HOT NTA APPLICATION ENHANCED METAL PHYTOEXTRACTION FROM CONTAMINATED SOIL

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Abstract. To increase the phytoextraction efficiency of heavy metals and to reduce the potential negative effects of mobilized metals on the surrounding environment are the two major objectives in a chemically enhanced phytoextraction process. In the present study, a biodegradable chelating agent, NTA, was added in a hot solution at 90°C to soil in which beans (*Phaseolus vulgaris* L., white bean) were growing. The concentrations of Cu, Zn and Cd, and the total phytoextraction of metals by the shoots of the plant from a 1 mmol kg^{-1} hot NTA application exceeded those in the shoots of plants treated with 5 mmol kg^{-1} normal NTA and EDTA solutions (without heating treatment). A significant correlation was found between the concentrations of metals in the shoots of beans and the relative electrolyte leakage rate of root cells, indicating that the root damage resulting from the application of a hot solution might play an important role in the process of chelate-enhanced metal uptake in plants. The application of hot NTA solutions did not significantly increase metal solubilization in soil in comparison with a normal application of solution of the same dosage. Therefore, the application of a hot NTA solution may provide a more efficient alternative in chemical-enhanced phytoextraction, although further studies of techniques of application in fields are sill required.

Key words: Hot NTA, phytoextraction, metals, beans, root damage

1. Introduction

The phytoextraction of toxic metals and metalloids from contaminated soils is

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becoming a competitive soil remediation technology because of its cost-effective and environmentally sound characteristics compared with other conventional technologies (Huang *et al.*, 1997; Garbisu and Alkorta, 2001). Plant biomass and metal concentrations in the harvestable parts of plants are the two determining factors for the success of this technique. To achieve higher biomass, some fast-growing crops such as corn, mustard and vetiver grass have been proposed for this process (Chen *et al.*, 2004a; Luo *et al.*, 2005). Metal solubility in soils can be improved by adding some chelating chemicals such as EDTA (ethylenediaminetetraacetic acid), EGTA [ethyleneglycol -bis (ß -aminoethyl ether), N, N, N', N-tetraacetic acid], CDTA (trans -1, 2 -diaminocyclohexane -N, N, N', N'-tetraacetic acid) and HEDTA (N-hydroxyethylenediaminetriacetic acid) (Blaylock *et al.*, 1997; Cooper *et al.*, 1999; Wu *et al.*, 1999; Shen *et al.*, 2002.).

Although EDTA has shown high efficiency in enhancing metal solubility and facilitating metal uptake in plants, the toxicity to plants and microorganisms, and the potential risk of leached metals associated with the application of EDTA because of its low biodegradability has excluded it as a good choice for use in practical applications (Bucheli-Witschel and Egli, 2001; Grčman *et al.*, 2003). Some easily biodegradable chelates such as NTA (nitrilotriacetate), EDDS (S,S-ethylenediaminedisuccinic acid), and organic acids such as citric acid have been tested for this purpose (Luo *et al.*, 2005; Meer *et al.*, 2005; Quartacci *et al.*, 2005; Luo *et al.*, 2006a).

It was reported that NTA can be degraded as fast as glucose and citric acid in soils, and can also rapidly be biodegraded under anaerobic conditions (Tiedje and Mason, 1974). Bucheli-Witschel and Egli (2001) observed a half-life of 2 to 7 d for NTA in sediments. In the last few years, the use of NTA has been proposed to enhance the uptake of heavy metals in soil phytoextraction (Kulli *et al.*, 1999; Kayser *et al.*, 2000).

Metal chelate complexes may enter plant tissues through breaks in the root endodermis and Casparian strips, and be rapidly transported to the shoots (Römheld and Marschner, 1981; Bell *et al.*, 1991). Our previous study showed some physical damage to the roots caused by hot water treatment, or the addition of acid can facilitate the uptake of metals by plants (Luo *et al.*, 2006b). The aims of this present study were: (i) to investigate whether soil amendments with biodegradable NTA and citric acid, in comparison to EDTA, added in hot solutions can further enhance the uptake of heavy metals by plants from metal-contaminated soils; and (ii) to further study, using hydroponic experiments, the possible mechanisms involved in NTA-induced metal accumulation in plants.

2. Materials and Methods

2.1. SOIL PREPARATION

Soil samples were collected from a disused agricultural field in the Yuen Long area of Hong Kong. The samples were sieved to pass through a 2 mm sieve and air-dried for one week. The soils were artificially contaminated with Cu (400 mg kg^{-1} of soil) in the form of $CuCO_3$ (copper carbonate); Pb (500 mg kg^{-1} of soil) as $Pb_3(OH)_2(CO_3)_2$ (lead hydroxide carbonate) and PbS (lead sulfide – galena, a common lead mineral in mining areas) at a Pb concentration ratio of 1:1; Zn (500 mg kg^{-1} of soil) in the form of $ZnCO_3$ (zinc carbonate) and ZnS (zinc sulfide) at a Zn concentration ratio of 1:1; and Cd (15 mg kg^{-1} of soil) in the form of $Cd(NO_3)_2{\cdot}4H_2O$ (cadmium nitrate). The basal fertilizers applied to the soil were 80 mg P kg^{-1} of dry soil, and 100 mg K kg^{-1} of dry soil as KH_2PO_4 (Shen *et al.*, 2002). After the addition of heavy metals, the soils were equilibrated for two months, undergoing seven cycles of saturation with deionized water and air-drying processes.

The electrical conductivity (EC) of the soil was measured using a conductivity meter on the soil extract, obtained by shaking soil with double-distilled water at a water-to-soil ration of 1:2 (w/v). The soil pH was measured by 0.01 mol of $CaCl_2$ at a 1:5 ratio (w/v) using a pH meter. The cation exchangeable capacity (CEC) of the soil was determined using the ammonium acetate saturation method. The soil texture, organic matter content, total N and field water capacity were measured by the procedures described by Avery and Bascomb (1982). The total metal concentrations were determined by ICP-AES (Perkin-Elmer Optima 3300 DV) after strong acid digestion (4:1 concentrated HNO_3 and $HClO_4$ (v/v)) (Li *et al.*, 2001). The selected physical and chemical properties of the soil used in the present study are presented in Table 1.

2.2. HOT EDTA, CITRIC ACID AND NTA TREATMENTS

Air-dried soils (500 g) were placed in plastic pots (12 cm i.d. x 12 cm in height). The moisture of the soil was maintained to near field water capacity by adding deionized water (DIW) on a daily basis. Seeds of beans (*P vulgaris* L. white bean) were sown directly in the soils. After germination, the seedlings were thinned to four plants per pot. On the 21st day after the beans were sown, EDTA, citric acid and NTA were applied in the form of 100 ml Na_2EDTA, citric acid and Na_3NTA solutions to the surface of the soils in two different ways (as heated and non-heated solutions) at rates of 0 (control), 1.0, 3.0 and 5.0 mmol kg^{-1} of soil. The three chemicals were all from BDH Laboratory Supplies (Poole U.K.), with a minimum assay of above 99.5%. The hot solution treatments were conducted by adding boiled solution to the soil in the pots, with the final temperature of the soils being about 40 ºC. Three replicates were conducted for each treatment. All of the experiments were operated in a greenhouse under natural light. Air temperatures ranged from 16 to 21 ºC. All of the plants were harvested 7 d after the treatment by cutting the shoots 0.5 cm above the surface of the soil. The shoots were washed with tap water, rinsed with DIW, and dried at 70 ºC in a drying oven to a constant weight for dry weight measurements. The dried plant materials were ground using an agate mill.

2.3. EXTRACTING METALS WITH DIFFERENT NTA SOLUTIONS

About 4.0 g of soil (based on dry weight) were placed in a 50-mL polypropylene centrifuge tube. NTA solutions at rates of 0, 1, 3 and 5 mmol kg^{-1} of soil were added to the soil (at a soil-to-water ratio of 1:5 (w/v)) at different temperatures of 25 ºC (control), 40 ºC, 60 ºC and 80 ºC, respectively. Every treatment was replicated three times. The suspension was shaken for 30 min. After the centrifugation, the supernatant was filtered through a 0.45 μm paper filter (Whatman [Maidstone, UK] 42), acidified with HNO_3 and analyzed for metal concentrations by ICP-AES (Perkin Elmer 3000DV).

2.4. ROOT PRETREATMENT WITH HOT WATER

Seeds of beans (*P vulgaris* L. white bean) were sterilized in 0.1% (w / v) $HgCl_2$ for 10 min, and rinsed four times in deionized water before being placed on filter paper for germination. After germination, the plants of the same size were selected and transferred to 2-L polyethylene vessels containing a modified 0.2-strength Rorison's nutrient solution (Hewitt, 1966) with the following composition (in μmol L^{-1}): 400 $Ca(NO_3)_2$, 200 $Mg(SO_4)_2$, 50 K_2HPO_4, 300 KCl, 9.2 H_3BO_3, 1.8 $MnSO_4{\cdot}4H_2O$, 0.21 $Na_2MoO_4{\cdot}2H_2O$, 0.31 $CuSO_4{\cdot}5H_2O$, 10 $ZnSO_4{\cdot}7H_2O$ and 10.8 Fe-EDTA at pH 6.0. Nutrient solutions were aerated continuously and renewed every two days. The plants were grown in a greenhouse where the temperature ranged from 17 ºC to 22 ºC.

After 7 d of the transplanting, different pretreatments were conducted to assess the effects of roots damaged by hot water on the accumulation of Cu in shoots. Five pretreatments were included: the roots were exposed in hot water at 30 ºC, 40 ºC, 50 ºC, 60 ºC and 80 ºC for 15 min. Plants that were not subjected to pretreatment in hot water (that were placed in a room where the temperature was about 20 ºC) were used as the control. After pretreatments, eighteen plants from every treatment were used to assess the membrane permeability of the roots (the relative electrolytic leakage) by measuring the electrical conductivity (Zhu *et al.*, 1990; Zhou and Leul, 1998). The root samples (0.5 g) were placed in a test tube containing 15 ml of deionized water, and the root tissue was immersed and vibrated at room temperature for 2 h. The conductivity of the solution was measured using a conductivity meter (DDS - 11A). After boiling the samples for 10 min, the conductivity was measured again when the solution had cooled to room temperature. The relative electrical conductivity (REC) was calculated as follows: REC = C_1 / C_2 × 100, where C_1 and C_2 were the electrolyte conductivities measured before and after boiling, respectively. The remaining eighteen plants from every treatment group were exposed to 500 µmol L^{-1} of Cu + 500 µmol L^{-1} of NTA solutions for 2 d (pH = 6.0). Cu and NTA were added in the forms of $CuSO_4{\cdot}5H_2O$, Na_3NTA solutions, respectively. Every treatment was replicated three times. At the end of these experiments, the shoots and roots were harvested for further chemical analysis. The effects of root damage on the accumulations of Pb, Zn and Cd were studied in the same way, whereby Pb, Zn and Cd were applied in the forms of $Pb(NO_3)_2$, $ZnSO_4{\cdot}7H_2O$ and $CdNO_3{\cdot}4H_2O$ solutions, respectively.

2.5. PLANT AND SOIL ANALYSIS

Subsamples of ground shoot samples (200 mg) were digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, by volume), and the major and trace elements in the solutions were determined with ICP-AES (Chen *et al.*, 2004b). Certified standard reference material (SRM 1515, apple leaves) from the National Institute of Standards and Technology, U.S.A., was used in the digestion and analysis as part of the QA/QC protocol. Reagent blank and analytical duplicates were also used where appropriate to ensure accuracy and precision in the analysis. The recovery rates were around 90 ± 5% for all of the metals in the plant reference material. The data reported in this paper were the mean values based on the results of the three replicated experiments. Statistical analyses of the experimental data, such as correlation and significant differences, were performed using SPSS® 11.0 statistical software.

3. Results

3.1. PLANT GROWTH

The dry mass yields of beans are shown in Fig. 1. When the three chelates were added in normal solutions, EDTA prohibited plant growth the most, followed by the NTA application. The depressed effects on plant growth increased with the dosage of chelate. The application of citric acid did not have any significant effect on the growth of the plants ($P < 0.05$). When the chelates were added in hot solutions, a significant decrease in the dry biomass yields was observed in all the plants, in comparison with the non-heated treatments ($P < 0.05$). On average, on the 7th day after the application of the chelates, the dry biomass of the shoots decreased by 18%, 19% and 15% in the hot EDTA, citric acid and NTA applications, respectively, in comparison with the results observed in the treatments using a normal chelate solution.

3.2. METAL CONENTRATIONS AND PHYTOEXTRACTIONS IN HOT EDTA, CITRIC ACID AND NTA TREATMENTS

Compared with the control group, there was no significant change in the concentrations of heavy metals in the shoots after the addition of citric acid (Fig. 2). In contrast to this, the application of EDTA and NTA to the soil significantly increased the concentrations of Cu, Pb, Zn and Cd in the shoots. Generally, NTA was comparable to EDTA in increasing the concentrations of Cu, Zn and Cd in shoots. For Pb, NTA was significantly less effective than EDTA, although an enhanced uptake was also observed.

When the chelates were applied as hot solutions to the soils, the concentrations of metals in the shoots of beans increased greatly in comparison with the chelates applied in normal solutions at the same dosage (Fig. 2). The concentrations of Cu ranged from 878 to 1460, 60 to 128, and 540 to 1250 mg kg^{-1} in the shoots of beans treated with hot EDTA, citric acid and NTA, respectively. These were 5.1 - 8.3, 3.2 - 7.2 and 4.7 - 7.3 times the concentrations of those with the normal chelates treatments without heating, and 26.5 - 44, 1.8 - 3.9 and 16.3 - 37.8 times that in the control group (with the application of hot water only), respectively. The highest Pb concentration of

998 mg kg^{-1} was found in the shoots of beans treated with hot EDTA at the rate of 5 mmol kg^{-1}. The average enhanced effects of hot EDTA, citric acid and NTA on the Pb shoot uptake were 12.9, 3.5 and 10.1 times greater than those in the corresponding chelate treatments without heating. With regard to Zn and Cd, when EDTA, citric acid and NTA were applied at rates of 1 - 5 mmol kg^{-1}, the concentrations of Zn and Cd in the shoots of the beans were about 8.2 and 21 times those of the controls.

Results on the total metal phytoextraction by the shoots of beans are shown in Table 2. The maximum phytoextraction of Cu, Zn and Cd was found in the heated NTA treatment, which increased 47-, 6.5- and 18- fold, respectively, compared with the control group (to which normal water was added). With regard to Pb, the plants treated with 5 mmol kg^{-1} of hot EDTA attained the maximum level of phytoextraction of approximately 136-fold that of the corresponding control group.

3.3. METAL DISSOLUTION STUDY WITH THE ADDITION OF HOT NTA

In order to examine the effects of temperature on metal solubility, soil was extracted with NTA solutions at different temperatures (see Fig. 3). The concentrations of water-soluble metals in soil were mainly dependent upon the chelate application dosage. The soluble metal concentrations increased as levels of NTA applied to the soil increased. At the same application dosage, no significant differences were observed in the concentrations of soluble metals between the treatments with hot NTA solutions and those with normal NTA solutions (Fig. 3).

3.4. EFFECTS OF PRETREATMENT WITH HOT WATER ON THE ACCUMULATION OF CU IN BEANS

The roots of beans were pretreated with hot water at different temperatures before they were exposed in solutions containing 500 µmol L^{-1} of Cu + 500 µmol L^{-1} of NTA. Two days after the exposure, the concentrations of Cu in shoots were measured (Fig. 4). The results showed that there was a significantly positive correlation between the Cu concentration in shoots and the relative electrolyte leakage rate of root cells ($R^2 = 0.93$, $n = 18$). Similar significantly positive correlation results with R^2 0.90, 0.84 and 0.89 were also obtained for Pb, Zn and Cd, respectively ($n = 18$).

4. Discussion

Chelate-enhanced phytoextraction has been proposed as an effective technology for the cleaning up of metal-contaminated soils (Huang *et al.*, 1997; Wu *et al.*, 1999, Luo *et al.*, 2005). The present study showed that by adding an easily biodegradable chelate of NTA in a hot solution to the soil, the concentrations of metals, specifically Cu, Pb, Zn and Cd, in the shoots of beans increased by 55, 45, 7.4 and 19-fold respectively compared to those of the control group (to which normal water had been applied). The total levels of metal phytoextraction in the shoots of the plant were also enhanced to 25, 20, 5.4 and 10 times those seen in the control group, despite a drop in the production of biomass. The enhancement in metal uptake after the application of hot NTA solution achieved in the present study was significantly higher than those previous studies on NTA applications (Kulli *et al.*, 1999; Kayser *et al.*, 2000; Robinson *et al.*, 2000; Wenger *et al.*, 2002; Meers *et al.*, 2004; Quartacci *et al.*, 2005). In addition, the concentrations and metal phytoextraction of Cu, Pb, Zn and Cd in the shoots of beans treated with 1 mmol kg^{-1} of hot NTA were significantly higher than those achieved in the treatment with normal NTA solution at a rate of 5 mmol kg^{-1} soil. The results indicate that for a given phytoremediation efficiency, the application dosage of NTA can be reduced to 1/5 (20%) if the NTA is applied in a hot solution instead of a normal solution. Thus, the application cost of this strategy can be greatly reduced and the potential risk associated with the application of NTA of metals leaching to the surrounding area can also be reduced accordingly. It should be noted that in the current study, the plants were grown in the pots for only 28 days, which differed greatly from the field conditions. First, the pot was a closed container, which limited the added chelate to a small space. Second, the metal uptake capacity of plant varied a lot at different growth stages. The young seedlings were usually more sensitive to the chelate application than those at the mature age. Therefore, further field experiments are essential to test this result before this technology can be adopted on a large scale.

In soils, most heavy metals have low phytoavailability because they are usually strongly associated with organic matter, Fe-Mn oxides, clays and precipitation as carbonates, hydroxides and phosphates (McBride 1994). Once the chelate is applied into soils, it will solubilize metals from the soils and transfer them to the roots, which is the so-called stage I process (Ensley *et al.*, 1999). The stage II process involving the enhanced transfer of the mobilized metals to the shoots will take place afterwards. As shown in Fig. 3, compared with the addition of a normal NTA solution, the application of NTA in a hot solution does not further improve the solubility of metals from soils, although the application of NTA in a hot or normal solution caused the metals to be much more soluble than was seen with the control group (to which NTA had not been applied). The less pronounced effect of temperature on the extraction of metals can be ascribed to the fact that the soils had been artificially contaminated. The absence of the effects of “aging” minimized mass transfer limitations and therefore the potential effect of temperature. Thus, the significantly higher metal uptake achieved in the hot NTA treatment than in the normal NTA treatment may be attributed to the possible enhanced transport of metal chelate complexes from soil solution to root xylems, and then to their translocation from the roots to the shoots of the plant with the transpiration stream.

A significantly positive correlation was found between the metal concentrations in the shoots of the beans and the relative electrolyte leakage rate of the root cells (Fig. 4), which meant that root damage could be helpful in the accumulation of metals in plant shoots. This result was consistent with our previous studies, where pretreatments on the roots of Indian mustard with MC (methanol-trichloromethane) solution, HCl and hot water before a combined treatment of Pb and EDTA dramatically increased the concentration of Pb in shoots compared with shoots that had not been pretreated (Luo *et al.*, 2006b). The enhanced translocation of metals from roots to shoots because of root damage can be explained by the breakdown of the root exclusion mechanism. Bell *et al.* (1991) suggested that the plant uptake of metal chelate complexes occurs at breaks in the root endodermis and Casparian strip. It is hypothesized that the hot solution firstly destroyed the physiological barrier(s) of plant roots that normally function to control the uptake and translocation of solutes. Then, the rapid equilibration of the soil solution with the sap of the xylem was achieved. After entering the xylem, metals would be translocated with the transpiration stream from the roots to shoots of the plant, leading to a high concentration of metals in the shoots. It has been reported that Pb can be absorbed and transferred as a Pb-EDTA complex in the presence of high concentrations of EDTA (Vassil *et al.*, 1998; Epstein *et al.*, 1999; Sarret *et al.*, 2001). In the process of hot NTA facilitated metal uptake, the metal might be transported in the form of a metal-NTA complex through the apoplastic route, as suggested by Wenger *et al.* (2003).

EDTA has been one of the most efficient chelating agents in increasing the uptake of metals, especially Pb (Blaylock *et al.*, 1997; Huang *et al.*, 1997, Cooper *et al.*, 1999). The chelate of NTA is usually shown to have a lower efficiency in solubilizing metals from soils and to be less effective in enhancing the uptake of metals by plants than EDTA (Shen *et al.*, 2002; Meers *et al.*, 2004; Meers *et al.*, 2005). In our present study, however, when NTA was applied in a normal solution, the concentrations and total phytoextraction of Cu, Zn and Cd in the shoots of beans were comparable to that achieved in the normal EDTA treatments at the same application dosage, although the data for Pb was far lower than that in the treatment of EDTA (Fig. 2 and Table 2). The higher metal uptake efficiency after the application of NTA may be attributed to different experimental conditions such as soil properties, metal concentrations and components in soil, and to chelate application dosages and methods. Chiu *et al.* (2005) found that NTA was more effective than EDTA in extracting Zn and Cu within the tested concentration of 20 mmol kg^{-1} of soil. Tandy *et al.* (2004) also reported that at pH 7, NTA showed higher extraction efficiency for Cu and Zn than EDTA.

Besides the screening of plant species, the selection of chelates and the optimization of the chelate application strategy will be very useful for increasing the uptake of metals by plants and reducing the potential risk to the surrounding environment, such as the leaching of metals, in the process of chemical-induced phytoremediation. It was reported that a split application of chelates is more effective than the application of single dosages in increasing the phytoextraction of metals from soils (Grčman *et al.*, 2001; Puschenreiter *et al.*, 2001; Shen *et al.*, 2002). Combining EDTA / NTA and glyphosate increased the concentration of Pb in plant tissues when glyphosate was added shortly before the plants were harvested (Ensley *et al.*, 1999 Kayser *et al.*, 1999). The combined application of EDTA and EDDS dramatically improved the uptake of Pb by corn (Luo *et al.*, 2006c). Applying an electric field around the plants in combination with the application of EDTA can also enhance the uptake of Pb by Indian mustard compared with the addition of EDTA only (Lim *et al.*, 2004). Recently, a new slow-release chelating agent application was reported, where solid EDTA was coated with a layer of silicate to slow down the mobilization of metals in the soil in order to match their uptake by the plant, and thus prevent excessive mobilization (Li *et al.*, 2005). The present study showed when NTA was applied in hot solutions at the rate of 1 mmol kg^{-1} of soil, the total metal uptakes of Cu, Zn and Cd were higher than those achieved in the normal EDTA application at a dosage of 5 mmol kg^{-1} of soil (see Table 2). For Pb, although the total phytoextraction observed at the treatment of 1 mmol kg^{-1} of hot NTA was lower than that achieved by the application of 5 mmol kg^{-1} of normal EDTA, it was still higher than that of 1 mmol kg^{-1} of normal EDTA (Table 2). This result indicates that if NTA were to be applied in a hot solution, the efficiency in enhancing metal uptake could exceed that of a normal EDTA treatment. In addition, the characteristic of easy biodegradability makes NTA more suitable than EDTA for metal phytoremediation. Another biodegradable chelate, EDDS, also showed high efficiency in metal phytoextraction, particularly when it was added in heated solutions (Luo *et al.*, 2007). However, EDDS is far more costly than NTA. Taking these factors into account, the application of a hot NTA solution might be a better alternative for chelate-enhanced metal phytoextraction.

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Table 1

The physicochemical properties of the soils used in the study

pH ($CaCl_2$)	7.12
Electrical conductivity at 25°C (μS cm^{-1})	262
Sand (%) > 0.05 mm	79.5
Silt (%) 0.05 - 0.001 mm	13
Clay (%) < 0.001 mm	7.5
N_{Total} (%)	0.15
Organic matter (%)	2.7
Cation exchange capacity (cmol kg^{-1})	4.2
Field water capacity (%)	39.7
Total metal concentration after amendment (mg kg^{-1})	
Cu	480
Pb	575
Zn	700
Cd	17

Table 2

Total phytoextraction (mg kg^{-1} soil) of Cu, Pb, Zn, and Cd in the shoots of beans 7 d after the application of EDTA, citric acid (CA) and NTA at different concentrations (mmol kg^{-1} soil)

Treatments	**Cu**	**Pb**	**Zn**	**Cd**
Water	70 ± 8.6a	8.6 ± 0.6a	482 ± 52a	6.6 ± 0.9a
Hot-water	110 ± 13a	13 ± 2.1a	468 ± 69a	8.8 ± 1.5a
1mM EDTA	359 ± 49ab	19 ± 2a	551 ± 80a	14.4 ± 2.8a
Hot-1mM EDTA	2600 ± 208cd	327 ± 40b	1910 ± 293c	77 ± 8b
3mMEDTA	622 ± 70b	126 ± 13ab	790 ± 54b	22.2 ± 3.6a
Hot-3mM EDTA	2630 ± 300cd	1140 ± 100	2420 ± 300c	105 ± 20c
5mM EDTA	700 ± 55b	358 ± 20b	926 ± 56b	28 ± 1a
Hot-5mM EDTA	3060 ± 245d	2100 ± 260c	2430 ± 315d	112 ± 19c
1mM CA	79 ± 8.2a	9.7 ± 0.5a	582 ± 70a	7.2 ± 5.5a
Hot-1mM CA	200 ± 24a	26 ± 3a	510 ± 65a	8.6 ± 0.7a
3mMCA	67 ± 7.5a	6.5 ± 0.8a	476 ± 35a	5.7 ± 0.6a
Hot-3mM CA	410 ± 56ab	19 ± 2.1a	586 ± 40a	10.2 ± 2.8a
5mM CA	67 ± 4.5a	8.4 ± 0.6a	493 ± 30a	6.2 ± 1.4a
Hot-5mM CA	385 ± 42ab	24 ± 3.5a	616 ± 75a	12.5 ± 2a
1mM NTA	427 ± 50ab	18.4 ± 2a	510 ± 80a	16 ± 2.5a
Hot-1mM NTA	1730 ± 150c	70 ± 5a	780 ± 64b	32.8 ± 4.8a
3mM NTA	585 ± 65b	29.3 ± 4.5a	586 ± 40a	17.4 ± 3a
Hot-3mM NTA	3310 ± 278d	340 ± 40b	777 ± 80b	117 ± 25c
5mM NTA	578 ± 25b	41 ± 6a	616 ± 50a	18.2 ± 3.8a
Hot-5mM NTA	3130 ± 480d	378 ± 48b	836 ± 86b	117 ± 25c

The values are means ± S.D. (n = 3); in the vertical direction the different small letters stand for statistical significance at the 0.05 level with the LSD test.

Figure legends:

Fig. 1. Effects of the application of EDTA, citric acid and NTA on the dry matter yields of beans. The values are means ± S.D. (n = 3).

Fig. 2. Effects of the application of chelates on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of beans. The values are means ± S.D. (n = 3).

Fig. 3. Effects of the application of NTA (mmol kg^{-1} soil) at different temperatures on the solubilization of Cu, Pb, Zn and Cd (mg kg^{-1} soil) in the soil. The values are means ± S.D. (n = 3).

Fig. 4. The correlation between the relative electrolyte leakage of roots and the concentration of Cu in the shoots of beans. Plants were pretreated with hot water at different temperatures, then exposed in solutions containing 500 µmol L^{-1} of Cu + 500 µmol L^{-1} of NTA for 2 d. The root cell electrolytic leakage (relative electrical conductivity) was measured immediately after the pretreatment with hot water.

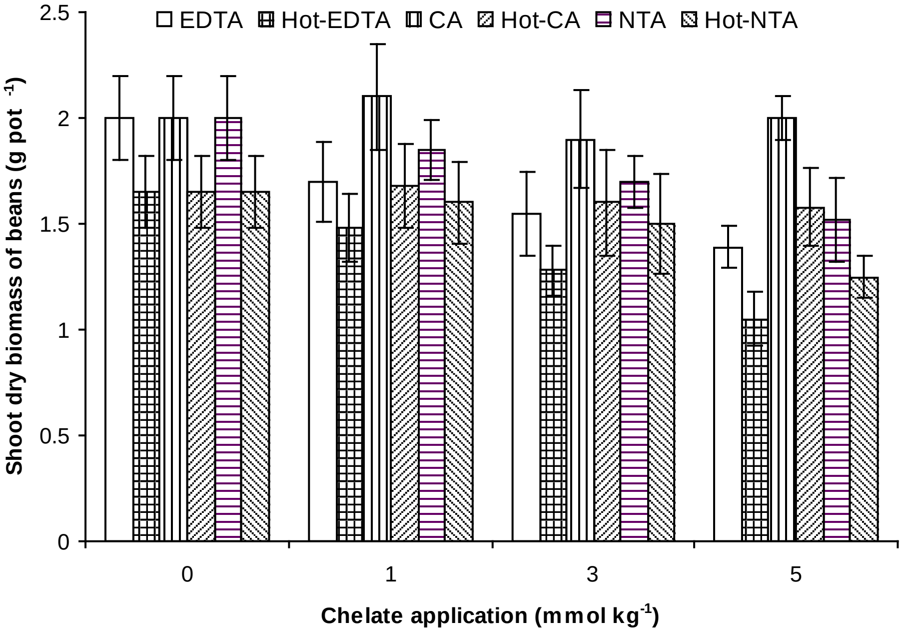

Fig. 1. Effects of the application of EDTA, citric acid and NTA on the dry matter yields of beans. The values are means ± S.D. (n = 3).

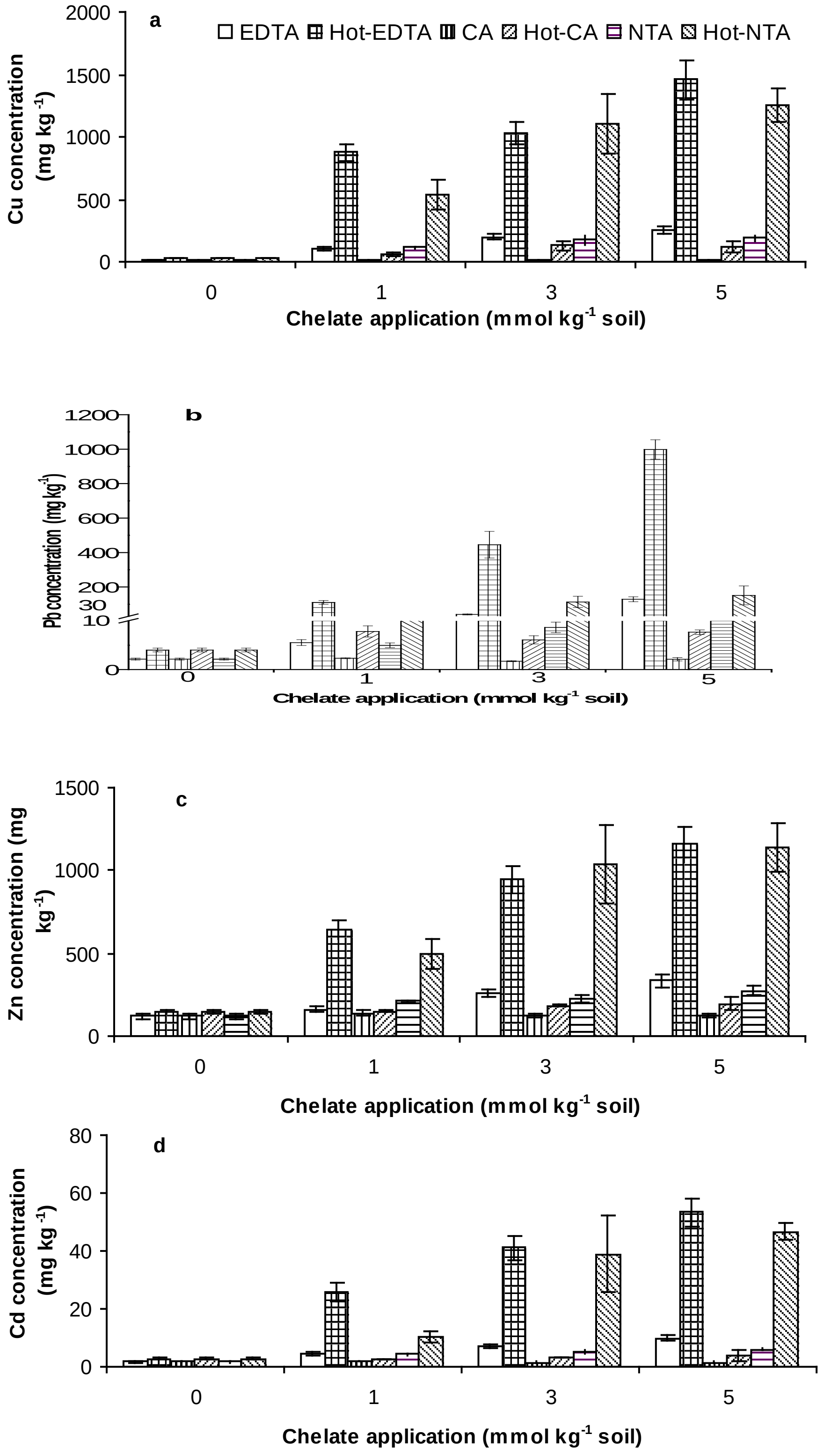

Fig. 2 Effects of the application of chelates on the concentrations of Cu (a), Pb (b), Zn (c), and Cd (d) in the shoots of beans. The values are means ± S.D. (n = 3).

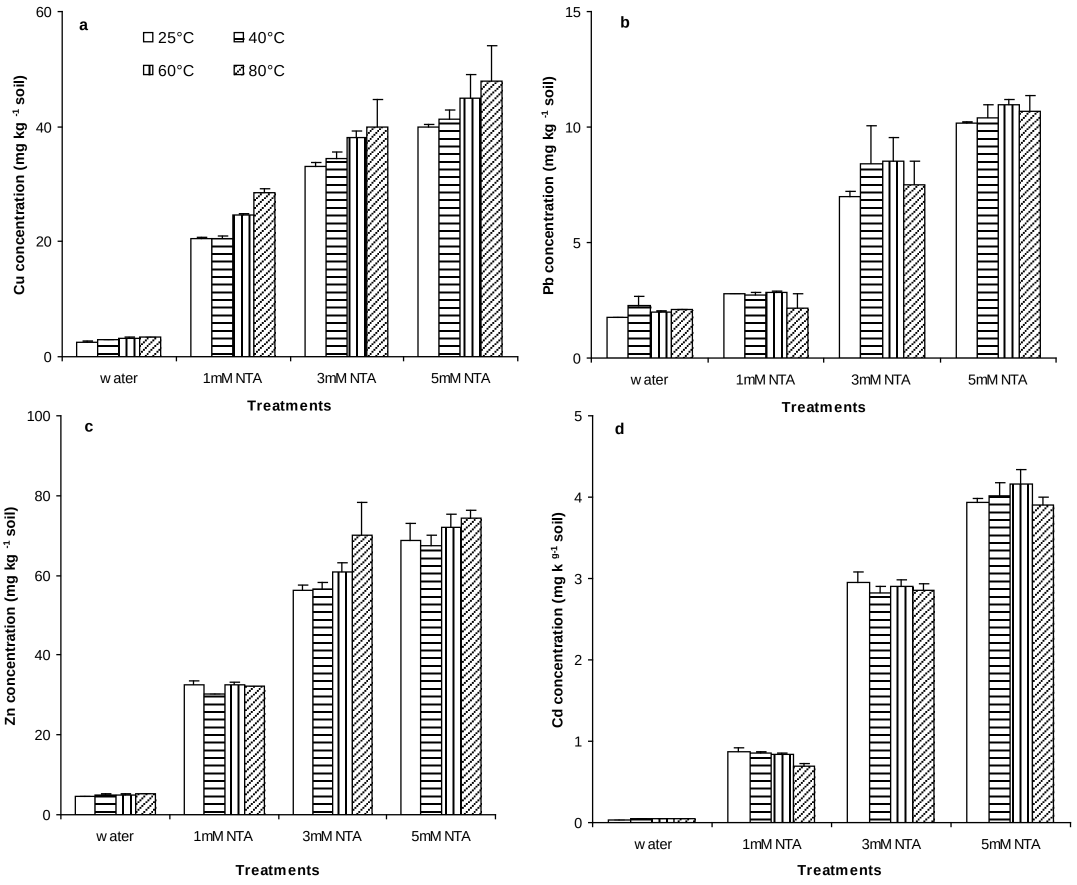

Fig. 3. Effects of the application of NTA (mmol kg^{-1} soil) at different temperatures on the solubilization of Cu, Pb, Zn and Cd (mg kg^{-1} soil) in the soil. The values are means ± S.D. (n = 3).

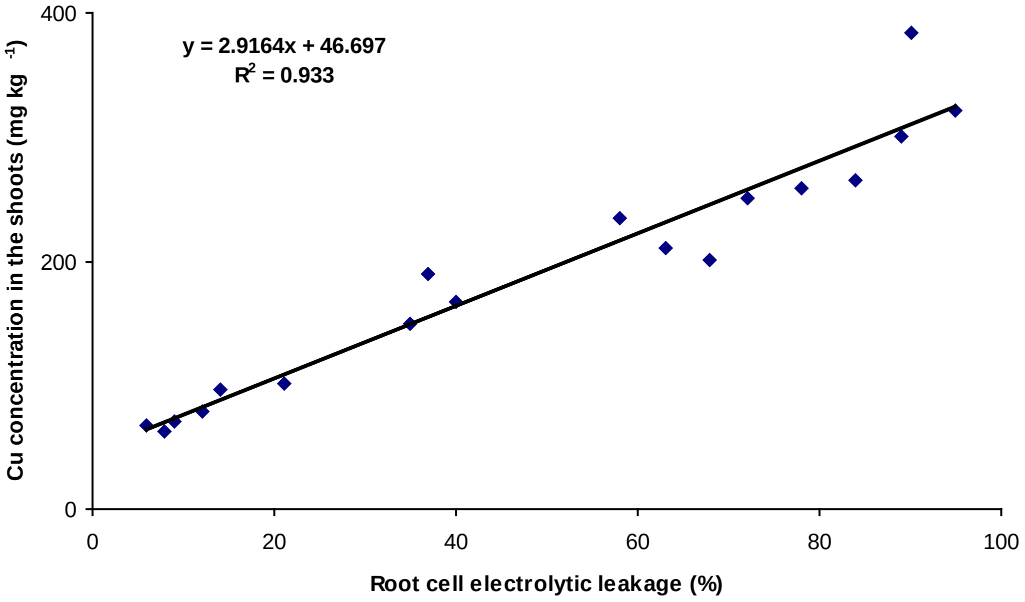

Fig. 4. The correlation between the relative electrolyte leakage of roots and the concentration of Cu in the shoots of beans. Plants were pretreated with hot water at different temperatures, then exposed in solutions containing 500 µmol L^{-1} of Cu + 500 µmol L^{-1} of NTA for 2 d. The root cell electrolytic leakage (relative electrical conductivity) was measured immediately after the pretreatment with hot water.