Biodegradable chelate enhances the phytoextraction of copper by *Oenothera picensis* grown in copper-contaminated acid soils

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A B S T R A C T

Oenothera picensis plants (Fragrant Evening Primrose) grow in the acid soils contaminated by copper smelting in the coastal region of central Chile. We evaluated the effects of the biodegradable chelate MGDA (methylglycinediacetic acid) on copper extraction by *O. picensis* and on leaching of copper through the soil profile, using an *ex situ* experiment with soil columns of varying heights. MGDA was applied in four rates: 0 (control), 2, 6 and 10 mmol plant^{-1}. MGDA application significantly increased biomass production and foliar concentration, permitting an effective increase in copper extraction, from 0.09 mg plant^{-1} in the control, to 1.3 mg plant^{-1} in the 6 and 10 mmol plant^{-1} treatments. With 10 mmol plant^{-1} rate of MGDA, the copper concentration in the leachate from the 30 cm columns was 20 times higher than in the control. For the 60 cm columns, copper concentration was 2 times higher than the control. It can be concluded that at increased soil depths, copper leaching would be minimal and that MGDA applications at the studied rates would not pose a high risk for leaching into groundwater. It can thus be stated that applications of MGDA are an effective and environmentally safe way to improve copper extraction by *O. picensis* in these soils.

1. Introduction

Prior to the establishment of environmental mining regulations in Chile, copper smelters released contaminated emissions directly into the atmosphere (Folchi, 2006). These emissions, composed of SO$_2$ and particulate matter rich in copper and other metals, have been deposited on the surrounding soils (De Gregori et al., 2003). One example of this situation is the smelter at Ventanas, in the Puchuncaví valley in central Chile. The soils around the smelter have a very acid pH and high concentrations of copper and other elements (Ginocchio, 2000; De Gregori et al., 2003; Neaman et al., 2009). Consequently, the ecosystems associated with these soils have become seriously degraded, reflected in the almost barren, semi-desert appearance of the surrounding landscape in which soil erosion processes are also evident (Sabatini et al., 1996; Ginocchio, 2000). Phytoextraction is a soil remediation technique that uses plants, preferably native species, for the extraction of metals and radionuclides. This technique requires plants that are tolerant to relatively high concentrations of metals, and also capable of absorbing and accumulating large quantities within their aerial biomass. Ideally, such species are hyperaccumulators, capable of accumulating over 1000 mg kg^{-1} of Cu, Pb, As and Zn (Baker and Brooks, 1989).

The success of the phytoextraction process is based on the capacity of the plant to extract the metal of interest and translocate it into its aerial biomass (Chen et al., 2003). The bioavailability of the metals in a given soil (the fraction which is available to be absorbed by an organism, NRC, 2003) is an important factor, as it often happens that high total concentrations of such metals are found in a soil, and yet, the bioavailable fraction is low. Under such conditions, plants do not absorb much metal, even when they have the capacity for increased accumulation (Ernst, 1996).

Chelates can be used to increase the bioavailability of metals in the soil. These compounds form complexes with the metal, producing soluble molecules that increase the metal concentration in the soil solution and thereby increase the amount available for absorption by roots. It is thought that these metal complexes, upon absorption, pass through the apoplasm of the roots and flow directly into the xylem. Carried by the pressure of evapotranspiration, they are translocated to the aerial biomass (Huang et al., 1997; Robinson et al., 2006; Cao et al., 2007). Synthetic chelates have been the most widely studied, principally ethylenediaminetetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) and ethylenediamine-N,N′-bis(2-hydroxyphenyl) acetic acid (EDDHA), among others. Previous studies have indicated that

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the addition of these chelates to contaminated soils increased the removal of metals by plants from 2 to 250 times more than from untreated soils (Grčman et al., 2001; Schmidt, 2003; Lou et al., 2007).

Despite their high efficiency, these traditional chelates also manifest the problem of environmental persistence. Consequently, this leads to longer periods of increased solubility for the metals during which they can leach into the groundwater or be carried by runoff into surface water courses (Nörtemann, 1999; Wu et al., 1999). Environmentally persistent chelates can also cause eutrophication due to their nitrogen-rich composition (Oviedo and Rodríguez, 2003; Carsten and Heinz-Jürgen, 2004). Some chelates, such as EDTA, are also toxic to photosynthetic organisms, inhibiting cell division, chlorophyll synthesis and production of biomass (Oviedo and Rodríguez, 2003).

Considering all of these negative effects, biodegradable chelates—whose reduced environmental persistence should also reduce the above-mentioned negative effects—have been suggested as an alternative. Chelates such as ethylenediamine disuccinic acid (EDDS), iminodisuccinic acid (IDSA), nitrilotriacetic acid (NTA) and methylglycinediacetic acid (MGDA) have been proposed (Grčman et al., 2001; Tandy et al., 2004). These chelates have a short half-life, in comparison with the traditional chelates whose persistence in the environment endures for years (Grčman et al., 2003; Tandy et al., 2004). MGDA in particular, known commercially as *Trilon M*, has the advantage of being more stable under a wide range of temperatures and pH than the other biodegradable chelates (BASF, 2007). Studies done by Robinson et al. (2006), Grčman et al. (2001) and Tandy et al. (2004) have demonstrated that the efficiency of biodegradable chelates is similar to traditional ones. Efficiency of these chelates mainly depends on the metal that is to be extracted and on the soil characteristics. In the case of copper, numerous studies show an advantage for biodegradable chelates over traditional synthetic ones (Grčman et al., 2003; Luo et al., 2004; Tandy et al., 2004; Cao et al., 2007; Cosciones et al., 2009). In terms of environmental risk, studies by Cao et al. (2007) and Grčman et al. (2003) have shown that the concentration of metals is significantly higher in the leachate from soils treated with the traditional chelate EDTA, than from soils treated with the biodegradable chelates MGDA or EDDS. However, the leaching of metals does not totally disappear with the use of biodegradable chelates, and this potential environmental risk must still be evaluated on a case by case basis.

Oenothera picensis is an easy to propagate, herbaceous plant identified in the area of the Ventanas smelter (González et al., 2008, classified as *Oenothera affinis*), having high biomass production and potential as a metallophytic (copper tolerant) species. The highest reported concentration of copper in shoots collected from the field is 614 mg kg^{-1} (González et al., 2008). On the other hand, the concentration of soluble copper (in 0.1 M KNO$_3$) in the surface soil horizons near the Ventanas smelter is higher (1.1–30 mg kg^{-1}, González et al., 2008) in comparison with other copper-contaminated sites (0.1–1.3 mg kg^{-1} in soils near the Chagres smelter, Ávila et al., 2007). However, it is possible that the concentration of copper in the plants could increase even more when the concentration of soluble metal in the soil is increased. Consequently, it is postulated that the application of a biodegradable chelate to the soils around the Ventanas smelter would allow an increase in the bioavailability of copper and could thus increase the extraction of metal by *O. picensis*, without producing extensive metal leaching.

The major objectives of this study are (1) to determine the effect of the application of MGDA on the extraction of copper by *O. picensis* in copper-contaminated acid soils, and (2) to evaluate the effects of the application of MGDA on the leaching of copper.

2. Materials and methods

An *ex situ*, open air soil column experiment was set up in polyvinyl chloride (PVC) tubes, near Quillota, Valparaíso Region, at the School of Agronomy of the Pontifical Catholic University of Valparaíso (approximately 30 km south-east of the Ventanas smelter).

Prior to the principal experiment, preliminary evaluations of the temporal dynamics of chelation and the effects of rainfall on copper leaching from this soil were done. For this purpose, the concentration of copper in the soil solution was determined for a period of 55 d after the chelate application.

2.1. Soil sampling

The soils used in this study were collected from the area of Los Maitenes, in the Puchuncaví valley, 3 km east of the Ventanas smelter (Universal Transverse Mercator coordinates: E 268662; N 6371115). These soils, derived from stabilized paleodunes (Cosio et al., 2007), have a sandy-loam texture (Muena et al., 2010) and have been classified as Entisols (Soil Survey Staff, 2003). Los Maitenes has been described as the area most affected by the deposition of particulate matter from the smelting operation (Pinochet et al., 1999; De Gregori et al., 2000). Approximately 0.2 m^3 of soil was collected from each of four depths: 0–15, 15–30, 30–45 and 45–60 cm. Soil was air-dried for approximately one week and then homogenized in a plastic-covered mixer.

2.2. Soil analysis

Samples from each soil layer were passed through a 2 mm sieve and oven-dried at 40 °C for 48 h. The total copper content was determined after the acid digestion of the samples with nitric and perchloric acid (adapted from Verlinden, 1982). To measure the pH, a solution of 0.1 M KNO$_3$ was used as an extractant, with a ratio of soil to extractant of 1:2.5 (Stuckey et al., 2008). Copper concentrations were determined using atomic absorption, with a detection limit of 0.001 mg L^{-1}. The water holding capacity was determined gravimetrically after saturation and drainage (ISO, 2003). The organic matter content was determined by the Walkley Black routine method (Sadzawka et al., 2006).

To assure the quality of the results, a standard reference was also analyzed for copper, zinc, and lead (SRM 2711, agricultural soil from Montana, from the National Institute of Standards and Technology). The difference between the values obtained in this study and the certified ones were less than 10%.

2.3. Chemical characteristics of the soil

The total copper concentration in the upper layer (0–15 cm) is 710 ± 69 mg kg^{-1}. This value is high in comparison with soils that are located further from the smelter (for example, 60 ± 1.7 mg kg^{-1} in Maitencillo, 11 km north of the Ventanas copper smelter, Muena et al., 2010). In the deepest layer (45–60 cm), the total copper concentration is 51 ± 9.2 mg kg^{-1}. The pH is strongly acidic in the upper layer (pH 4.8 ± 0.04) and gradually becomes moderately acidic (pH 5.7 ± 0.12) in the deepest layer. Neaman et al. (2009) attribute this to the sandy loam texture of the topsoils that allowed rapid water infiltration but did not allow equilibrium to be reached between the rain water and the soil constituents. Another factor restricting the leaching of protons and metals is the low rainfall in this area, which is characteristic of a semi-arid Mediterranean zone, with an average annual precipitation of 300 mm (Cosio et al., 2007).

The total concentrations of Zn and Pb in the soil (Supplementary material, Table 1) are below the acceptable concentrations

according to international standards (e.g. 300 mg kg^{-1} for both metals according to Council of the European Communities (2009). However, they are high when compared to the background concentrations in uncontaminated soils in central Chile (for example, 29 ± 0.43 mg kg^{-1} and 85 ± 0.72 mg kg^{-1} of Pb and Zn, respectively, in Maitencillo; Muena et al., 2010).

2.4. Sampling and propagation of plant material

Seeds from various *O. picensis* individuals were collected from Los Maitenes. The seeds were propagated in a 1:1 mix of perlite and peat, under shade (T° min/max 7–29 °C), and irrigated daily.

2.5. Chelate

The chelate used in this study was methylglycinediacetic acid (MGDA), commercially known as liquid *Trilon M*, in the form of trisodic salt (Na$_3$MGDA). This chelate degrades at a rate of 90 to 100% within 14 d (Brenntag Canada Inc., 2009). In comparison, EDTA shows no degradation after 30 d (Brenntag Canada Inc., 2007).

Research on the metal extraction efficiency of MGDA has found it to be similar to EDTA (Borowiec et al., 2007; Cao et al., 2007), considerably decreasing the quantity of metal leached through the soil profile, but without the negative effects on rhizosphere microorganisms (Grěman et al., 2003; Chen et al., 2005). As with other biodegradable chelates, MGDA is currently being used to replace synthetic chelates, which are becoming less widely used, in some cases by legislation, due to increasing knowledge of their harmful environmental effects (for example, European Chemical Bureau, 2004).

2.6. Temporal dynamics and effects of MGDA on copper leaching

A preliminary study was done to evaluate the effect of the chelate and of rainfall on copper leaching from the soil. The test was done at the School of Agronomy, in PVC columns of 60 cm long, 20 cm diameter. The soil was packed into the columns layer by layer, with three replicates per treatment. Soils were then saturated to 100% of their water holding capacity. Rhizons (soil solution extractors) with a pore diameter of 0.1 μm (Rhizosphere Research Products, www.rhizosphere.com), were installed at 10, 25 and 40 cm depths (3 at each depth) and then connected to 20 mL syringes (Fig. 1). After 4 h (the necessary drainage time for these columns according to previous observations), the soil solution was collected by disconnecting the filled syringes from the Rhizons. The samples were acidified (with concentrated HCl, 10 μL per 10 mL) and the concentration of soluble copper was determined. This first sample corresponded to the soluble copper concentration of the soil solution before the application of a chelate.

On the second day, 5 mmol of MGDA dissolved in 200 mL of water was applied to each column. The syringes were reconnected to the Rhizons; 4 h later the solution was extracted, acidified, and the soluble Cu concentration was determined. For 55 d, Rhizon samples were collected daily, with applications of 250 mL of water to replace the sample volume removed (maximum 180 mL d^{-1}) and the humidity lost to evapotranspiration (47 to 66 mL d^{-1} in Quillota, Ferreyra et al., 2009).

On days 16 and 17, 10 mm of water were added to each column to simulate an episode of light rainfall. On days 18 and 31, 32 mm of water were added to simulate episodes of heavier rainfall, similar to the heaviest rainfalls recorded in the area for the previous year (32 mm in Quillota in 2007; Pontificia Universidad Católica de Valparaíso, 2009). This was one way to evaluate the effect of the chelate on potential for soil leaching under the harshest conditions observed in this area (for Los Maitenes, the highest recorded
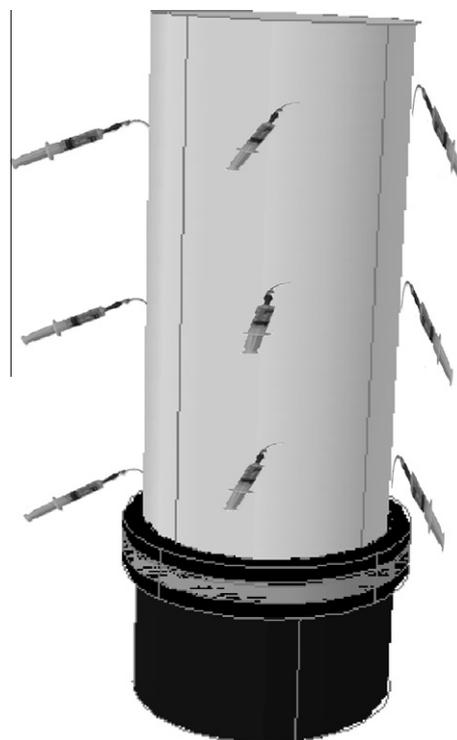

Fig. 1. Diagram of Rhizon placement for evaluation of the effects of MGDA applications and rainfall on copper leaching through soil columns.

rainfall was 12 mm in 1 h in 2007, Corporación Nacional del Cobre (CODELCO, 2009).

2.7. Induced phytoextraction trials

The soil layers were packed into 20 cm diameter PVC columns of 30, 45 and 60 cm height (Fig. 2). For each MGDA rate, there were three replicates for the column of each height (total 36 columns, Fig. 3). The different column heights were included in order to collect leachate from different soil depths as a simulation of the leaching that could take place at the given depths in the field. To collect the leachate, a series of meshes was set at the base of each tube, consisting of plastic mesh (65%, cells of 2 × 3 mm), nylon stocking (pantyhose with cells of approximately 0.5 mm), and plastic mesh (65%, cells of 2 × 3 mm). Additionally, a 20 cm PVC recipient of equal diameter was attached under each column, and sealed with plastic film (Fig. 2). For the biomass and plant copper concentration analyses, each column was considered a replicate of the same treatment, regardless of height, as *O. picensis* roots do not penetrate below 20 cm in the first year of growth (previous field observations). Thus, there were 9 replicates for each MGDA application rate (Fig. 3).

The soil in the columns was wetted to 80% of water holding capacity and one-month-old seedlings of *O. picensis* were transplanted. The trial took place in the open air to simulate natural conditions and began in mid-autumn (April), along with the first rains, which ended 9 months later, at the beginning of summer (December). Total precipitation during the experimental period was 320 mm (Pontificia Universidad Católica de Valparaíso, 2009). Only in the case of clear evidence of soil drying, at the end of the rainy period, 200 mL of water was applied to each column, every 3 d. The maximum and minimum temperatures during this period fluctuated between 0.4 and 29 °C (Pontificia Universidad Católica de Valparaíso, 2009).
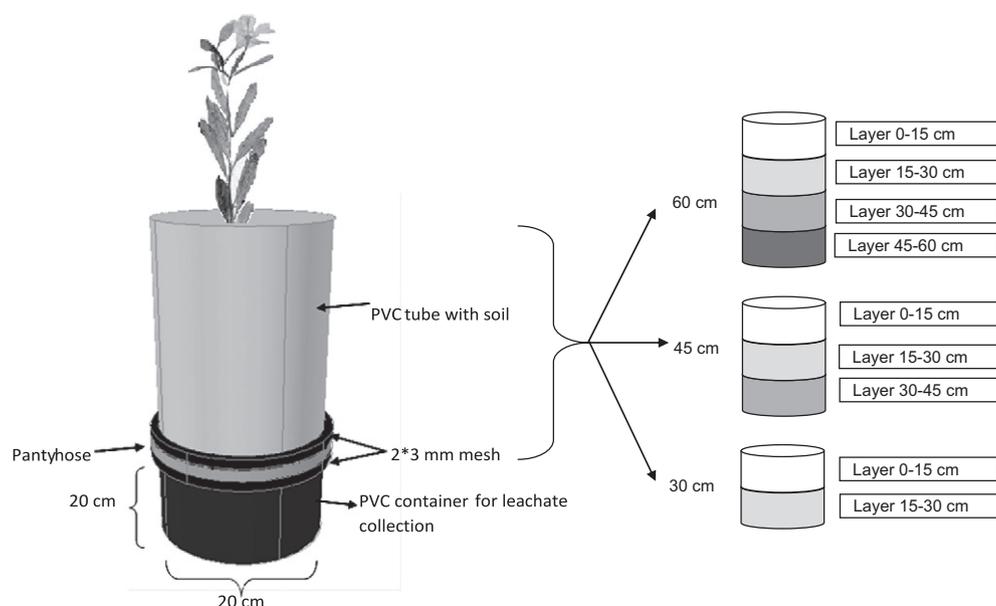
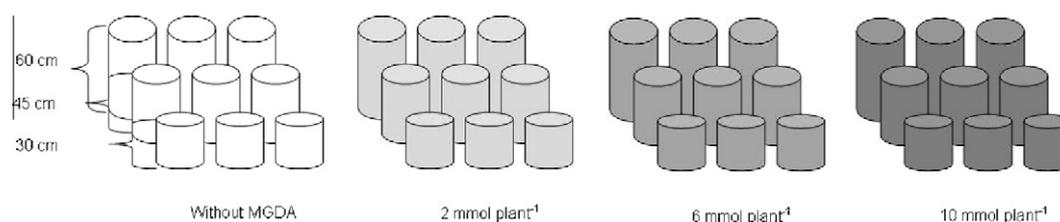**Fig. 2.** Diagram of the assembly of the experimental unit.

Fig. 3. Diagram of the experimental design.

After 7 months of plant growth, MGDA was applied. Based on the results of other studies (Shen et al., 2002; Fernandez, 2005), three MGDA rates were used: 1, 3, and 5 mmol plant^{-1}, plus a control with no MGDA. Application of MGDA at the same rates (1, 3, and 5 mmol plant^{-1}) was done one month after the first application, with the objective of maintaining a more constant level of bioavailable metal in the upper soil layer. Thus, the total MGDA rates were: 2, 6, and 10 mmol plant^{-1}, plus a control with no MGDA. The lapse between the first and second applications was defined based on the results from the preliminary experiment, indicating that one month after treatment with 5 mmol plant^{-1} the availability of the chelate in the upper soil layers had begun to decrease.

The repeated application used in this experiment was based on reports of increased metal accumulation in shoots achieved by other researchers following this method (Shen et al., 2002). This is due to the fact that repeated applications of chelate in small concentrations solubilize only a fraction of the metal that the plant is capable of absorbing. Alternately, a single, more concentrated application can solubilize a large amount of metal in the soil, making it impossible for the plants to completely absorb all of it, and thus provoking leaching of the non-absorbed fraction (Shen et al., 2002).

2.8. Analysis of plant material

One month after the second MGDA application, the plants were harvested and separated into their component structures (roots, leaves, and stems). The aerial biomass was washed with the following sequence: tap water, HCl 0.1 M, distilled water (Madejón et al., 2002). Roots were washed in the following sequence: tap water, HCl 0.1 M, distilled water, EDTA 0.05 M and distilled water (Ginocchio et al., 2002). The samples were then dried for 48 h at 60 °C and total biomass was determined. The dried material was then ground in a stainless steel mill. The total copper concentration in each type of plant structure was analyzed using atomic absorption spectrophotometry using routine methods (Sadzawka et al., 2007). Effective copper extraction for each plant was calculated by multiplying the total biomass by the concentration of copper in the aerial biomass of each individual.

Quality control of the plant analysis was measured against two reference materials for copper (tomato 1573a and spinach 1710a from the National Institute of Standards and Technology). The difference between the values obtained in this study and the certified ones were less than 10%.

2.9. Leachates analysis

One month after the second MGDA application, the leachates were collected from the base receptacles. The leachates were filtered through a 0.2 μm syringe filter, and acidified with concentrated HCl (10 μL for each 10 mL), after which they were analyzed for soluble copper concentration with atomic absorption spectrophotometry.

2.10. Statistical analysis

The differences between treatments were determined statistically for the following variables: aerial biomass production, foliar copper concentrations, and total Cu extraction. For this purpose,

analysis of variance (ANOVA) with a Tukey's test at 95% confidence was performed, using the Minitab for Windows software program. However, it was not possible to complete the statistical analysis for copper concentrations in the leachates from columns at the end of the study because, in some cases, leachate was obtained from only one replicate.

3. Results and discussion

3.1. Dynamics of copper leaching with MGDA

After MGDA application, the concentration of soluble copper in the upper soil layer (0–15 cm) increased from 0.23 mg L^{-1} on day 0, up to a maximum of 135 mg L^{-1}, 5 d after the chelate was applied (Fig. 4). After that, the soluble copper concentration gradually decreased until day 15. This coincides with the results seen by Römkens et al. (2002), in which 0.01 M EGDA (Ethylene glycol diacetate acid) was applied to a sandy soil. Through extraction of the soil solution with lysimeters at 60 cm depth, the maximum concentration of soluble Cu was found between 2 and 5 d after application.

From day 0 until day 15, the chelate application showed no effect on the concentration of soluble Cu in the second layer (15–30 cm). Leaching of copper was observed from the first layer down to the second, on days 16 and 17, during the simulation of the two light rains (10 mm). Consequently, the concentration of soluble Cu decreased in the first layer and increased in the second (Fig. 4).

On day 18, increased leaching of copper was observed from the first layer down to the second, after the simulation of a heavy rain (32 mm), with a sharp increase in soluble Cu concentration in the second layer. On day 19, the soluble Cu concentration in the second layer (66 mg L^{-1}) surpassed that of the first (24 mg L^{-1}). Afterwards, from day 20 to day 32, the soluble Cu concentration gradually decreased in both layers. On day 32, after the second heavy rain (32 mm) simulation, leaching from the first layer to the second was almost imperceptible (Fig. 4). After this point, soluble Cu concentrations in both layers decreased by day 55, when they had reached values similar to the initial measurements. Thus, under the conditions of this study, the period of chelating action of a single application of MGDA lasted for 55 d. The concentration of soluble Cu in the third layer (30–45 cm) did not vary during the evaluation period, maintaining values near 0.004 mg L^{-1} (the detection limit). The application of MGDA definitely increased the solubility of copper in the first layer. However, it did not induce
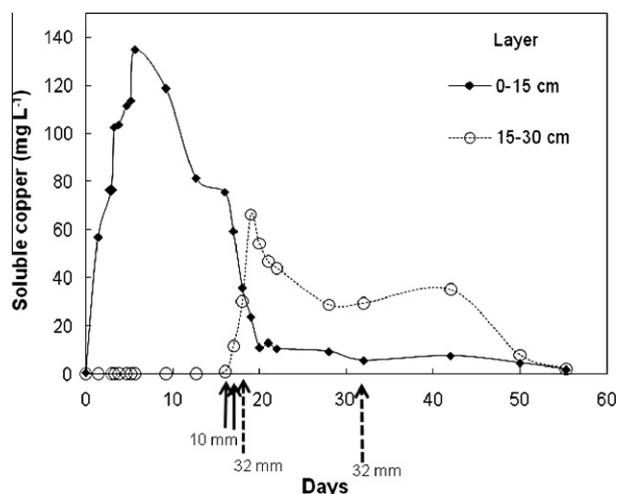
leaching of metal into the deeper layers without an extra water supply. The addition of water caused the leaching of MGDA chelated copper from the first layer to the second. Therefore, future field applications of this chelate would be best applied once the period of the heaviest rains has passed, in the spring. This deduction coincides with that of Römkens et al. (2002) in their study of EGDA on sandy soils.

3.2. Effect of MGDA on copper extraction and biomass production of O. picensis

The application of MGDA at concentrations of 6 and 10 mmol plant^{-1} significantly increased the biomass production of O. picensis ($p = 0.013$), while the effect of 2 mmol plant^{-1} treatment was not significant (Fig. 5). However, there were signs of light stress (browning and chlorosis) in the 6 and 10 mmol plant^{-1} treatments. Biomass production was 1.5 times greater in the 6 and 10 mmol plant^{-1} treatments than in the control, although there was no significant difference between these two treatments. It would therefore be advisable to evaluate the effects of increasing concentrations of MGDA on O. picensis biomass, in order to determine the maximum concentration that could be tolerated without impairing growth and development.

In a study by Lou et al. (2007), vetiver (Vetiveria zizanioides, a metal tolerant species) showed an increase in biomass after the application of HEDTA in soils that were contaminated with Pb, Cu and Zn. Grěman et al. (2001) reported that there was no variation in the biomass of Brassica rapa after application of 11 mmol plant^{-1} of EDTA in Pb-contaminated soils. In another study, Grěman et al., 2003 observed no change in biomass of Trifolium pratense with application of 11 mmol plant^{-1} of EDTA or EDDS in Pb-contaminated soils. In contrast, Shen et al. (2002) reported significant reductions in biomass production for Brassica olarecea, Vigna radiata and Triticum sp. after applying 3 mmol plant^{-1} of EDTA in Pb-contaminated soils.

It is reasonable to expect that Cu tolerant species, such as O. picensis and V. zizanioides, would tolerate higher metal concentrations without showing diminished biomass production, than would intolerant species, from which it would be more likely to expect symptoms of toxicity.

Copper is an essential metal for plant growth and development, although it is also potentially toxic. Copper participates in numerous physiological processes and is an essential cofactor for many metalloproteins; however, problems arise when excess copper is present in cells. Excess copper inhibits plant growth and impairs important cellular processes (i.e., photosynthetic electron transport). Since copper is both an essential cofactor and a toxic element, involving a complex network of metal trafficking pathways, different strategies have evolved in plants to appropriately regulate its
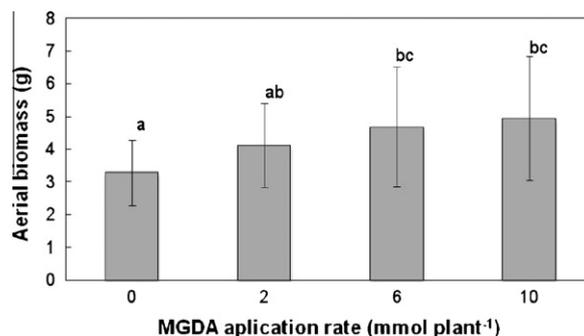
Fig. 4. Temporal variation of copper solubility after the application of 5 mmol of MGDA. The amount of water applied to the column is indicated.

Fig. 5. Effect of the MGDA application on the aerial biomass production of O. picensis. Vertical bars indicate the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

homeostasis as a function of the environmental copper level. Such strategies must prevent accumulation of the metal in the freely reactive form (metal detoxification pathways) and ensure proper delivery of this element to target metalloproteins. The mechanisms involved in the acquisition of this essential micronutrient have not been clearly defined although a number of genes have recently been identified which encode potential copper transporters (Yruela, 2005).

The Cu concentration in the leaves and stems increased significantly with the concentration of MGDA application ($p = 0.002$ for stems and $p < 0.001$ for leaves, Fig. 6). The Cu concentrations were significantly higher in the 6 and 10 mmol plant^{-1} treatments, than in the control, and the 2 mmol plant^{-1} treatment. The average Cu concentration in the plants was 9 times higher in the 10 mmol MGDA treatment than in the control (from 29 ± 5.9 mg kg^{-1} to 260 ± 99 mg kg^{-1}) (data not shown in Fig. 7). The Cu concentration in the roots was not affected by the treatments, with an average of 656 ± 605 mg kg^{-1}.

Data of plants sampled in the field (González et al., 2008) have shown Cu concentrations in the aerial biomass of 614 ± 4.6 mg kg^{-1}, a much higher level than was observed in this study. However, as the plants in the field corresponded to individuals of variable ages and cycles of growth, it can be supposed that the foliar Cu concentration of plants could increase with age. In addition, the possibility that the field samples were contaminated due to the strong adherence of Cu particles to the trichomes of *O. picensis* leaves has not been discarded.

Coincidentally, in a similar study by Meers et al. (2004) and Lombi et al. (2001), foliar Cu concentrations in *Zea mays* were 2 to 13 times higher with the use of EDTA at rates similar to those of the present study, and 2.2 and 1.2 times higher with the use of DTPA and NTPA at similar rates.
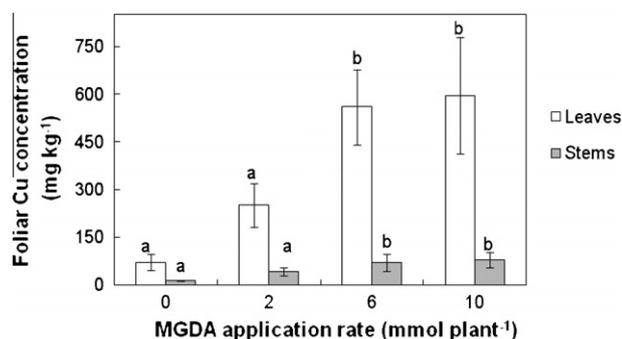

Fig. 6. Effect of the MGDA application on copper concentrations in stems and leaves of *O. picensis*. Vertical bars indicate the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).
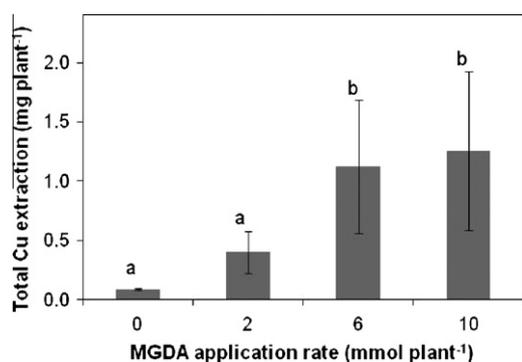

Fig. 7. Effect of the MGDA application on the total Cu extraction by *O. picensis*. Vertical bars indicate the standard deviation. Different letters indicate significant differences between treatments ($p < 0.05$).

Phytoextraction of copper was increased, on average, 14 times for the 6 and 10 mmol plant^{-1} MGDA treatments in relation to the control ($p < 0.01$, Fig. 7). This increase in Cu extraction would allow a notable reduction in the time needed for remediation of a given surface area of contaminated soil if no toxicity appears at the next time.

3.3. Effect of MGDA application on leaching of copper

The copper concentration of the leachate from the control was very low, 0.004 mg L^{-1} in the 30 cm columns, and undetectable in the higher columns (<0.001 mg L^{-1}). The Cu concentration in the leachate from the 30 cm columns was 16 to 20 times higher with the application of MGDA, reaching a maximum of 0.075 mg L^{-1} at 10 mmol plant^{-1}. The Cu concentration in the leachate from the 45 cm columns was 4–16 times higher than the control, reaching a maximum of 0.02 mg L^{-1} with 10 mmol plant^{-1}. The Cu concentration in leachate from the 60 cm columns was up to 2 times higher than in the control. There was a clear decrease in the Cu leachate with increasing soil column heights.

Even though there was an overall increase in Cu leaching with the application of MGDA, these Cu concentrations could be considered low, as the maximum allowable amount for irrigation water in Chile, according to NCh 1333, is 0.2 mg L^{-1} (Instituto Nacional de Normalización, 1978). Nonetheless, the comparison with an irrigation water standard could lead to an error, as from the moment that the leachate passes beyond the effective root zone, it is no longer a factor for irrigation, but it does constitute a potential risk for groundwater contamination.

Currently, there is no Chilean legal standard for metal concentrations in groundwater. Yet it should be noted that aquatic organisms are much more sensitive than terrestrial species to copper toxicity, with toxicity generally occurring between 6.9 and 10.8 μg L^{-1} (Adriano, 2001). In this study, a concentration of 8 μg L^{-1} of soluble copper in the leachate was obtained from the 60 cm columns, with the 10 mmol plant^{-1} treatment. This concentration is within the toxic range for aquatic organisms. However, there would be a dilution factor for this leachate upon reaching the water table that should also be considered.

The Cu concentration of the leachate from the 60 cm columns was 6–10% of the concentration of the leachate obtained from the 30 cm columns. Considering this and the fact that the water table in the study area is at least 11 m below the surface (personal communication from the inhabitants of this area), it is unlikely that the leachate would reach depths greater than 1 m, under the evaluated conditions. Consequently, it is possible to affirm with a high degree of certainty, that the application of MGDA at the rates used in this study would not endanger the groundwater quality in the areas near the Ventanas smelter.

On the other hand, the leaching of copper that has been solubilized by the chelate is triggered by rainfall (Fig. 4), so it can be deduced that, in the case of little or no precipitation, leaching would be even less than in this study. The climate of central Chile is characterized by a concentration of the rainfall in winter (Di Castri, 1981). Therefore it is recommended that chelate applications will be done in the spring, after the heaviest rains have passed (coinciding with the period of maximum vegetative growth for this species), in order to minimize the risk of leaching.

4. Conclusion

Copper extraction by *O. picensis* in Cu-contaminated acid soils was increased 14 times by the addition of 6 to 10 mmol plant^{-1} of MGDA. Although the application of MGDA did increase the

leaching of copper, this leaching was drastically diminished with depth, making it less likely to contaminate groundwater.

The application of this biodegradable chelate is recommended for the soils near the Ventanas smelter, as it will considerably reduce the time necessary for phytoextraction of metal from this soil.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.03.015.

References

Adriano, D., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risk of Metals, second ed. Springer Verlag, New York, New York.

Ávila, G., Gaete, H., Morales, M., Neaman, A., 2007. Reproducción de *Eisenia foetida* en suelos agrícolas de áreas mineras contaminadas por cobre y arsénico. Pesq. Agropec. Bras. 42, 435–441.

Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements: a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81–108.

BASF, 2007. Data Sheet: Trilon M Types. Ludwigshafen, Germany, p. 14.

Borowiec, M., Polanska, P., Hoffmann, J., 2007. Biodegradability of the compounds introduced with microelement fertilizers into environment. Polish J. Chem. Technol. 9, 38–41.

Brenntag Canada Inc., 2007. Data sheet BASF Trilon B (EDTA), liquid, p. 5.

Brenntag Canada Inc., 2009. Data sheet BASF Trilon M, liquid, p. 5.

Cao, A., Carucci, A., Lai, T., La Colla, P., Tamburini, E., 2007. Effect of biodegradable chelates on heavy metals phytoextraction with *Mirabilis jalapa* and its associated bacteria. Eur. J. Soil Biol. 43, 200–206.

Carsten, S., Heinz-Jürgen, B., 2004. Impact of aminopolycarboxylates on aquatic organisms and eutrophication: overview of available data. Environ. Toxicol. 19, 620–637.

Chen, Y.X., Lin, Q., Luo, Y.M., He, Y.F., Zhen, S.J., Yu, Y.L., Tian, G.M., Wong, M.H., 2003. The role of citric acid on the phytoremediation of heavy metal contaminated soil. Chemosphere 50, 807–811.

Chen, Y., Luang, Y., Wu, W., Lin, Q., Xue, S., 2005. Impacts of chelate-assisted phytoremediation on microbial community in the rhizosphere of a copper accumulator and non-accumulator. Sci. Total Environ. 356, 247–255.

Corporación Nacional del Cobre (CODELCO), 2009. Datos Meteorológicos de la Estación Central: temperatura, viento, humedad y precipitaciones. Puchuncaví, Chile.

Coscienes, A., Abreu, C., Gabrielli dos Santos, G., 2009. Chelatesto solubilize heavy metals from oxisols contaminated by the addition of organic and inorganic residues. Sci. Agric. 66, 64–70.

Cosio, F., Negrón, M., Gastó, J., Villate, G., 2007. Distritos y Sitios de la Provincia Templada Secoestival Nubosa o Valparaíso: secano de la costa. RC Suelo Nutr. Veg. 7, 38–61.

Council of the European Communities, 2009. Corrigendum to the Council Directive 86/278/EEC of 12 June 1986 on the Protection of the Environment, and in Particular of the Soil, When Sewage Sludge is used in Agriculture. Document Number 31986L0278R(01). <http://eur-lex.europa.eu/RECH_celex.do> (accessed 24.01.11).

De Gregori, I., Fuentes, E., Rojas, M., Pinochet, H., Potin-Gautier, M., 2003. Monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile. J. Environ. Monitor. 5, 287–295.

De Gregori, I., Lobos, G., Lobos, S., Pinochet, H., Potin-Gautier, M., Astruc, M., 2000. Copper and selenium in rainwater, soils and alfalfa from agricultural ecosystems of Valparaíso region, Chile. J. Environ. Monitor. 45, 131–146.

Di Castri, F., 1981. Mediterranean-type shrublands of the World. In: Di Castri, F., Goodall, D.W., Specht, R. (Eds.), Mediterranean-Type Shrublands. Elsevier, Amsterdam, The Netherlands, pp. 1–52.

Ernst, W.H., 1996. Bioavailability of heavy metals and decontamination of soil by plants. Appl. Geochem. 11, 163–167.

European Chemical Bureau, 2004. European Union Risk Assessment Report: Tetrasodium Ethylenediaminetetraacetate (Na$_4$EDTA). <http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/na4edtareport062.pdf> (accessed 21.01.11).

Fernandez, D., 2005. Fitoextracción inducida de Cr en un suelo calcáreo. Máster Experimental en Ciencias Farmacéuticas. Departament de Productes Naturals, Biología Vegetal i Edafología. Universidad de Barcelona, Barcelona, 143 pp.

Ferreyra, R., Selles, G., Gil, P., 2009. Asfixia radicular: evidencia y mitigación en paltos. Revista Fedefruta 124, 5–11.

Folchi, M.A., 2006. Historia ambiental de las labores de beneficio en la minería del cobre en Chile, siglos XIX y XX. Tesis de doctorado. Departamento de Economía y de Historia Económica. Universidad Autónoma de Barcelona, Barcelona, 727 pp.

Ginocchio, R., 2000. Effects of a copper smelter on a grassland community in the Puchuncaví Valley, Chile. Chemosphere 41, 15–23.

Ginocchio, R., Rodríguez, P., Badilla-Ohlbaum, R., Allen, H., Lagos, G., 2002. Effect of soil copper content and pH on copper uptake of selected vegetables grown under controlled conditions. Environ. Toxicol. Chem. 21, 1736–1744.

González, I., Muena, V., Cisternas, M., Neaman, A., 2008. Acumulación de cobre en una comunidad vegetal afectada por contaminación minera en el valle de Puchuncaví, Chile central. Rev. Chil. Hist. Nat. 81, 279–291.

Grēman, H., Velikonja-Bolta, S., Vodnik, D., Kos, B., Leštan, D., 2001. EDTA enhanced heavy metal phytoextraction: Metal accumulation, leaching, and toxicity. Plant Soil 235, 105–114.

Grēman, H., Vodnik, Š., Velikonja-Bolta, S., Leštan, D., 2003. Ethylendiaminedissuccinate as a new chelate for environmentally safe enhanced lead phytoextraction. J. Environ. Qual. 32, 500–506.

Huang, J.W., Chen, J., Berti, W.R., Cunningham, S., 1997. Phytoremediation of lead contaminated soil: role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol. 31, 800–805.

Instituto Nacional de Normalización, 1978. Norma Chilena Oficial NCh 1333. Requisitos de calidad del agua para diferentes usos. Santiago, Chile, 12 pp.

ISO, 2003. Soil Quality-determination of the Effects of Pollutants on Soil Flora-Part 1: Method for the Measurement of Inhibition of Root Growth. ISO 11269-2. International Organization for Standarization, Geneva, Switzerland.

Lombi, E., Zhao, F.J., Dunham, S.J., McGrath, S.P., 2001. Phytoremediation of heavy metal-contaminated soils: Natural hyperaccumation versus chemically enhanced phytoextraction. J. Environ. Qual. 30, 1919–1926.

Lou, L.-Q., Ye, Z.H., Wong, M.H., 2007. Solubility and accumulation of metals in chinese brake fern, vetiver and rostrate sesbania using chelating agents. Int. J. Phytorem. 9, 325–343.

Luo, C., Shen, Z., Li, X., 2004. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59, 1–11.

Madejón, P., Murillo, J., Marañón, T., Cabrera, F., López, R., 2002. Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain). Sci. Total Environ. 290, 105–120.

Meers, E., Hopgood, M., Lesage, E., Vervaeke, P., Tack, F.M., Verloo, M.G., 2004. Enhances phytoextraction: in search of EDTA alternatives. Int. J. Phytorem. 6, 95–109.

Muena, V., González, I., Neaman, A., 2010. Efectos del encalado y la fertilización nitrogenada sobre el desarrollo de *Oenothera picensis* en un suelo afectado por la minería del cobre. RC Suelo Nutr. Veg. 10, 102–114.

Neaman, A., Reyes, L., Trolard, F., Bourrié, G., Sauvé, S., 2009. Copper mobility in contaminated soils of the Puchuncaví valley, central Chile. Geoderma 150, 359–366.

Nörtemann, B., 1999. Biodegradation of EDTA. Appl. Microbiol. Biotechnol. 51, 751–759.

NRC, 2003. Bioavailability of contaminants in soils and sediments: Processes, tools and applications. Commission on Bioavailability of Contaminants in Soils and Sediments. National Academy Press, Washington, DC, USA.

Oviedo, C., Rodríguez, J., 2003. EDTA: the chelate under environmental scrutiny. Quim. Nova 26, 901–905.

Pinochet, H., De Gregori, I., Lobos, G., Fuentes, E., 1999. Selenium and copper in vegetables and fruit grown on long-term impacted soils from Valparaíso region, Chile. Bull. Environ. Contam. Toxicol. 63, 327–334.

Pontificia Universidad Católica de Valparaíso, 2009. Datos Meteorológicos de los años 2007–2008. Estación Meteorológica de la Facultad de Agronomía, Quillota, Chile.

Robinson, B., Schulin, R., Nowack, B., Roulier, S., Menon, M., Clothier, B., Green, S., Mills, T., 2006. Phytoremediation for the management of metal flux in contaminated sites. For. Snow Landsc. Res. 80, 221–234.

Römkens, P., Bowman, L., Japenga, J., Draaisma, C., 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils. Environ. Pollut. 116, 109–121.

Sabatini, F., Mena, F., Vergara, P., 1996. El conflicto ambiental de Puchuncaví bajo democracia XII, 30–40.

Sadzawka, A., Carrasco, M., Grez, R., Mora, M., Flores, H., Neaman, A., 2006. Métodos de Análisis Recomendados para los Suelos de Chile. Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Sadzawka, A., Carrasco, M.A., Demanet, R., Flores, H., Grez, R., Mora, M.L., Neaman, A., 2007. Métodos de Análisis de Tejidos Vegetales. Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Schmidt, U., 2003. Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J. Environ. Qual. 31, 1939–1954.

Shen, Z.-G., Li, X.-D., Wang, C.-C., Chen, H.-M., Chua, H., 2002. Lead phytoextraction from contaminated soil with high-biomass plant species. J. Environ. Qual. 31, 1893–1900.

Soil Survey Staff, 2003. Keys to Soil Taxonomy. United States Department of Agriculture, Natural Resources Conservation Service, Washington, USA.

Stuckey, J., Neaman, A., Ravella, R., Komarneni, S., Martínez, C.E., 2008. Highly charged swelling mica reduces free and extractable Cu levels in Cu-contaminated soils. Environ. Sci. Technol. 42, 9197–9202.

Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., Nowack, B., 2004. Extraction of heavy metals from soils using biodegradable chelating agents. Environ. Sci. Technol. 38, 937–944.

Verlinden, M., 1982. On the acid decomposition of human blood and plasma for the determination of selenium. Talanta 29, 875–882.

Wu, J., Hsu, F.C., Cunningham, S., 1999. Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. Environ. Sci. Technol. 33, 1898–1904.

Yruela, I., 2005. Copper in plants. Braz. J. Plant Physiol. 17, 145–156.