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ORIGINAL PAPER

Effect of soil amendments on phytoextraction potential of *Brassica juncea* growing on sewage sludge

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Abstract A pot experiment was conducted to investigate the influence of elemental sulfur, gypsum and chelating agent (Ethylenediaminetetraacetic acid) on copper, zinc, nickel, cadmium, chromium and lead uptake by *Brassica juncea* from sewage sludge. Addition of sulphur acidified the sludge, which caused the pH decrease to 5.4 with an initial pH 6.7. The shoot and root biomass were increased with sulfur addition, while decreased with Ethylenediaminetetraacetic acid addition. Applications of Ethylenediaminetetraacetic acid and sulfur resulted in a considerable increase in copper and lead concentrations in the plant. The highest root concentration of copper obtained to be 110 mg/kg dw at Ethylenediaminetetraacetic acid treatment. For sulfur treatment, lead concentrations in shoots indicated almost high concentrations 77 mg/kg, about twofold increases relative to roots (34 mg/kg). The Transportation Index of all studied metals were quite low ($TI < 0.5$), whereas the Bioaccumulation Factor values were much higher, varied from 0.01 to 9.67. Furthermore, the plant showed better Bioaccumulation Factor for copper and lead metals in both shoot and root. The efficiency to remove copper and lead from sludge is high in this plant. As a result, elemental sulfur will be effective amendment for phytoextraction of heavy metals from sewage sludge.

Keywords Biosolid Ethylenediaminetetraacetic acid · Elemental sulfur · Gypsum · Heavy metal removal · Hyperaccumulator plant

Introduction

The application of sewage sludge in agriculture is generally considered the best option of management, because it offers the possibility of recycling plant nutrients, provides organic material to the soil, and improves the soil's aggregate stability, porosity and water infiltration rate (Torri and Lavado 2008). However, the presence of high concentrations of toxic metals in sludge poses a constraint on the application of this sludge's to the agricultural land. Therefore, heavy metal removal from sewage sludge is considerable to minimize prospective risks during and after the application. Unfortunately, existing remediation methods for heavy metal removal from sludge or soils are expensive and disruptive (Pogrzeba et al. 2004). In recent years, efforts have focused on the remediation strategies that are less expensive and less destructive than current approaches (Singh and Sinha 2005; Gupta and Sinha 2007a, b; Pogrzeba et al. 2004; Xiaomei et al. 2005; Samake et al. 2003). Phytoremediation, defined as the use of plants to remove pollutants from the environment, which is a promising technology for remediation of contaminated soils and perhaps for the removal of metals from sludge. Unfortunately, the success of phytoremediation depends on solubility of heavy metals and ability of plant to uptake and translocate the heavy metals to the upper parts (Turgut et al. 2004). Several chelating agents, such as CDTA, DTPA, EDDHA, EDDS, EDTA, EGTA, HEDTA and NTA have been studied for their ability to dissolve metals and enhance the uptake of metals by plants. However, these chemicals have limitations due to their negative effects on plant and soil properties (Kaplan et al. 2005; Wenger et al. 2002; Kayser et al. 2000; Catherine et al. 2006; Cui et al. 2004a, b; Wang et al. 2006; Robinson et al. 1999).

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The use of natural elements such as S has, therefore, been suggested for alleviation of these constraints and for soil acidification to increase the solubility of metals in contaminated soils (Kayser et al. 2001; Cui et al. 2004a, b). Thus, the objectives of the study were to determine the heavy metal removal of *Brassica juncea* from sewage sludge and to evaluate the effect of soil amendments such as S and gypsum on the uptake and translocation of heavy metals. EDTA was used as the model chelator to examine enhanced metal mobilization and distribution of heavy metals within the plant tissues. The experiment was carried out in the climatic conditions of Sakarya, Turkey. The experiment was carried out from March 10, 2009 to March 10, 2010.

Materials and methods

Sewage sludge characteristics

Raw, undigested and dewatered sewage sludge (20 % DW) was obtained from Adapazari Municipal Waste Water Treatment Plant. The physicochemical properties of the sludge were: pH 6.7, EC 1998 µS/cm, organic matter content 54 %, total N 4.10 %, P 3.15 %, K 0.12 %, CEC 8.53 cmol (+)/kg, organic carbon 31.21 %, C/N 7.61. Heavy metal content of the sewage sludge was Cu 19 mg/kg, Zn 1435 mg/kg, Cr 243 mg/kg, Ni 79 mg/kg, Pb 34 mg/kg, Cd 3 mg/kg.

Plant and cultivation

B. juncea seeds were sown at a rate of six in the plastic pots containing 4.5 kg of raw sewage sludge and thinned to three individuals in each pot, 1 week after germination. Pots were placed in an open-air condition at an average temperature of 20–25 °C. The tap water was provided to the pots to keep the top layer moist and avoid leaking from the pots. Plant was harvested after 1 year of growing periods.

Experimental design and treatments

The experiment was carried out according to the randomized plot design with four replicates. At the beginning of the experiments, different amounts of elemental sulfur (S) (0, 78, 156, 312, 625 mmol/kg) were used to adjust sludge pH to desired levels based on a preliminary incubation experiment. Sludge pH was monitored periodically by taking 10 g soil and measuring pH. Changes in sludge pH under different treatments of S were shown in Table 1. Sludge was thoroughly mixed every day to ensure equal distribution of S and to accelerate the S oxidation process. Incubation was terminated when pH did not change for 3 consecutive weeks. It took 9 weeks to reach the final pH (5.4) for S amendment (Table 1). Four different applications, which were the equivalents of elemental, finely ground sulfur at a rate of 312 mmol/kg (fresh weight) of sludge (T1); gypsum applied at 1.8 g per pot in consideration of 1 ton per hectare (T2); EDTA applied at a rate of 5 mmol/kg (T3) and control (T4).

Table 1 Changes in sludge pH under different treatments of S

Treatment	16 days	28 days	36 days	50 days	65 days
S_0	6.7	6.9	6.6	5.6	6.6
$S_{0.25}$	6.7	6.1	6.1	5.4	6.1
$S_{0.5}$	6.5	6.0	6.0	5.4	5.7
S_1	6.4	5.9	5.8	5.4	5.4
S_2	6.6	5.6	5.0	3.0	2.7

Sampling and analysis

Oven dried (70 °C), ground and sieved with 2 mm mesh size sludge samples were used in the analysis. Sludge pH was measured with a pH-meter at the 1:5 (w/v) ratio of sludge water suspension (Kalra and Maynard 1991). The electrical conductivity (EC) was measured with an EC electrode at the 1:5 (w/v) sludge water suspension (Kalra and Maynard 1991). Organic matter (OM) and organic carbon (%) were measured according to Walkley and Black method (Ryan et al. 2001). Total N was determined by the Kjeldahl method (Kalra and Maynard 1991). Phosphorus was measured spectrophotometrically according to Bingham method (Pierzynski 2000) and potassium was obtained at ICP-MS (Perkin Emler, Optima 2100 DV) by employing ammonium acetate method (Ryan et al. 2001). Cation exchange capacity was obtained according to $BaCl_2$ extraction method (Ross 2001). Sludge samples (~250 mg) were digested in a Microwave Digestion System (MWS-3, DAP 60S) using 6 ml of HNO_3 (65 %), 1 ml of H_2O_2 (30 %) acid mixture and then heavy metal contents were determined with an ICP-MS (Perkin Emler, Optima 2100 DV).

After harvest of the plant materials, plants were washed with tap water, rinsed with deionized water and finally blotted dry. Plants were separated in roots and shoot with a stainless steel scissor and then oven-dried at 78 °C to constant weight. Dry weights of the shoots and roots were measured and then samples were ground into fine powder, sieved through 2 mm sieve (Kalra and Maynard 1991). Samples (~250 mg) were digested in a microwave oven (MWS-3, DAP 60S) using 6 ml of HNO_3 (65 %), 1 ml of H_2O_2 (30 %) acid mixture and then analyzed for heavy metal concentrations with an ICP-MS (Perkin Emler, Optima 2100 DV) (Kalra and Maynard 1991).

Data analysis

Two indicators were calculated to evaluate the plant for phytoextraction purposes. The Bioaccumulation Factor (BF) was calculated for root and shoot by dividing the trace-element concentration in plant tissues (mg/kg) at harvest by the initial concentration of the element in the sludge (mg/kg) in which the plant grew. The Transportation Index (TI $C_{shoots} = C_{roots}$) was calculated for total plant from the compartment concentrations of heavy metals to evaluate the plant for phytoextraction purposes, and in particular, the plant ability to translocate the heavy metals from roots to the harvestable aerial part (Ghosh and Singh 2005).

Measured data for the sewage sludge and plant were analyzed statistically using two ways ANOVA analysis procedure to compare the treatment effects on heavy metal removal of plant. Treatment means were compared with a least significant differences test (LSD) at $p < 0.05$.

Results and discussion

Sludge characteristics after harvesting

From the obtained results, agronomic characteristics of sewage sludge, such as pH, EC, CEC and organic matter content, were in acceptable range and all favorable for subsequent plant growth (McFarland 2000). Additional fertility characteristics, such as NPK levels, organic carbon and C/N ratio, were also fairly consistent.

The total concentrations of Cd, Cr, Cu, Ni, Pb and Zn in the sewage sludge were much lower than the limits recommended by the Turkish Soil Pollution Control Regulations for agricultural usage. On this account, sewage sludge was classified as an excellent amendment for agricultural use. Unfortunately, sludge generally contains excessive Cd, Ni and Zn compared to the European Union standards; therefore, long-term and unrestricted applications of sludge have led to an increase in soil heavy metal content. Thus, removal of heavy metal from sewage sludge by hyperaccumulator plants could be a promising technology.

Heavy metal content of sewage sludge after harvesting was obtained as 3 mg/kg Cu, 1100 mg kg^{-1} Zn, 85 mg/kg Cr, 20 mg/kg Ni, 21 mg/kg Pb, and 2 mg/kg Cd. Obtained results showed that 65–70 % of the heavy metal was removed by *B. juncea*. 30–35 % of the residual metal content was remained at sludge.

Sludge pH decreased in all the treatments with application of S compared to the control. Robinson et al. (1999) reported that 5 g of elemental, finely ground sulfur per kilogram of soil was added with an initial soil pH 6.9 and the soil pH decreased to 5.5 within 15 weeks. Kayser et al. (2000) reported that adding 36 mol S m^{-2} to the soil led to a decrease in soil pH from 7.2 to 6.9. In the present study, adding S also acidified the sludge, which caused the pH decrease to 5.4 with an initial pH 6.7 within 9 week just as in the incubation experiments (Table 1).

Plant yield

Shoot and root biomass of *B. juncea* influenced by different treatments were shown in Fig. 1. Treatments by plant interaction had significant effects on yield of *B. juncea* ($p < 0.001$). Compared to the control, the growth and aboveground biomass of *B. juncea* were significantly enhanced by S addition. Plant dry biomass ranged from 35 to 43 g/pot and highest yield obtained at the S application (lowest pH treatment) (Fig. 1). Cui et al. (2004a, b) reported that root and shoot weight in tested plants significantly decreased with S application and also Kayser et al. (2001) reported that shoot weight in some plants did not change significantly with S application. However, in the present study, application of S increased the plant biomass. One of the possibilities was that the decrease of sludge pH may cause an increase in the solubility and bioavailability of the nutrients. However, another reason might be that different plants have different sensitivity to the toxicity of amendments. Nonetheless, application of EDTA chemical to sludge causes a decrease on the plant yield (Fig. 1). EDTA applied 2 weeks before harvest and both root and shoot yields were obtained lower compared to control, and the effect was more pronounced on the shoots as obtained at Jiang et al. (2003). At root biomasses, there were no significant differences between yields of EDTA treated and control plants, and root and shoot yields of all plants declined with application of EDTA (Fig. 1). At the gypsum treatment, no significant differences in biomasses were observed to control application.

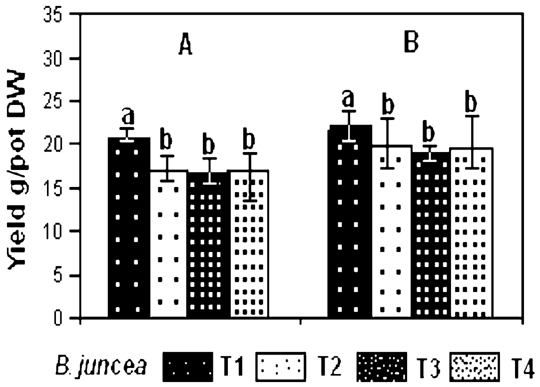

Fig. 1 Shoot and root biomass of *B. juncea* influenced by different treatments: **a** root and **b** shoot (T1: sulfur, T2: gypsum, T3: EDTA, T4: control treatments) (*Vertical bars* indicate standard deviation, $n = 4$)

Heavy metal accumulation in plant tissues

Heavy metal concentrations (mg/kg dw) of *B. juncea* at different treatments were shown in Fig. 2. Overall, the total metal accumulation (sum of the metal in the root and shoot) in plant was in the order of Zn > Pb > Cu > Cr > Ni > Cd, which varied from one part to another (Fig. 2).

The highest zinc concentration was obtained 681 mg/kg root dw at EDTA treatment, nearly threefold higher than the concentrations in shoots (251 mg/kg dw). The second was obtained at S treatment as 379 mg/kg root dw (Fig. 2). Applications of EDTA and S resulted in a considerable increase in the Cu and Pb concentrations in the plant. The concentration of Cu in roots reached 110 mg/kg dw at EDTA treatment (Fig. 2). The concentration of Cu in the sewage sludge is 19 mg/kg, and the obtained root Cu concentration is 8 times higher than this. The second highest Cu accumulation was obtained at S treatment as 93 mg/kg root dw (Fig. 2). Plant samples exhibit Cu concentrations higher than 20 mg/kg, considered by Kabata-Pendias (2001), as the limit for toxicity. But generally, the concentration of Cu in shoots is lower than in roots. As has been observed for all tested metals except for Pb, Cu concentrated in the roots and was not moved to the upper part of the plant.

It is well known that Pb is an immobile metal in soil, since it readily forms a precipitate with a low aqueous solubility within the soil matrix, and in many cases it is not readily bioavailable. In addition, many plants retain Pb in their roots via sorption and precipitation with only minimal transport to the aboveground harvestable plant portions (Cui et al. 2004a, b). In the present study, at the EDTA treatment, the Pb concentration in shoots was as obtained 82 mg/kg, representing about twofold increases relative to the roots (42 mg/kg). For the S treatment, the Pb concentrations in shoots were representing almost high concentrations, 77 mg/kg, about twofold increases relative to the roots (34 mg/kg). The concentration of Pb in sewage sludge is 34 mg/kg, while *B. juncea* exhibit Pb concentration is well above this value, and therefore, tested plant appears to accumulate Pb in the tissues (Fig. 2).

The best results for Ni phytoextraction were obtained at roots (66 mg/kg) for EDTA treatment followed by the shoots (12 mg/kg). The highest concentration of Cd was found in roots (3 mg/kg) despite the concentration of Cd in the plant tissues was below the sewage sludge Cd concentration level (3 mg/kg). The highest concentration of Cr was found in roots (170 mg/kg). Almost, obtained Cr concentrations were below the sewage sludge Cr concentrations (243 mg/kg) as obtained Cd concentrations (Fig. 2).

Bioaccumulation factor (BF) and Transportation index (TI) from sludge to plant were shown in Table 2. Translocation of metals was restricted from root to shoot, as among the plant tested; the concentrations of heavy metals in roots were higher ($p < 0.01$) than the shoots. Unfortunately, a primary concern for plants grown in sewage sludge is the accumulation of metals in aboveground tissues (Xiaomel et al. 2005). Therefore, from the obtained BF and TI values, plant did not hyperaccumulate zinc in the tissues and did not translate to the upper parts (BF < 1,

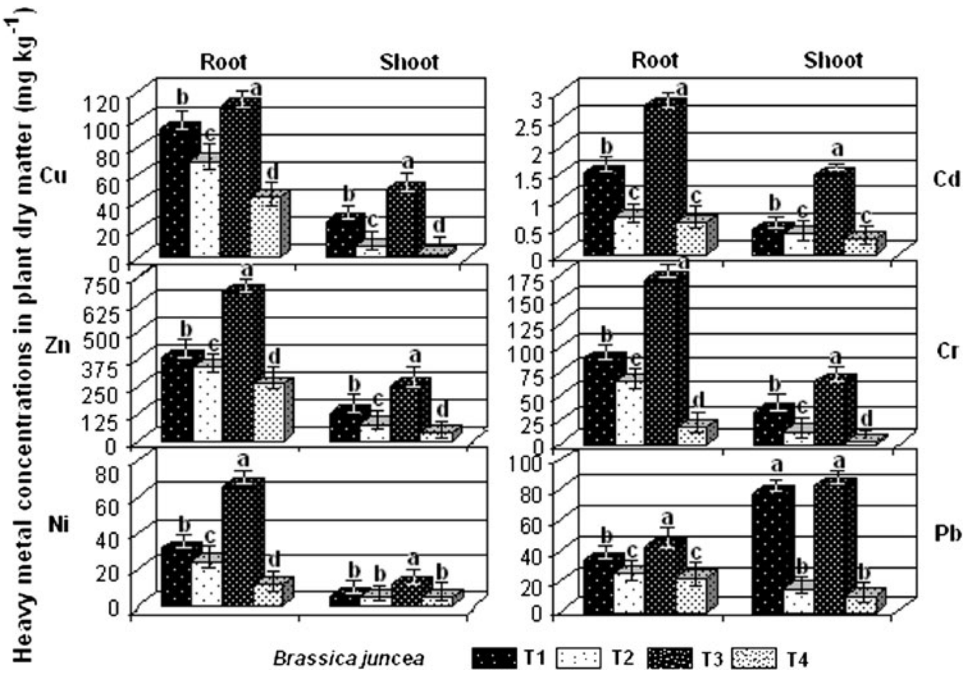

Fig. 2 Heavy metal concentrations (mg kg^{-1} dw) of *B. juncea* at different treatments (T1: sulfur, T2: gypsum, T3: EDTA, T4: control treatments) (*Vertical bars* indicate standard deviation, $n = 4$)

Table 2 Bioaccumulation factor (BF) and Transportation index (TI) from sludge to plant

Treatments	Cu		Zn		Ni		Cd		Cr		Pb	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
BF												
Sulfur	5.01b	1.43b	0.26b	0.08b	0.39b	0.07b	0.44b	0.14b	0.36b	0.14b	1.87b	2.82b
Gypsum	3.72c	0.41c	0.23b	0.05c	0.30c	0.05b	0.21c	0.11b	0.26c	0.05c	0.73c	0.43c
EDTA	9.67a	2.63a	0.47a	0.17a	0.83a	0.14a	0.81a	0.43a	0.69a	0.26a	3.59a	5.63a
Control	2.34d	0.13d	0.18c	0.02c	0.14d	0.05b	0.17d	0.08c	0.07d	0.01c	0.64d	0.01d
TI												
Sulfur	0.28a		0.33a		0.17b		0.32c		0.38a		1.51a	
Gypsum	0.11b		0.22b		0.17b		0.57a		0.19b		0.59b	
EDTA	0.27a		0.37a		0.17b		0.53b		0.38a		1.56a	
Control	0.05c		0.15c		0.37a		0.52b		0.10c		0.01c	

Values followed by different letters are significantly different for $p = 0.05$

TI < 1), since a little amount of Zn reached the shoots (Table 2). Furthermore, the highest BF was observed in root tissues at S and EDTA treatments, 5.01 and 9.67 for Cu, respectively (Table 2). Obtained BF values (>1) for shoots at the S and EDTA treatments show the suitability of this plant for phytoextraction of Cu from sewage sludge. Unfortunately, plants do not have the ability to translate Cu from root to shoot (TI < 1). BF values for shoots and roots at S and EDTA treatments show the usefulness of this plant in removal of Pb from sewage sludges. Especially at S and EDTA treatments, remarkable Pb translocations to the upper parts of the plant were observed; BF values were 2.8 and 5.63, respectively (Table 2). From the obtained TI values, plant can translate Pb to the upper parts at only S and EDTA treatments (TI > 1) (Table 2). Except Cu and Pb, no significant difference was found between the obtained bioaccumulation factors for the other tested metals. Maximum BF values were observed for Cu and Pb. It was observed that *B. juncea* removed maximum amount of Cu and Pb from sewage sludge on the basis of high BF value for both root and shoot at EDTA and S treatments. Similarly, plant studied do not appear to accumulate Cd, Cr and Ni (BF < 1) (TI < 1). Maximum transport was observed at Pb, with a TI maximum variation ranging from 0.01 to 1.56 (Table 2). For the other heavy metals obtained in plant tissues were <1, indicating that the root metal concentrations were always high.

Heavy metal removal efficiencies within plant tissues

It is well known that a good phytoextraction method depends on plants producing sufficient biomass while accumulating high concentrations of heavy metals (Cui et al. 2004a, b). In the present study, the metal removal efficiencies increased with the application of S, as obtained metal concentrations at the tissues. As for Pb removal, the shoot removal efficiency obtained was higher than roots. In fact, it is lower at shoots for the other metals. Because of high biomass, production of *B. juncea* has excellent potential for Pb and Cu phytoextraction. Therefore, the removal of heavy metal with application of gypsum had no significant impact compared to control. The addition of EDTA had no negative effect on yield of *B. juncea*, but increased metal accumulation by *B. juncea* directly translated into equivalent increases in metal removal.

Conclusion

Results show that the addition of elemental S was a suitable strategy for the removal of heavy metals from the sewage sludge, such as an alternative to chemical amendment EDTA. Sludge pH decreased with S application and the accumulation of Cu and Pb was dramatically increased in tested plant compared to control treatment. From the results it could be concluded that *B. juncea* accumulated significantly high quantities of toxic metals in the upper parts of the plant. Overall, 65–70 % of the sludge heavy metal was removed by *B. juncea*. The TI of all studied metals in shoot metal concentration/root metal concentration (S/R) was quite low (TI < 0.5), whereas the BF values were much higher in plant metal concentration/sludge metal concentration, i.e., BF varied from 0.01 to 9.67. Further, plant showed better BF for Cu and Pb metals both shoot and root. According to Ghosh and Singh (2005), a plant under accumulator category as the ratio of metal concentration in the plant to that in the soil is >1. From the obtained results, the efficiency to remove the Cu and Pb from sludge is high in this plant. As a result, elemental S when applied at the right concentration will be an effective amendment for the phytoextraction of heavy metals from sewage sludges' without posing any detrimental impact on the environment.

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