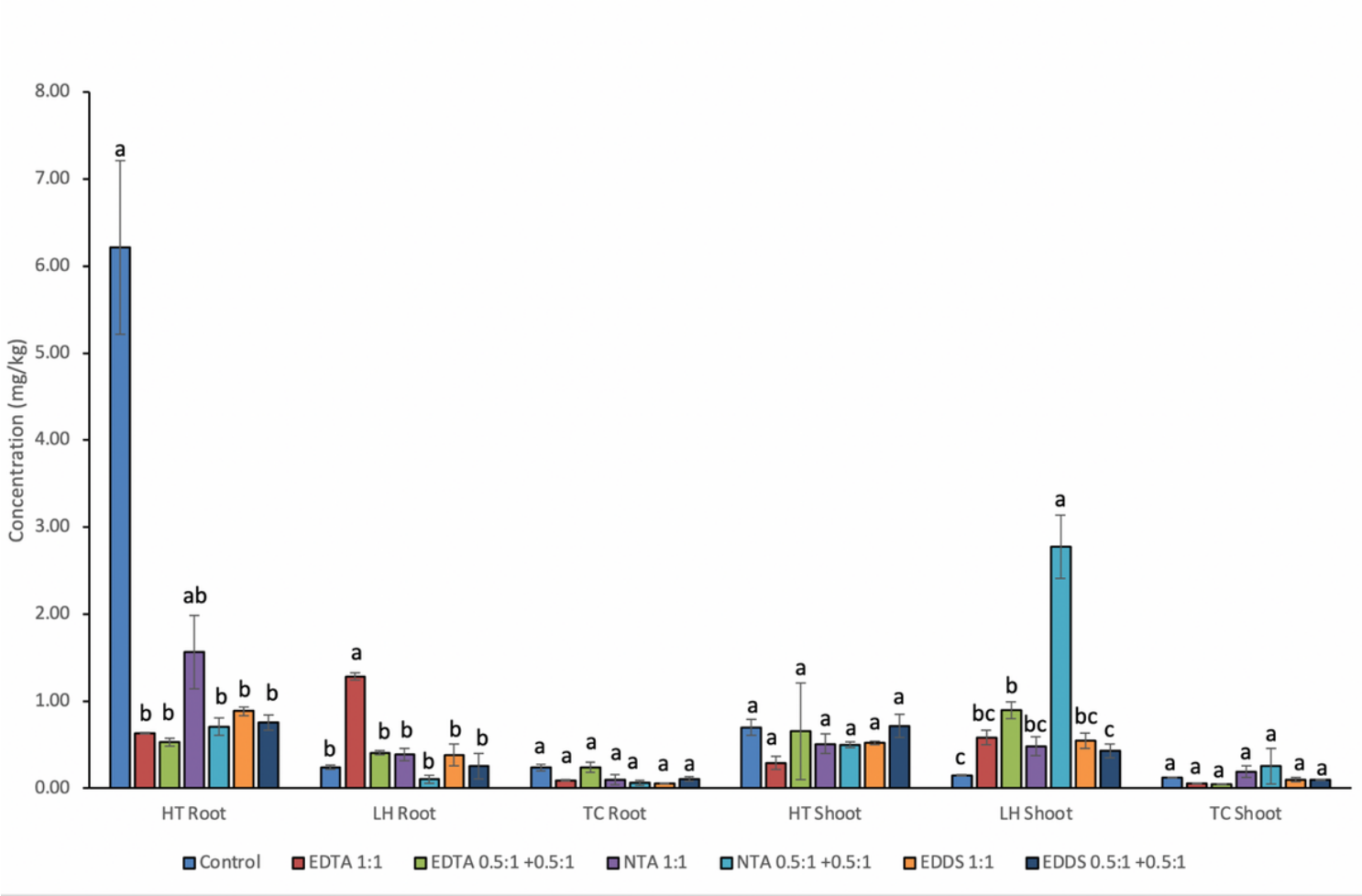

Figure 5

Effects of the application of chelates on the uptake of As in the roots and shoots of the ryegrass. Values are means ±SD (n = 3). Different letters indicate significant ($p < 0.05$) difference from other treatments; values are in the order a > b > c

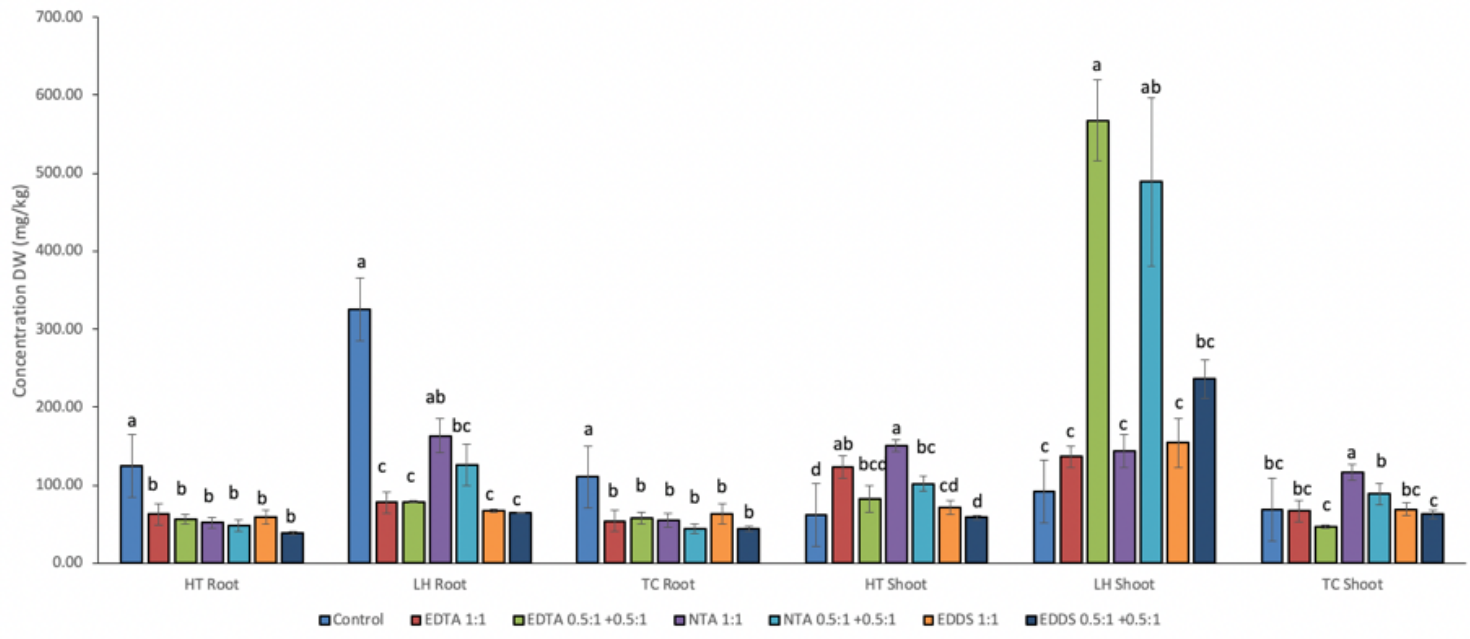

Figure 6

Effects of the application of chelates on the uptake of Zn in the roots and shoots of the ryegrass. Values are means ±SD (n = 3). Different letters indicate significant ($p < 0.05$) difference from other treatments; values are in the order a > b > c

Supplementary Files

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