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Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids

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Abstract

Heavy metals should be removed in soil for the safety of the environment, and phytoremediation, which is the use of plants to remove contaminants from the environment, can be useful in rehabilitating polluted sites. Chelating agents such as ethylenediamine tetraacetic acid (EDTA) have been used in different situations in phytoremediation to enhance the extraction of heavy metals by plants from soil. The objectives of the study were (1) to assess the availability of heavy metals in soil injected with biosolids and in composted biosolids with or without EDTA amendment, (2) to determine the efficacy of EDTA on uptake of heavy metals by sunflower plants from soil with biosolids and composted biosolids, and (3) to investigate whether EDTA applied in the previous season can reduce growth of the next crop. A laboratory and two greenhouse experiments were conducted at Kansas State University. In the laboratory, five EDTA doses were applied into the flasks with 5 g of either soil injected with biosolids (soil) or composted biosolids (compost). The soil and EDTA solution in flasks were shaken by electronic shaker for 4 h. The solution was decanted 2 h after shaking and then tested for the concentration of heavy metals by inductively coupled plasma emission spectrometry (ICP-ES). In the greenhouse, sunflower (*Helianthus annuus*) plants were grown in soil with biosolids and in the composted biosolids. At the flowering stage, EDTA salt at 0, 1.0, and 2.0 g/kg soil or compost was applied. Plant organs were separated and dried at the end of the trial. The concentrations of heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) were analyzed using ICP-ES. After harvesting the first greenhouse experiment and extraction of the roots, the pots were refilled with the same soil or compost and five sunflower seeds were sown to establish a second greenhouse experiment. The emergence of seedlings and plant growth was determined. In the laboratory experiment, heavy metal concentration in the solution was increased by EDTA in both soil with biosolids and the composted biosolids. The solution from composted biosolids which received EDTA had a higher concentration of heavy metals than solution from soil injected with biosolids. EDTA increased the accumulation of Cd and Ni in the roots of plants grown in the composted biosolids. Essential heavy metal (Cu, Fe, Mn, Zn) concentrations were increased in the organs of the plants grown in the composted biosolids where EDTA at 2.0 g/kg soil was applied. The concentrations of all seven heavy metals in the plant organs were not affected by EDTA in soil injected with biosolids. The high EDTA dose of 2.0 g/kg soil reduced seedling emergence and height of the plants grown in the composted biosolids.

Keywords: Biosolids; Ethylenediamine tetraacetic acid; Heavy metals; Sunflower; Chelate; Phytoremediation

1. Introduction

Pollution of the environment by heavy metals poses a threat to surface water and groundwater, which are used as the main sources of drinking water by many people in the world. Heavy metals in the soil may enter the human food web through plants. Phytoremediation, which is the use of green plants to remove metals from the soil or environment, is an environmentally sustainable approach to remediate moderate, diffuse, and shallow metal contamination (Salt et al., 1998; Hammer and Keller, 2002).

Land application of biosolids, either as liquid sludge or composted material, often adds heavy metals into the soil. The city of Manhattan, Kansas in the United States of America (USA) has been injecting aerobic digested biosolids below the soil surface for 25 years, while the city of Topeka, Kansas in USA has been piling composted biosolids near its waste water treatment plant from the 1980s. The composted biosolids in Topeka are often given away to gardeners, who spread the

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material on lawns, flowerbeds or vegetable plots. In other USA states, composted biosolids are sold as fertilizers because biosolids are rich in plant nutrients. Although utilization of biosolids as crop fertilizers has gained momentum in some states in the USA, there are still organized groups which lobby against the use of biosolids or sludge as fertilizers for growing food crops because biosolids may harbour health risk substances such as heavy metals.

Extraction of heavy metals by plants is usually limited by availability of metals to plants in the soil (Stanhope et al., 2000). Biosolids or soil with biosolids have high organic matter (McBride, 1994 [121–164]; Calace et al., 2002), which increases complexation of metals with organic matter and renders the metals unavailable to plants for uptake (Xia et al., 1997; Yoo and James, 2002). High organic matter (O.M.) content and cation exchange capacity (CEC) are some of the most important soil factors that determine the bioavailability of metals to plants (Liphadzi and Kirkham, 2005). Several studies showed that chemical amendments such as chelating agents increase metal availability and uptake by plants (Blaylock et al., 1997; Huang et al., 1997; Wu et al., 1999; Kirkham, 2000; Madrid et al., 2002). However, most of the studies in which EDTA was used in phytoremediation involved artificial spiking of soil with heavy metal salts, and the time for metals to react with O.M. and other metals was usually limited. In case of the metals in biosolids or sludge the metals had more time to react with each other and with the biosolids (Liphadzi and Kirkham, 2005). Therefore, heavy metals spiked into the soil might be more easily extracted by plants and chelated by EDTA than the heavy metals sorbed by biosolids.

Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for phytoremediation because it has a strong chelating ability for different metals and it also increases the bioavailability and plant uptake of the metals in the soil (Salt et al., 1998; Huang et al., 1997; Norvell, 1991). About 80% of the total soil metal is solubilized and becomes available for phytoextraction when EDTA is applied (Haag-Kerwer et al., 1999). Synthetic chelating agents such as EDTA allow plants which are not considered as hyper-accumulators to be usable for phytoremediation purposes, because chelates induce plants to take up more heavy metals than they normally accumulate (Liphadzi et al., 2003).

Application of EDTA on soil at the flowering or maturity stages has been recommended by Salt et al. (1998) and Sun et al. (2001), because solubilized metals and EDTA salt can be toxic to plants and thus hinder plant growth and phytoextraction of heavy metals. Moreover, a few studies showed that EDTA is relatively stable in the soil; it may remain in the soil for a long time (Hong et al., 1999; Nortemann, 1999; Satroutdinov et al., 2000). Therefore, we postulated that if EDTA remains in soil after the removal or harvesting of the plants, growth and development of plants grown later on the same soil or land might be inhibited by EDTA salt residues in the soil. There is no study, which investigated the effect of EDTA residues on development and growth of the plants grown after (in succession) on the same soil or piece of land that received EDTA in the previous season. The objectives of the study were (1) to assess the availability of heavy metals in soil injected with biosolids and in composted biosolids with or without EDTA amendment, (2) to determine the efficacy of EDTA on uptake of heavy metals by sunflower plants from soil with biosolids and composted biosolids, and (3) to investigate whether EDTA applied in the previous season can reduce growth of the next crop.

2. Materials and methods

Three experiments were conducted in 2001, one in the laboratory and two in the greenhouses at Kansas State University in the United States of America. Soil injected with biosolids for 25 years and composted biosolids were used in all three trials. The soil was collected from the Manhattan Biosolids Farm in Kansas, and was sampled from five farm sites at the 0–0.5 m soil depth. The soil of the Manhattan biosolids farm is mapped as a Haynie very fine sandy loam and is classified as coarse-silty, mixed, calcareous, mesic typic undifluvent. Injection of biosolids at the Manhattan Biosolids Farm is done annually below the soil surface at a depth between 15 and 30 cm. The City of Manhattan, Kansas began applying biosolids or sewage sludge to the city owned farm in 1976. The composted biosolids were collected from a piling site near the waste water treatment plant in Topeka, Kansas, USA. The waste water treatment plant in Topeka began piling composted biosolids in the 1980s. The chemical analysis of soil with biosolids from the Manhattan Biosolids Farm and the composted biosolids from Topeka is presented in Table 1. The concentration of heavy metals and other elements were

Table 1
Chemical analysis of soil from the Manhattan Biosolids Farm (soil injected with biosolids) and composted biosolids from the Topeka Wastewater Treatment Plant

	Soil injected with biosolids [a]	Composted biosolids
O.M %	2.1	16.4
CEC (meq/100 g)	13.2	32.8
Elec. conduc (dS/m)	4.4	13.5
pH	6.2	6.2
Salinity	High	Extremely high

Salinity	High		Extremely high	
	Total	Extractable	Total	Extractable
	mg/kg		mg/kg	
Na	– [b]	89	– [b]	182
Ca	–	2446	–	8338
K	–	324	–	448
Mg	–	241	–	610
Cd	0.8	–	4.2	–
Cu	16.7	6.4	190	46
Fe	8773	81	15,719	135
Mn	167	4	469	97
Ni	8.93	–	17	–
Pb	27	–	139	–
Zn	31	12	227	120

[a] Soil was sampled at depth between 0–30 cm.
[b] Data not available (not analysed).

higher in the composted biosolids than in the soil with biosolids. Salinity was extremely higher in the composted biosolids as compared to soil with biosolids.

2.1. Laboratory trial

In the laboratory, 5 g of either soil injected with biosolids or composted biosolids was weighed into 100-ml glass flask. The tetrasodium salt of EDTA, at five doses, was dissolved in deionized water and added to the flasks with either soil or compost. The EDTA treatments were 0 g EDTA/kg soil or compost, 0.5 g EDTA/kg soil or compost, 1.0 g EDTA/kg soil or compost, 2.0 g EDTA/kg soil or compost, and 3.0 g EDTA/kg soil or compost. Treatments were replicated five times. After the EDTA salt solution was added into the flask, the soil and EDTA solution were shaken by an orbital shaker for 4 h to enable the EDTA salt to solubilize and bind metals in soil with biosolids and the composted biosolids. The sediments in the solution were then given time to settle at the bottom of the flask before the solution was decanted into test tubes. The decanted solution was filtered and tested for the concentrations of three toxic heavy metals, cadmium (Cd), nickel (Ni), and lead (Pb) and four essential heavy metals copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) using inductively coupled plasma emission spectrometry (ICP-ES). The detection limits for water in mg/L were Cd, 0.005; Cu, 0.20; Fe, 1.00; Mn, 0.60; Ni, 0.10; Pb, 0.10; and Zn, 0.10.

2.2. First greenhouse trial

In the greenhouse experiment, pots were filled with 6000 g of dry soil from the biosolids farm or composted biosolids. The inside of the pots was lined by a plastic bag to prevent the loss of heavy metals with drainage water. Five sunflower (*Heliathus annuus*) seeds were planted 5-cm deep below the soil surface on 27 October 2001. After two weeks, seedlings were thinned to 2 plants per pot. Plant height was measured throughout the growing period on 54 DAP, 58 DAP, 61 DAP, 64 DAP (days after planting). The tetra sodium salt of EDTA at 0 g/kg medium, 1.0 g/kg medium, and 2.0 g/kg medium (soil with biosolids or composted biosolids) was applied 54 DAP when plants had developed flower heads. Treatments were replicated four times. During the first 3 weeks of the trial, 30 ml of water was irrigated onto each pot per week, and thereafter increased to 60 ml per pot per week.

At the end of the experiment, plant organs were separated and the total plant biomass was measured. All roots were extracted from soil by the wet sieving method. The concentrations of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, Zn) in the plant organs were analyzed using ICP-ES. A method similar to that of Sposito et al. (1982) was used to analyze soil for total concentrations of Cd, Cu, Fe, Mn, Ni, Pb and Zn. 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 of 4 M HNO_3 at 80 °C. We used 2 g samples, but added 20 ml of 4 M HNO_3 and heated the mixture for 20 h at 80 °C in a water bath. The extract was analyzed using ICP-ES.

2.3. Second greenhouse trial

After harvesting and extraction of the roots of the plants in the first greenhouse trial, the pots were emptied and then refilled with similar soil with biosolids or composted biosolids that was in the pot during the first greenhouse trial. As a common practice, EDTA salt is applied to soil when plants are matured, usually at flowering to take advantage of a huge plant biomass for metal accumulation and to delay the toxic effect of the EDTA salt and solubilized metals to plants. Again five sunflower seeds were planted at 5 cm soil depth. The number of seedlings emerged above the soil surface were counted on 15 DAP (days after planting), 22 DAP, and 27 DAP. Plant height was measured during the growing period on 15 DAP, 27 DAP, and 55 DAP.

2.4. Data analysis

Differences between treatments were determined by analysis of variance (ANOVA) using a statistical analysis system (SAS Institute, 1998). Treatment means were compared using least significant difference (LSD) at the 5% level of significance.

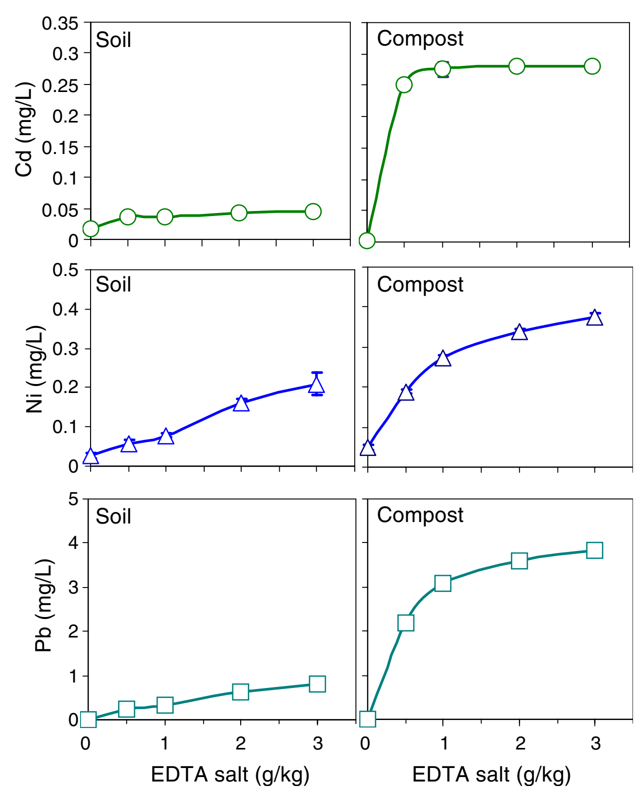

Fig. 1. Concentration of toxic heavy metals (Cd, Ni, Pb) in the solutions of soil injected with biosolids (here referred to as soil) and composted biosolids (here referred to as compost) at different EDTA application rates.

3. Results and discussion

3.1. Concentration of heavy metals in the soil or compost solution (bioavailability)

The tetrasodium salt of EDTA increased the concentration of the toxic heavy metals (Cd, Ni, and Pb) in the solutions of both soil with biosolids and the composted biosolids (Fig. 1). In both media, Ni and Pb concentrations increased in the solution with increase of EDTA doses. It seems that EDTA application rates higher than 3.0 g/kg would still increase the amount of soluble Ni and Pb in both growing media. However, EDTA doses above 0.5 g/kg did not further increase Cd concentration in the solutions of both soil injected with biosolids and composted biosolids. This suggested that it was not beneficial to the process of Cd phytoextraction to increase doses of EDTA above 0.5 g/kg soil or composted biosolids because similar amounts of Cd were solubilized at all three doses above 0.5 g/kg soil. This can be attributed to the fact that Cd is weakly adsorbed by soil and other sediments, which makes it more easily leached to groundwater (Basta and Sloan, 1999; McLaughlin et al., 2000; Perronnet et al., 2000). With EDTA, the concentrations of the three toxic heavy metals in the composted biosolids were higher than in soil with biosolids, which could be due to the different metal concentrations in the two media (Table 1). Without EDTA, the concentration of the toxic heavy metals in solution of the composted biosolids was similar to that in solution from the soil injected with biosolids.

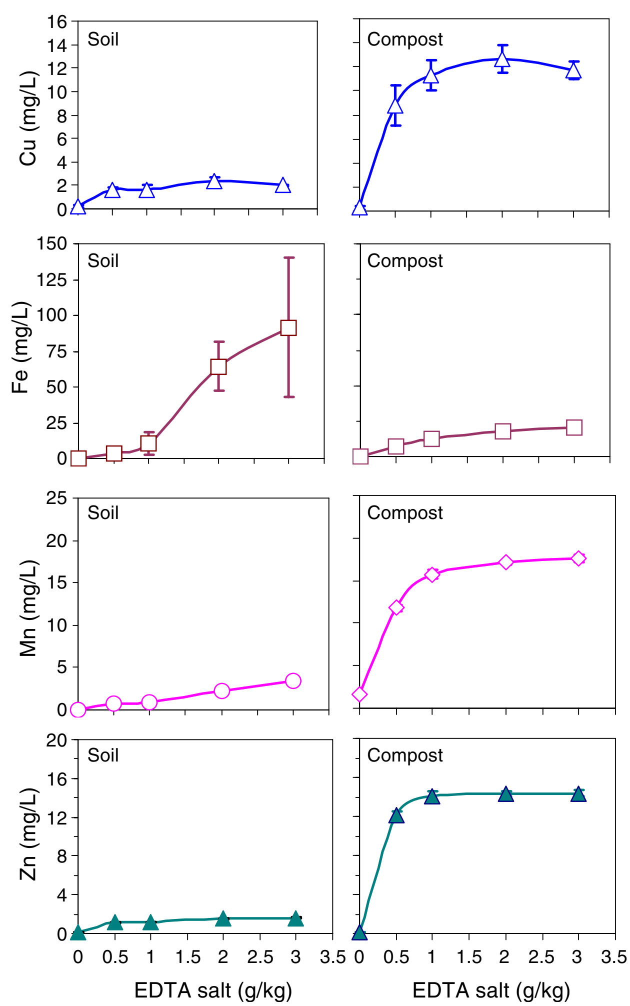

Fig. 2. Concentration of essential heavy metals (Cu, Fe, Mn, Zn) in the solutions of soil injected with biosolids and composted biosolids at different EDTA application rates.

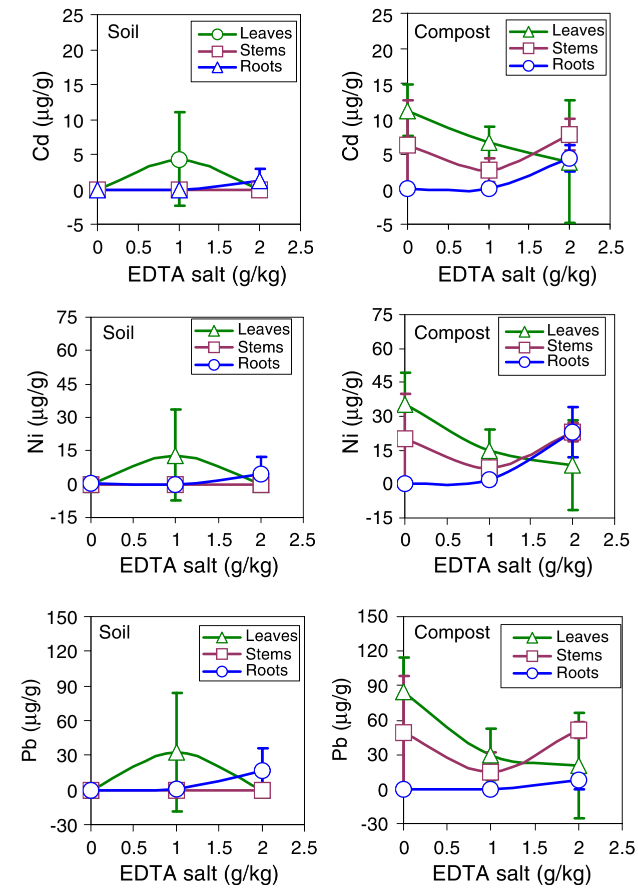

Fig. 3. Concentration of toxic heavy metals (Cd, Ni, Pb) in leaves, stems, and roots of sunflower plants grown in soil with biosolids and in the composted biosolids amended with different EDTA doses.

The concentrations of essential heavy metals (Cu, Fe, Mn and Zn) were increased by EDTA in the solution of the composted biosolids (Fig. 2). However, in soil injected with biosolids, Cu, Mn and Zn concentrations were slightly affected by EDTA application. Their concentrations in solution remained relatively low even when EDTA was increased to 3.0 g/kg soil. In the composted biosolids, all four essential heavy metals increased in the solution as a result of EDTA-facilitated solubilization. EDTA doses of 2.0 and 3.0 g/kg compost resulted in similar concentrations of heavy metals as 1.0 g EDTA/kg compost. This indicated that the maximum solubilization of heavy metals in the composted biosolids could be achieved with EDTA doses between 0.5 and 1.0 g/kg soil. This is consistent with studies by Robinson et al. (1999), Kirkham (2000) and Madrid et al. (2002), which stated that EDTA at rates between 0.5 and 1.0 g/kg soil optimized bioavailability and uptake of heavy metals by plants in phytoremediation. The concentrations of Cu, Mn, and Zn were higher in the composted biosolids solution than in the solution of soil injected with biosolids. It is not known why Fe concentration was low in the composted biosolids solution compared to the solution of soil injected with biosolids, especially when the chemical test in Table 1 has shown a higher Fe concentration in the composted biosolids than in soil injected with biosolids.

3.2. Plant uptake

3.2.1. Toxic heavy metals

The amount of heavy metals taken up by plants is presented in Fig. 3. The concentrations of heavy metals in plant organs were not affected by EDTA in plants grown on soil injected with biosolids. In plants grown on the composted biosolids, 1.0 g EDTA/kg compost reduced the concentrations of toxic heavy metals (Cd, Ni, and Pb) in the leaves and stems. However, when the EDTA dose was increased to 2.0 g/kg compost, the concentrations of the Cd and Ni in the roots increased to levels greater than the concentrations known to be toxic to plants. According to Kirkham (1975), Alloway (1995), Fageria et al. (2002), and Liphadzi and Kirkham (2005), the toxic concentrations of heavy metals in µg per g in plants are Cd, 0.1; Ni, 10; and Pb, 30. Natural metal hyperaccumulators allocate a lower concentration of heavy metal to the roots compared with leaves and stems (Baker et al., 1994) because the primary target of heavy metal toxicity is the root system (Godbold et al., 1984). In general, the three toxic heavy metals in the composted biosolids tended to accumulate more in the leaves at low EDTA application rates of 1.0 g/kg compost or without EDTA.

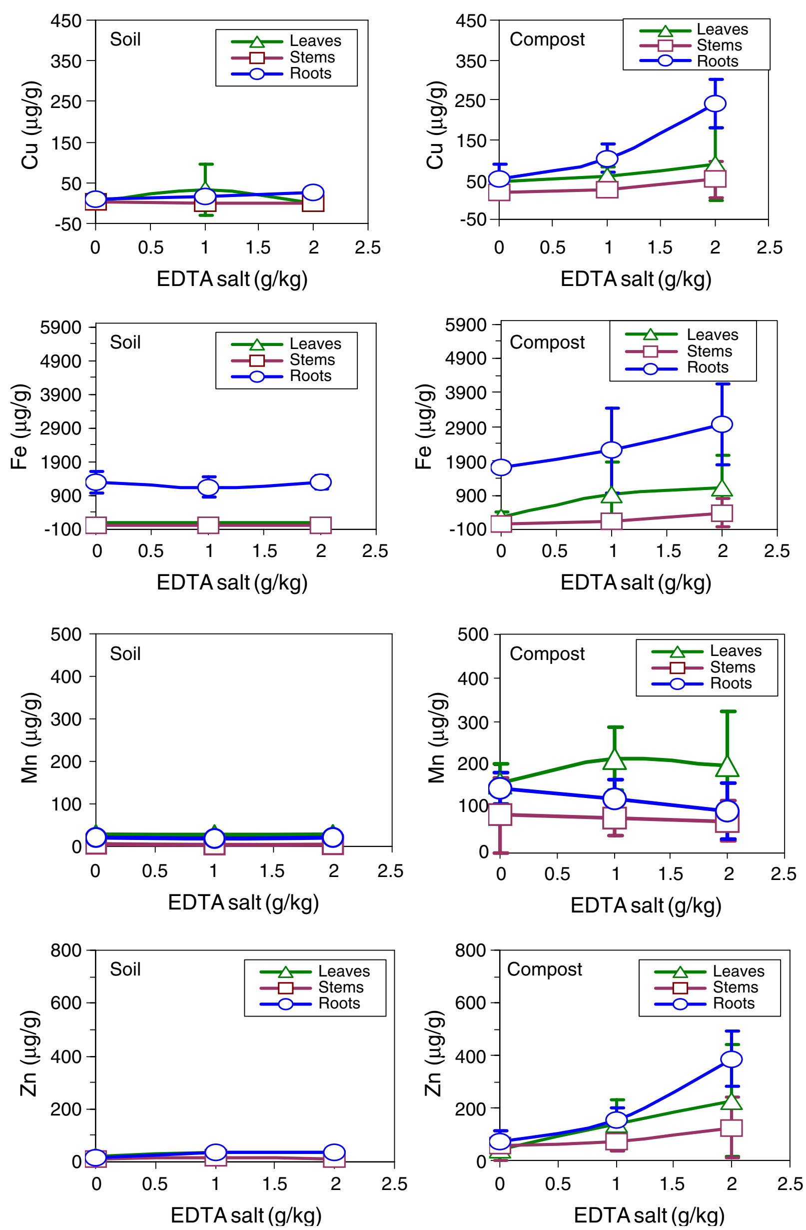

Fig. 4. Concentration of essential heavy metals (Cu, Fe, Mn, Zn) in leaves, stems, and roots of sunflower plants grown in soil with biosolids and in the composted biosolids amended with different EDTA.

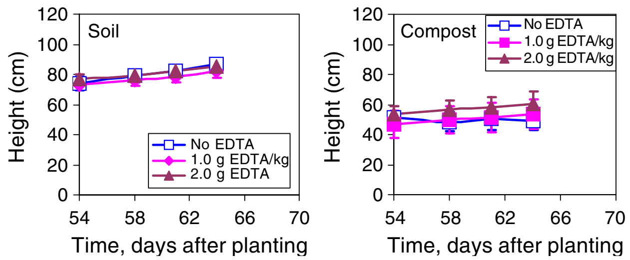

Fig. 5. Height of sunflower plants grown in soil injected with biosolids or the composted biosolids amended with EDTA salt at different doses.

3.2.2. Essential heavy metals

The concentrations of essential heavy metals in the plant organs are presented in Fig. 4. EDTA had no effect on the uptake and accumulation of heavy metals by plants grown in soil injected with biosolids. In plants grown on the composted biosolids, the tetrasodium salt of EDTA at 2.0 g/kg compost increased the accumulation of Cu, Fe, and Zn in the roots. The accumulations of these three heavy metals in the roots were also higher than the concentrations reported to be toxic to plants, which in μg per g are: Cu, 20; Fe, 1000; Zn; 100 (and Mn, 300) [Kirkham (1975), Alloway (1995), Fageria et al. (2002), Liphadzi and Kirkham (2005)]. The roots tended to accumulate more essential heavy metals (with the exception of Mn) than other plant organs at various EDTA application rates. The plants seemed to have had difficulties in translocation of these heavy metals from the roots to the aerial organs, a situation which exacerbates root damage as a result of high toxic concentrations of heavy metals. Root and plant growth are affected by high concentrations of heavy metals in the roots because roots are also the main producers of cytokinin (Crozier et al., 2000 [800]).

3.3. Plant growth and development

3.3.1. Plant height and weight — first greenhouse trial

Height of the plants grown on soil injected with biosolids and those on composted biosolids is presented in Fig. 5. Plants in the composted biosolids were generally stunted (55 cm) compared to plants grown in soil with biosolids (75 cm). Stunting of plant grown in the composted biosolids might have been due to the extreme salinity status of the growth medium (Table 1) and high accumulation of heavy metals in the roots. EDTA had no effect on plant height in both media. In both media, plant height did not change over 10 days (from 54 to 64 days after planting). Fresh and dry weights of the plants per pot were not affected by EDTA in both media. The average total plant fresh weights were 65 g/pot in soil injected with biosolids and 57 g/pot in the composted biosolids, while the mean plant dry weights were 8 g/pot in soil injected with biosolids and 7 g/pot in the composted biosolids.

3.3.2. Seedling emergence in the second greenhouse trial

The effect of EDTA on seedling emergence of the plants in succession is presented in Fig. 6. In soil injected with biosolids, EDTA had no effect on seedling emergence and growth. However, the number of seedlings was reduced by 40% (3 out of 5 seedlings emerged) in the composted biosolids in pots treated with 2.0 g EDTA/kg compost. Poor emergence of seedlings in the composted biosolids with 2.0 g EDTA/kg might have been due to the combined effects of high EDTA dose, extreme salinity, and high amount of other chemical elements in the composted biosolids.

4. Conclusions

The study indicated that without EDTA, the availability of heavy metals in the solution of soil with biosolids (low O.M. and low CEC) was similar to that in the solution of the composted biosolids because heavy metals were complexed by O.M. in the composted biosolids (high O.M. and CEC). However, EDTA solubilized heavy metals that were complexed by O.M., particularly in the composted biosolids. EDTA at 0.5 g/kg was sufficient to free most of the heavy metals from the binding sites in soil injected with biosolids, whilst 1.0 g EDTA per compost was needed to optimize solubilization of metals in the composted biosolids. This study indicated that a high amount of EDTA was needed in the composted biosolids to make most of the metals available for plant uptake. However, high metal concentrations could not guarantee enhanced uptake by plants. Leaves and stems of plants grown in the composted biosolids tended to accumulate more toxic heavy metals (Cd, Ni, and Pb) without EDTA than with EDTA. High EDTA application rates exacerbated the toxicity of the chemical

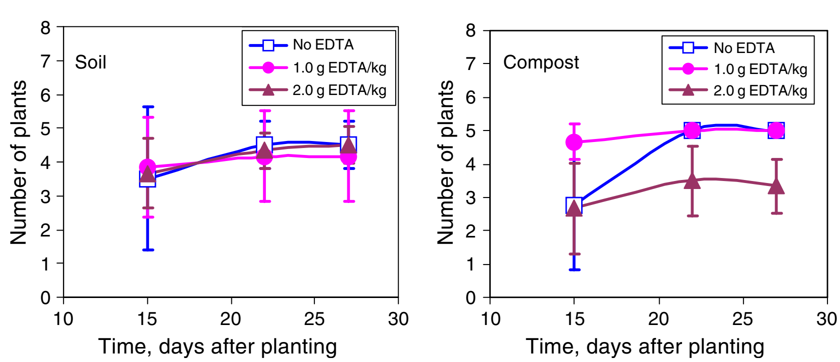

Fig. 6. Number of sunflower seedlings that emerged above the surface of soil injected with biosolids and of the composted biosolids amended with different application rates of EDTA salt.

substances or salts to plants in the composted biosolids, which resulted in poor seedling emergence, plant growth and thus metal uptake.

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