Uptake and Translocation of CuEDDS Complexes by *Brassica carinata*

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The knowledge of the mechanisms that underlie metal complex uptake may lead to the development of new strategies for enhancing metal phytoextraction. As metals such as copper are actively taken up by roots, by inhibiting the proton driving force it is possible to obtain preliminary indications on the metal complex uptake mechanism. For this, Cu, EDDS, and Cu-EDDS uptake kinetics of *Brassica carinata* excised roots incubated in 30 and 150 μM solutions of either the metal, the chelant, and the complex were determined in the presence or not of the ATPase inhibitor vanadate. Following both Cu and CuEDDS treatments, metal uptake was negatively influenced by vanadate, whereas EDDS uptake did not, suggesting that Cu and the chelant did not enter the roots in their complexed form but by two different routes. The incubation in the same solutions of *B. carinata* intact plants showed that, differently from Cu, EDDS was largely translocated to shoots, but its low concentration resulted in a Cu to EDDS molar ratio ranging from 2 to 4 depending on metal complex concentration in the solution confirming that the uptake pathways of the two compounds were different.

Introduction

Chelant-enhanced phytoextraction aims to clean up metal-polluted soils by stimulating plants to accumulate the contaminating metal(s) in the harvestable parts owing to the addition of chelating agents to the soil. This technique exerts its effects by solubilizing target metals from soil and making them more available for plant uptake and translocation to shoots (*1*, *2*). The EDTA structural isomer (S,S)-*N*,*N*′-ethylenediamine disuccinic acid (EDDS) has received attention as a potential replacement for EDTA as it is a strong chelant and unlike EDTA it is easily biodegradable (*3*).

Differently from the active mechanisms involved in the uptake of essential metals such as Cu and Zn, it is widely accepted that synthetic chelants and their metal complexes pass through the root cortex and reach the xylem along a fully nonselective apoplastic pathway. It is unlikely that the anionic metal−ligand complexes pass through the cell membrane due to its large size and to the fact that no specific transporters are known. In spite of this, complexes can cross the Casparian strip barrier at the root tip where it is not fully differentiated or damaged by lateral roots formation, by high chelant concentrations and seedling transplantation. This nonselective passive apoplastic uptake allows the complex to reach the xylem and to be translocated to the shoots using transpiration as the main driving force (*4*−*8*). However, a not fully understood selective route of uptake was also suggested (*9*). According to this pathway, complexes actively move through some endodermal passage cells adjacent to the Casparian strip to the other side of the strip and then (again) extracellularly to the xylem.

An active mechanism is also at the basis of the split-uptake mechanism by which only free metal ions are taken up by roots (*8*). As the free metal ion activity decreases, metals are released from the complex to compensate for the shift in the equilibrium, maintaining a supply for their uptake in the rhizosphere. Indeed, Fe-EDTA is known to dissociate before plant uptake (*8, 10*).

Hydroponic solutions are useful research tools because of the easiness in which toxic elements and chelants are manipulated to investigate metal complex uptake into the roots and translocation to the above-ground parts (*4, 6, 7, 11*). In addition, the use of equimolar or high concentrations of the chelant in the solution eliminates free metal availability. However, hydroponic trials alone cannot be transferred directly to phytoextraction in the field where many soil factors affect the process.

To improve Cu phytoextraction effectiveness a better understanding of the key mechanisms of chelant-stimulated metal acquisition is needed. To predict soil-plant transfer of metals the understanding of the uptake mechanisms of metal complexes is fundamental for enhancing metal phytoextraction potential and for applying the more appropriate technology for chelant distribution (doses and application times respectful of the environment). The aim of this work was to investigate the short-term influx of free Cu ions, EDDS, or CuEDDS complexes into excised roots of *Brassica carinata* at equimolar concentrations of both the metal and the chelant. Inhibitory experiments concerning selective metal uptake mechanism and solute leakage measurements were also carried out. In addition, on whole plants the translocation of Cu and CuEDDS complexes from roots to the above-ground parts was studied.

Experimental Section

Plant Growth. Seeds of *Brassica carinata* cv 180 were surface sterilized for 10 min with NaClO (about 2% of active chlorine) and sown directly in polystyrene trays with holes containing wet expanded clay. The trays were placed in plastic bins filled with 6 L of a continuous aerated nutrient solution (*2*). Plants were grown at 16-h photoperiod, 400 μmol m^{-2} s^{-1} photon flux density, 23 ± 1 °C temperature, and 70−75% relative humidity.

Experimental Setup. In order to select the Cu concentrations to be used in the experimental solutions 3-week-old plants were collected from the nutrient solution, and excised roots were incubated in experimental solutions at increasing Cu concentrations (0.25−300 μM Cu as $CuSO_4$) for 30 min in the above-reported conditions. In the experimental solutions all micronutrients other than Cu and $NH_4H_2PO_4$ were omitted to avoid competition among metals and precipitation of copper phosphate. At the end of the treatments adsorbed metals were desorbed from roots by soaking them in 5 mM $Pb(NO_3)_2$ for 30 min under continuous stirring and rinsed with distilled water. Roots were then oven-dried for metal determination.

In Experiment I plants were collected from the nutrient solution, and the excised roots were incubated separately in experimental solutions containing Cu (as $CuSO_4$), EDDS (as Na_3EDDS), or CuEDDS at two concentrations of both the metal and the chelant (30 and 150 μM) for increasing time

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(0−12 h) at the growth conditions above-reported. Control excised roots were incubated in the nutrient solution (0.12 μM Cu). In order to inhibit ATPase activity 500 μM Na_3VO_4 and 50 mM KCl were added to each experimental solutions before excised roots were incubated. At the end of the treatments metals were desorbed and rinsed with distilled water. Roots were then frozen in liquid N_2 and stored at −80 °C until EDDS analysis or oven-dried for Cu determination.

In Experiment II whole plants collected from the nutrient solution were incubated in controlled conditions for 48 h using both the experimental solutions (Cu, EDDS, or CuEDDS in the presence or not of 500 μM vanadate) at the concentrations reported for Experiment I. Control plants were incubated in the nutrient solution containing 0.12 μM Cu. After desorption of metals from roots by 5 mM $Pb(NO_3)_2$ for 30 min under continuous stirring shoots were cut 1 cm above roots, and shoots and roots were collected after washing with distilled water. Shoots and roots were then frozen in liquid N_2 and stored at −80 °C until EDDS analysis or oven-dried for Cu determination.

Copper Analysis. Shoots and roots were oven-dried at 110 °C for 24 h. Dried ground material was then microwave digested with a mixture (3:1, v/v) of HNO_3 (65%) and H_2O_2 (30%) in a capped Teflon pressure digestion vessel at 200 °C for 20 min and analyzed for Cu using a Perkin-Elmer Optimal DV 2100 ICP OES. Standards (National Institute of Standards and Technology, MD, USA) and reagent blanks were run with all samples to ensure accuracy and precision in the analyses (*12*). The standard reference material used was SRM 1570a (spinach leaves).

EDDS Analysis. For EDDS analysis frozen shoots and roots were first lyophilized. The dry material was then extracted in a mortar with 50% ethanol (1:5, w/v), transferred to a test tube, and sonicated for 2 h with a Bransonic 3510 ultrasonic apparatus (Branson, Danbury, CT, USA) at room temperature to avoid degradation or cyclization of EDDS (*13*). After centrifugation at 3000 *g* for 3 min, the supernatant was filtered through 0.45 μm nylon syringe filters (Millipore). EDDS derivatization and analysis were carried out as described by Metsärinne et al. (*14*). Analyses were carried out by HPLC with a Waters HPLC system consisting of two 515 pumps and a 2487 programmable UV detector. EDDS was separated using a Waters Spherisorb ODS2 C18 reverse-phase column (25 × 0.46 cm, 5 μm) and detected at 254 nm wavelength. A 15% methanol and 85% tetrabutylammoniumbromide (0.02 M) eluent at a flow rate of 1 mL min^{-1} was used as the mobile phase. Identification of EDDS was obtained by comparison of its retention time with that of standards. Quantification of EDDS was obtained by comparing calibration curves. Chromatogram analysis was performed by Millennium 32 software (Waters).

Relative Leakage Ratio Measurement. Relative leakage ratio (RLR) was evaluated by determining solute release from roots (*15*). Solute release was measured by determining diffusate conductivity using a Janway 4010 Conductivity Meter. RLR was expressed as the ratio of conductivity of solute leakage after 24 h to total conductivity following liquid N_2 treatment.

Determination of ATPase Activity. The H^+-ATPase hydrolytic activity of root microsomal fractions was carried out following the rate of NADH oxidation at 340 nm (*16*).

Statistical Analysis. All statistical analysis was carried out with Costat 6.4 (CoHort software). The error bars represent the standard deviation of the mean of three independent experiments ($n = 3$) each analyzed twice. The effects of experimental factors were evaluated by one-way ANOVA, and comparisons between means were carried out using the LSD test at the significance level of $P \leq 0.05$.

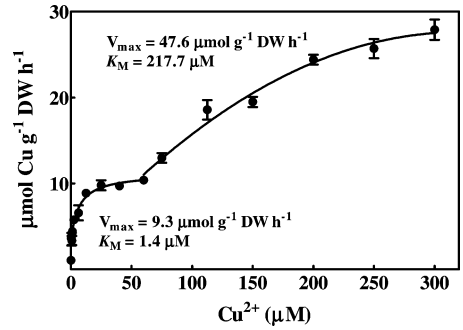

FIGURE 1. Copper uptake by *Brassica carinata* excised roots incubated for 30 min in solutions containing increasing concentrations of Cu. Data points are means ± SD ($n = 3$).

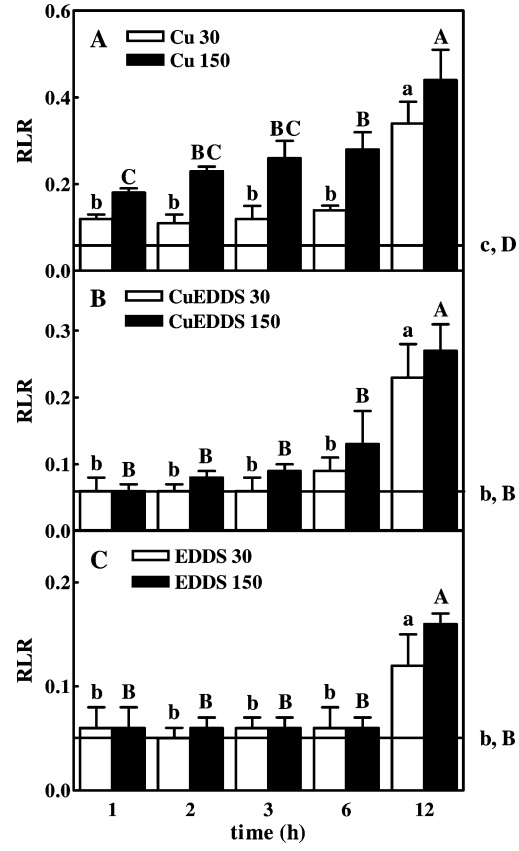

FIGURE 2. Relative leakage ratio (RLR) of *B. carinata* excised roots incubated for increasing time in solutions containing 30 or 150 μM Cu, CuEDDS, or EDDS. Results are means ± SD ($n = 3$). For each treatment means followed by the same letter are not significantly different by ANOVA (LSD test, $P \leq 0.05$). The horizontal lines represent the mean value of control roots (0.12 μM Cu) throughout the treatment and related significance.

Results

Concentration-Dependent Uptake of Copper. Excised roots of *B. carinata* showed two concentration-dependent uptake mechanisms for free Cu^{2+} ions (Figure 1) which kinetics fitted well to a typical Michaelis−Menten curve ($R^2 = 0.98$). A first uptake saturation curve showing a K_M value of 1.4 μM was observed at Cu concentrations up to 60 μM, whereas a second more flat curve was present at higher concentrations (K_M = 217.7 μM).

Experiment I. *Root Solute Leakage.* The relative leakage ratio of excised roots incubated for 12 h in solutions containing 30 or 150 μM Cu, CuEDDS, or EDDS is reported in Figure 2. Compared to control roots, free Cu ions caused an early leakage of solutes at both concentrations (already

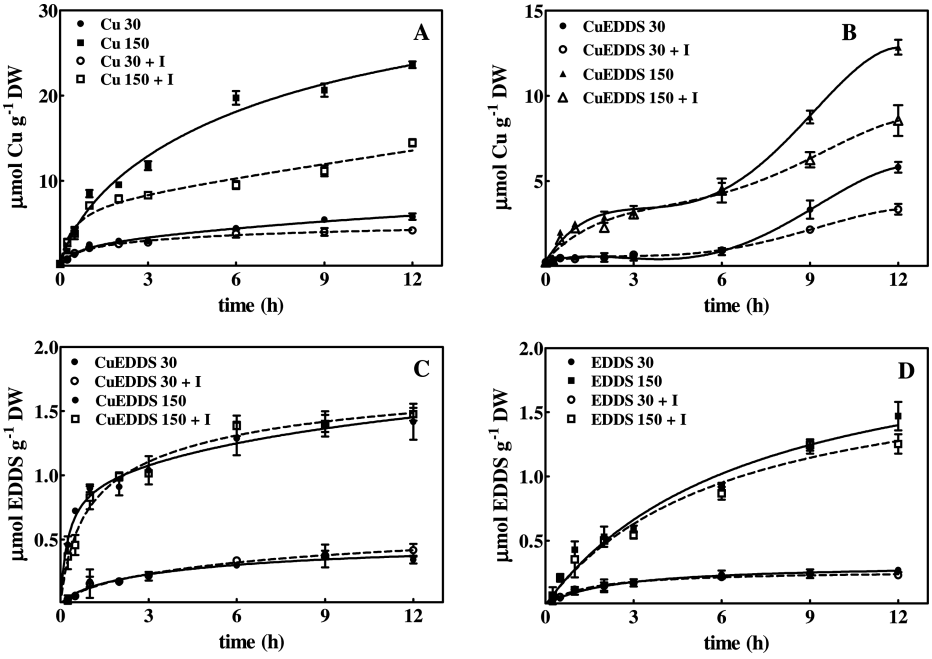

FIGURE 3. Copper and EDDS uptake by *B. carinata* excised roots incubated for increasing time in 30 or 150 μM Cu (A), CuEDDS (B, C), or EDDS (D) solutions in the presence or not of vanadate (I, inhibitor). Data points are means $\pm$ SD ($n = 3$).

after 1 h of incubation) showing a continuous increase during time (Figure 2A), the 150 μM incubation resulting in a higher leakage. In contrast, CuEDDS and EDDS treatments resulted less toxic inducing a significant membrane leakage only at 12 h (Figure 2B,C).

Root Copper Influx. In order to select the vanadate concentration to be used in the experimental solutions the ATPase inhibitor was added at increasing doses, and RLR of roots was determined after 12 h of incubation. Up to 500 μM vanadate no significant differences in comparison with the control were observed (data not shown), concentration beyond which an enhanced solute leakage occurred. At 500 μM vanadate (the concentration selected for the inhibitory experiments) an about 90% inhibition of ATPase activity was found.

Uptake of free Cu ions by excised *B. carinata* roots was monitored over 12 h (Figure 3A). The influx displayed a saturation curve at both concentrations in the presence or not of vanadate. At both concentrations metal uptakes resulted in a rapid initial phase (up to 1 h) followed by a steady-state phase in which the rate of influx progressively lowered (150 μM) or remained almost constant (30 μM). The inhibition of Cu uptake induced by vanadate was observed at different times (6 h at the lowest and 1 h at the highest metal concentration). At the end of the incubation the addition of vanadate reduced Cu uptake by 28 and 40% in the 30 and 150 μM treatments, respectively. The time course of copper influx into CuEDDS-treated roots (Figure 3B) showed a trend somewhat different from that observed for Cu ions. In this treatment the initial influx phase was followed by an almost constant uptake up to 6 h, after which a second influx period occurred. Also in this case the uptake of the metal into excised roots was reduced by vanadate (Figure 3B). The inhibition rates at 12 h were 42 and 33% in the 30 and 150 μM CuEDDS treatment, respectively. Compared to free Cu ion incubation, the lowest complex concentration in the absence of vanadate resulted in a similar final metal level, whereas at the highest concentration there was a 1.8-fold reduction (from 23.6 to 12.8 μmol Cu g^{-1} DW).

Root EDDS Influx. In both CuEDDS and EDDS treatments the chelant influx into roots showed a similar behavior at both concentrations (Figure 3C,D) reaching at the end of the incubation period almost the same values. The presence of

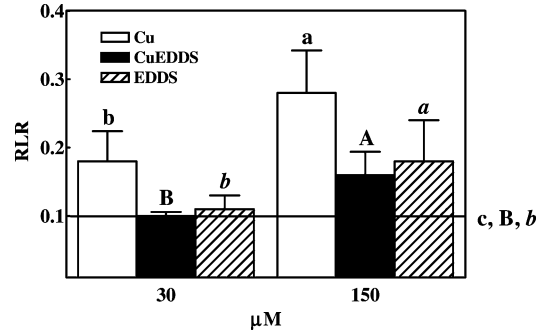

FIGURE 4. Root relative leakage ratio (RLR) of *B. carinata* intact plants incubated for 48 h in solutions containing 30 or 150 μM Cu, CuEDDS, or EDDS. Results are means $\pm$ SD ($n = 3$). For each treatment means followed by the same letter are not significantly different by ANOVA (LSD test, $P \leq 0.05$). The horizontal line represents the mean value of control roots (0.12 μM Cu) and related significance.

vanadate did not influence EDDS uptake. The CuEDDS treatment resulted in chelant molar concentrations about 10- and 17-fold lower (at 30 and 150 μM, respectively) compared to those detected for Cu in the same treatment (Figure 3B,C). Following CuEDDS incubation a linear relationship between metal and chelant uptake was observed up to 6 h ($R^2 = 0.87$ and $R^2 = 0.91$ at 30 and 150 μM, respectively), time after which chelant influx stopped whereas Cu one continued.

Experiment II. *Solute Leakage.* The root RLR values of intact plants incubated for 48 h at 30 and 150 μM Cu, CuEDDS, or EDDS are shown in Figure 4. Compared to control plants, free Cu ions induced a significant solute leakage at both levels. On the contrary, CuEDDS and EDDS alone treatments caused an enhancement of the RLR value only at the highest concentration. In any case, the increase in the leakage was relatively slight not exceeding the value of 0.3.

Plant Copper Uptake. In comparison with control plants, Cu incubation caused an increase in root metal concentration (6- and 76-fold at 30 and 150 μM treatments, respectively), whereas in shoots the enhancement was more than halved (Figure 5A). The presence of the inhibitor vanadate drastically

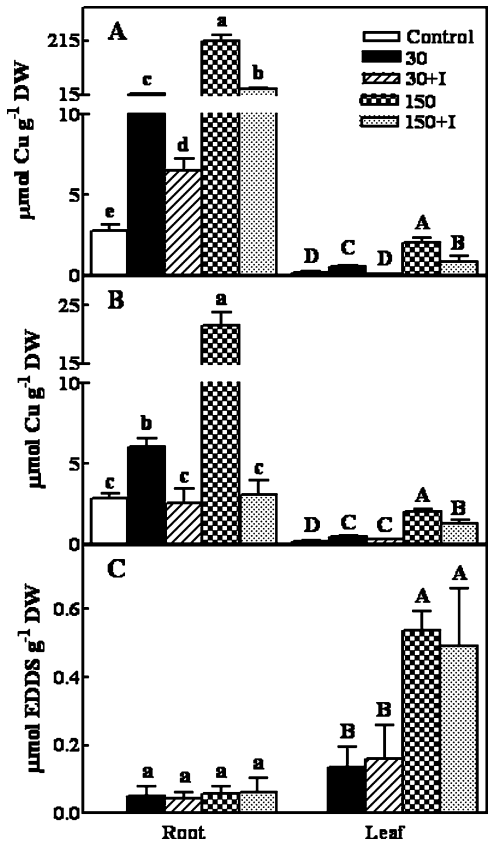

FIGURE 5. Copper and EDDS concentrations in roots and shoots of *B. carinata* intact plants incubated for 48 h in 30 or 150 μM Cu (A) or CuEDDS (B, C) solutions in the presence or not of vanadate (I, inhibitor). Results are means ± SD (n = 3). For each plant organ means followed by the same letter are not significantly different by ANOVA (LSD test, $P \leq 0.05$).

reduced copper concentrations in roots (−64 and −83%, respectively) and shoots. Treatments did not increase metal translocation to shoots which remained constant at a value of about 3% of total copper.

Also in the CuEDDS treatment an increase in root and shoot copper concentrations was observed when compared to control plants (Figure 5B). However, at 30 and 150 μM CuEDDS the amount of metal in roots was 67 and 90% lower than that detected following Cu incubation, whereas shoot copper concentrations did not show differences. As a consequence the amount of copper translocated to the shoots increased to about 10%. As for Cu incubation, vanadate inhibited metal uptake by roots at almost the same percentages.

Plant EDDS Uptake. In the CuEDDS treatment chelant uptake by roots did not show differences at both concentrations (Figure 5C). On the contrary, in shoots an about 7-fold increase at the highest metal complex concentration was observed. Compared to copper EDDS was present in much lesser amounts both in roots and in shoots. In addition, it was prevalently detected in shoots (74 and 95% of total concentration, respectively).

Discussion

Copper and EDDS Influx into Excised Roots. The determination of metal influx kinetics is a fundamental step toward the modeling of soil-plant transfer in polluted environments. In nutrient acquisition two steps have to be distinguished: i) passive binding of ions within the free space of the cell wall and ii) active uptake of ions into the cells across membranes. The latter step might be mediated by multiphasic mechanisms (*17*), and for its correct understanding removal of ions from the apoplasm by suitable desorption solutions is necessary. In addition, time course studies on trace metal uptake showed that it should be measured over periods sufficiently short to reflect inward unidirectional flux (*17, 18*) and to avoid modifications of uptake kinetics that are expected to occur in the range of metal toxicity due to structural damages (*19*). For these reasons an incubation time of 30 min was chosen to study concentration-dependent influx of Cu. The use of excised roots is justified by the fact that in this case metal influx into roots is not affected by its translocation to the shoots.

The change in the influx pattern when the external Cu concentration was higher than 60 μM (Figure 1) might be explained in terms of a biphasic mechanism, consisting of a high-affinity uptake system present at lower metal concentrations and a low-affinity system active at higher concentrations. The existence of a biphasic or multiphasic kinetic pattern for Cu uptake by roots of several species was already observed and related to active mechanisms (*17, 20*). Copper absorption is most likely mediated by the high-affinity Cu^+ transporter COPT1 and the Cu^{2+} transporter ZIP2 as recently described for *Arabidopsis* (*21, 22*). The hyperbolic shape of the two kinetic curves (Figure 1), both showing a saturable component, suggests that Cu uptake - in the tested concentration range - was metabolically driven by root plasma membrane carrier proteins. The K_M value (1.4 μM) derived from the first curve of the biphasic kinetics fell within the lower micromolar range described for the Ctr1 family of transporters, which COPT1 belongs to (*22*). The high K_M observed in the second phase likely originated from an inactivation of the binding sites responsible for the active transport (*17*). The calculated kinetic parameters K_M and V_{max} were not much different from those observed for intact barley roots incubated for 2 h in solutions containing Cu in the range 1.6−315 μM (*23*).

The 30 and 150 μM copper concentrations, representative of the two influx mechanisms (Figure 1), were selected being them the concentrations at which CuEDDS caused, after 12 h of incubation, progressive significant increases in solute leakage compared to control excised roots. It was previously observed (*24*) that Cu induced in the elongation zone of roots both rhizoderm and outer cortex ruptures after 12 h of exposure to low levels of Cu (about 1−2 μM).

It was reported that metal complexes are less phytotoxic than free metal ions as they reduce the interactions of metals with plant metabolic targets (*7, 12*). Free Cu, which is a redox metal responsible for oxidative damage to cellular metabolism, resulted in a much higher and earlier solute leakage compared to CuEDDS (Figure 2A,B), the latter showing increased RLR values only after 12 h compared to the control. In contrast to uncomplexed EDTA, which reduced plant growth and transpiration by removal of essential divalent ions from the plasma membrane (*11, 12*), in this study free protonated EDDS resulted in a low toxicity to excised roots (Figure 2C), likely due to the lowest concentrations and shortest time of exposure. Similarly, hydroponically grown sunflower did not show any reduction of shoot and root biomass compared to the control when treated with 500 μM EDDS (*7*).

The proton-pumping ATPase (H^+-ATPase) of the plant plasma membrane generates the proton electrochemical gradient that is necessary to activate ion and metabolite secondary transport. Vanadate, a potent inhibitor of P-type ATPases, is thought to mimic P_i and block the enzyme in its E2 form preventing the solute release. The time course influxes of both Cu and EDDS following H^+-ATPase inhibition by vanadate suggest that whereas the metal was taken up by an active mechanism, the chelant entered the roots by a nonselective passive mechanism (Figure 3). Indeed, following

both Cu and CuEDDS treatments, metal influx was negatively influenced by vanadate suggesting the involvement of an active mechanism. The different times at which the inhibition was detected (1 and 6 h for Cu and CuEDDS treatments, respectively) might be explained by the fact that, besides the time needed to inactivate ATPases, the metal complex had likely to be split and free Cu ions released before active uptake occurred. This uptake route was previously observed for another redox metal such as iron following FeEDTA treatment (*9, 11*) but not for Pb and Zn complexes which cannot be split through the reduction or oxidation of the metals. Compared to CuEDDS treatment, the highest RLR values following free Cu incubation (Figure 2A,B) did not significantly influence Cu uptake mechanism. A saturation pattern of copper uptake, comparable with the uptake pattern of rice roots, was already observed in cell suspension cultures of bean incubated for 28 h in solutions containing Cu concentrations up to 2 mM. The saturation time was reached at significantly different time intervals depending on the metal concentration (*25*). Starting from 16 h a linear enhanced uptake of the metal was detected in both treatments (data not shown) as a consequence of a disorganized root system induced by Cu - and to a lesser extent by EDDS - toxicity, which allowed a large nonselective influx.

In contrast to Cu uptake, EDDS was not influenced by vanadate throughout the two treatments (CuEDDS and EDDS) suggesting in both cases a passive mechanism (Figure 3C,D). At both levels, CuEDDS incubation resulted in chelant concentrations about 1 order of magnitude lower than those detected for copper (Figures 3A,B). Even if in the presence of equimolar concentrations of both the metal and the chelant in the solution, a remarkable higher Cu influx was observed suggesting that in the first 12 h of CuEDDS incubation, EDDS and Cu did not enter the roots in their complexed form but mainly by two different routes and confirms that chelant influx was due to an apoplastic pathway (*5, 9*) as suggested by the similar amounts of EDDS in the two treatments. The saturation pattern of the curves observed after about 9 h of incubation were likely linked to the attainment of the chelant binding capacity of root apoplasm. Similarly to Cu, the large linear influx of the chelant during CuEDDS incubation starting from 16 h (data not shown) was likely due to membrane alterations and tissue disorganization induced by the toxicity of both the metal and the chelant. When Cu molar concentrations were plotted against EDDS ones throughout the CuEDDS treatment, a linear correlation up to 6 h was obtained at both 30 and 150 μM levels. The Cu to EDDS molar ratios, ranging from 2.3 to 2.5, further suggest that the metal and the chelant were taken up by different mechanisms.

Copper and EDDS Uptake by Intact Plants. Intact plants treated for 48 h with Cu, CuEDDS, or EDDS showed root solute leakage values lower than those determined for excised roots incubated at the same concentrations for 12 h (Figures 2 and 4). All the treatments resulted in a very slight toxicity to roots even at the highest concentrations. It may be suggested that, differently from excised roots, during the incubation period intact roots were able to activate defense/repair antioxidative mechanisms which allowed functioning of membranes and cell metabolism (*3, 15*).

At both concentrations, CuEDDS incubation determined a lower metal accumulation in roots compared to Cu likely due to both reduced membrane disorganization - as evidenced by the low root leakage - and/or the absence of free Cu ions in the solution (Figure 5A,B), which might have limited uncontrolled Cu inflow. According to other hydroponic studies (*7, 26, 27*), Cu uptake in the presence of EDDS was reduced compared to free Cu. As the hypothesis of a switch to an apoplastic less effective route (*28*) is not supported by the vanadate-induced inhibition of Cu uptake an active mechanism in the CuEDDS treatment may be suggested. Large apoplastic uptake of metal complexes is a function of the complex concentration in the solution (*7*) as well as metal and plant species. Above a threshold chelant concentration, a linear relationship between the solution concentration and the amount taken up is expected due to damage to membranes of root cells which normally function to control the uptake and translocation of solutes (*11, 29*).

Following both Cu and CuEDDS incubation, shoot metal concentrations showed similar values (Figure 5A,B), even though Cu translocation in the CuEDDS treatment was, at 30 and 150 μM level, 3- and 10-fold higher, respectively, compared to the metal alone treatment. The low copper accumulation in shoots of Cu and CuEDDS-treated plants might be related to the low RLR values and the consequent slight damage to root cells which did not allow uncontrolled inflow of solution into the stele by the apoplastic pathway (*30*). In addition, the reduced metal translocation suggests that at the two concentrations used nonselective uptake of Cu in the presence of the chelant did not occur or did not exceed active uptake along the symplastic pathway (*28*). The low translocation value of Cu-treated plants (about 3%) is in accordance with that observed for hydroponically grown sunflower (*7*), whereas the highest translocation found in sunflower following CuEDDS treatment (about 40% in comparison with 10% of the present study) may be due to a higher chelant concentration (500 μM) and a longer duration (6 days).

The presence of EDDS in different plant organs was already observed (*5*−*7*). Differently from Cu, the chelant was largely translocated to shoots (Figure 5C), but its very low concentration resulted in a Cu to EDDS molar ratio ranging from 2 to 4 depending on metal complex concentration (Figure 5B,C). This may be considered a further indication that the uptake pathways of the two compounds were different and that only a reduced fraction of total absorbed Cu was complexed by free EDDS after passing into the xylem and then transported to the shoots (*31*). Alternatively, it cannot be turned out the possibility that part of the metal might have been taken up in the complexed form. Shoot chelant uptake increased in proportion to the dissolved EDDS, whereas root uptake did not (Figure 5C). To explain the root behavior it may be suggested that the chelant was adsorbed to the roots which become saturated at relatively low EDDS concentrations (*6*).

Acknowledgments

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Supporting Information Available

Additional text. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Meers, E.; Tack, F. M. G.; Verloo, M. G. Degradability of ethylenediamine disuccinic acid (EDDS) in metal contaminated soils: Implications for its use in soil remediation. *Chemosphere* **2008**, *70*, 358–363.

(2) Quartacci, M. F.; Irtelli, B.; Baker, A. M. J.; Navari-Izzo, F. The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica carinata*. *Chemosphere* **2007**, *68*, 1920–1928.

(3) Quartacci, M. F.; Irtelli, B.; Gonnelli, C.; Gabbrielli, R.; Navari-Izzo, F. Naturally-assisted metal phytoextraction by *Brassica carinata*: Role of root exudates. *Environ. Pollut.* **2009**, *157*, 2697–2703.

(4) Nowack, B.; Schwyzer, I.; Schulin, R. Uptake of Zn and Fe by wheat (*Triticum aestivum* var. Greina) and transfer to the grains in the presence of chelating agents (ethylenediaminedisuccinic acid and ethylenediaminetetraacetic acid). *J. Agric. Food Chem.* **2008**, *56*, 4643–4649.
(5) Tandy, S.; Schulin, R.; Nowack, B. Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration. *Environ. Sci. Technol.* **2006**, *40*, 2753–2758.
(6) Tandy, S.; Schulin, R.; Nowack, B. The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers. *Chemosphere* **2006**, *62*, 1454–1463.
(7) Bell, P. F.; McLaughlin, M. J.; Cozens, G.; Stevens, D. P.; Owens, G.; South, H. Plant uptake of ^{14}C-EDTA, ^{14}C-Citrate and ^{14}C-Histidine from chelator-buffered and conventional hydroponic solutions. *Plant Soil* **2003**, *253*, 311–319.
(8) Leštan, D.; Luo, C.; Li, X. The use of chelating agents in the remediation of metal-contaminated soils: A review. *Environ. Pollut.* **2008**, *153*, 3–13.
(9) Tanton, T. W.; Crowdy, S. H. The distribution of lead chelate in the transpiration stream of higher plants. *Pestic. Sci.* **1971**, *2*, 211–213.
(10) Vassil, A. D.; Kapulnik, Y.; Raskin, I.; Salt, D. E. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol.* **1998**, *117*, 447–453.
(11) Hernández-Allica, J.; Garbisu, C.; Barrutia, O.; Becerril, J. M. EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants. *Environ. Exp. Bot.* **2007**, *60*, 26–32.
(12) Markert, B. *Instrumental Element and Multi-Element Analysis of Plant Samples - Methods and Applications*; John Wiley & Sons: Chichester, New York, Brisbane, Toronto, Singapore, 1996.
(13) Kolleganov, M.; Kolleganova, I. G.; Mitrofanova, N. D.; Martynenko, L. I.; Nazarov, P. P.; Spitsyn, V. I. Influence of cyclization on *N, N'*-ethylenediaminedisuccinic acid on its complex forming properties. *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1983**, *32*, 1167–1175.
(14) Metsärinne, S.; Tuhkanen, T.; Aksela, R. Photodegradation of ethylenediaminetetraacetic acid (EDTA) and ethylenediamine disuccinic acid (EDDS) within natural UV radiation range. *Chemosphere* **2001**, *45*, 949–955.
(15) Sgherri, C.; Quartacci, M. F.; Navari-Izzo, F. Early production of activated oxygen species in root apoplast of wheat following copper excess. *J. Plant Physiol.* **2007**, *164*, 1152–1160.
(16) Briskin, D. P.; Reynolds-Niesman, I. Determination of H^+/ATP stoichiometry for the plasma membrane H^+-ATPase from red beet (*Beta vulgaris* L.) storage tissue. *Plant Physiol.* **1991**, *95*, 242–250.
(17) Lidon, F. C.; Henriques, F. S. Effects of increasing concentrations of Cu on metal uptake kinetics and biomass yields. *Soil Sci.* **1992**, *154*, 4–49.
(18) Reid, R. J.; Smith, F. A. Measurement of calcium fluxes in plants using ^{45}Ca. *Planta* **1992**, *186*, 558–566.
(19) Bravin, M. N.; Le Merrer, B.; Denaix, L.; Schneider, A.; Hinsinger, P. Copper uptake kinetics in hydroponically-grown durum wheat (*Triticum turgidum durum* L.) as compared with soil's ability to supply copper. *Plant Soil* **2010**, *331*, 91–104.
(20) Marquenie-van der Werff, M.; Ernst, W. H. O. Kinetics of copper and zinc uptake by leaves and roots of an aquatic plant *Elodea nuttallii*. *Z. Pflanzenphysiol. Bd.* **1979**, *92*, 1–10.
(21) Burkhead, J. L.; Reynolds, K. A. G.; Abdel-Ghany, S. E.; Cohu, C. M.; Pilon, M. Copper homeostasis. *New Phytol.* **2009**, *182*, 799–816.
(22) Yruela, I. Copper in plants: acquisition, transport and interactions. *Funct. Plant Biol.* **2009**, *36*, 409–430.
(23) Veltrup, W. Concentration dependent uptake of copper by barley roots. *Physiol. Plant.* **1976**, *36*, 217–220.
(24) Kopittke, P. M.; Blamey, F. P. C.; Menzies, N. W. Toxicities of soluble Al, Cu and La include ruptures to rhizodermal and root cortical cells of cowpwa. *Plant Soil* **2008**, *303*, 217–227.
(25) Veltrup, W.; Austenfeld, F.-A. The uptake of copper by cell suspension cultures of *Phaseolus vulgaris* L. cv. SAXA. *Plant Cell Rep.* **1981**, *1*, 31–33.
(26) Wenger, K.; Gupta, S. K.; Furrer, G.; Schulin, R. The role of nitrilotriacetate in copper uptake by tobacco. *J. Environ. Qual.* **2003**, *32*, 1669–1676.
(27) Taylor, G. J.; Foy, C. D. Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum*. *Can. J. Bot.* **1985**, *63*, 1271–1275.
(28) Nowack, B.; Schulin, R.; Robinson, B. H. Critical assessment of chelant-enhanced metal phytoextraction. *Environ. Sci. Technol.* **2006**, *40*, 5225–5232.
(29) Luo, C.; Shen, Z.; Baker, A. J. M.; Li, X. A novel strategy using biodegradable EDDS for the chemically enhanced phytoextraction of soils contaminated with heavy metals. *Plant Soil* **2006**, *285*, 67–80.
(30) Collins, R. N.; Merrington, G.; McLaughlin, M. J.; Knudsen, C. Uptake of intact zinc-ethylenediaminetetraacetic acid from soil is dependent on plant species and complex concentration. *Environ. Toxicol. Chem.* **2002**, *21*, 1940–1945.
(31) Luo, C.; Shen, Z.; Li, X. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. *Chemosphere* **2005**, *59*, 1–11.

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