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Mechanistic insight into the interactions of EDDS with copper in the rhizosphere of polluted soils

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Abstract

The interactions between S,S-ethylenediaminedisuccinic acid (EDDS) and trace metals/major elements in the soil affect the bioavailability of metals in contaminated soils and their subsequent phytoextraction efficiency. This study aimed to investigate the macroscopic and molecular-level interactions of EDDS with Cu in the rhizosphere and non-rhizosphere of a Cu-polluted agricultural soil. A multi-interlayer rhizobox planted with ryegrass was used to simulate the transport of EDDS and Cu from the non-rhizosphere to rhizosphere soils. The results showed that EDDS (5 mM kg^{-1}) significantly dissociated Cu (285–690 fold), Fe (by 3.47–60.2 fold), and Al (2.43–5.31

fold) from the soil in comparison with a control group. A combination of micro-X-ray fluorescence, X-ray absorption near-edge structure spectroscopy, and sequential extraction analysis revealed that EDDS primarily chelated Cu from the adsorbed fraction in goethite probably due to the dissolution of Fe oxides promoted by EDDS. Moreover, as facilitated by ryegrass transpiration, CuEDDS was moved from the non-rhizosphere to rhizosphere and accumulated in ryegrass. *In situ* processes of Cu extraction and transport by EDDS in the rhizosphere were further elucidated with chemical speciation analysis and geochemical modeling methods.

Keywords: Cu-contaminated soil; Rhizosphere; Soil organic matter (SOM); MINTEQ modeling; μ-XRF and μ-XANES.

Capsule

EDDS can significantly dissociated Cu from soil matrix and CuEDDS can move from the non-rhizosphere to rhizosphere and accumulated in ryegrass due to plant transpiration.

1. Introduction

Elevated concentrations of Cu have been found in soils around the world, which may be harmful to soil ecology, food safety, and human health. Among soil remediation methods, phytoextraction is a low-cost and environmentally friendly technology that utilizes plants to extract metals from soils (Kumar et al., 1995). To improve phytoextraction efficiency, various chelates have been evaluated, including biodegradable S,S-ethylenediaminedisuccinic acid (EDDS), which has been demonstrated to be effective at mobilizing Cu from a relatively stable fraction in contaminated soils (Quartacci et al., 2007; Attinti et al., 2017; Beiyuan et al., 2017), and facilitating Cu uptake as a CuEDDS complex primarily by damaging root passage cells (Niu et al., 2011; Doblas et al., 2017; Zhao et al., 2018). Furthermore, EDDS may limit potential risks of leached metals in fields due to its rapid biodegradation in soils (Epelde et al., 2008; Meers et al., 2008; Wang et al., 2012).

The efficiency of EDDS-assisted phytoextraction is critically affected by the sufficient interaction of EDDS with Cu and other components of soils, and it is controlled by EDDS concentration, solution pH, reaction duration, and physical and chemical properties of the soil (Yip et al., 2009; Yan and Lo, 2011; Fabbricino et al., 2016). EDDS can directly complex with weakly bound metals and promote the release of strongly bound metals at a relatively slow rate through EDDS-promoted soil dissolution (Zhang et al., 2010), effectively increasing the phytoextraction efficiency of trace metals. However, the extracted major cations (*e.g.,* Fe, Ca, Al, and Mn) from soil by EDDS may compete with trace metals for complexation with EDDS and have been shown to lower the extraction efficiency of target metals in several soil washing tests (Komárek et al., 2009; Tsang et al., 2009; Yang et al., 2012). In addition, for field-contaminated soils, soil aging favors strong sequestration of metals to soil components and lowers the extraction efficiency of EDDS (Tsang et al., 2007). However, the exact interactions of EDDS with targeted metals and soil major components using field-contaminated soils during the phytoextraction process remain large unknown, especially in the plant rhizosphere zone.

Plant growth may influence the interactions of EDDS with metals in rhizosphere soils, but few studies have focused on this aspect. Plant transpiration is known to create a water potential gradient in soils near roots and continuously drives the water flow from bulk soils to root surfaces (Jackson et al., 2000; Carminati, 2012). This water flow may bring soluble EDDS from far- to near-root zones and may subsequently enhance the convective transport of metals, which has been ascribed to the formation of metal-EDDS complexes, as well as the increased metal solubility in soils. To test this hypothesis, we employed a multi-interlayer rhizobox, which has been used to successfully traced the biochemical changes in rhizosphere soils (Tao et al., 2003; Kim et al., 2010), to investigate the role of plants in the mobilization and transport behavior of Cu in EDDS-assisted phytoextraction of a real contaminated soil.

The current study aimed to: (1) characterize the effects of EDDS on Cu extraction, speciation, and transport in rhizosphere soils, particularly under the influence of plant growth; and (2) improve understanding of the Cu extraction mechanisms by EDDS from field-contaminated soils. Advanced synchrotron-based spectroscopy, such as micro X-ray fluorescence (μ-XRF) and X-ray absorption near-edge structure (XANES), was employed to elucidate the *in situ* distribution and speciation of Cu in a soil matrix with or without the use of EDDS.

2. Materials and methods

2.1 Soil collection

Soil sample from a depth of 0–20 cm was collected from the farmland in the vicinity of an abandoned Cu mine (32°03’N, 118°47’W) in the town of Tangshan in Nanjing, Jiangsu, China. Copper pollution in this area was derived from dust and wastewater irrigation from mining operations (Qin et al., 2012) and posed a high health risk of Cu transfer to human beings through the food chain (Wu et al., 2011). Soils were air-dried, mixed thoroughly, and sieved through a 2 mm plastic mesh for further use. Basic physico-chemical characteristics of the soils (Table S1) were determined using standard analytical methods (Li et al., 2001; Cater and Gregorich, 2006).

2.2 Experiment set-up

A multi-interlayer rhizobox (Fig. S1) was made with slight modifications based on Wang et al. (2002). The dimensions of the rhizobox were 80 mm × 130 mm × 130 mm (length × width × height). Six compartments were divided in the rhizobox by nylon mesh (< 25 μm) at a defined distance. The leftmost compartment grown with ryegrass was marked as the rhizosphere, and adjacent compartments next to the rhizosphere were named according to the distance from this rhizosphere, such as non-rhizosphere (0–1 cm). The rhizobox restricted plant roots in the rhizosphere compartment by the nylon mesh while allowing soil pore water, root exudates, and soil microfauna to transfer through each compartment (Xie et al., 2012). Small drain holes (1 mm diameter) were made in the bottom of the rhizobox. Each rhizobox was packed with a total weight of 960 g of soil.

Ryegrass seeds were sterilized with 95% ethanol for 15 min, washed thoroughly with deionized water, and then germinated on moist filter paper for 7 d (Zhao et al., 2018). About 80 seedlings were transferred to each rhizobox, and the soil humidity was kept at 60% maximum water holding capacity. Deionized water was added to the rhizobox every day to maintain soil humidity. Rhizoboxes were placed in a climate growth chamber at 60% humidity, 25 °C /20 °C day/night, with a 16-h photoperiod per day (325 μmol photons m^{-2} s^{-1}). Each rhizobox was wrapped with aluminum foil to prevent the growth of algae. After three weeks of ryegrass growth, a total of 40 mL of 120 mM EDDS was evenly added to the top surface of the compartments in the rhizobox, which equaled the dosage of 5 mM EDDS kg^{-1} soil that is frequently used in EDDS-assisted phytoextraction (Luo et al., 2005; Duquène et al., 2009; Almaroai et al., 2013). One set of rhizoboxes was un-planted and received EDDS at the same dosage as a control. One set of rhizoboxes was planted without EDDS as a control. Three replicates were employed for each treatment. Following the use of EDDS for 7 d, the ryegrass was harvested, separated into roots and shoots, and washed with deionized water. The soil from each compartment was collected, homogenized, and stored at 4 °C or -20 °C until further analysis.

2.3 Analytical methods

2.3.1 Soil solution extraction and analysis

Chemical analysis was carried out on soil extracts to mimic the conditions in a soil solution. Fresh soil (2.5 g) was extracted with deionized water in a 1:10 (w: v) ratio on a dry weight soil basis using an end-over-end shaker at 50 rpm for 2 h (Séguin et al., 2004). The suspensions were then centrifuged for 10 min at 8000 rpm, and filtered through 0.45-μm cellulosic membranes. Chemical analysis included determining pH, electrical conductivity (EC), anions content (Cl^-, F^-, NO_2^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}) by an ion chromatography, and total dissolved organic carbon content (DOC) by a Shimadzu TOC analyzer. Copper, Zn, Pb, Al, Ca, Fe, Mg, Mn, K, and Na were analyzed with ICP-AES (Agilent 700 series) and ICP-MS (Agilent 7700 series) after digestion of soil extracts with concentrated HNO_3 (Cui et al., 2017). Ionic strength (IS) of the soil extracts was calculated from EC with the empirical relationship IS ＝ 0.13 EC (Vulkan et al., 2000). EDDS derivation and analysis in soil extracts were performed based on Katata et al. (2006). Generally, EDDS was converted into FeEDDS, separated using a reversed-phase Inertsil ODS-3 C18 column (5 μm 4.6 * 259 mm), and detected at 254 nm using a Waters HPLC 2487. The mobile phase consisted of 10% methanol and 90% tetrabutylammoniumbromide (0.02 M) with the pH adjusted to 4.0 using formic acid. The flow rate was set at 1 mL min^{-1}. To exclude the exogenous DOC derived from EDDS, the "natural" DOC was named and calculated from the difference between total DOC and EDDS-contributed DOC (Koopmans et al., 2008).

2.3.2 Modeling of metal and EDDS speciation

The software Visual MINTEQ version 3.1 was used to calculate the EDDS and metal speciation in soil extracts from different compartments of the rhizobox. Input data included the concentration of EDDS, dissolved metals (e.g., Cu, Zn, Pb, Ni, Fe, Mn, Ca, Mg and Al), anions (e.g., Cl^{-}, F^{-}, NO_2^{-}, NO_3^{-}, SO_4^{2-} and PO_4^{3-}), and dissolved organic matter (DOM). Parameters, such as solution pH and ionic strength, were also considered. The stability constants of most metal-EDDS complexes metals were obtained from Tandy et al. (2006), and AlEDDS was acquired using data from Koopmans et al. (2008). The DOM concentration was set at twice of the concentration of "natural" DOC, and the composition of DOM was assumed to be 50% fluvic acid, and 50% humic acid (Yip et al., 2009a). The binding of metals to DOM were modeled using the NICA-Donnan model with generic parameters (Milne et al., 2003). Iron and aluminum were allowed to precipitate when exceeding the solubility of $Fe(OH)_3$ (log Ksp = 2.69, 25°C) and $Al(OH)_3$ (log Ksp = 8.29, 25°C), respectively (Sjöstedt et al., 2010).

2.3.3 Metal fractionation in soil by sequential extraction

The speciation of trace metals in soil was determined by a classic sequential extraction procedure (SEP) (Li et al., 1995). The concentration of trace metals (Cu, Zn, and Pb) in solution was determined using either ICP-AES (Agilent 700 series) or ICP-

MS (Agilent 7700 series). According to this sequential extraction procedure, five operation-defined chemical forms of trace metals were obtained including exchangeable, carbonate bound/specifically adsorbed, iron and manganese oxide bound, organic matter bound, and residual metals.

2.3.4 Metal distribution and speciation in soil by μ-XRF, μ-XANES and bulk-XANES

μ-XRF and μ-XANES experiments were conducted in ambient conditions at beamline 15U at the Shanghai Synchrotron Radiation Facility (SSRF), China. Freeze-dried soil was grounded to less than 50 μm and dispersed on tape before microprobe analysis. A selected sample area (1500 × 1500 μm) was scanned with a step size of 50 μm. The fluorescence signals of Cu, Fe, Si, Al, Mn, Ca, and K were selectively acquired with a dwell time of 1.5 s using a one-element Si drift detector. Elemental images from the XRF data were produced using Igor Pro 6.0 software (IGOR). Two hot spots of Cu identified from the XRF image were selected for collecting Cu K-edge μ-XANES spectra (Cui et al., 2019).

Acquisition of Cu K-edge bulk-XANES data for soil and standards was done on beamline 01C1 at the National Synchrotron Radiation Research Center (NSRRC), Taiwan. CuO, CuS, CuCl, $CuSO_4$, $CuCO_3$, $Cu_3(PO_4)_2$, $Cu(OH)_2$ and $Cu(CH_3COO)_2$ standards were obtained from Sigma-Aldrich and measured in a transmission mode (Fig.S7). Cu-goethite, Cu-ferrihydrite, Cu-humic acid, and Cu-clay (montmorillonite) were prepared according to Strawn and Baker (2008), and CuEDDS was prepared according to Shi et al. (2008). These standards and soil samples were measured in a fluorescence mode (Fig.S7). In addition, Fe K-edge bulk-XANES spectra of soils and standards were collected at beamline 16A1 at the NSRRC. The XANES sample preparation and data processing were described in our previous studies (Cui et al., 2017;

Cui et al., 2018). More details on data acquisition and linear combination fitting (LCF) process can be found in the supplementary information.

3. Results and discussion

3.1 Effects of plants on EDDS distribution in rhizobox

The distribution of EDDS in soil extracts from the rhizobox is shown in Fig. 1. The concentration of EDDS showed no difference ($p < 0.05$) in soil extracts from different compartments of the unplanted rhizobox (Fig. 1). Compared to the initial application dosage (4.8 mmol), 15% of the EDDS (0.74 mmol) was lost in soil extracts from the rhizobox as calculated in Fig. 1. The loss of EDDS should not have been the result of the adsorption of EDDS on the surface of the contaminated soil, since the adsorption is negligible at pH of 8 (Fig. S2) (Koopmans et al., 2008; Yip et al., 2009b). Instead, the decrease may have been due to EDDS degradation after application to soils for 7 d. Although it has been reported that an initial lag phase ranging from 7 to 32 d is required before EDDS degradation, the length of the lag phase can vary with soil type and metal concentration (Tandy et al., 2006a; Meers et al., 2008). EDDS is a nitrogen containing compound, and soil microbes can decompose EDDS into a potential nitrogen source as a fertilizer (Vandevivere et al., 2001; Fang et al., 2017). Our results showed that NH_4^+ and NO_3^- were higher in soils with EDDS than those without EDDS (Table S2), which provided solid evidence for the degradation of EDDS.

The total amount of EDDS (3.92 ± 0.10 mmol) in soil extracts in the planted rhizobox was generally consistent with those from the unplanted rhizobox (4.06 ± 0.12 mmol), as summarized in Fig. 1 ($p < 0.05$), suggesting that the degradation rate of EDDS was not affected by ryegrass growth. However, the distribution of EDDS in rhizobox compartments was greatly altered by planted ryegrass (Fig. 1). The concentration of EDDS increased substantially in the rhizosphere and decreased in the non-rhizosphere after planting ryegrass. In addition, the increase of EDDS in the rhizosphere (1.43 ± 0.13 mmol) generally corresponded to the loss in the non-rhizosphere (1.30 ± 0.10 mmol) ($p < 0.05$). These results indicated that EDDS was transported from the non-rhizosphere to the rhizosphere of ryegrass, probably driven by the continuous plant-transpiration-induced water flow towards the root surface. Similarly, in light of a previous study on soil nutrients, soluble nutrients (*e.g.,* Ca, Mg, and NO_3^-) were also observed to move to the rhizosphere through the transpiration stream induced by plants (Moritsuka et al., 2000). Although ryegrass can absorb some EDDS through the plant transpiration stream, the uptake amount by plants is usually marginal (accounting for about 0.3% of total EDDS in soils, Tandy et al., 2006b). Therefore, EDDS gradually accumulated in the rhizosphere compartment.

The interaction of EDDS with soil components is known to be a kinetic process. In light of previous works regarding soil washing, most of trace metals can be extracted by EDDS within 24 h, and this process has been shown to reach equilibrium after 48−72 h (Yip et al., 2009; Yang et al., 2012). However, the kinetic results from EDDS-assisted phytoextraction should be different from EDDS-assisted soil washing in a well-agitated condition. In phytoextraction, EDDS requires more time to penetrate into soil particles or aggregates to extract metals. Therefore, in our study, it was expected that the plant-transpiration-induced water flow towards roots should contain EDDS in both free and complexed forms. Furthermore, the transport of uncomplexed EDDS to the rhizosphere may intensify the chemical interactions of EDDS with metals in rhizosphere soils (see discussion below).

3.2 Copper extraction and distribution in a rhizobox

Copper was negligible in soil extracts of the planted rhizobox without EDDS (Fig. 2a), and there was no great difference in soluble Cu between the rhizosphere and non-rhizosphere ($p < 0.05$). This result suggested that during the short period of cultivation (21 d), ryegrass could not mobilize Cu significantly from soils naturally via root exudates. After EDDS application, the concentration of Cu in the rhizobox increased remarkably by 285–690 fold (Fig. 2a). The concentrations of Zn and Pb also increased in soil extracts after the use of EDDS, but to a significantly lesser extent (5.97–70.0 fold for Zn and 0.79–66.1 fold for Pb, respectively) in comparison with Cu (Fig. S3). The greater solublization of Cu relative to Zn and Pb by EDDS was also reported elsewhere (Quartacci et al., 2007; Koopmans et al., 2008) due to the higher affinity of Cu for EDDS (Log K CuEDDS = 20.46, log K ZnEDDS = 15.34 and log K PbEDDS = 14.46) (Orama et al., 2002; Tandy et al., 2006a), and the lower total concentration of Zn and Pb in the tested soil (Table S1) (Yan et al., 2010). Despite this, Cu concentration in soil extracts from the rhizosphere was higher than the non-rhizosphere by 2.07–3.49 fold.

SEP results showed that the distribution of Cu in original soils followed the sequence of Fe/Mn oxides fraction (37.8%) > residual fraction (35.9%) > organic matter fraction (21.5%) > carbonate fraction/specifically adsorbed (4.8%) > exchangeable fraction (0.08%) (Fig. 2b). This result was similar to a previous investigation from the same sampling site (Wu et al., 2011). The growth of ryegrass alone did not obviously affect the fractionation of Cu in the rhizobox over a short period, since ryegrass only slightly modified the Cu fractionation in e-waste soils over almost 3 years (Cui et al., 2017).

After the application of EDDS to the planted rhizobox, the Cu fractionation in soil was greatly changed (Fig. 2c), while no significant influence from EDDS was observed on Zn and Pb fractionation (Fig. S3). Generally, EDDS reduced Cu from the carbonate/specifically adsorbed (F2), Fe/Mn oxides bound (F3), and organic matter bound fractions (F4), and accordingly increased the exchangeable Cu fraction (F1). As affected by plant growth, the change of Cu fractionations showed a large difference in the rhizosphere and non-rhizosphere after EDDS application to the rhizobox for 7 d. On the one hand, the quantity of EDDS-reduced Cu from F2, F3, and F4 increased with decreasing distance from the rhizosphere. This result was due to the transport of uncomplexed EDDS from the non-rhizosphere to the rhizosphere as mentioned above, because metal extraction efficiency usually increases with an increasing concentration of free chelant (Yan et al., 2010). On the other hand, considering the mass balance of Cu in each compartment, the quantity of EDDS-reduced Cu from F2, F3, and F4 should have equaled to the increase of F1 in soils. In contrast, the reduction of F2, F3, and F4 was higher than the increase of F1 in the non-rhizosphere but lower in the rhizosphere. The result was caused by the convective transport of exchangeable Cu (F1) from the non-rhizosphere to the rhizosphere via plant transpiration stream. Our mass balance calculations revealed that 33 mg of exchangeable Cu was transported convectively from the non-rhizosphere to the rhizosphere, 0.58 mg of exchangeable Cu was absorbed by ryegrass from the rhizosphere, and 3.42 mg of exchangeable Cu leached away from the bottom of the rhizobox.

Collectively, plant transpiration worked as a driving force for EDDS transport from the non-rhizosphere to the rhizosphere, which subsequently increased the extraction and transport of Cu in the rhizosphere. However, the amount of Cu accumulated by ryegrass only accounted for 0.5% of the total soluble Cu in the rhizosphere, suggesting that the limiting step of Cu phytoextraction was root absorption.

The absorption of solubilized Cu can be controlled by the strength of plant transpiration, since plants mainly uptake CuEDDS via the apoplastic pathway (Niu et al., 2011).

3.3 Mineral and organic matter dissolution

Chelants not only extract heavy metals from soils, but also promote the dissolution of minerals (*e.g.,* Fe, Mn, Ca, and Al oxides) through the destabilization of metal-oxygen bonds (Nowack et al., 2002). The dissolved cations from minerals often bind with a chelant, and thus reduce the extraction efficiency of trace metals (Tandy et al., 2004; Manouchehri et al., 2006; Komárek et al., 2009; Yip et al., 2009). Our results showed that EDDS obviously increased the dissolution of Fe (3.47−60.2 fold) and Al (2.43−5.31 fold) from the soil in comparison with a control without EDDS (Fig. 3a and b). The potential competition with Cu from dissolved Fe and Al for complexation with EDDS is assessed in the subsequent speciation simulation section below.

The “natural” DOC was calculated from the difference between the total DOC and the EDDS-contributed DOC in a soil solution. An increase of “natural” DOC (2.00–4.44 fold) was observed in the EDDS treated rhizobox (Fig. 3d). EDDS has been reported to mobilize soil organic matter (SOM) in soil solution in different soils (Hauser et al., 2005; Koopmans et al., 2008; Yang et al., 2012). As soil organic matters often adsorb on Fe/Al oxides, EDDS may cause the dissolution of organic matters at the same time with Fe/Al oxides (Tsang et al., 2007).

Similar to the Cu distribution in soil extracts (Fig. 2a), the concentration of mineral cations (except Al) and “natural” DOC in the rhizosphere soil extract was higher than the non-rhizosphere extract by a factor of 3.03 (Fe), 1.13 (Ca), and 2.34 (“natural” DOC), respectively (Fig. 3). These results can be associated with plant-transpiration-induced water flow. On the one hand, the transport of extra EDDS towards roots may should have intensified the dissolution of rhizosphere soils and increased the dissolution of Fe and organic matters (Yan et al., 2010). Moreover, the high concentration of EDDS should have enhanced the dissolution of Ca in the rhizosphere, while the low concentration of EDDS should have even decreased soluble Ca in non-rhizosphere soils (Fig. 3c). This was supported by Luo et al. (2005), which found that chelants at different dosages exhibited various effects on soluble Ca in soils. On the other hand, the plant transpiration stream should have also brought dissolved Fe and natural “DOC” from the non-rhizosphere soil towards the rhizosphere. However, the extraction of minerals from soil is not only controlled by the available quantity of EDDS, but also by the availability of mineral cations. Aluminum in this soil was mostly present as aluminosilicates forms, which were involved in the lattices of AlO_4 tetrahedrons and difficult to extract using EDDS. Therefore, EDDS-solubilized Al was low and showed no difference between the rhizosphere and the non-rhizosphere soils (Fig. 3b).

3.4 Modelling of EDDS and metal speciation in soil extracts

The speciation of EDDS and metals in a soil solution may reveal the potential process regarding metal competition in solution (Yip et al., 2009a). In our study, MINTEQ modeling showed a high affinity of EDDS for Cu, although the species of EDDS and Cu varied slightly with the relative distance from the rhizosphere (Fig. 4). In the rhizosphere and near-rhizosphere soils (0−1 cm) (EDDS/Cu >1), EDDS was primarily present as CuEDDS (85.8%–93.7%), with a minor proportion being ZnEDDS (5.3%–5.8%), free EDDS (0%–4.0%), and other metal-EDDS complexes (*e.g.*, CaEDDS and FeEDDS, 0%–4.4%). Copper was completely chelated with EDDS in the rhizosphere and near-rhizosphere (0−1 cm of the non-rhizosphere) compartments. In the non-rhizosphere compartment (1–6 cm, EDDS/Cu < 1), EDDS was almost entirely complexed with Cu. For Cu, a portion of Cu (6%-16%) was associated with DOM in addition to CuEDDS, consistent with a previous study at low concentrations of EDDS (Tandy et al., 2006a).

The low percentage of EDDS complexed with major cations (*e.g.*, FeEDDS, CaEDDS and AlEDDS) (Fig. 4) indicated that the competition from major cations for EDDS posed little influence on Cu extraction in this study. This was because Cu-EDDS is a strong complex with a large ionic potential and a compact quinquedentate structure, which is difficult to dissociate or exchange via other metals (Tsang et al., 2009). Moreover, the solubilized major elements, such as Fe or Al are prone to hydrolyzing in soil extracts at pH 8 (Yip et al., 2009; Yang et al., 2012). Our results showed that Fe was primarily present as Fe-DOM and $Fe(OH)_3$ colloidal (Fig. S4). In addition, Ca was mostly present as free ions or in metal complex with DOM, and Al was mostly present as $Al(OH)_3$ colloidal in all compartments (Fig. S4).

3.5 Copper distribution and speciation in soils by μ-XRF, μ-XANES, and bulk-XANES

The distribution and speciation of metals in a soil matrix plays a vital role in the efficacy of EDDS for metal extraction. As mentioned in previous studies, weakly-bound metals (*e.g.,* exchangeable and carbonate fractions) are the first to be released, followed by strongly-bound metals (*e.g.*, Fe/Mn oxides and organic matter bound fractions), and residual (silicate bound) metals are not extractable by chelants (Guo et al., 2010; Fabbricino et al., 2016). Although SEP has been widely used in the soil analysis of metal fractionation, the term metal fractionation is operationally defined by chemical extraction, and the accuracy is impacted by non-specific dissolution by extraction agents, incomplete dissolution of the target phase, and resorption of solubilized metals

(Calmano et al., 2001; Kirpichtchikova et al., 2006). In contrast, the non-destructive synchrotron based techniques such as μ-XRF, μ-XANES, and bulk-XANES can provide more reliable information on the distribution and speciation of metals due to their high sensitivity and selectivity for target elements (Majumdar et al., 2012; Cui et al., 2018) .

3.5.1 Copper distribution in soils by μ-XRF

μ-XRF analysis of rhizosphere soil with or without EDDS (Fig. 5A and B) showed the distribution pattern of Cu and other elements, which helped to identify the hosting phases of Cu in soils. The results showed that Cu was heterogeneously distributed in the soil matrix with several hotspots in the scanned area.

In the rhizosphere soil without EDDS, the position of the Cu hotspot overlapped with the Fe hotspot, and correlation analysis revealed that the intensity of Cu and Fe was highly correlated (R = 0.795, $p < 0.01$; Fig. 5A). Iron K-edge XANES demonstrated that Fe was present as crystalline goethite (67%) and amorphous ferrihydrite (33%) (Fig. S8 and Table S5), and these Fe oxyhydroxides have been known to be important sink for Cu in soil (Peacock and Sherman, 2004; Yang et al., 2014).

Copper also showed a moderate correlation with Si (R = 0.423, $p < 0.01$) and Al (R = 0.355, $p < 0.01$), although the X-ray fluorescence intensities of Si and Al were low (Fig. 5A). Silicon and Al were mainly derived from soil aluminosilicates including quartz, feldspars, and clay minerals. Actually, Fe oxides often precipitate on the surface of clay minerals (Goldberg, 1989), which was supported by our SEM-EDX analysis on the contaminated soil (Fig. S6). Therefore, Cu adsorbed on iron hydroxides can be also spatially correlated with Si and Al in clay minerals. Moreover, clay minerals are capable of directly adsorbing Cu (Martínez-Villegas and Martínez, 2008). Nevertheless, the correlation coefficients of Cu with Si and Al were much lower than the correlation coefficient with Fe due to the presence of phases containing Si and Al with little association to Cu, such as quartz and feldspars. In spite of this, the Cu hotspot was separated from the K, Ca, and Mn hotspots (Fig. S5A), indicating that little Cu was associated with K-feldspars, carbonates, and Mn oxides in the contaminated soil (Yang et al., 2014).

The application of EDDS to the rhizobox generally did not affect the associations between Cu and other elements in the rhizosphere soils (Fig. 5B and Fig. S5B). Correlation coefficients of Cu with Fe, Si, and Al were lower compared to those without EDDS treatment, which may have been either the result of the mobilization of Cu by EDDS from hotspots or the heterogeneity of soil samples (Kirpichtchikova et al., 2006).

3.6 Copper speciation in soils by μ-XANES, and bulk-XANES

Although the extraction process of Cu from soils by EDDS could be inferred from our SEP results, only limited information on the exact molecular transformation of Cu can be provided by this operationally defined method. The XANES technique was thus employed to elucidate the exact species of Cu in soils, via characterizing the valance and bonding environment of Cu. In the original planted rhizobox without EDDS treatment, the LCF analysis of Cu K-edge μ-XANES and bulk-XANES showed that Cu was primarily adsorbed on clay (49%–65%), followed by goethite (35%–51%) in both the rhizosphere and non-rhizosphere soils (Fig. 6 and Table 1). The results were consistent with the correlation analysis of Cu to Fe, Si, and Al by XRF (Fig. 5). Clays can capture Cu on their surface in interlayers or mineral lattices during the weathering or pedogenesis process (Tenginkai et al., 1991; Minkina et al., 2016). Moreover, goethite is effective soil component for binding with Cu, and form an innersphere complex (Grossl et al., 1994). The importance of SOM on Cu complexation has often been revealed in organic soils (Jacobson et al., 2007; Strawn and Baker, 2008; Strawn and Baker, 2009; Xu et al., 2018). However, Cu standards representing Cu associated with SOM (*e.g.*, Cu-humic acid and Cu acetate) (Fig. S7) contributed little to Cu species in this soil, which was primarily due to the low content of soil organic matters from mining sites (Table S1) (Yang et al., 2014). In addition, Cu standards representing readily labile fractions for extraction (*e.g.*, $CuSO_4$, $Cu(OH)_2$, and $CuCO_3$) were absent in this soil, which was in agreement with the low percentage (4.8%) of Cu in the exchangeable and carbonate/specifically adsorbed fractions by SEP (Cui et al., 2017).

In the EDDS-treated rhizobox, μ-XANES and bulk-XANES of the rhizosphere soil indicated the formation of CuEDDS (22%–38%), the decrease of Cu-goethite, and the unchanged percentage of Cu-clay species in the rhizosphere soil in comparison with the original soil (Fig. 6 and Table 1). However, the bulk-XANES of non-rhizosphere soil showed no significant change after EDDS addition (Fig. 6 and Table 1). Thus, it can be inferred that the transport of CuEDDS from the non-rhizosphere to the rhizosphere occurred.

The Cu speciation analysis of the EDDS-treated rhizobox indicated that EDDS-extracted Cu from rhizosphere soils was primarily from Cu-goethite. Similarly, SEP analysis also showed that the reduction of Cu associated with Fe/Mn oxides accounted for 73% of the EDDS-mobilized Cu from rhizosphere soils (Fig. 2). There were two mechanisms potentially responsible for Cu extraction by EDDS indicated by these results. First, EDDS could be directly complexed with readily available Cu from soils according to thermodynamic favorability (Yip et al., 2009b). Alternatively, strongly-bound metals tend to be released during the disintegration of soil structures caused by

EDDS-promoted dissolution of soil minerals and organic matters (Tsang et al., 2007; Zhang et al., 2010). In this field-contaminated soil with little readily available Cu, the second pathway should have been more important for the mobilization of Cu that was strongly bound to Fe oxides and clay minerals (Fig. 6 and Table 1). In view of the limited dissolution of clay minerals, as supported by small amount of soluble Al in soil extracts (Fig. 3b), EDDS was difficult to extract the proportion of Cu adsorbed on clays. Instead, EDDS was expected to mobilize the proportion of Cu bound with goethite, as the EDDS-promoted dissolution of Fe was substantial (Fig. 3a). On the one hand, this dissolution may disintegrate the structure of goethite (Yip et al., 2010), resulting in the exposure of internal Cu to solution EDDS for direct complexation. Similarly, the surfactant TX100 was found to assist the dispersion of clayed sediments, thus exposing Zn for EDTA extraction (Yuan et al., 2010). On the other hand, the FeEDDS formed during Fe dissolution was able to further extract Cu from the soil surface through metal exchange, forming CuEDDS. The Fe displacement from FeEDDS by Cu was corroborated by the previous observation of substantial formed CuEDDS after the application of FeEDDS to soils for 1 d (Ylivainio, 2010). The displaced Fe may be resorbed to soils, or form Fe-DOM and $Fe(OH)_3$ colloids in alkaline soil solutions (Fig. S4c). Therefore, our results suggested that EDDS-promoted the dissolution of Fe oxides in this soil, facilitating the extraction of Cu from soil.

3.7 Implications for chelant-assisted phytoextraction

In chelant-assisted phytoextraction, EDDS can form soluble complex with the strongly-bound metals in soils and facilitate the transport of Cu from the non-rhizosphere to the rhizosphere. This mechanism was revealed in our study with new evidence from the combined *in situ* approaches of μ-XRF and XANES. Our results showed that the phytoextraction efficiency was limited by the absorption capacity for CuEDDS of plant roots from the rhizosphere soil solution. In light of this, further improvement methods, such as using plant growth regulators (*e.g.*, plant hormones) or plant growth promoting bacteria (PGPB), can be employed to assist plant growth, enhance the plant transpiration rate, and thus, increasing the plant absorption of CuEDDS via plant-transpiration-induced water flow. On the other hand, the combined approaches of μ-XRF and XANES can be applied to the analysis of interactions between other natural or synthetic chelants and metals in phytoextraction, and these results will be helpful for identifying the controlling factors for the metal phytoextraction process.

4. Conclusion

This study investigated the major interactions and key mechanisms of EDDS with Cu-polluted soils in the rhizosphere of ryegrass using a multi-interlayer rhizobox. After an even surface application of EDDS to a planted rhizobox, EDDS was found to have been transported from the non-rhizosphere soil to the rhizosphere after 7 d, probably via plant transpiration-induced water flow. Correspondingly, the transported EDDS increased the solubilization of soil Cu in the rhizosphere compared to the non-rhizosphere and facilitated Cu transport from the non-rhizosphere to the rhizosphere. EDDS primarily extracted Cu that was adsorbed on goethite from rhizosphere soils, probably due to EDDS-promoted dissolution of iron oxides. Then, the solubilized Cu primarily formed a CuEDDS complex with EDDS in the rhizosphere and non-rhizosphere soil extracts, and mineral cations like Fe, Al, and Ca did not compete with Cu for complexation with EDDS. The understanding of the interactions between EDDS and Cu in the rhizosphere is helpful for identifying the controlling factors on metal phytoextraction and providing further methods for improved phytoextraction practice in the field.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at the journal website.

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Table 1. Copper species (%) in the original and EDDS-treated soil from rhizosphere and non-rhizosphere soils using µ-XANES and bulk-XANES analysis using a linear combination fit.

Samples	Cu-Goethite	Cu-Clay	CuEDDS	R-factor	Reduced chi-square
Original soil					
Spot 1 Rhizo	35	65		0.0117	0.0024
Spot 2 Rhizo	51	49		0.0059	0.0011
Bulk Rhizo	42	58		0.0039	0.0008
Bulk Non-rhizo	41	59		0.0025	0.0005
EDDS treated soil					
Spot 3 Rhizo		62	38	0.0256	0.0050
Spot 4 Rhizo	14	54	31	0.0057	0.0012
Bulk Rhizo	25	52	22	0.0018	0.0004
Bulk Non-rhizo	48	52		0.0060	0.0012

Figure captions

Fig. 1. Concentrations of EDDS in soil extracts from different compartments of a rhizobox.

Fig. 2. Concentrations of Cu in soil extracts (a) from different compartments of a rhizobox; sequential extraction fractionations of Cu without (b) or with (c) EDDS treatments of soils from the different compartments of a ryegrass planted rhizobox. Orange dashed lines in b and c represent the total concentration of Cu in the original soil by SEP.

Fig. 3. Concentrations of Fe (a), Al (b), Ca, (c) and “natural” DOC (d) in soil extracts from the different compartments of a rhizobox. The “natural” DOC was derived from the total detected DOC with the subtraction of the DOC involved in EDDS.

Fig. 4. Calculated speciation of EDDS (a) and Cu (b) in soil extracts using Visual MINTEQ from different soil compartments and the trend (solid line) of the molar ratio of EDDS/Cu in extracts.

Fig. 5. Elemental distribution and correlation in an area of 1.5 mm × 1.5 mm from rhizosphere soil without (A) and with (B) treatment of EDDS by μ-XRF. Four hotspots marked with numbers were selected for μ-XANES analysis.

Fig. 6. Copper K-edge XANES spectra of standards and rhizobox soil samples with or without EDDS treatment, with red dashed lines as the linear combination fitting results. The linear combination fitting results are reported in Table 1. “Rhizo” refers to rhizosphere soil, “Non-rhizo” refers to non-rhizosphere soil, “RhizoE” refers to rhizosphere soil treated by EDDS, and “Non-rhizoE” refers to non-rhizosphere soil treated by EDDS.

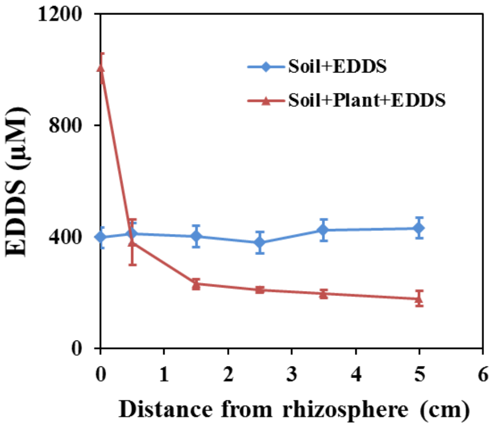
1200
800
400
0
EDDS (μM)
0
1
2
3
4
5
6
Distance from rhizosphere (cm)
Soil+EDDS
Soil+Plant+EDDS

Fig. 1.

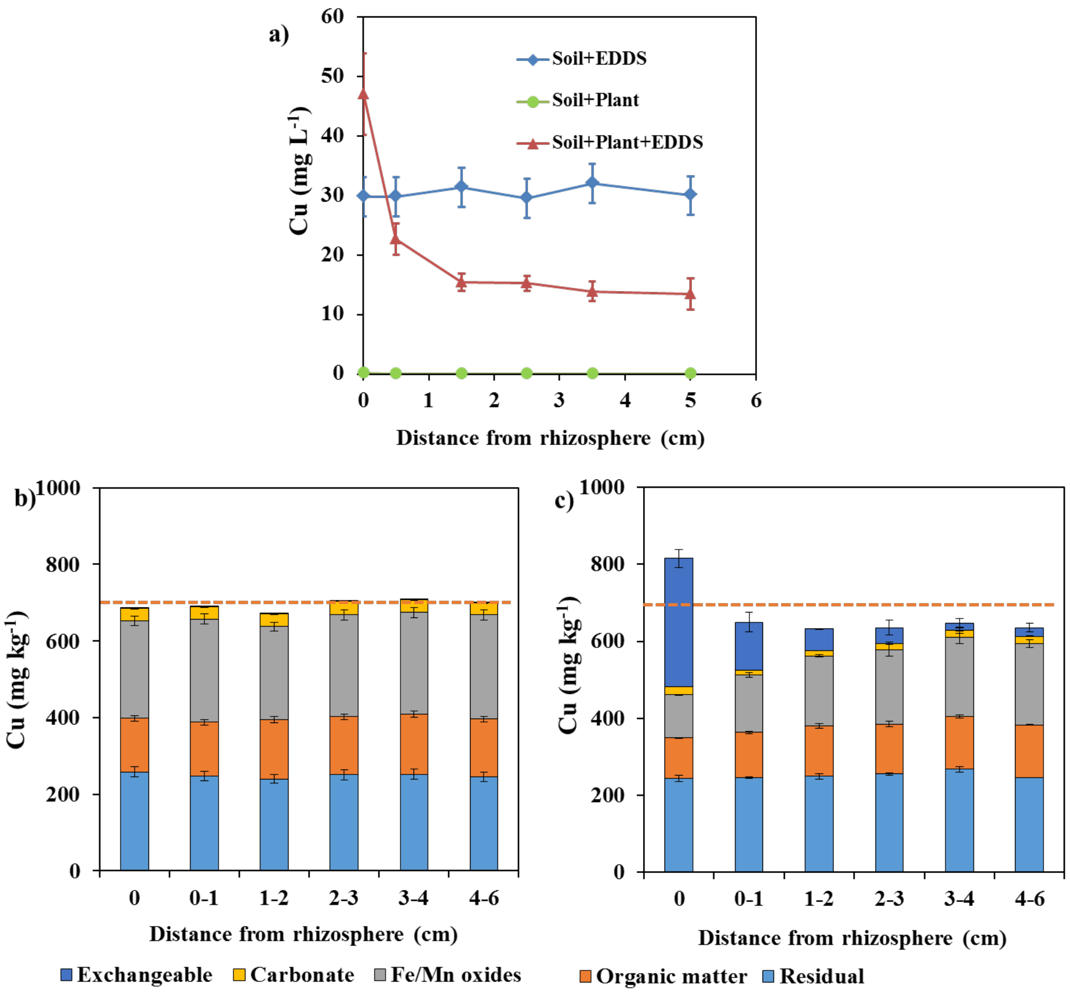
a)
Cu (mg L^{-1})
60
50
40
30
20
10
0
Soil+EDDS
Soil+Plant
Soil+Plant+EDDS
0
1
2
3
4
5
6
Distance from rhizosphere (cm)
b)
1000
800
600
400
200
0
Cu (mg kg^{-1})
0
0-1
1-2
2-3
3-4
4-6
Distance from rhizosphere (cm)
c)
1000
800
600
400
200
0
Cu (mg kg^{-1})
0
0-1
1-2
2-3
3-4
4-6
Distance from rhizosphere (cm)
Exchangeable
Carbonate
Fe/Mn oxides
Organic matter
Residual

Fig. 2.

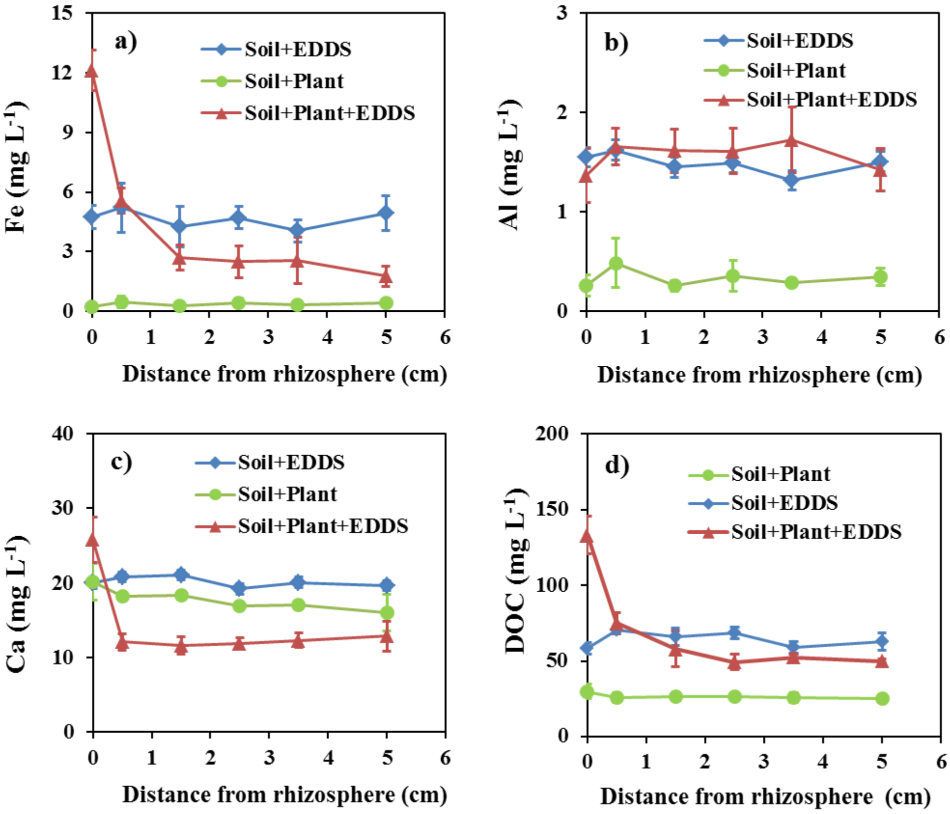
a)
Fe (mg L-1)
15
12
9
6
3
0
Soil+EDDS
Soil+Plant
Soil+Plant+EDDS
0
1
2
3
4
5
6
Distance from rhizosphere (cm)
b)
Al (mg L-1)
3
2
1
0
Soil+EDDS
Soil+Plant
Soil+Plant+EDDS
Distance from rhizosphere (cm)
c)
Ca (mg L-1)
40
30
20
10
0
Soil+EDDS
Soil+Plant
Soil+Plant+EDDS
Distance from rhizosphere (cm)
d)
DOC (mg L-1)
200
150
100
50
0
Soil+Plant
Soil+EDDS
Soil+Plant+EDDS
Distance from rhizosphere (cm)

Fig. 3.

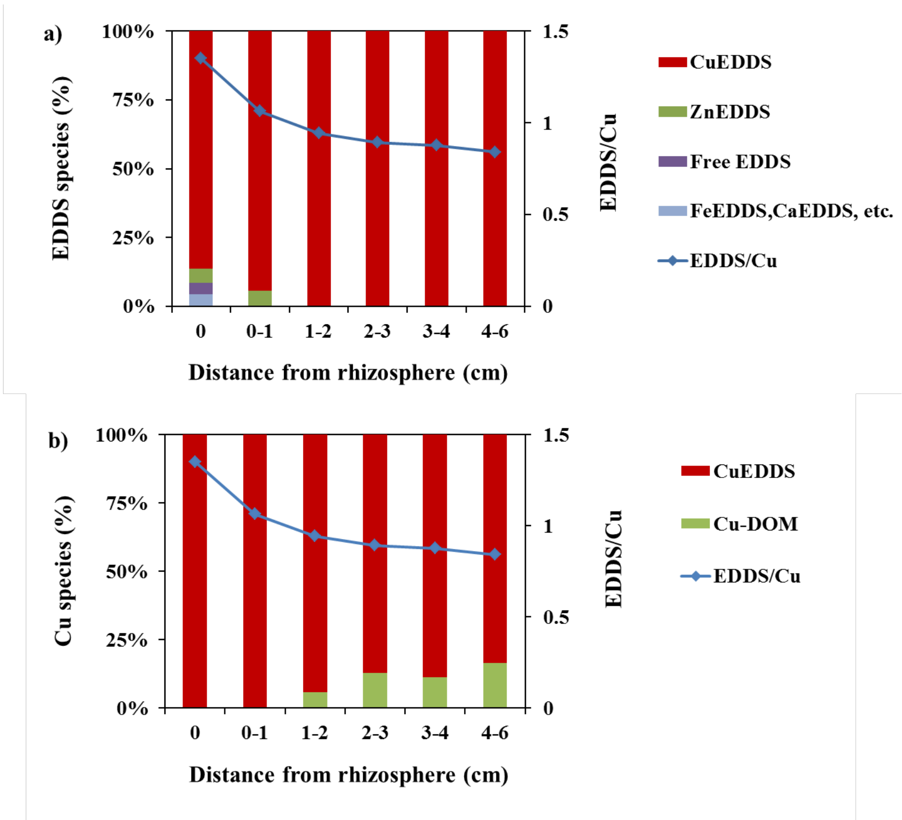
a)
EDDS species (%)
100%
75%
50%
25%
0%
0
0-1
1-2
2-3
3-4
4-6
Distance from rhizosphere (cm)
1.5
1
0.5
0
EDDS/Cu
CuEDDS
ZnEDDS
Free EDDS
FeEDDS,CaEDDS, etc.
EDDS/Cu
b)
Cu species (%)
100%
75%
50%
25%
0%
0
0-1
1-2
2-3
3-4
4-6
Distance from rhizosphere (cm)
1.5
1
0.5
0
EDDS/Cu
CuEDDS
Cu-DOM
EDDS/Cu

Fig. 4.

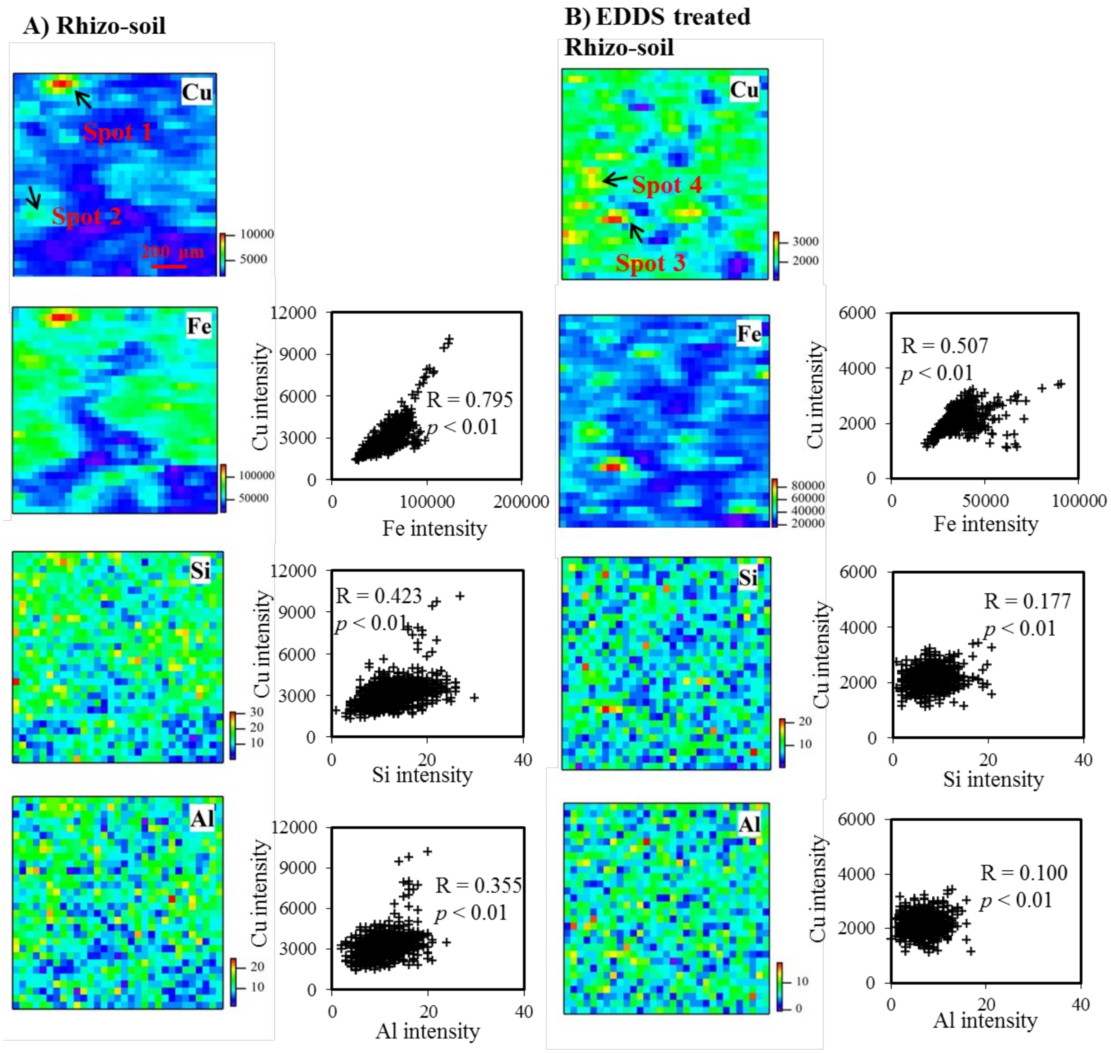
A) Rhizo-soil
B) EDDS treated Rhizo-soil
Cu
Spot 1
Spot 2
200 μm
10000
5000
Spot 4
Spot 3
3000
2000
Fe
100000
50000
80000
60000
40000
20000
Si
30
20
10
Al
Cu intensity
Fe intensity
Si intensity
Al intensity
R = 0.795
p < 0.01
R = 0.423
p < 0.01
R = 0.355
p < 0.01
R = 0.507
p < 0.01
R = 0.177
p < 0.01
R = 0.100
p < 0.01
12000
9000
6000
3000
0
100000
200000
4000
2000
50000
40

Fig. 5.

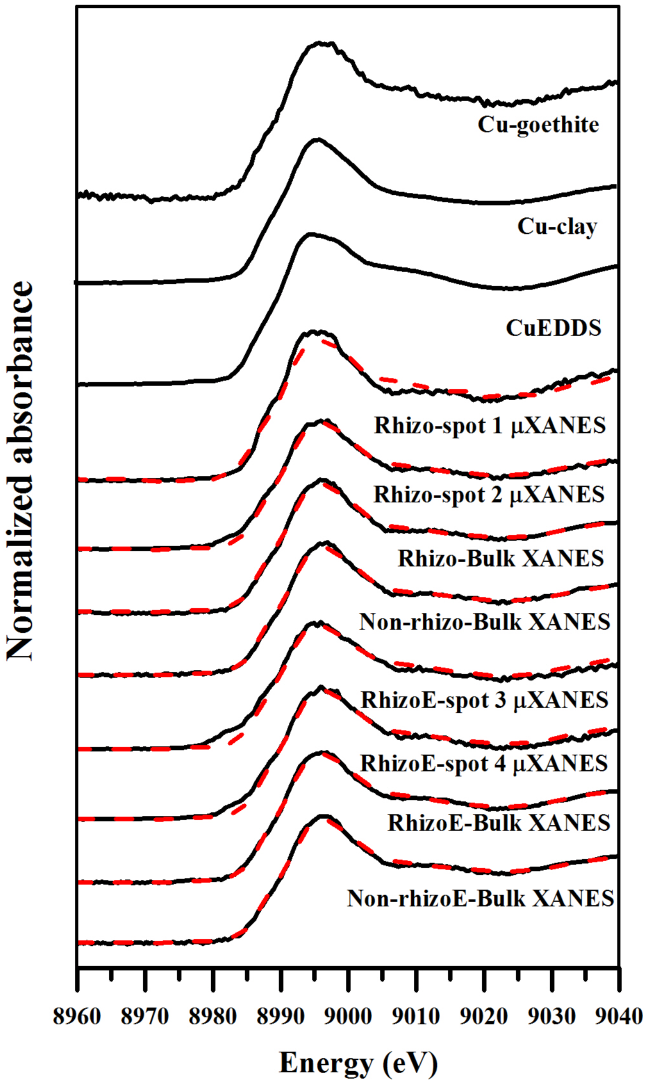
Normalized absorbance
Cu-goethite
Cu-clay
CuEDDS
Rhizo-spot 1 µXANES
Rhizo-spot 2 µXANES
Rhizo-Bulk XANES
Non-rhizo-Bulk XANES
RhizoE-spot 3 µXANES
RhizoE-spot 4 µXANES
RhizoE-Bulk XANES
Non-rhizoE-Bulk XANES
8960
8970
8980
8990
9000
9010
9020
9030
9040
Energy (eV)

Fig. 6.