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Partitioning and Accumulation of Heavy Metals in Sunflower Grown at Biosolids Farm in EDTA-facilitated Phytoremediation

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ABSTRACT

A field trial was conducted on a farm that annually received biosolids for 25 years in Manhattan, Kansas, in the USA. The aims of the trial were to investigate translocation and accumulation of heavy metals in organs of sunflower (*Helianthus annuus*) in EDTA-facilitated phytoremediation and to determine if plant density affects translocation and accumulation of heavy metals in sunflower plant parts. Two plant densities of 20,000 and 60,000 plants per hectare were grown on the biosolids farm. Four EDTA application rates, 0, 0.5, 1.0, and 2.0 g per kg soil as treatments were applied during the flowering stage and treatments were replicated four times. Plant organs were harvested separately at the end of the growing period, and the plant samples were analysed for the concentration of heavy metals. The concentration of toxic heavy metals (Cd, Ni, and Pb) in the roots decreased as a result of EDTA application but increased in aerial plant parts. High transpiration rate of the upper leaves in plants at 60,000 plants per ha, indicated by low stomatal resistance, enabled plants to retranslocate most of the toxic heavy metals from the roots to the upper leaves. However, plants grown at 20,000 plants per ha had reduced toxic metals in upper leaves because most of its metals could not be translocated to upper leaves due to high stomatal resistance in those leaves. EDTA had little or no effect on the concentration of essential metals (Cu, Fe, Mn, Zn) in plant roots. However, high plant density enhanced the accumulation of Zn in the top half of the stem.

Keywords: ethylenediamine tetra-acetic acid, heavy metals, phytoremediation, retranslocation, stomatal resistance, sunflower

INTRODUCTION

Sewage sludge or biosolids have been applied to land for decades in the USA for disposal purposes and to use as a soil conditioner and fertilizer (Kirkham 1975). Long-time spreading of sludge on land, however, can result in high concentrations of heavy metals in soils, because many metals present in sewage are removed by sewage treatment and are concentrated in the biosolids (Raskin *et al*. 1994; Hargreaves *et al*. 2008). The long-term land application of biosolids permitted under the Part 503 regulation of the U.S. Environmental Protection Agency may cause a gradual buildup of metal concentrations in amended soils over time (Granato *et al*. 2004). The City of Manhattan, Kansas, has a biosolids farm where sludge has been injected annually since 1976. The city was one of the first to use sub-surface injection of liquid biosolids. Subsurface injection is a popular method of disposal because the biosolids are not visible, and, furthermore, odors, pests, pathogens, and runoff are reduced, while plant nutrients are conserved (Kirkham 1983).

There are concerns that plants grown on these soils may accumulate heavy metals in amounts toxic to man and animals eating the crop and that arable land for cultivation may shrink due to stringent environmental laws limiting food production on contaminated lands (GrČman *et al*. 2001; Alkorta *et al*. 2004; Liphadzi and Kirkham 2006). In addition, the metals could runoff and pollute surface water, move to ground water through cracks, or be ingested by children playing on biosolids-fertilized soil (Lombi *et al*. 2001; Alkorta *et al*. 2004; Liphadzi and Kirkham 2005). For environmental safety, the heavy metals should be removed and phytoremediation can be an effective remediation technology for low to medium metal polluted soils (Schmidt 2003).

Phytoremediation is not only cheaper in comparison to conventional engineering methods, but it also is an *in situ* technology that preserves physical properties of the soil so that the remediated land may remain productive. Addition of chelating agents for phytoextraction is advocated for enhancing the clean-up of soil contaminated by heavy metals (Blaylock *et al*. 1997; Huang *et al*. 1997; Thayalakumaran *et al*. 2000; Nowack *et al*. 2006). In the experiment carried out by Wu *et al*. (2003), in which the effects of EDTA and low molecular mass organic acids (citric, oxalic, and malic) on heavy metals in soil solution were investigated, low molecular mass organic acids had a very small effect on metal concentrations in soil solution compared to EDTA. The use and effectiveness of ethylenediamine tetra-acetic acid (EDTA) in phytoremediation of heavy metals has been reported in numerous papers in the literature (Blaylock *et al*. 1997; Huang *et al*. 1997; Brooks 1998; Salt *et al*. 1998; Wu *et al*. 1999; Kirkham 2000; Lasat 2002; Liphadzi *et al*. 2006). EDTA prevents precipitation and sorption of the metals, thereby maintaining their availability for plant uptake through the formation of chelates (Salt *et al*. 1998). EDTA forms chelates with both transition-metal ions and main group ions, and according to Haag-Kerwer *et al*. (1999), about 80% of the total soil metal is solubilised and becomes available for plant uptake after EDTA application. The enhanced phytoextraction results in high metal concentrations in plants (Salt *et al*. 1998; Deram *et al*. 2000). Metal-laden plants are usually incinerated into ash and placed in a confined disposal site or landfill. If the metals are valuable, they can be extracted from the ash and recycled (Anderson *et al*. 1998). However, the challenge of using EDTA in phytoextraction is that mobilized heavy metals in soil pore water could pose an environmental risk in the form of groundwater contamination (Lombi *et al*. 2001; Madrid *et al*. 2003).

So far, little or no attention has been given to the

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distribution and accumulation of heavy metals in the bottom and top leaves and stem of the plant grown on heavy-metal laden soil. If large amounts of heavy metals accumulate in the bottom leaves, which usually senesce and fall to the ground in most of the annual plants, the benefits of remediating contaminated sites and the amount of metals mined out by plants can be reduced because the metal-laden leaves might fall back to the ground that may result in the pollution of soil. The same can happen with heavy metals accumulated in the stem (stalks) after harvesting, because combine harvesters usually cut the upper (top) part of the stem closer to the head and leave the other part of the stem on the field. Thus, there are leaves and stem parts with heavy metals that fall to the ground surface and return heavy metals back into soil when decomposed, if not removed. However, removal of the fallen leaves from the ground surface for safe disposal can be laborious and expensive. Therefore, understanding partitioning and accumulation of heavy metals in plant parts (old and young) should assist in managing the plant materials on land sites during phytoremediation.

In spite of the availability of a large body of literature on phytoextraction, there is paucity of data and insight with regard to the partitioning of heavy metals among the bottom (usually old) and upper (mostly young) plant organs, especially during chelant-facilitated phytoremediation. Most phytoextraction studies have investigated metal concentration in plant parts, but overlooked the partitioning of the metals between upper and bottom plant parts. It has been widely reported that plants store mineral nutrients in matured leaves and then translocate the mobile nutrients to new growing tissues, where they are needed for growth and development. When the bottom leaves are old, prior to abscission, plant nutrient elements are moved out of those leaves to the new growing leaves and other organs, while immobile elements such as Fe remain in the abscising old leaves (Devlin and Witham 1983; Hikosaka 2005). However, the fate of toxic heavy metals and metal-chelant complexes is not well known.

The aims of the study were to determine the partitioning and accumulation of heavy metals in the bottom and upper (top) leaves and stems of sunflower plants during chelant-facilitated phytoremediation, and to investigate if plant population density affects phytoextraction of heavy metals from soil.

MATERIALS AND METHODS

The experiment was conducted at the Manhattan, KS, Biosolids Farm, located on the southeast side of the city along the Kansas River. The farm, described by Kirkham (1983), has been in operation since 1976. The soil at the farm is a Haynie very fine sandy loam (coarse-silty, mixed, superactive, calcareous, mesic Mollic Udifluvents) with pH of 6.6 and exchangeable Ca of 2446 mg kg^{-1} soil. The concentration of heavy metals in the biosolids and the farm soil are presented in **Table 1**.

Two areas at the farm were assigned for this trial. In each area, 16 1-m^2 plots were measured in a symmetrical pattern (4 plots going east-west direction and four plots in the north-south direction), and 1 m separated each 1-m^2 plot. Sunflower (*Helianthus annuus* L. Red River Commodities, hybrid '2582') seeds were planted on 4 July 2001. One area was planted with 20 000 plant/ha and the other with 60 000 plant/ha. Sunflower was considered for this study because it has high tolerance to heavy metals and is, therefore, to a certain extent able to extract surpluses that originate from soil manipulation (Schmidt 2003; Alkorta *et al*. 2004). According to Schmidt (2003), using plants like sunflower on low to moderately polluted soils is economically viable for farmers because, on one hand, additional benefits such as oil for biodiesel can be obtained while, on the other hand, the crop reduces metal concentrations of the polluted soils.

On 31 August (58 days after planting), ethylenediamine tetraacetic acid as EDTA Na_4. $2H_2O$ (ICN Biomedicals, Inc., Aurora, Ohio) was applied on soil surface at three rates of 42.2, 84.4 and 168.8 g/L water. These amounts of EDTA were equivalent to 0.5, 1.0, and 2.0 g EDTA per kg of soil. The application rates of EDTA are normally 0.3-6 g EDTA per kg soil (Nowack *et al*. 2006). EDTA was applied uniformly on the soil surface, following procedures described by Vogeler *et al*. (2001). EDTA solutions and irrigation water were kept within each plot by soil-constructed ridges about 10 cm tall built around each plot. The targeted soil depth to which EDTA was applied was the top 25 cm, and a bulk density of 1.35 Mg/m^3 was used in the calculation for the amount of EDTA to add as described in Liphadzi *et al*. (2003). Control plots received only 1 L of water.

Each plot was watered at planting and then only when the soil became dry, at which time 20 cm^3 was added to each plot. Approximate irrigation times were 13 July, 22 July, 7 and 17 August, and 3 and 14 September.

Stomatal resistance was measured on 01 September between 11:00 and 02:00 hr on the adaxial (upper) and abaxial (below) surfaces of recently matured leaves with a transient AP4 Porometer (Delta-T Devices, Cambridge, England; obtained from Decagon Devices, Pullman, WA).

The plant parts were harvested separately on 6 October, 2001 at the maturity stage and were divided into four parts: lower stems (stems nearer soil surface) and upper stems; lower leaves and upper leaves (lower leaves were the older leaves on the bottom part of stems). Plant parts were dried in an oven at 70°C for 72 hours, and the samples were analyzed for total amounts of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) using inductively coupled plasma-atomic emission spectroscopy (ICP-ES). The concentration of heavy metals in grains was not analyzed because when EDTA is applied to soil at flowering, plants usually die or get stunted before grains can fully develop. Heavy metals in plants were determined on a 0.25-g samples of dried and ground material from plant organs digested in 8 mL of a 1:1 mixture of HNO_3/$HClO_4$ and then diluted to 25 mL with deionized water and mixed as described in Liphadzi *et al*. (2003). The solution was analyzed for the heavy metals using ICP-ES. Detection limits in µg/g for the ICP-ES were Cd, 0.05; Cu, 0.20; Fe, 1.00; Mn, 0.60; Ni, 0.10; Pb, 0.10; and Zn, 0.10.

After harvest soil samples were taken from each plot at the upper 50 cm soil depth. Total concentration of the heavy metals in the soil was analyzed using a method similar to that of Sposito *et al*. (1982). In their method, total concentration of the heavy metals in the soil is determined on filtered extracts obtained from 2 g samples, which are digested overnight with 12.5 mL 4 M HNO_3 at 80°C. We used 2 g samples, but added 20 mL 4 M HNO_3 and heated the mixture for 18 h at 85°C in a water bath. The extract was analyzed using ICP-ES. **Tables 2** and **3** show the concentrations of heavy metals in the roots and in soils after harvest.

Table 1 Total concentration of heavy metals in biosolids and soil from Manhattan, Kansas and normal heavy metal concentration in plants (Kirkham 1975; Alloway 1995; Bastian 1997; Fageria *et al*. 2002).

Heavy metal	Manhattan, KS, biosolids (µg/g)	Concentration in Manhattan biosolids farms' soil (mg/kg)	Toxic concentration in soil (µg/g)	Normal concentration in plants (µg/g)	Toxic concentration in plants (µg/g)
Cd	3.9	0.8	3	0.1	5
Cu	395	17	60	5	20
Fe	15708	8773	None	50	1000
Mn	650	167	1000	30	300
Ni	19	8.9	100	0.1	10
Pb	72	27	100	0.1	30
Zn	221	31	70	20	100

Table 2 Concentration of essential and toxic heavy metals in the roots of sunflower plants grown on a long-term biosolids farm in chelant facilitated phytoremediation.

EDTA (g kg^{-1} soil)	Essential heavy metals in the roots (µg/g)							
	20 0000 plants ha^{-1}				60 000 plants ha^{-1}			
	Cu	**Fe**	**Mn**	**Zn**	**Cu**	**Fe**	**Mn**	**Zn**
0	9.6 a	787 a	20.7 a	13.2 b	12.3 a	1100 a	27 a	9.9 b
0.5	7.1 b	693 a	16.4 a	20.1 a	4.5 b	1123 a	22 a	15.2 a
1.0	7.5 b	873 a	19.8 a	20.6 a	8.4 a	1033 a	21 a	18.7 a
2.0	9.7 a	741 a	18.2 a	21.2 a	6.7 a	1096 a	21 a	17.3 a
EDTA (g kg^{-1} soil)	**Toxic heavy metals in the roots (µg/g)**							
	20 0000 plants ha^{-1}			60 000 plants ha^{-1}				
	Cd	**Ni**	**Pb**	**Cd**	**Ni**	**Pb**		
0	1.15 a	5.4 a	10.1 a	1.4 a	7.9 a	15.2 a		
0.5	1.15 a	5.6 a	11 a	0.5 b	1.9 b	4.3 b		
1.0	0 b	0 b	0 b	0.05 c	0.45 bc	0.6 c		
2.0	0.15 b	1.1 b	1.4 b	0 c	0 c	0 c		

Means followed by the same letter are not statistically different at the 0.05 level. Only column means are compared.

Table 3 Total concentration of heavy metals in mg. kg^{-1} in the top 50 cm of soil after phytoremediation with sunflower grown at two plant densities.

Metal	Plant. ha^{-1}	EDTA (g.kg^{-1} soil)			
		0	**0.5**	**1.0**	**2.0**
Cd	20,000	0.74 ± 0.06	0.62 ± 0.02	0.66 ± 0.1	0.63 ± 0.02
	60,000	0.81 ± 0.05	0.67 ± 0.04	0.67 ± 0.03	0.63 ± 0.01
Cu	20,000	14.2 ± 1.5	13.2 ± 1.8	13.9 ± 1.4	11.8 ± 0.9
	60,000	16.9 ± 2.3	15.5 ± 1.5	16.3 ± 1.1	13.7 ± 0.8
Fe	20,000	8937 ± 194	9006 ± 293	8998 ± 212	8842 ± 275
	60,000	9641 ± 301	9607 ± 290	9580 ± 177	9329 ± 125
Mn	20,000	137 ± 5.4	138 ± 5.9	142 ± 1.7	144 ± 6
	60,000	146 ± 12.9	143 ± 9.5	135 ± 6.3	151 ± 15
Ni	20,000	8.5 ± 0.2	8.2 ± 0.04	8.3 ± 0.4	8.1 ± 0.5
	60,000	9 ± 0.3	8.6 ± 0.3	8.34 ± 0.2	8.3 ± 0.3
Pb	20,000	21.2 ± 1.1	21.1 ± 0.9	21.1 ± 1.2	19.5 ± 1
	60,000	23 ± 1.4	21.9 ± 1.2	22.4 ± 0.7	20.7 ± 0.6
Zn	20,000	26.6 ± 2.2	26.6 ± 2.2	26 ± 1.6	23.4 ± 1.2
	60, 000	29.4 ± 2.5	26.7 ± 1.7	27.3 ± 1.1	25 ± 0.8

± standard error (n=4)

The experiment was a completely randomized block design with four replications. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS 1998) computer program. The least significant differences among treatment means were tested at the $P < 0.05$ level of probability.

RESULTS

Heavy metal data are presented and discussed in this paper either as essential or toxic metals, although it is widely accepted that most microelements are toxic to plants in large quantities irrespective of their essentiality for structural or physiological functions. Some microelements are generally essential in all plant species, while others are essential in specific plant species (Alkorta *et al*. 2004). Although we discuss Ni in this paper as a toxic element, we are aware that Ni is essential in certain plant species such as pecan (*Carya illinoinensis*) because it activates RNase A - Urease and appears to assist in the catabolism of stored N forms being translocated in spring xylem sap to growing points (Bai *et al*. 2006; Wood *et al*. 2006).

Heavy metals in leaves

The total concentrations of toxic heavy metals (Cd, Ni, and Pb) in leaves were variably partitioned between the bottom (old) and top (young) leaves in both 20,000 and 60,000 plant population densities (**Fig. 1**). The concentration of toxic heavy metals in the top leaves increased with increase of EDTA application rate to 1.0 g per kg soil. However, top leaves at high plant density accumulated significantly higher amounts of the three toxic heavy metals at EDTA rates less than 2.0 g/kg soil, even in control plots, compared to plants at 20,000 plant per ha. At 2.0 g per kg soil application rate, EDTA did not affect the accumulation of toxic heavy metals in the top leaves of plants grown at 20 000 plants per hectare but significantly reduced the concentration of toxic heavy metals in top leaves of plants grown at 60,000 plants per hectare. The concentration of toxic heavy metals in the

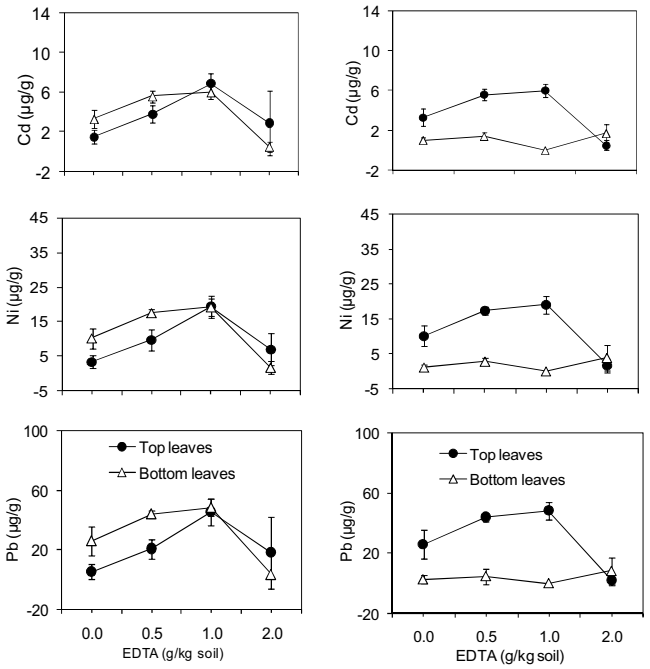

Fig. 1 Partitioning of toxic heavy metals in the top and bottom leaves of the sunflower plant grown at two population densities and treated with different amounts of EDTA salt. Left hand side graphs: plant density = 20,000 plants/ha; right hand side graphs: plant density = 60,000 plants/ha.

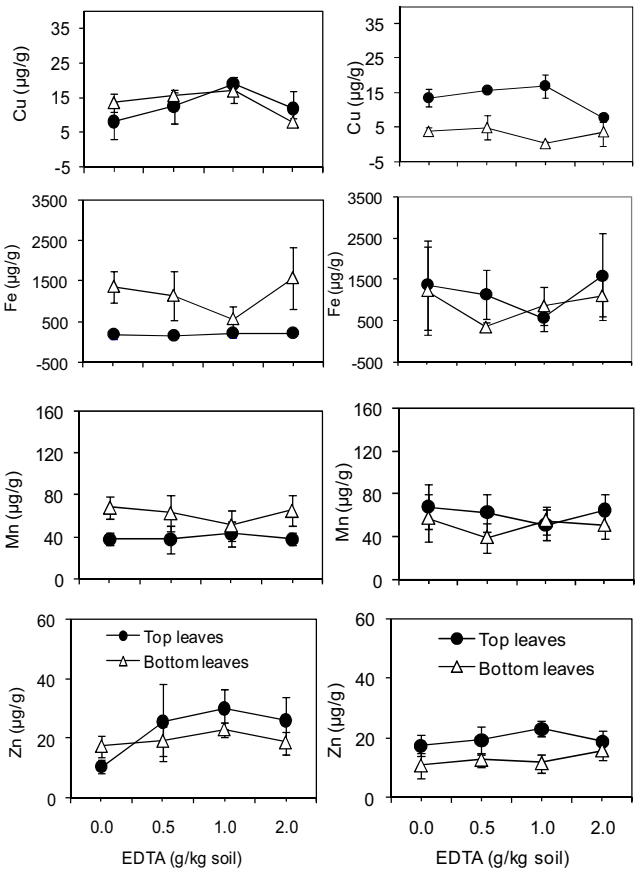

Fig. 2 Partitioning of the essential heavy metals in the top and bottom leaves of the sunflower plant grown at two population densities and treated with different amounts of EDTA salt. Left hand side graphs: plant density = 20,000 plants/ha; right hand side graphs: plant density = 60,000 plants/ha.

bottom leaves increased in plots that received EDTA at 0.5 and 1.0 g per kg soil at 20,000 plants per ha (**Fig. 1**, left). However, EDTA at 2.0 g/kg soil reduced the accumulation of toxic heavy metals in the bottom (old) leaves at 20,000 plants per hectare, while no effect of EDTA was noticed at 60,000 plants per ha.

In the control and 0.5 g per kg EDTA treatments, the concentration of toxic heavy metals in the bottom (old) leaves was higher than in the top (young) leaves in plants grown at 20,000 plants per hectare (**Fig. 1**). Contrary to that, the top leaves at 60,000 plants per ha had higher concentration of the three toxic heavy metals than the bottom leaves. However, similar amounts of heavy metals in µg g^{-1} were accumulated only in the top leaves at 1.0 g EDTA per kg at both low and high plant densities: Cd, 6; Ni, 20; and Pb, 40 (**Fig. 1**). At both plant densities, the highest accumulation of toxic heavy metals in both top and bottom leaves generally occurred at the EDTA treatment rate of 1.0 g per kg soil. However, the high plant population density of 60,000 plants per ha seemed to have depressed the accumulation of the toxic heavy metals in their bottom leaves in favour of depositing them in the upper leaves.

The accumulation of essential heavy metals, Cu and Zn was enhanced only in the top leaves due to application of 1.0 g EDTA per kg soil, while Fe and Mn concentrations in all leaves (top and bottom) were not affected by EDTA at both plant densities (**Fig. 2**). However, 2.0 g EDTA per kg soil reduced the concentration of Cu in the top leaves at 60,000 plants per ha (**Fig. 2**, right). Plants tended to accumulate the mobile essential metals (Cu and Zn) in top leaves at a plant density of 60,000 plants per ha. Compared to those at 20,000 plants per ha, bottom leaves at 60,000 plants per ha tended to contain lower concentrations of Cu and Zn. Neither EDTA nor plant population density affected the concentrations of Mn and Fe in all leaves. However, at 20,000 plants per ha higher concentration of Mn and Fe accumulated in the bottom (old) leaves compared to the top (young) leaves (**Fig. 2**, left), and this is comparable to the results in the Page *et al*. (2006) study in which redistribution of Fe and Mn was found to be limited in plants because Fe and Mn tend to remain in older (bottom) leaves.

Table 4 shows adaxial and abaxial stomatal resistances. For all treatments, stomatal resistances of plants grown at 60 000 plants per ha were less than those of plants grown at 20 000 plants per ha.

Heavy metals in the stem

The concentrations of three toxic heavy metals (Cd, Ni, and Pb) in the stem are presented in **Fig. 3**. EDTA at 1.0 g per kg soil increased the accumulation of toxic heavy metals in the top half of the stem in plants grown at 20 000 plants per ha and in the bottom half of the stem at 60,000 plants per ha (**Fig. 3**). Accumulation of Cd, Ni, and Pb in the bottom stems at 60,000 plants per ha exceeded accumulation in similar plant parts by 4, 17, and 40 times at 20,000 plants per hectare when 1.0 g EDTA per kg soil was applied. At low population density (20,000 plants per ha), EDTA enhanced the accumulation of toxic heavy metals in the top part of the stem (**Fig. 3**, left), while at high population density (60,000 plants per ha) EDTA promoted accumulation of toxic heavy metals in the bottom parts of the stem (**Fig. 3**, right). The concentrations of the toxic heavy metals in the bottom half of stems at 20,000 plants per ha were not affected by EDTA. However, there were slight increases that occurred as a result of 2.0 g EDTA per kg application in bottom stems of plants grown at 60,000 plants per ha.

The concentrations of essential heavy metals (Cu, Fe, Mn, and Zn) in the stem were not affected by EDTA treatments, with the exception of Cu in the bottom part of stem at 60,000 plants per ha (**Fig. 4**).

Heavy metals in roots and soil

The concentration of heavy metals in the roots and soil after plant harvest are presented in **Tables 2** and **3**. At both densities, plant roots grown on plots that received 1.0 g EDTA per kg soil had lower concentration of toxic heavy metals as compared to roots from control treatments (**Table 2**) indicating that EDTA reduced accumulation of toxic heavy metals (Cd, Ni, Pb) in plant roots. In fact, application of 1.0 and 2.0 g EDTA per kg soil reduced toxic metal concentrations in the root to levels below 1.0 µg/g at 60,000 plants per ha. However, the concentrations of essential heavy

Table 4 Adaxial and abaxial stomatal resistance of sunflower leaves grown on soil that received biosolids for more than 25 years in EDTA-facilitated phytoextraction. The measurements were taken on 01 September, 24 days after EDTA application (n=4).

EDTA (g/kg soil)	Stomatal Resistance (s.cm^{-1}) ‡			
	20,000 plants/ha		60,000 plants/ha	
	Adaxial	Abaxial	Adaxial	Abaxial
0	1.93 ± 0.88	2.62 ± 2.41	1.54 ± 0.71	1.71 ± 0.53
0.5	2.81± 0.92	3.61 ± 4.64	2.74 ± 0.93	1.39 ± 0.89
1.0	1.40 ± 0.85	2.47 ± 2.56	1.11 ± 0.34	1.10 ± 0.32
2.0	1.69 ± 0.95	1.91 ± 1.14	1.38 ± 0.40	1.28 ± 0.54

‡ Maximum and minimum temperatures on 01 September were 29 and 13 °C.
± Standard deviation

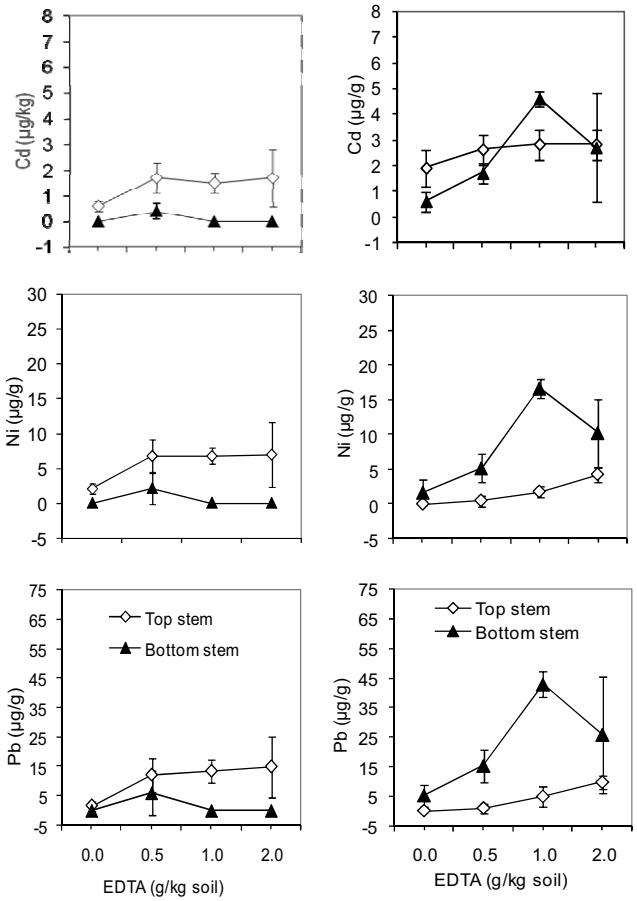

Fig. 3 Partitioning of the three toxic heavy metals in the top and bottom part of the stem of the sunflower plant grown at two population densities and treated with different amounts of EDTA salt. Left hand side graphs: plant density = 20,000 plants/ha; right hand side graphs: plant density = 60,000 plants/ha.

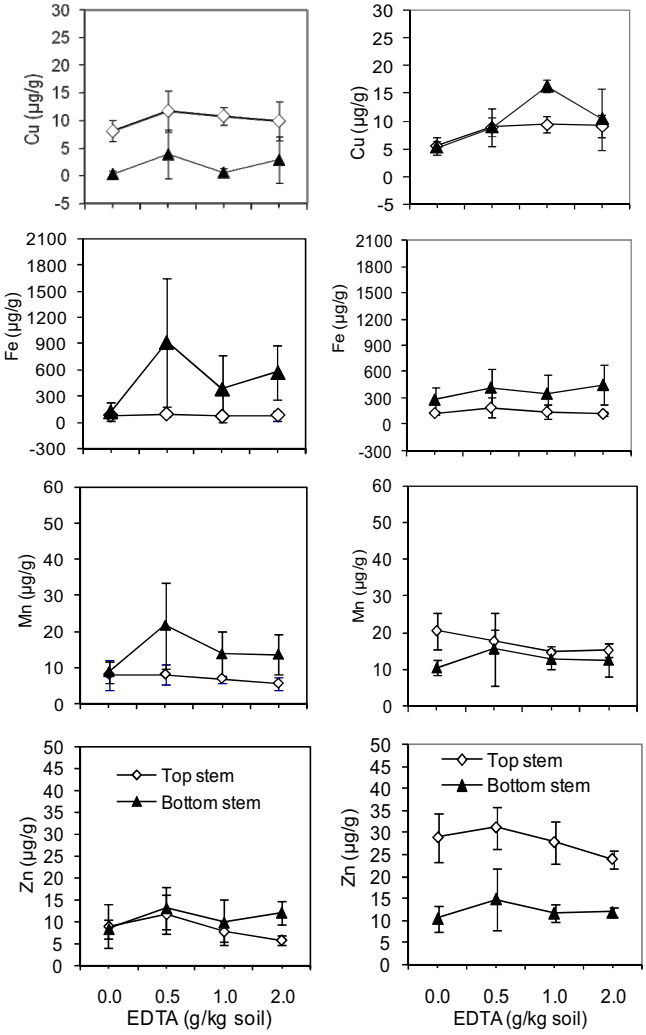

Fig. 4 Partitioning of the four beneficial (essential) heavy metals in the top and bottom part of the stem of the sunflower plant grown at two population densities and treated with different amounts of EDTA salt. Left hand side graphs: plant density = 20,000 plants/ha; right hand side graphs: plant density = 60,000 plants/ha.

metals, with the exception of Cu, remained high in the roots at both plant densities due to EDTA. The concentration of Cu in the roots decreased as a result of 0.5 EDTA application at both 20,000 and 60,000 plant per ha. There was a tendency of increase of Zn accumulation in the roots with increase of EDTA application rate.

The concentration of heavy metals in soil (0-50 cm below soil surface) is presented in **Table 3**. Soil from plots that had 60,000 plants per ha had higher concentrations of heavy metals compared to plots that that had lower plant density (20,000 plants per ha).

DISCUSSION

The mobilized toxic metals from the roots at 20,000 plants per ha were translocated mainly to the leaves, and very little went to the stems (**Figs. 1, 3**). In plants at 60,000 plants per ha, the ultimate sinks for the toxic metals were top (young) leaves and the bottom stems, and both sinks had similar amounts of each of the toxic heavy metals (**Figs. 1, 3**). Although the essential metals were variably mobilized and translocated within the plant, distribution of these metals was probably in accordance to physiological needs of the cells or organs. Similar observations were reported by Kusoto *et al*. (1992) in their heavy-metal study involving grain crops. Unlike toxic heavy metals, plants did not avoid accumulating high amounts of essential heavy metals in their roots probably because the metals were required by the plants and were not harmful to the roots for plants to store there to meet future demands. The amount of essential heavy metals in the roots at both plant densities was within normal ranges (**Tables 1, 2**).

Effect of plant density

When EDTA of less than 1.0 g per kg soil was applied, high concentration of toxic heavy metals (Cd, Ni, and Pb) and two essential heavy metals (Cu and Zn) accumulated in the bottom leaves of plants grown at 20,000 plants per ha, whereas in plants at 60,000 plants per ha, those metals accumulated in top leaves. Both Fe and Mn, which are known to be less mobile in plants, were not affected by either EDTA or plant density. This is probably because the total concentrations of these essential nutrient elements were abundant in the soil to such an extent that mobilization of these metals by EDTA could not enhance their uptake by roots and translocation in the plant.

The elevated amounts of Cd, Cu, Ni, Pb, and Zn in the top leaves of plants at 60,000 plants per ha relate to the low stomatal resistance measured in those leaves. At 20,000 plants per ha, the stomatal resistances (s cm^{-1}) of the control treatment on 01 September were higher (adaxial, 1.93; abaxial, 2.62) than of the plants grown at 60,000 plant per ha (adaxial, 1.54; abaxial, 1.74) (**Table 4**). Similar differences occurred even when various amounts of EDTA were applied. Chelant-metal complexes are mainly carried up the plant in the transpiration stream to the shoots where transpiration occurs (Gleba *et al*. 1999). Metal accumulation in the leaves is thought to be driven primarily by mass flow caused by transpiration, which serves as a natural pump that assists in the uptake and translocation of metals and their chelate up the plant through the xylem (Salt *et al*. 1998).

High stomatal resistance, which results in low transpiration rate, caused low retranslocation of toxic heavy metals from the bottom leaves to the top leaves at 20,000 plants per ha. When EDTA of less than 1.0 g per kg soil was applied, the top stems at 60,000 plants per ha accumulated a far smaller amount (50-95% less) of Cd, Ni, and Pb than the upper leaves, which indicates that high transpiration rate in upper leaves forced retranslocation of metals from the bottom plant parts to those leaves. Similar transpiration effects could not be observed when more than 1.0 g EDTA per kg soil was applied because this high amount of EDTA was probably toxic to the plants. EDTA can be harmful to plants, because at high concentrations it is toxic and behaves like a detergent (Sillanpaeae 1996; Dirilge 1998; Shahandah and Hossner 2000).

When less toxic EDTA rates (less than 1.0 g per kg soil) were applied, absorbed toxic metals in the roots at 60,000 plants per ha were retranslocated to the bottom half of stems where they accumulated in high concentration, whereas these heavy metal were retranslocated to the upper stems in plants at 20,000 plants per ha, bypassing bottom stems as the second storage site or sink. This probably indicates that although EDTA could assist in translocation of metals from the roots to the stems, any further translocation of metals to the leaves (away from the main xylem in the stem) needs facilitation by a high transpiration rate in the top leaves.

The lower stomatal resistance in plants at 60,000 plants per ha than in plants at 20,000 plants per ha was probably due to close plant spacing (higher plant density) which provides a close leaf canopy that limited penetration of solar radiation to the soil surface and thus reduced soil surface evaporation. The reduced evaporation on the soil surface at 60,000 plants per ha might have conserved plant available water that plants took up with metals and chelate. However, the plants at 20,000 plants per ha had more exposed soil surface that was prone to high soil surface evaporation demands due to a small leaf canopy provided by a lower plant density.

EDTA and metal uptake

1. Toxic heavy metals

EDTA mobilized the toxic heavy metals (Cd, Ni, and Pb) in the soil and facilitated their translocation from roots to the shoots of the plant. Although toxic metals are usually confined to the roots with minimal transport to the shoot (Jarvis and Leung 2001; Alkorta *et al*. 2004), 1.0 g EDTA per kg soil at 20,000 plants per ha and 2.0 g EDTA per kg soil at 60,000 plants per ha facilitated the movement of nearly all toxic metals from roots to the above ground parts (**Table 2**). This is desirable because above ground plant parts can be harvested and burned into manageable ashes. Vassil *et al*. (1997) found that the majority of Pb directly measured in the xylem of *B. juncea* was transported in coordination with EDTA. No translocation of toxic metals into the shoots in the absence of a chelant has been reported (Nowack *et al*. 2006). Although several studies have shown that EDTA promotes more accumulation of Pb in plants (Huang *et al*. 1997; Schmidt 2003), the other two toxic metals also accumulated in the plants as result of EDTA application. Furthermore, the Pb-EDTA complex has a high stability constant that slows down its degradability in soil and plant, and hence makes it moveable in a plant with minimum toxic effect to the plant. Plants are expected to accumulate high Pb in EDTA enhanced phytoextraction because EDTA has a high affinity for the Pb (Blaylock *et al*. 1997; Salt *et al*. 1998).

2. Essential heavy metals

EDTA was not necessary to mobilize essential metals (Cu, Fe, Mn, and Zn) in soil and to assist in the translocation of these metals in the plant because there was no difference in metal concentration between EDTA and control treatments in the roots (**Table 2**). Actually, plant organs in some control treatments accumulated higher concentrations of these essential heavy metals than those in the EDTA treatments (**Figs. 2, 4**). These nutrient elements are integral constituents of enzymes or involved in the activation of enzymes. Copper activates ascorbic oxidase and pheloxidase; Fe is a component of heme proteins, ferredoxin, and Fe-S proteins; Mn is needed for activation of enzymes in the Krebs cycle, photosynthesis, and O_2 evolution; Zn activates carbonic anhydrate and glutamate dehydrogenase. Large amounts of Zn, a mobile element, were taken up by plants and moved to the leaves even in the control treatment without EDTA, a phenomenon similar to what was reported by Nowack *et al*. (2006). Sunflower plants took up essential micronutrients probably in accordance to their physiological requirements, and EDTA had little impact on uptake and translocation of essential heavy elements metals in the plant. However, in the presence of multiple metals in the soil, metal chelation might be plant- and metal-specific and is subject to inhibition (Chen and Cutright 2001).

SUMMARY AND CONCLUSIONS

The accumulation of large amounts of toxic heavy metals in the bottom (old) leaves of plants grown at a low plant density (20,000 plants/ha) and in bottom stems at a high plant density of 60,000 plants per ha indicates that plant spacing or plant density can be manipulated to determine the sinks of toxic heavy metals in EDTA facilitated phytoremediation. However, the same cannot be done with the essential heavy metals because physiological requirement of plants for these nutrient elements determines where they are retranslocated, and this may not need facilitation by EDTA as observed in **Figs. 2** and **3**. Most of the leaves that fall to the ground are bottom, old leaves (Hikosaka 2005). Since these leaves accumulate small concentrations of toxic heavy metals in plants grown at a high plant density (60,000 plants/ha), it is recommended to grow sunflower at high plant density to reduce the amount of toxic metals that go back to soil with bottom leaves.

The use of a sunflower crop on agricultural holdings in phytoremediation has some economic benefits to a farmer, because, while reducing the heavy metals in the soil, oil for biofuel can be attained from the harvested grains. Seeds for planting the next crop in the next season can be produced in low to medium contaminated land, and that enables the farmer to save money that could be spent on seeds. According to Robinson *et al*. (2003), phytoremediation should be combined with a profit making operation that is unaffected by elevated plant heavy metal loadings.

The use of EDTA for phytoextraction of heavy metals in phytoremediation can be environmentally unsafe because mobilized metals can leach to groundwater (Schmidt 2003; Thayalakumaran *et al*. 2003) that many people use as a source of drinking water. Therefore, other chelating agents that are considered less harmful to the environment such as citric acid, nitrolotriacetic acid (NTA), and ethylenediamine dissucinic acid (EDDS) should be considered in the phytoremediation of biosolids farms with elevated concentrations of heavy metals (Alkorta *et al*. 2004; Nowark *et al*. 2006). However, phytotoxicity and leaching of metals can be reduced by gradual application of small doses of a chelant during the growth period, and placing them into the root zone of crops as suggested by Schmidt (2003) and Alkorta *et al*. (2004).

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