Plant uptake and leaching of copper during EDTA-enhanced phytoremediation of repacked and undisturbed soil

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Abstract

EDTA-enhanced phytoremediation of copper contaminated soil was evaluated. Up to 740 μg g^{-1} of Na$_2$H$_2$ EDTA in solution was added to repacked soil columns, and intact cores of a sandy loam of volcanic origin, that was growing *Agrostis tenuis*. The soil contained up to 400 μg g^{-1} of copper due to a history of fungicide spraying. EDTA application increased the herbage copper concentration of the grass growing in repacked soil from 30 to 300 μg g^{-1}, but the same application to an intact core only brought about an increase from 10 to 60 μg g^{-1}. More copper accumulated in the herbage when the EDTA was applied in numerous small doses than in just one or two larger amounts. Calculation of the concentration of copper in the water taken up by the grass revealed this to be two orders of magnitude lower than that in the soil solution. As a result of the EDTA applications, about 100 times more copper was leached than was taken up by the herbage. This means that a strategy for managing leaching losses needs to be part of any plan for EDTA-enhanced phytoremediation.

Introduction

Contamination of land with heavy metals has become a problem in some industrial and agricultural areas. Copper is one such contaminant in many soils around the world, as it is used as an active ingredient in fungicides in orchards. Also, along with chromium and arsenic, it is used as a timber preservative. Typically the concentration of copper in the soil at contaminated sites in New Zealand ranges from 100 to 8000 μg g^{-1} (Roberts et al., 1996). Copper is usually strongly bound to soil organic matter and thus relatively unavailable to living organisms. However if the soil adsorption sites in the soil become saturated with copper, it then becomes toxic for microbes and plants, resulting in decreased soil fertility (Merry et al., 1983). This threshold concentration depends on the organism, soil pH, and the organic matter and clay content. The guideline for the maximum acceptable concentration of copper in horticultural soils in Australia by the NSW Environmental Protection Agency is 100 μg g^{-1}. This is well below the levels of contaminated soils in New Zealand. Using plants to remediate such sites has been proposed as a relatively inexpensive process, as compared to conventional engineering-based solutions. This so-called phytoremediation has been suggested as an economic and environmentally sound method for extracting heavy metals from moderately polluted soils (Brooks, 1998; Cunningham et al., 1996).

Although several conditions must be met in order for phytoremediation to be effective, the bioavailability of the target metal in the soil is a critical requirement for plant uptake to occur. Usually little copper is taken up by plants, mainly due to its low concentration in the soil solution. This lack of bioavailability is a result of copper- forming complexes with organic matter,

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as well as being sorbed onto oxides and clays, and being occluded by oxides and clay lattices (McLaren and Crawford, 1973). This low solution concentration also means that the mobility of copper is limited. Therefore copper is usually concentrated in the surface soil at contaminated sites.

Chelates have long been used in nutrient solutions to increase the solubility and plant availability of trace elements. They have also been used to remediate contaminated soils using soil-washing techniques (Tuin and Tels, 1990; Yu and Klarup, 1994). More recently, many glass-house studies and a few field studies (Kayser et al., 2000) have demonstrated the potential for chelates to enhance the plant uptake of metals that exist in soil predominantly in a sorbed state. Different metals have been targeted, either individually or in a multi-metal contaminated environment. Amongst the metals that have been targeted individually are Pb (Blaylock et al., 1997; Cooper et al., 1999; Epstein et al., 1999), Zn (Ebbs and Kochian, 1998), Cs (Lasat et al., 1998), U (Huang et al., 1998) and Au (Anderson et al., 1998). Furthermore Lombi et al. (2001), Grčman et al. (2001) and Kayser et al. (2000) have evaluated chelate-assisted phytoremediation in a multi-metal contaminated environment.

Enhanced-phytoremediation makes use of high-biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by the chelates. *Brassica juncea* is one plant that produces high rates of biomass under field conditions, and also has the capacity to accumulate substantial metal concentration in its shoots (Kumar et al., 1995; Blaylock et al., 1997). Of the chelates tested for this enhanced technology, ethylene diamine tetraacetic acid (EDTA) has been found to be the most effective for increasing the uptake of various metals (Blaylock et al., 1997; Brooks, 1998; Ebbs and Kochian, 1998; Epstein et al., 1999; Grčman et al., 2001; Huang et al., 1997; Lombi et al., 2001), including copper (Deram et al., 2000).

In situ application of such chelates can increase the risk of water pollution by promoting uncontrolled metal solubilisation and leaching. Although some of the studies referred to above mention the risk of target metal being leached below the root zone to the ground water, little attention has been given to measuring or predicting such leaching. The exception is the paper of Grčman et al. (2001). While they found that EDTA did enhance heavy metal uptake by *Brassica rapa*, it also resulted in the loss, by leaching of 38, 10 and 56%

of the total Pb, Zn and Cd, respectively, present in their repacked soil columns.

Clearly the potential mobility of chelated metals needs to be evaluated before there is widespread acceptance of this technology. The main objective of our study was to investigate the relative importance of plant uptake and leaching of copper following the addition of EDTA.

The key to chelate-induced phytoremediation is to maintain an increased bioavailability of the target metal long enough for plants to take it up. Hence the soluble complex which the target metal forms with EDTA needs to be relatively stable. Although EDTA itself is quite stable in soil (Hong et al., 1999), many metal–EDTA complexes are not (Jardine et al., 1993; Szecscody et al., 1994). In particular, Cu-EDTA can react with soil Fe over time, leaving the more stable Fe-EDTA in solution whereupon the Cu is released and re-adsorbed by the soil (Thayalakumaran et al., 2003a,b). Therefore a second objective of this study was to compare the effect on the plant copper uptake of numerous small EDTA applications over time, as compared to just two widely-spaced applications.

Except for the study of Kayser et al. (2000), the chelate-induced phytoremediation studies conducted so far have all used repacked soil columns. Kayser et al. (2000) conducted a field study to investigate nitrilotriacetic acid (NTA)-enhanced phytoremediation of soil contaminated with Cu, Zn and Cd by the emissions from a nearby brass smelter. They reported that the NTA-amended soil increased the solubility of Zn, Cd and Cu by factors of 21, 58 and 9, respectively, but plant accumulation of these metals was only increased by a factor of 2–3. They did not investigate any leaching of the NTA-complexed metals. Water movement and root growth in repacked soil may not be the same as in intact soil with its quite different pore geometry. Hence, the third objective was to compare EDTA-induced copper uptake by grass grown in repacked soil columns, with that of grass in intact soil cores.

Materials and methods

A copper-tolerant grass from Parys Mountain, Amlwch, Anglesey, UK, *Agrostis tenuis* was used in this study. A copper-tolerant grass was used, rather than a copper hyperaccumulator, such as the *Haumanistrum* spp. These identified hyperaccumulators have a small biomass, and are not tolerant of temperate climatic conditions, as they are native of the Democratic Re-

public of Congo (Deram et al., 2000). A preliminary test showed *Brassica juncea,* a commonly used plant for enhanced phytoremediation, can suffer from severe phytotoxicity when EDTA mobilizes the copper.

The soil used was collected from a site near Opotiki, New Zealand, that had been used to grow passion fruit for the preceding 10 years. The top 20 mm had become contaminated with copper due to the legacy of a heavy use of copper-based fungicide sprays. The soil was Opotiki sandy loam, a Typic Udivitrand Ashy Thermic in Soil Taxonomy. The top 100 mm had a bulk density of 0.9 Mg m^{-3}, a relatively high cation exchange capacity of 22 cmol$_c$ kg^{-1}, and an organic matter content of 12–16%. The pH of the soil in a 1:2.5 water suspension was 5.6.

Two experiments were conducted, which we will refer to as I and II. Experiment I used repacked soil columns, while Experiment II used intact soil cores. The soil for Experiment I was collected from the top 100 mm of the A horizon in the copper-contaminated orchard and passed through a 2-mm nylon sieve while still moist. A depth of 150 mm of soil was packed at a bulk density of 0.75 Mg m^{-3} into plastic tubes with an internal diameter of 100 mm and a nylon mesh base. The average acid-extractable (conc. HNO$_3$) copper content in the sieved soil was 276 μg g^{-1}. The intact cores for Experiment II were collected carefully from the same orchard site. After the removal of the above ground vegetation, plastic cylinders 150 mm long and 100 mm in diameter were gently pressed into the soil with minimal disturbance to the soil, and the cores retrieved by excavation. The bulk density ranged from 0.91 to 0.96 Mg m^{-3}. Several smaller 35-mm diameter soil cores were taken adjacent to the larger cores to measure the copper distribution in the soil profile. The cores and repacked columns were placed in a glasshouse and seeds were sown into the surface soil.

In all cases, EDTA application commenced 4 weeks after seedling emergence. The EDTA solution applied was 0.01 *M* Na$_2$H$_2$EDTA, which had been raised to pH 6.0 with KOH to ensure optimal plant growth as well as mobility of the Cu-EDTA complex. To ameliorate the soil dispersion effect likely to be caused by the sodium, 0.01 *M* CaCl$_2$ was also added to the EDTA solution. Where there were nine or 18 applications of this solution, the EDTA solution was mixed with the required amount of irrigation water and applied to the column or core. However, when there were only one or two EDTA applications, the EDTA solution was applied without mixing with water.

In Experiment I, three repacked columns were used. The first column was kept as a control with no EDTA addition. The second column had a total of 1800 μmol of EDTA sprinkled on to it in two applications of 900 μmol, the second application was 16 days after the first. The third column also received a total of 1800 μmol, but in 18 applications, each of 100 μmol, and applied on alternate days. As the columns contained soil with a dry mass of 880 g, the 1800 μmol of EDTA added corresponded to 0.74 mg of Na$_2$H$_2$EDTA per gram of dry soil.

Experiment II involved five intact soil cores. The experimental treatments used in Experiment I were imposed on three of the cores. The other two cores both had 900 μmol of EDTA added to them. This was added as a single application to one core, and as nine 100 μmol applications applied at 4-day intervals to the other core.

Every day the water loss from each of the columns and cores was measured, and water applied to correct for the weight loss. No leachate emerged from the base of the columns or cores, as the soil water content was just brought back to 'pot capacity' each day. Forty-four days after EDTA was first applied, the grass was harvested from all columns and cores and placed in a drying oven at 80 °C until a constant weight was achieved. Samples were analysed for copper as described below.

After harvesting, the columns and cores were leached with about seven liquid-filled pore volumes (PV) of water under unsaturated conditions. The apparatus used to do this is described by Magesan et al. (1995). In brief, a disk permeameter sat atop each soil column to allow solution entry at a pre-set pressure potential. Unsaturated control was maintained by a bubbling tower and a vacuum system. A similar apparatus underneath maintained the same pressure potential at the base. A pressure head of −100 mm was maintained at the top and bottom of each core or column. Thin layers of sand ensured good contact between the soil and the porous plates. The leachate was collected and analysed for copper and iron as described below. Then the soil in each column or core was cut into six segments. The soil in each segment was homogenised, sub-sampled, and analysed as described below.

The plant material analysed for copper was prepared in the following manner. Sub-samples (1 g) of grass were weighed into 10-mL borosilicate test tubes. The tubes were heated in a muffle furnace overnight at 500 °C. The next day 5 mL of warm 2 *M* HCl was

added to each tube, mixed in with a vortex mixer, and then the solution was stored for analyses. The acid-extractable copper concentrations in the soil were determined by digesting the soil. To do this about 0.2 g of sieved soil was weighed into boiling tubes. Ten mL of concentrated HNO_3 were then added and the mixture boiled until a final volume of 3 mL was reached. A further 10 mL of concentrated HCl were then added and the mixture again evaporated to 3 mL. The solution was made up to 10 mL, filtered and stored for analyses (Deram et al., 2000).

All samples were analysed for copper using flame atomic adsorption spectroscopy (FAAS).

Results and discussion

The dry matter yields at harvest are shown in Table 1, and these ranged from an equivalent of 331–433 $g\ m^{-2}$. There was no observable decrease in yield attributable to EDTA application, probably because the grass had been allowed to establish and grow for about 45 days before the EDTA was applied. During the 44-day period between the first application of EDTA and harvest, the evapotranspiration from the columns and cores ranged from 10 to 13 mm day^{-1} on a pot cross-sectional basis. Because of the overhang of the plants, the effective leaf area was large accounting for such high rates of water use on an area basis. There was no observable decrease in transpiration after the addition of EDTA.

In the repacked soil columns, pre-mixing of the soil meant that the initial soil copper concentration was reasonably uniform, although some variation was observed in the amount of total acid-extractable copper (Table 1). In contrast the copper in the intact cores was concentrated near the surface, as copper binds strongly to organic matter and therefore has limited mobility. As we had no way of non-destructively measuring the initial copper distribution in the cores, we used the data from the smaller cores taken close to each large core to infer the copper distribution in the larger cores. However due to soil variability, this did not necessarily give an exact measure of the total amount of initial copper in each large core. We found the total amount of copper by adding the amount of copper leached and the amount left in the soil after leaching. To get the correct mass balance for each core we used these total values, in conjunction with the shape of the distribution found in the smaller cores, to infer the initial copper distribution in each large

core. Again some variation was observed in the total amount of copper in the cores, as expected in the field (Table 1). On average the repacked columns contained about 50% more copper than in the intact cores.

The 1800 μmol of EDTA applied to the contaminated soil corresponded to 740 $\mu g\ g^{-1}$ in the repacked columns and 670 $\mu g\ g^{-1}$ in the intact cores. This EDTA was sufficient to complex about half of the acid-extractable copper present, assuming a 1:1 molar reaction between copper and EDTA. However, EDTA forms a soluble complex with not only copper but with most other heavy metals, and has a strong affinity for iron when applied to soil. Therefore we expected less than half of the copper to be brought into solution by the EDTA.

Figure 1 shows that the addition of EDTA to the copper contaminated soil resulted in a substantial increase in the copper concentration in the leaves of *A. tenuis,* in both the repacked columns and intact cores. In the absence of EDTA, copper uptake was minimal. The highest herbage copper-concentration measured was 300 $\mu g\ g^{-1}$ and this is comparable to the 472 $\mu g\ g^{-1}$ measured by Aseni et al. (1999) in *Erica andevalensis*, a metal-tolerator, grown in crushed copper ore containing 1926 $\mu g\ g^{-1}$ of copper and with 2000 $\mu g\ g^{-1}$ of EDTA added. But our value was well below the 2500 $\mu g\ g^{-1}$ found in *Arrhenatherum elatius*, also a metal-tolerator, by Deram et al. (2000) after the addition of EDTA at a rate of 500 $\mu g\ g^{-1}$ to a copper ore with a total concentration of just 188 $\mu g\ g^{-1}$. These variations in leaf copper concentration could be due to differences in the form of copper present in the substrate, the age of the copper in the soil, or to differences between plant species. Copper ores mostly contain copper in inorganic form, whereas the copper in orchard topsoil is most likely to be bound to organic matter.

The herbage copper concentration was much higher for the repacked columns than for the intact cores (Figure 1). In part this can be attributed to more copper being present in the repacked soil than intact cores (Table 1). But we suggest it was mainly due to a greater spatial separation between the copper adsorption sites and the roots in the intact cores, as compared to the repacked soil columns. In intact cores, the humus responsible for the copper adsorption sites tends to be more associated with the clay in the soil's matrix inside the aggregates, while roots tend to grow in the interstitial larger cracks and bio-pores. In contrast, in the repacked soil columns, the roots would have a more uniform distribution. Additionally, the physical

Table 1. Summary of results

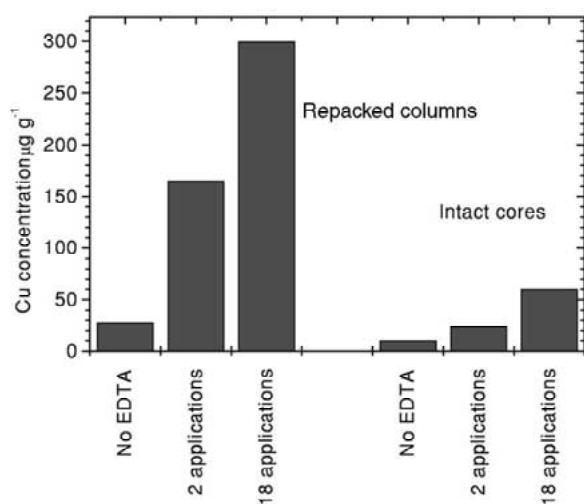|	Number of EDTA applications	Initial copper in soil (μmol)	EDTA applied (μmol)	Copper leached (μmol)	Iron leached (μmol)	Dry matter [DM] produced (g)	Copper in herbage [Conc $\times$ DM] (μmol)
Experiment I	0	3831	0	5	2	3.3	1.54
(Repacked)	2	3075	1800	480	788	3.0	7.8
	18	3522	1800	363	686	3.1	14.9
Experiment II	0	3453	0	1	1	3.0	0.4
(Intact)	1	2484	900	109	467	2.6	0.9
	9	2597	900	169	407	2.7	1.0
	2	2120	1800	115	1045	3.2	1.2
	18	1720	1800	340	816	3.4	3.2

Figure 1. Copper accumulation in shoots of *A. tenuis* grown on copper contaminated repacked soil columns and intact soil cores in response to the addition of EDTA (1800 μmol per column, or core).

disturbance caused by sieving of the soil for repacked columns would make some of the copper adsorption sites more accessible.

The amount of copper uptake by the plants also varied with the amount and frequency of EDTA application (Table 1). As might be expected, the addition of 1800 μmol of EDTA induced more uptake from repacked soil columns than did the addition of 900 μmol. But also, when 1800 μmol of EDTA was applied in 18 small aliquots rather than two large doses, the copper concentration in the herbage (Figure 1) and total amount of copper uptake (Table 1) doubled, both in the repacked column and intact core.

The reasons for this are not clear. However it is possible that the application of 900 μmol of EDTA on one day produced such high local concentrations of copper in the soil solution near the surface that the roots became inactive there. Thus they may have taken up most of their water from lower down where there was little EDTA, and so available copper, in the soil solution. This explanation is consistent with the finding that the uptake difference due to this treatment was less pronounced when only 900 μmol of EDTA was applied either as one application or in nine aliquots (Table 1).

Thus far, the discussion has mainly centered on plant uptake. Enhanced-uptake occurred because EDTA brought copper into the soil solution. But if this copper is not all taken up by the plants, there is the risk that it could leach into the groundwater. To assess this risk, following plant harvest we measured the amount of copper leached from each of the columns and cores when 7 PV, about 500 mm, of water were applied. Table 1 shows that between 10 and 20% of the copper in the soil was leached from the columns and cores that received EDTA. A lot of iron was also leached. About 23–58% of the EDTA applied apparently was complexed with iron when it was leached from soil. Figures 2 and 3 show the copper distributions in the columns and cores after leaching. Most of the copper from the top 30 mm has been brought into solution by the EDTA, and then it was leached downward. The final copper concentration was about 84 μg g^{-1} on the surface soil in the two repacked columns to which EDTA had been applied. This concentration is similar to the 90 μg g^{-1} of copper that Thayalakumaran et al. (2003a, b) found remaining in contaminated Opotiki
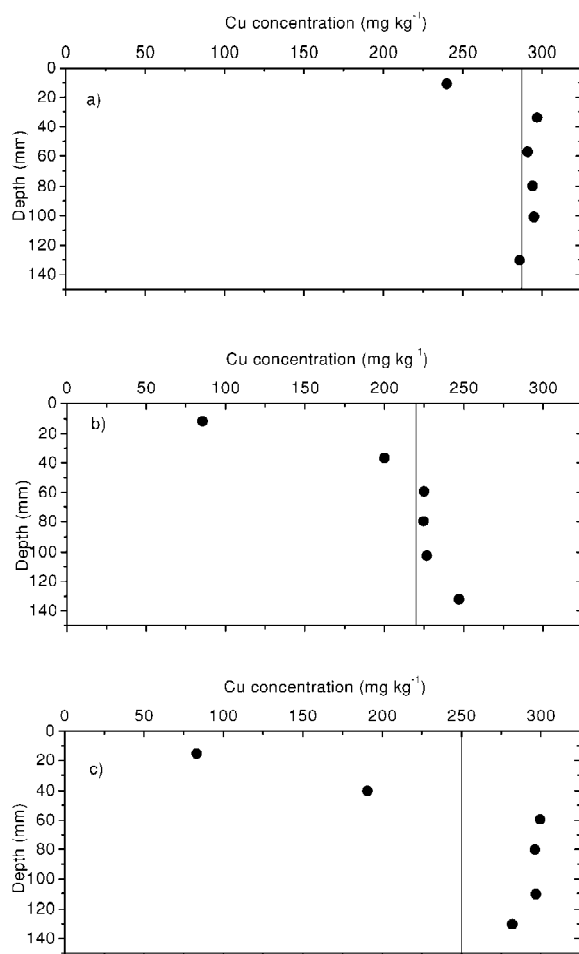
Figure 2. The initial copper concentration (—) and the final measured copper concentrations (●) in the repacked soil columns in Experiment I. (a) Control column; (b) two EDTA applications; and (c) 18 EDTA applications.

sandy loam after leaching with an excess of EDTA solution. The higher copper concentration measured in the top 30 mm of the intact cores could be due to the amount of EDTA applied not being enough to solubilise the higher initial copper concentration at the surface soil.

Beyond a depth of 80 mm, the final copper concentration in the soil was greater than the initial concentration in all columns and cores apart from the controls (Figures 2 and 3). We attribute this increase to the resorption of EDTA-solubilised copper. Another study on the same soil has shown that with EDTA residence times of a week or more, CuEDTA slowly reacts with iron in the soil to form FeEDTA. The once-mobilised copper ions are then adsorbed by the soil (Thayalak-

umaran et al., 2003a, b). The negligible amount of copper measured in the leachate at the end of the 7 PV of leaching confirmed that the increased copper concentration below 80 mm depth was not due to incomplete leaching of the copper present in the soil solution. Between 58 and 70% of the applied EDTA was leached in association with either copper or iron. The rest of the EDTA probably was complexed with calcium or the small amount of zinc in the soil. Alternatively it could have been adsorbed or broken down in the soil.

Comparing the amount of copper uptake by the grass, with the amount leached, provides a risk assessment of how effective an EDTA-enhanced phytoremediation might be in terms of the added benefit of removing copper by plant uptake, relative to the increased chance of polluting the groundwater. For the two repacked columns with EDTA added, 25 and 62 times more copper was leached than was taken up by plants. This uptake-leaching ratio was even higher for the intact cores, ranging from 99 to 169. EDTA-enhanced phytoremediation is unlikely to be risk-free in humid environments, unless a drainage system can be installed and the leachate collected and recycled, or unless drainage can be otherwise minimized. If the leachate were collected, then it rather than plant uptake would provide the major Cu removal pathway. Alternatively there may be scope for using deep-rooted, higher water-use trees to reduce drainage.

A simple calculation allows us to address the interesting question of to what extent the grass roots might exclude copper in the soil solution. As an example, we consider the repacked soil column with the highest copper concentration in the leaves. Over the 50 days between the first EDTA application and harvest, 450 mm or 3.5 kg of water was lost. We can reasonably assume that this was mostly lost via transpiration. The *A. tenuis* leaves took up 14.8 μmol, or 941 μg of copper. Therefore the average copper concentration in the water entering the roots must have been 0.27 μg g^{-1}. A total of 363 μmol, or 23 mg of copper was leached from that column after harvest. We consider this an approximate estimate of the Cu in the soil solution over the 50 days. The volume of soil in the column was 1.2 L, and the water content at 'pot capacity' was 0.5 m^3 m^{-3}. So the column would have typically contained 600 g of water. Thus we can estimate the average copper concentration in the soil solution as being 23 mg/600 g, or 38 μg g^{-1}. Looked at this way, the copper concentration in the water entering the roots was, 0.27/38, or 0.007, some
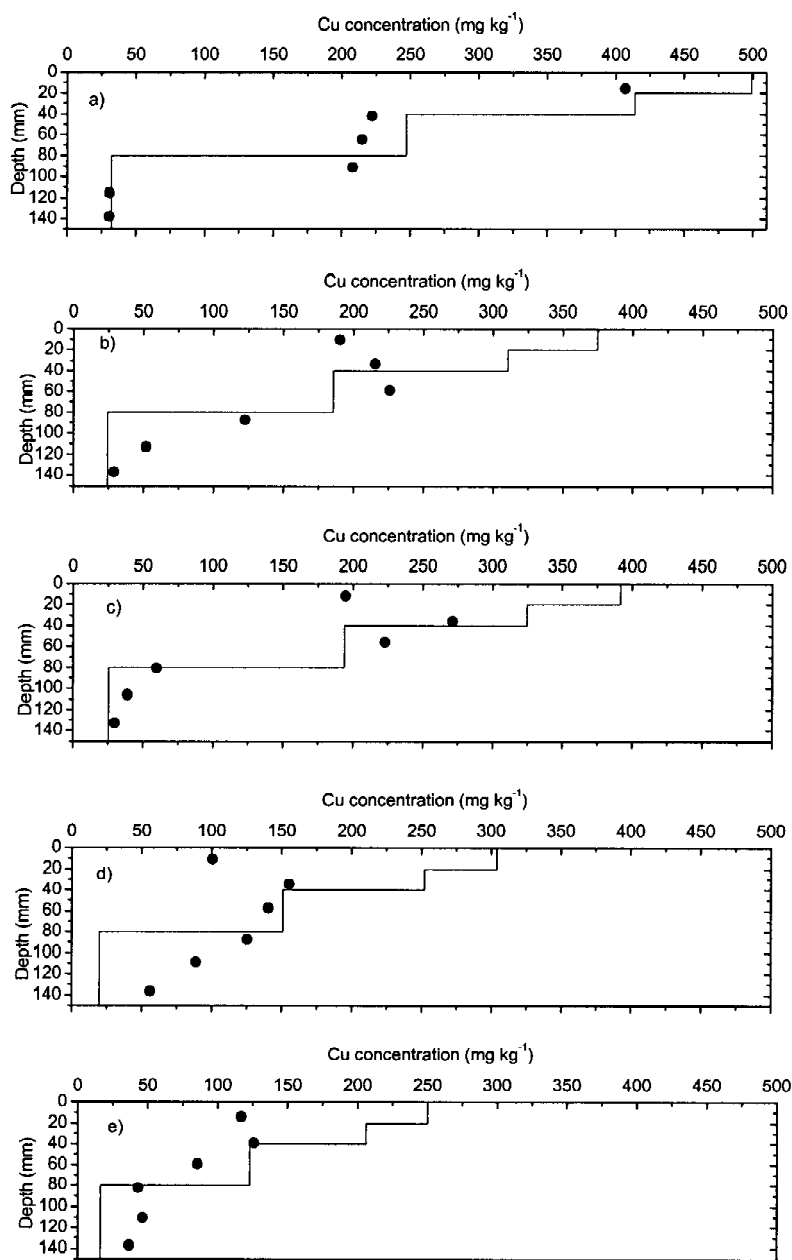
Figure 3. The initial copper concentration (—) and the final measured copper concentrations (●) in the soil cores in Experiment II. (a) Control core; (b) one EDTA application of 900 μmol; (c) nine EDTA applications totalling 900 μmol; (d) two applications totalling 1800 μmol; and (e) 18 EDTA applications totalling 1800 μmol.

two orders of magnitude less than the concentration in the soil solution. Hence the grass was quite effective at screening out the copper in the soil solution. This high filter efficiency poses a limitation on the phytoremedi-ation of copper-contaminated soils using *A. tenuis*, unless leachate recycling can be used to allow the plant repeated opportunities to take up the copper.

The current study was set up as a pilot study, to look at both, the uptake of copper following various application rates of EDTA, and the risk of leach-ing, treatments were not replicated. As most of the

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differences found were large and consistent across treatments, critical inferences can be made.

Conclusions

EDTA application of 1800 μmol to 0.9 kg of copper-contaminated soil in a repacked column growing *A. tenuis*, increased the shoot copper concentration from 30 μg g^{-1} to 300 μg g^{-1}. However the EDTA-enhanced phytoremediation was less effective in the intact cores, in which the same EDTA application to 1.0 kg of soil only increased the herbage copper concentration from 10 to 60 μg g^{-1}. More copper accumulated in the herbage when the EDTA was applied in numerous small doses, rather than in just one or two larger amounts. The concentration of copper in the water taken up by the grass was two orders of magnitude lower than that in the soil solution.

After the EDTA application 25 to 169 times more copper was leached than was taken up by the herbage. This means that a strategy for managing leaching losses needs to be part of any plan for EDTA-enhanced phytoremediation.

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Section editor: A.J.M. Baker