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Roles of *Bacillus megaterium* in Remediation of Boron, Lead, and Cadmium from Contaminated Soil

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Phytoremediation is an attractive, economical alternative to soil removal and burial methods to remediate contaminated soil. The objective of this study was to investigate the effects of adding different rates of Bacillus megaterium *on the capacity of* Brassica napus *plants to take up boron (B), lead (Pb), and cadmium (Cd) from polluted soils under field conditions. Field experiments were conducted using a randomized complete block design with control (without pollution and* B. megaterium *application) and B, Pb, and Cd in two doses (0 and 100 mg* kg^{-1}*),* B. megaterium *with four doses (no application and* 10^8 *cfu* B. megaterium ml^{-1} *sprayed at 50 ml* $plot^{-1}$*, 100 ml* $plot^{-1}$*, 150 ml* $plot^{-1}$*). Results indicated that soil pollution treatments significantly decreased seed (SDMY), shoot (SHDMY), root (RDMY), and total dry-matter yield (TDMY) of plants at 42.9, 3.8, 62.6, and 23.4% for B-polluted treatment; 25.8, 8.7, 17.6, and 14.2% for Pb-polluted treatment; and 33.2, 7.0, 14.0, and 16.4% for Cd-treatment without* B. megaterium *application, respectively. However, the application of* B. megaterium *ameliorated the negative effects of B, Pb, and Cd at 41.4, 52.7, and 10.9% for B; 24.4, 21.6, and 4.9% for Pb; and 22.8, 22.0, and 3.3% for Cd, respectively. The potentially bioavailable and relatively available fraction of soil B, Pb, and Cd increased with increases in the* B. megaterium *application but total fraction and stable fraction decreased. It is concluded that the seed and shoot parts of* B. napus *can be used as hyperaccumulators for plant B, Pb, and Cd remediation according to remediation factors but the shoot is the biggest part of the plant, and thus an important portion of the plant to remove B, Pb, and Cd from the B-, Pb-, and Cd-contaminated soils. To decrease desired concentration for 8 mg B* kg^{-1}*, 4 mg Pb* kg^{-1}*, and 3 mg Cd* kg^{-1} *in the active rooting zone of soil, approximately 2, 6, and 21 years would be necessary with only 150 ml* $plot^{-1}$ B. megaterium*–sprayed soil cultivated with* B. napus*, respectively.*

Keywords *Bacillus megaterium*, heavy metal, PGPR, phytoremediaiton, remediation factors

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Introduction

Some heavy metals, including manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), molybdenum (Mo), and boron (B), are essential or beneficial micronutrients for plants, animals, and microorganisms, whereas others such as cadmium (Cd), lead (Pb), and nickel (Ni) have no known biological or physiological function. Lead, Ni, and Cd amounts in the soil are important because high quantities of them can decrease crop production due to the risk of bioaccumulation in the plant and food chain (Schmidt 2003; Nowack, Schulin, and Robinson 2006). Other trace elements, such as B, can be extremely toxic to plants, because the concentration range between deficiency and toxicity is narrow. Boron toxicity is a common problem that can limit plant growth and yield on soils of arid and semi-arid environments throughout the world (Nable, Bãnuelos, and Paull 1997). In arid and semi-arid areas, B toxicity results from high levels of B in soils and from additions of B via irrigation water, saline soil, fertilizers, wastes from surface mining, fly ash, and industrial chemicals (Akar 2007; Aydin and Çakir 2009).

Industry, agriculture, extensive mining, and military operations have led to the accelerated release of metals into the ecosystem, causing serious environmental problems and posing health risks to plants and animals, including humans. Therefore, the development of a remediation strategy for metal-contaminated soils is urgent for environmental conservation and human health (Abou-shanab, Angle, and Chaney 2006). Metal-contaminated soil can be remediated by physical, chemical, or biological techniques (Mulligan, Yong, and Gibbs 2001). Phytoremediation offers significantly more benefits than conventional technology to accumulate heavy metals from the soil due to its less expensive, sustainable, and safer characteristics for humans and the environment (Fischerova et al. 2006). However, slow growth, low biomass of plants in heavy metal–contaminated soil, and low metal bioavailability may limit the efficiency of phytoremediation (Burd, Dixon, and Glick 2000).

Brassica napus, belonging to the *Brassica* family, is recognized as a fast-growing metal-accumulating species and thus a good candidate for induced phytoextraction (Prasad and Freitas 2003). Within the *Brassica* genus, *Brassica juncea* was tolerant for individual Cd, chromium (Cr), Cu, Ni, Pb, and Zn pollution conditions (Kumar et al. 1995; Salt et al. 1995; Ebbs et al. 1997; Liphadzi and Kirkham 2005), but *Brassica napus* and *Raphanus sativus* were moderately tolerant for multiply contaminated Cd, Cr, Cu, Ni, Pb, and Zn soils (Marchiol et al. 2004). That is, these species could be used as safely and successfully in polluted-soil remediation. Because phytoextraction is a long-term technology, fields undergoing phytoremediation need to be kept productive to achieve economically viable and socially acceptable decontamination. The use of energy and/or biodiesel crops as heavy-metal phytoextraction plants would give contaminated soil a productive value and decrease remediation costs (Kos, Grcman, and Lestan 2003).

To estimate effects and potential risks associated with elevated elemental concentrations that result from natural weathering of mineral deposits or from mining activities, the fraction of total elemental abundances in water, sediment, and soil that are bioavailable must be identified. Bioavailability is the proportion of total metals that are available for incorporation into biota (bioaccumulation). Total metal concentrations do not necessarily correspond with metal bioavailability (Davis et al. 1994).

The solubility of heavy metals in soil tends to be low due to complexation with adsorption on clays and silicate minerals, organic components, and precipitation as phosphates, carbonates, and hydroxides (McBride 1994). The solubility of the heavy metals can be increased by adding synthetic chelators such as ethylenediamine-di-o-hydroxyphenylacetic acid, ethylenediaminetetraacetic acid, and nitrilotriacetate. These compounds have been used to enhance the solubility of metals in soils and their subsequent uptake, but they are not easily biodegradable and are nonselective (Evangelou et al. 2007). Thus, alternative methods are needed.

One possibility to enhance metal bioavailability is the use of soil microorganisms and plant root–associated bacteria (Kamnev and van der Leile 2000). These bacteria exert beneficial effects on plant growth and development and therefore may be used as biofertilizers for agriculture. The use of rhizobacteria in combination with plants is expected to provide high efficiency in phytoremediation (Whiting, de Souza, and Terry 2001). In particular, plant growth–promoting rhizobacteria (PGPR) are now considered to play an important role in phytoremediation technologies (Mayak, Tirosh, and Glick 2004). Success of PGPR in remediation is attributed to more rapid breakdown of organic matter, enhanced availability of nutrients, and improved soil properties, and these effects are mostly explained by the release of metabolites directly stimulating growth. All the mechanisms by which PGPRs promote plant growth are not fully understood, but may include the ability to produce plant hormones, such as auxins, cytokinins, gibberellins, and inhibit ethylene production; symbiotic N_2 fixation; solubilization of inorganic phosphate; mineralization of organic phosphate and/or other nutrients; antagonism against phytopathogenic microorganisms by production of siderophores; the synthesis of antibiotics, enzymes, and/or fungidical compounds; competition with detrimental microorganisms; and helping plants acquire sufficient microelements for optimal growth (Esitken et al. 2003, 2006; Caballero-Mellado et al. 2007).

Although some soil bacteria–assisted phytoremediation has been studied (Whiting, de Souza, and Terry 2001; Zaidi et al. 2006; Dell'Amico, Cavalca, and Andreoni 2008), there is little information on the potential of *Bacillus megaterium* bacteria. *Bacillus megaterium* bacterium, generally considered a soil microbe, is Gram-positive and has great potential for phytoremediation of metal-polluted sites. Thus, PGPR such as *B. megaterium* may be of particular interest as they have the advantages of being relatively protected from the competitive, high-stress environment of the soil and can have the capacity to control pathogens and promote plant establishment via increasing resistance under adverse conditions (Saleem et al. 2007).

When microbes are used to bioremediate a contaminated site, plant-associated bacteria potentially can be used to improve phytoextraction activities by altering the solubility, availability, and transport of heavy metals, and nutrients as well, by reducing soil pH and releasing chelators (Ma et al. 2011). Among the metabolites produced by PGPR, siderophores play a significant role in metal mobilization and accumulation (Rajkumar et al. 2010). Recently, Cr and Pb were found to be released into the soil solution after soil was inoculated with some of PGPR (Braud et al. 2009). The concept of inoculating seeds/rhizospheric soils with selected metal-mobilizing bacteria to improve phytoextraction in metal-contaminated soils has merit (Tak et al. 2012).

For phytoremediation to be successful, the selection of plant species that are efficient in metal accumulation is of primary importance. More than 400 species of plants have been reported to be the hyperaccumulators of elements such as Ni, Cd, Pb, cobalt (Co), and selenium (Se) in greenhouse experiments (Reeves and Baker 2000). Nearly all research on the phytoremediation of heavy metal–contaminated soils has been focused on determining accumulator plant species. Hence, little attention has been paid to the potential use of *B. megaterium* as a tool for enhancing heavy-metal availability in the phytoremediation process in field conditions.

Materials and Methods

Study Site

This study was conducted at the Agricultural Research Station of Ataturk University located in Erzurum, Turkey (long. 39° 55′ N, lat. 41° 16′ E) during the summer periods (late May to late September) of 2008 and 2009. Its altitude is 1835 m. The soil was classified as an Aridisol with parent materials mostly consisting of volcanic, marn, and lacustrin transported material (Soil Survey Staff 1992). The experimental region has a semi-arid climate. During the growing period, the mean maximum temperature was 22 °C in both years, whereas the mean minimum temperatures were 6.1 °C in 2008 and 5.5 °C in 2009. The mean relative humidity, wind speed, and daily sunshine, total precipitation, and total evaporation amounted to 51.32%, 2.82 m s^{-1}, 11.23 h, 33.18 mm, and 388.7 mm in 2008 (20 May to 29 Sept.) and 55.76%, 3.50 ms^{-1}, 10.67 h, 51.05 mm, and 448 mm in 2009 (28 May to 10 Oct.), respectively.

Bacterial Strain, Culture Conditions, Media, Treatment, and Trial Design

Bacteria were grown on nutrient agar (NA) for routine use and maintained in nutrient broth (NB) with 15% glycerol at –80 °C for long-term storage. For this experiment, the bacterial strains were grown on nutrient agar. A single colony was transferred to 500-ml flasks containing NB and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Darmstadt, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^8 cfu ml^{-1}, and the resulting suspensions were sprayed to polluted soil cultivated with *B. napus* plants.

Field experiments were conducted using a randomized complete block design with control (without pollution and *B. megaterium* application), and B, Pb, and Cd with two doses (0 and 100 mg kg^{-1}), *B. megaterium* with four doses (no application, 10^8 cfu *B. megaterium* ml^{-1} sprayed at 50 ml $plot^{-1}$, 100 ml $plot^{-1}$, 150 ml $plot^{-1}$) in 2008 and 2009. Each plot according to the study plan was treated with 0 and 100 mg Pb kg^{-1} from lead nitrate [$Pb(NO_3)_2$], 100 mg Cd kg^{-1} from CdN_2O, and 15 mg B kg^{-1} from disodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) as separately. These concentrations were selected according to U.S. Environmental Protection Agency (USEPA) maximum permissible limits concentrations and World Health Organization standards (USEPA 1993; WHO 1996). The soil contaminations were performed by adding a specific amount of heavy metals; they were dissolved in deionized water and applied to each plot. The wetting–drying mixing process was repeated to ensure soil equilibrium for a 1-month period. After the incubation period, soluble fraction, exchangeable-bound fraction, carbonate-bound fraction, metal oxide–bound fraction, organic matter–bound fraction, silicate-bound fraction, total fraction, and diethylenetriaminepentaacetic acid (DTPA)–available parts of Pb, Cd, and B concentrations of soil at 0–20 cm deep were determined, and data are given later. Soils contaminated with heavy metals were treated with basal fertilizer. A basal dressing of nitrogen (N) (120 kg ha^{-1} as ammonium nitrate) was applied and incorporated into the seedbed. Nitrogen was split into two applications: half with sowing and the remaining half at the beginning of stem elongation. All plots received phosphorus (P) at 60 kg P ha^{-1} as triple superphosphate at sowing in both years. Canola seeds (*Brassica napus* cv. Licosmos) were sown at 10 kg ha^{-1} (plant density of 90 plant m^{-2}) on 19 April 2008 and 29 April 2009. Plots were overseeded to extract heavy-metal contamination more easily in the phytoremediation technique. The area of each plot was 6 m^2 consisting of four rows 5 m long and 1.2 cm wide. A 2.0-m space was left between the plots to prevent water movement from one plot to another during irrigation. Herbicides or pesticides were not applied to the field experiment in either year. Weeds were controlled with hand weeding as needed. Plots were irrigated five times during both growing seasons. Good-quality underground water with an electrical conductivity (EC) of 0.28 dS m^{-1}, sodium adsorption ratio of 0.40, and pH of 7.4 was used for surface irrigation. The moisture content (0 to 60 cm soil depth) was increased to field capacity after planting and soil moisture contents at 0- to 30-cm and 30- to 60-cm soil depths were determined daily with a time domain reflectometer (TDR 300; Spectrum Technologies, East Plainfield, Ill.). When the moisture content fell below 23.5% as Pw, a total of 32.4 mm irrigation water was applied to the soil based on an effective root depth of 60 cm. The total amount of irrigation water was 470.2 mm in 2008 and 395.4 mm in 2009. The plants were harvested on 27 September 2008 and 29 September 2009. Fifteen plants were collected randomly by hand pulling from the center except for two rows of the corner, and the following growth and yield component variables were recorded for each plot. Soil particles at the root surface were removed by washing with water.

Plant and Soil Analysis

After plant yield was recorded, the plant sample was separated as root, shoot, and seed. Each part of the plant was analyzed for B, Cd, and Pb content to assess the relationship between the part of the plant and mineral content. Tissue B, Cd, and Pb were determined after wet digestion of dried and ground subsamples using a nitric acid (HNO_3)–hydrogen peroxide (H_2O_2) acid mixture (2:3 v/v) with three steps (first step: 145 °C, 75% RF, 5 min; second step: 180°C, 90% RF, 10 min; and third step: 100 °C, 40% RF, 10 min) in a microwave (Berghof Speedwave microwave digestion equipment MWS-2; Berghof, Eningen, Germany) (Mertens 2005). Tissue B, Cd, and Pb were determined on an inductively coupled plasma spectrophotometer (Perkin–Elmer, Optima 2100 DV, ICP/OES, Shelton, Conn., USA). After heavy-metal amendments and plant harvest, soil samples from each plot were taken over a 0- to 30-cm depth to determine baseline soil properties, heavy-metal pollution degree, and soil element fractions according to sequential extraction and remediation parameters. Soil samples were air dried, crushed, and passed through a 2-mm sieve before physical and chemical analysis (AOAC 2005). Some chemical and physical properties of the soil are given Table 1. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0) according to Sumner and Miller (1996). The Kjeldahl method (Bremner 1996) was used to determine organic N while plant-available P was determined by using the sodium bicarbonate method of Olsen et al. (1954). Electrical conductivity (EC) was measured in saturation extracts according to Rhoades (1996). Soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to McLean (1982). Soil organic matter was determined using the Smith–Weldon method according to Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 (Thomas 1982) was used to determine exchangeable cations. Microelements in the soils were determined by diethylenetriaminepentaacetic acid (DTPA) extraction methods (Lindsay and Norvell 1978).

Sequential Extraction Procedures

Lead, Cd, and B distribution of soil initial parameters and before/after B, Pb, and Cd pollution were analyzed for heavy-metal distribution using the sequential extraction procedure

Table 1

Chemical and physical properties of the surface sampled (0–30 cm) averaged over 2 years (2008 and 2009) prior to land preparation for B, Cd, and Pb response trials for canola (*Brassica napus* L.) growth (n = 10)

Soil properties	Units	Mean ± Sd
Sand	%	32.1 ± 2.40
Silt	%	38.3 ± 2.60
Clay	%	29.6 ± 2.20
CEC[a]	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	24.1 ± 3.20
Organic C	g kg^{-1}	5.0 ± 0.50
pH	1:2.5 w/v	7.9 ± 0.20
$CaCO_3$	g kg^{-1}	20.0 ± 2.90
Total N	g kg^{-1}	1.6 ± 0.40
Olsen P	mg kg^{-1}	5.2 ± 1.40
EC[b]	dS m^{-1}	1.8 ± 0.20
Exchangeable K	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	2.6 ± 0.10
Exchangeable Ca	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	16.2 ± 2.40
Exchangeable Mg	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	2.8 ± 0.30
Exchangeable Na	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	0.31 ± 0.10
Available Fe	mg kg^{-1}	1.55 ± 0.01
Available Mn	mg kg^{-1}	1.90 ± 0.07
Available Zn	mg kg^{-1}	1.20 ± 0.03
Available Cu	mg kg^{-1}	1.10 ± 0.08
Available Cd	mg kg^{-1}	4.55 ± 0.80
Available Pb	mg kg^{-1}	0.21 ± 0.03
Available b	mg kg^{-1}	0.32 ± 0.03

[a] Cation exchangeable capacity.
[b] Electrical conductivity.

developed by Tessier, Campbell, and Bisson (1979). Half of a gram (0.5 g) of soil was treated and fraction 1, the water-soluble fraction (WSF) was extracted by adding 10 ml water (pH 6) to 0.5 g of soil, shaking for 3 h at 25 ± 1 °C, and centrifuging at 3000 rpm for 5 min. Fraction 2, the exchangeable bound fraction (EBF), was extracted by adding 10 ml 0.1 M magnesium chloride ($MgCl_2$) (pH 6) to the residue from the first step, shaking for 3 h at 25 ± 1 °C, and centrifuging at 3000 rpm for 5 min. Fraction 3, the carbonate-bound fraction (CBF), was extracted by adding 10 ml 0.1 M sodium acetate (NaOAc)/acetic acid (HOAc) (pH 5) to the residue from the second step, shaking for 3 h at 25 ± 1 °C, and centrifuging at 3000 rpm for 5 min. Fraction 4, the metal oxide–bound fraction (MOBF), was extracted by adding 10 ml of 0.1 M hydroxylamine (NH_2OH) hydrochloric acid (HCl) (pH 3) to the residue from the third step, shaking for 3 h at 60 ± 1 °C, and centrifuging at 3000 rpm for 5 min. Fraction 5, the organic matter–bound fraction (OMBF), was extracted by adding 3 ml HNO_3 and 7 ml H_2O_2 (pH 2) to the residue from the fourth step, shaking for 3 h at 85 ±1 °C, and centrifuging at 3000 rpm for 5 min. Fraction 6, silicate-bound fraction (SBF), was extracted by adding 10 ml $HNO_3 + H_2O_2$ + hydrofluoric acid (HF) (3:5:2,v/v) to the residue from the fifth step, shaking for 5 h at 190 ± 1 °C, and centrifuging at 4000 rpm for 5 min.

Remediation Parameters

Many factors determine the effectiveness of phytoremediation in remediating metal-polluted sites. The translocation factor (Zayed, Gowthaman, and Terry 1998), bioconcentration factor (Mun, Hoe, and Koo 2008; Marques, Rangel, and Castro 2009), bioaccumulation factor (Vysloužilová, Tlustoš, and Száková 2003), phytoextraction potential (Kos, Grcman, and Lestan 2003; Kos and Leštan 2003), transfer index (Paiva, Carvalho, and Sıqueıra 2002), transfer factor (Lubben and Sauerbeck 1991), enrichment factor (Kisku, Barman, and Bhargava 2000), remediation time (Robinson et al. 2006), and remediation factor (Vysloužilová, Tlustoš and Száková 2003) were calculated as follows:

Translocation factor (TLF) = [(Metal concentration in the shoots, mg kg^{-1}, and grain, mg kg^{-1}) / Metal concentration in the roots, mg kg^{-1})]

Exchangeable bioconcentration factor (BCF$_{\mathrm{Exc}}$) = [(Metal concentration in plant tissue (root or shoot or grain), mg kg^{-1}) / (Exchangeable element concentration of soil at harvest mg kg^{-1})]

Total bioconcentration factor (BCF$_{\mathrm{T}}$) = [(Metal concentration in plant tissue (root or shoot or grain), mg kg^{-1}) / (Total element concentration of soil at harvest mg kg^{-1})]

Exchangeable bioaccumulation factor (BAF$_{\mathrm{Exc}}$) = [(Metal concentration in plant grain, mg kg^{-1} + Metal concentration in plant shoot, mg kg^{-1}) / (Exchangeable element concentration in the soil at initial times mg kg^{-1})]

Total bioaccumulation factor (BAF$_{\mathrm{T}}$) = [(Metal concentration in plant grain, mg kg^{-1} + Metal concentration in plant shoot, mg kg^{-1}) / (Total element concentration in the soil at initial times, mg kg^{-1})]

Phytoextraction potential (PP) = [(Metal concentration in plant grain (mg kg^{-1}) × grain yield (ton ha^{-1})) + (Metal concentration in plant shoot (mg kg^{-1}) × shoot yield (ton ha^{-1}))]

Transfer index (TI) = [(Metal concentration in the shoot × shoot yield) + Metal concentration in the grain × grain yield)] / [(Metal concentration in the shoot × shoot yield + (Metal concentration in the grain × grain yield) + Metal concentration in the root × root yield)] × 100

Total transfer factor (TF$_{\mathrm{T}}$) = [(Metal concentration (in plant grain + shoot + root), mg kg^{-1}) / (Total metal concentration in the soil harvest time, mg kg^{-1})]

Exchangeable transfer factor (TF$_{\mathrm{Exc}}$) = [(Metal concentration (in plant grain + shoot + root)), mg kg^{-1} / (Exchangeable metal concentration in the soil harvest time mg kg^{-1})]

Enrichment factor (EF) = [(Contaminated site (Metal concentration in plant grain, mg kg^{-1} + Metal concentration in plant shoot, mg kg^{-1} + Metal concentration in plant root, mg kg^{-1}) / (Uncontaminated site (Metal concentration in plant grain, mg kg^{-1} + Metal concentration in plant shoot, mg kg^{-1} + Metal concentration in plant root, mg kg^{-1})]

Remediation time (RT) = (Metal concentration in soil requested level mg kg^{-1} × soil mass, ton ha^{-1}/ (Metal concentration in the shoot, mg kg^{-1} × shoot yield, ton ha^{-1}) + Metal concentration in the grain, mg kg^{-1} × grain yield, ton ha^{-1} (metal pollution occurs only in the active root zone, namely the top 20-cm soil layer assuming a soil bulk density of 1.3 t m^{-3})

Remediation factor (RF) = [(Metal concentration in plant grain, mg kg^{-1} × grain yield, ton ha^{-1}) + (Metal concentration in plant shoot, mg kg^{-1} × shoot yield, ton ha^{-1}) / (Total metal concentration in soil, mg kg^{-1} × soil mass ton ha^{-1})]

Statistical Analysis

Each of the treatments was repeated in four times. Data averaged over 2 years were first evaluated by a two-way analysis of variance (ANOVA) (SPSS Inc., Chicago, Ill., USA) with a linear model component for treatment and time, and treatment by time interactions were analyzed. When annual data were pooled, the "year × treatment interaction" term was insignificant for most of the evaluated parameters. The group means were compared by the least significant difference (LSD) option at $P \leq 5\%$.

Results

Dry-Matter Yield of Plants

The 2 years of field trials showed that soil pollution treatments including B, Cd, and Pb significantly affected seed (SDMY), shoot (SHDMY), root (RDMY), and total dry-matter yield (TDMY) of plants (Table 2). Compared to the unpolluted treatment, SDMY, SHDMY, RDMY, and TDMY decreased by 42.9, 3.8, 62.6, and 23.4% for B-polluted treatment; 25.8, 8.7, 17.6, and 14.2% for Pb-polluted treatment; and 33.2, 7.0, 14.0, and 16.4% for Cd-polluted treatment without *Ba. megaterium* application, respectively. However, the application of *B. megaterium* to contaminated soils significantly affected SDMY, RDMY, and TDMY of the plants and ameliorated negative effects of the heavy metals. These amelioration ratios of SDMY, RDMY, and TDMY of the plants were 41.4, 52.7, and 10.9% for B; 24.4, 21.6, and 4.9% for Pb; and 22.8, 22.0, and 3.3% for Cd, respectively. On the other hand the *B. megaterium* application increased SHDMY of plant, and increasing ratios were 24.2, 6.1, and 9.5% greater than the control$_1$ for B, Cd, and Pb, respectively.

Efficiency of **Bacillus megaterium** *Agents in Enhancing Soil B, Pb, and Cd Desorption from the Soil Fraction*

Sequential extraction procedures have been widely used to quantify the distribution of heavy metals in contaminated soils. Generally, WSF, EBF, DTPA, and CBF are considered readily or potentially bioavailable, MOBF and OMBF are considered relatively stable, and SBF is entrapped within the crystal structure of the minerals and thus is the least labile fraction. After a 1-month period of heavy metal amendments to the plot, B, Pb, and Cd pools of polluted soil increased linearly with B, Pb, and Cd application. The readily or potentially bioavailable B, Pb, and Cd fractions accounted for 5.3, 5.5, and 1.9% of the total B, Pb, and Cd pools, whereas the relatively stable and least labile fractions accounted for 10.2 and 12.7%, 32.6 and 37.2%, and 44.2 and 33.4% of total B, Pb, and Cd pools, respectively (Table 3).

Exchangeable bound fraction and CBF, MOBF, OMBF, SBF, and TF fractions of Cd, B, and Pb amended soil decreased to 37, 21, 11, 32, 18, and 19% for Cd; 17, 13, 9, 6,

Table 2

Effects of *B. megatarium* application on B, Pb, and Cd concentrations and yields of seed, shoot, and root of canola (*Brassica napus* L.) under growth with different B, Pb, and Cd additions (2-year average mean)

Parameter		Control	0 ml plot^{-1}	50 ml plot^{-1}	100 ml plot^{-1}	150 ml plot^{-1}
B						
Concentration (mg kg^{-1})	Seed	13.89e*	23.31d	31.63c	35.17a	33.04b
	Shoot	22.42e	30.17d	56.61c	70.36b	81.12a
	Root	40.47e	54.47d	65.71c	78.85b	94.16a
Yield (kg ha^{-1})	Seed	3770a	2150e	2430c	2550b	2210d
	Shoot	5590c	5380e	5530d	5890b	6940a
	Root	910a	340e	480c	550b	430d
	Total	*10270A*	*7870E*	*8440D*	*8990C*	*9150B*
Pb						
Concentration (mg kg^{-1})	Seed	0.27e	3.65d	5.85c	6.83a	6.68b
	Shoot	0.32d	4.29c	6.88b	8.04a	7.86a
	Root	0.27e	3.65d	5.85c	6.83a	6.68b
Yield (kg ha^{-1})	Seed	2870a	2150e	2200c	2310b	2170d
	Shoot	5860c	5350e	5760d	5980b	6220a
	Root	510a	420c	470b	430c	400c
	Total	*9240A*	*7920E*	*8420D*	*8720C*	*8790B*
Cd						
Concentration (mg kg^{-1})	Seed	0.04e	0.39d	0.95c	1.15b	1.51a
	Shoot	0.07e	0.72d	1.72c	2.10b	2.75a
	Root	0.38e	3.98d	6.14c	7.22b	9.82a
Yield (kg ha^{-1})	Seed	2980a	1990e	2520c	2820b	2300d
	Shoot	5260c	4890d	5470b	5200c	5760a
	Root	500a	430b	440b	400c	390d
	Total	*8740A*	*7310C*	*8430B*	*8420B*	*8450B*

Note. Different letters within rows indicate significant differences at $P \leq 0.05$ for bacteria application doses.

4, and 7% for B; and 15, 9, 11, 9, 2, and 7% for Pb in the planted soil with *Br. napus* and without *B. megaterium* application, respectively. Readily or potentially bioavailable, relatively stable, and least liable (SBF) fractions accounted for 13.3, 13.4, and 19.7%; 17.4, 21.1, and 25.1%; and 30.2, 32.6, and 26.5% of total B, Pb, and Cd pools, respectively, without *B. megaterium* application (Table 3).

On the other hand, the application of *B. megaterium* significantly increased B, Pb, and Cd availability in the soils and affected distribution of element fraction. The greatest B, Pb, and Cd uptakes of plants were determined with 150 ml plot^{-1} doses *B. megaterium*. Values for EBF, CB, MOBF, OMBF, SBF, and TF of the soil increased to 14, 31, 22, 41, 21, and 24% for Cd; 8, 24, 21 19, 7, and 15% for B; and 18 20, 22, 22, 5, and 14% for Pb, with 150 ml plot^{-1} doses *B. megaterium* application, respectively.

Table 3

Effects of *B. megatarium* on water-soluble fraction (WSF), exchangeable-bound fraction (EBF), carbonate-bound fraction (CBF), metal oxide–bound fraction (MOBF), organic matter–bound fraction (OMBF), silicate-bound fraction (SBF), total fraction (TF), and diethylenetriaminepenta acetic acid (DTPA)–available parts of Cd-, B-, and Pb-amended soil (mg kg^{-1}) (2-year average mean)

			PGPR application (10^8 cfu ml^{-1})			
Element fraction	Initial	B, Pb, and Cd amendments to soil[a]	0 ml plot^{-1}	50 ml plot^{-1}	100 ml plot^{-1}	150 ml plot^{-1}
B						
DTPA	0.32	0.96	0.43	0.64	0.74	0.99
WSF	0.32	0.28	0.25	0.24	0.34	0.44
EBF	6.06	10.67	8.83	7.54	8.42	9.78
CBF	15.20	39.61	34.46	33.56	32.85	29.86
MOBF	29.12	52.00	47.34	45.63	45.12	41.02
OMBF	7.97	32.91	30.80	29.32	26.72	26.35
SBF	56.75	90.14	86.31	85.37	85.02	83.84
TF	152.85	298.81	275.47	267.10	262.87	253.36
Pb						
DTPA	0.21	0.64	0.43	0.54	0.75	0.86
WSF	0.11	0.49	0.12	0.12	0.33	0.53
EBF	7.50	17.80	15.21	16.95	17.71	14.60
CBF	20.46	52.29	47.72	46.48	45.49	41.35
MOBF	47.78	82.46	73.56	70.90	70.11	63.74
OMBF	39.85	67.35	61.09	58.15	53.01	52.28
SBF	113.98	127.27	124.51	123.15	122.64	120.95
TF	257.99	390.51	361.91	354.68	347.41	335.12
Cd						
DTPA	4.95	8.61	5.81	6.03	7.21	9.15
WSF	0.11	0.97	0.32	0.11	0.54	1.25
EBF	2.48	10.44	6.58	6.41	7.35	8.93
CBF	2.91	30.79	24.35	23.72	23.21	21.10
MOBF	2.58	40.26	35.90	34.61	34.22	31.11
OMBF	3.44	3.88	2.64	2.51	2.29	2.26
SBF	8.18	42.63	34.58	34.20	34.06	33.59
TF	24.53	160.59	129.97	126.46	126.60	121.71

[a] After 1 month of incubation with B-, Pb-, and Cd-contaminated soil.

Notes. WSF, water-soluble fraction; DTPA, DTPA-extractable fraction; EBF, exchangeable-bound fraction; CBF, carbonate-bound fraction; MOBF, metal oxide–bound fraction; OMBF, organic matter–bound fraction; and SBF, silicate-bound fraction.

Efficiency of **Bacillus megaterium** *Agents Uptake of B, Pb, and Cd in Seed, Shoot, and Roots Part of Plant*

The application of *B. megaterium* to B-, Pb-, and Cd-contaminated soils significantly affected plant B, Pb, and Cd concentrations and B, Pb, and Cd uptakes of the plants. Boron, Pb, and Cd concentration in the plant seed, shoot, and root increased with increases in *B. megaterium* application of doses. The Pb, Cd, and B concentrations of seed, shoot, and root parts of plant from control to 150 ml plot^{-1} *B. megaterium* applications varied in ranges 3.65–6.83, 0.4–1.50, and 23.43–33.00 mg kg^{-1} for seed; 3.65–6.83, 3.98–9.82, and 54.60–94.16 mg kg^{-1} for roots; and 4.29–7.85, 0.71–2.75, and 30.16–81.12 mg kg^{-1} for shoots, respectively. In all *B. megaterium* application the rate was constant; it was the number of applications that changed the B, Pb, and Cd concentrations in the root to make them about 2–3 times greater than those in the shoots and about 3–4 times greater than those in the seeds (Table 2). Although, the root had the greatest B, Pb, and Cd concentrations, the shoot is a more important portion of the plant for B, Pb, and Cd removal in contaminated soil because it is the biggest part of the plant (Table 2). Thus, the greatest B, Pb, and Cd uptakes were observed from plant shoots followed by seed and root.

Effects **Bacillus megaterium** *on B, Pb, and Cd Remediation Factors of* **Brassica napus** *Grown in Contaminated Soil*

Translocation Factor (TLF). The TLF is a ratio that indicates the ability of a plant to translocate metals from its roots to its harvestable part of plant. This parameter influences how readily the extracted B, Pb, and Cd can be harvested. Metals that are accumulated by plants and largely stored in the roots of plants have TLF values of less than 1. Greater TLF values indicate translocation to the aerial parts of the plant. The TLF of a metal is calculated using the two formulae for the exchangeable and total metal concentrations.

The PGPR application affected the TLF_{exc}. and TLF_{T}, and the greatest TLF_{exc}. was obtained from in plants grown in soil treated with 150 ml plot^{-1} doses of *B. megaterium* application, but TLF_{T} was obtained from 100 ml plot^{-1} doses of *B. megaterium*. Two years of field trials showed that *Br. napus* had high TLF_{exc}. values (>1) for B, Pb, and Cd, taking into consideration exchangeable concentration of metal, but the only high TLF_{T} value was determined for B, taking into consideration total metal concentration of soil (Table 4).

Bioconcentration Factor (BCF). The BCF of a metal is calculated using the two formulae for the exchangeable and total metal concentrations. This parameter was used to determine the quantity of B, Pb, and Cd absorbed by the plant from the soil according to initial degree of soil pollution. The BCF represents the ability of parts of *Br. napus* to extract heavy metals from the soil. The application of *B. megaterium* affected the BCF_{Exc} and total BCF_{T} values for B, Pb, and Cd of the plant shoot, root, and seed, except for BCF_{T} values of B, Pb, and Cd of the plant seed and shoot. The BCF_{Exc} and total BCF_{T} values increased with increasing application concentration of the *B. megaterium* application concentration. The greatest BCF_{Exc} and BCF_{T} values for B, Pb, and Cd were obtained from plant root, followed by shoot and seed, in that order with two doses of *B. megaterium* application treatments (Table 4). BCF_{Exc} and total BCF_{T} for shoot, root, and seed were greater in B than the Pb and Cd. By comparing BCF_{Exc} and total BCF_{T}, researchers can compare the ability of different plants in taking up metals from soils and translocating them to the shoots. *B. napus* had a high BCF_{Exc} value (>1) for B but not BCF_{T}.

Bioaccumulation Factors (BAF). The BAF of metals indicate the quantity of metal absorbed by part of the plant from the soil according to the soil pollution degree at initial time. The *B. megaterium* application altered both the BAF_{Exc}. and total BAF_{T} values.

Table 4

Effects of *B. megatarium* application on remediation component of canola (*Brassica napus* L.) under growth with different B, Pb, and Cd amendments (2-year average mean)

Doses ml plot^{-1}	RF	PP	BCF exc seed	BC$_T$ seed	BCF exc shoot	BCF$_T$ shoot	BCF exc root	BCF$_T$ root	BAF$_T$	BAF exc	TI	TF$_T$	TF exc	TLF$_T$	TLF exc	EF	RT
B																	
Control	23.0d	0.32c	1.49c	0.05b	2.40b	0.08c	4.33b	0.14b	13.96c	16.29d	89.31b	0.26e	8.22b	0.90c	0.5d	1.00d	6.2a
0	26.0d	0.40c	2.49b	0.08b	3.23b	0.10c	5.83b	0.19b	23.41b	26.54c	89.71b	0.37d	11.54b	0.98c	1.1c	1.41c	5.2b
50	55.3c	0.70b	3.96a	0.11a	7.09a	0.20b	8.23a	0.23a	31.81a	38.72b	92.45a	0.54c	19.29a	1.34a	2.9b	2.00b	2.8c
100	74.5b	0.94a	3.95a	0.13a	7.89a	0.25b	8.85a	0.28a	35.39a	43.06a	93.86a	0.66b	20.69a	1.34a	3.1b	2.40ab	2.0d
150	80.2a	1.03a	3.19a	0.12a	7.84a	0.30a	9.10a	0.35a	33.30a	40.88a	93.86a	0.78a	20.12a	1.21b	3.7a	2.71a	1.7e
Pb																	
Control	8.2c	0.10d	0.02d	0.01^{ns}	0.77^{ns}	0.04^{ns}	0.13b	0.01^{ns}	0.30c	1.05d	78.71c	0.04c	0.92c	0.26c	0.4d	1.00c	7.6a
0	15.3c	0.12c	0.26c	0.01^{ns}	0.87^{ns}	0.04^{ns}	1.68a	0.07	3.68b	4.52c	87.98b	0.12b	2.81b	0.67b	0.9c	3.10b	7.0a
50	19.4b	0.16b	0.37b	0.02^{ns}	0.92^{ns}	0.04^{ns}	1.55a	0.07	5.88a	6.77b	89.36a	0.14ab	2.84b	0.83a	1.8b	3.49a	5.4b
100	22.5a	0.19a	0.41a	0.02^{ns}	0.94^{ns}	0.05^{ns}	1.68a	0.09	6.87a	7.77a	90.28a	0.15a	3.03a	0.80a	2.0a	3.88a	4.7c
150	23.6a	0.18a	0.36b	0.02^{ns}	0.78^{ns}	0.05^{ns}	1.53a	0.09	6.72a	7.46a	90.17a	0.16a	2.68b	0.74a	2.3a	3.80a	5.1c
Cd																	
Control	1.5c	0.00c	0.01c	0.01^{ns}	0.01^{ns}	0.01^{ns}	0.06c	0.00	0.04c	0.05c	71.88b	0.01c	0.08c	0.28b	0.4e	1.00e	815.8a
0	2.6c	0.01c	0.06b	0.01^{ns}	0.12^{ns}	0.01^{ns}	0.65b	0.03	0.40b	0.51b	71.53b	0.04b	0.83b	0.28b	0.7d	10.54d	90.9b
50	6.4b	0.02b	0.16a	0.01^{ns}	0.29^{ns}	0.01^{ns}	1.03a	0.05	0.96b	1.24a	81.52a	0.08b	1.48a	0.43a	1.1c	18.24c	32.7c
100	7.3b	0.03a	0.17a	0.01^{ns}	0.31^{ns}	0.02^{ns}	1.06a	0.06	1.16a	1.46a	80.69a	0.09b	1.53a	0.45a	1.9b	21.68b	27.4d
150	11.7a	0.03a	0.18a	0.01^{ns}	0.33^{ns}	0.02^{ns}	1.18a	0.09	1.53a	1.84a	82.68a	0.12a	1.70a	0.43a	2.1a	29.16a	20.2e

Notes. Different letters within columns indicate significant differences at $P \leq 0.05$ for bacteria application doses; ns, not significant; RF, remediation factor (%); PP, phytoextraction potential (kg ha^{-1}); BCF$_{Exc.}$, bioconcentration factor (seed or shoot or root) according to exchangeable soil element (B, Pb, or Cd); BCF$_T$, bioconcentration factor (seed or shoot or root) according to total soil element (B, Pb, or Cd); BAF$_{Exc.}$, bioaccumulation factor according to exchangeable soil element (B, Pb, or Cd); BAF$_T$, bioaccumulation factor according to total soil element (B, Pb, or Cd); TI, transfer index (%); TF, transfer factor; TLF, translocation factor; EF, enrichment factor; and RT, temediation time (year).

The greatest BAF_{Exc}. and total BAF_T values were obtained from two applications only in B- and Pb-contaminated soils. For Cd the greatest BAF values were obtained after three applications of *B. megaterium*. The BAF_{Exc}. and total BAF_T values for B were greater than those for Pb and Cd (Table 4). The BAF_{Exc} value indicates that the plants may be of use in the short term for remediation of polluted soil, whereas the BAF_T value indicates that may be of use in the long term as well.

Phytoextraction Potential (PP). The PP represents the total amount of heavy metals extracted per ha of soil in a single phytoextraction cycle. The application of *B. megaterium* affected the PP for B, Pb, and Cd. The PP increased with increasing application of *B. megaterium*. The B, Pb, and Cd PP of *B. napus* plants in one growing season were up to 0.32, 0.10, and 0.01 in the $control_1$ treatment, but 1.03, 0.18. and 0.03 in the three doses of *B. megaterium* application treatment (Table 4). Compared to B, Pb, and Cd PP values, the B value was greater than those for Pb and Cd.

Transfer Index (TI). The TI suggested by Paiva, Carvalho, and Siqueira (2002) indicates the ratio of metal uptake of harvestable part of plant to total plant uptake. The application of *B. megaterium* affected the TI (Table 4). The greatest TI values for B, Pb, and Cd were with two doses *B. megaterium* treatment.

Transfer Factor (TF). The ability of a species to translate metal from the soil to its shoots was estimated using the TF. The TF of a metal is calculated using two formulae for the exchangeable and total metal concentration in the harvest time. The *B. megaterium* application affected the TF of plants. The TF values of the plant increased with increasing doses of *B. megaterium* (Table 4). The greatest TF_T and $TF_{exc.}$ values for B, Pb and Cd were obtained in treatments with two doses in both exchangeable and total concentration in the soil. High TF_T and TF_{exc} values are used as criteria for selecting species. TF_{exc} and TF_T values were 8.22–0.26 for B, 0.92–0.04 for Pb, and 0.08–0.01 for Cd, whereas TF_{exc} and TF_T increased to 20.69–0.66 for B, 3.03–0.15 for Pb, and 1.53–0.09 for Cd with *B. megaterium* application. The greatest TF_{exc} and TF_T were obtained from B, followed by Pb and Cd.

Enrichment Factor (EF). The application of *B. megaterium* affected the EF of plant for B, Pb, and Cd. The EF of plant increased with increasing application of *B. megaterium*. The B, Pb, and Cd PP values of *B. napus* plant in one growing season were up to 1.41, 3.10, and 10.54 in the control treatment and 2.71, 3.80, and 29.16 in the three-dose *B. megaterium* application treatment, respectively (Table 4). This high enrichment factor (>1) indicates greater availability and distribution of metals in the polluted soil and thereby increasing the average heavy metal concentration in cultivated *B. napus* plant.

Remediation Time (RT). The application of *B. megaterium* altered the RT for B, Pb, and Cd from contaminated soil. The RT values of B, Pb, and Cd decreased with three applications of the *B. megaterium* application; 6.2, 7.6, and 815 years were needed for remediation time without *B. megaterium*, but these periods reduced to 1.7, 5.1, and 20 years with three doses *B. megaterium* (Table 4). Boron has the lowest RT, followed by Pb and Cd.

Remediation Factor (RF). The application of *B. megaterium* changes the RF for B, Pb, and Cd from contaminated soil. The RF values of B, Pb, and Cd increased with increasing application concentration of the *B. megaterium* application concentration. The greatest RF values for B, Pb and Cd were obtained with three doses of *B. megaterium*, and these values were 80.2, 23.6, and 11.7%, respectively (Table 4).

Discussion

Dry-Matter Yield and Uptake of B, Pb, and Cd

Increases in B, Pb, and Cd contents of plant tissues may have resulted in a significant decrease in biomass accumulation by different crops. The reduction in dry-matter yield due to B, Pb, and Cd application is in agreement with the findings of Lehoczky, Szabados, and Martha (1996), Turan and Angın (2004), Turan and Esringu (2007), Angın et al. (2008), Dursun et al. (2010), and Esringü and Turan (2012). Yield reductions in mustard plants have been attributed to the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis, inhibition of various enzyme activities, and induction of oxidative stress including alterations in enzymes of the antioxidant defense system (Sandalio et al. 2001). On the other hand, high metal accumulation may be attributed to a well-developed detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on appropriate ligands such as organic acids, proteins, and peptides in the presence of enzymes that can function at high level of metallic ions and metal exclusion strategies of plant species (Ghosh and Singh 2005).

Brassica species are well known as metal accumulators and especially *B. napus* has been investigated for several years for the accumulation of a range of metals. However, the harvestable parts might be utilized only for industrial purposes and not for human or animal consumption, and similar results were reported by Banuelos, Zambrzuski, and Macke (2000), Belimov et al. (2005), Ghosh and Singh (2005).

Biomass production plays an important role in a general phytoremediation strategy for heavy-metal removal from contaminated soil, if plant extraction and plant accumulation of heavy metal are to be considered as the principle pathways from removal of heavy metal from contaminated land. In this study *B. megaterium* had the two positive effects for phytoremediation of B-, Pb-, and Cd-polluted soil: increasing availability of B, Pb, and Cd in the soil and amelioration of negative effects of heavy-metal toxicity to some extent on dry-matter yield (DMY) of the plant.

Boron, Pb, and Cd Desorption from the Soil Fraction

Application of *B. megaterium* at 10^8 cfu ml^{-1} 50, 100, and 150 ml plot^{-1} doses significantly increased B, Pb, and Cd desorption periods from soil by *B. napus* plants. When *B. megaterium* application among the soil element fractions was evaluated, there were significant differences in its ability to stimulate soil element pools for B, Pb, and Cd. The potentially bioavailable fraction of B, Pb, and Cd increased with increasing *B. megaterium* application but the stable and least liable fraction decreased. The greatest increase was determined from three doses *B. megaterium* application (10^8 cfu ml^{-1}), and the increase ratios for potentially bioavailable and relatively fractions were 28.4 and 22.7% for B fraction, 22.0 and 24.9% for Pb fraction, and 33.16 and 18.1% for Cd fraction, respectively. The stable, least liable fractions decreased by 8.9, 9.7, and 3.8% for B, Pb, and Cd. These results are in line with those of Roane (1999), Cheung and Gu (2005), Mohapatra, Siebel, and Alaerts (1993), Vary (1994), Wu et al. (2006), Pobell-Selenska (1999), and Abou-Shanab et al. (2008), who reported that the presence of *B. megaterium* bacteria in the rhizosphere area increases the concentrations of Zn, Cu, Pb, Cd or Cr in plants and improves the interactions between plants and beneficial rhizosphere microorganisms, which can enhance biomass production, tolerance of the plants to heavy metals, and accumulation capacity of several [Mn, Co, Cd, Ni, Cu, Zn, mercury (Hg), Pb, uranium (U), radon (Ra), polonium (Po)] heavy metals.

Remediation Factors of **Brassica napus** *Grown in Contaminated Soil*

We considered several indicators that provided estimates of the plant efficiency at B, Pb, and Cd removal and also the period of time necessary to remove all soil B, Pb, and Cd, considering annual cultivation cycles.

Brassica napus can be used as hyperaccumulator plant for B, Pb, and Cd remediation according to remediation factors. Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TLF values <1, with greater values indicating translocation to the aerial part of the plant. In our study, results showed that our plant has a high TLF_{Exc} factor (>1) for B, Pb, and Cd in three doses of *B. megaterium* application, but a TLF_T factor of >1 for B. High root-to-shoot translocation of these metals indicated that these plants have vital characteristics to be used in phyto-extraction of these metals as indicated by Zhang et al. (2002), Ghosh and Singh (2005), Lázaro, Kiddb, Martýńeza (2006), and Fayiga and Ma (2006). Tolerant plants tend to restrict soil–root and root–shoot transfers and therefore have much less accumulation in their biomass, whereas hyperaccumulators actively take up and translocate metals into their aboveground biomass.

Plants exhibiting BCF_{Exc} and total BCF_T values less than 1 are unsuitable for phytoextraction (Fitz and Wenzel 2002). A few species growing at the site were capable of accumulating heavy metals in the roots, but most of them had low BCF values, which means limited ability of heavy-metal accumulation and translocation by the plants. According to the BCF_{Exc} values, this plant may be used in the short term to remediate polluted soil, and the BCF_T values indicate that it may be useful in the long term as well.

Taking into account exchangeable and total amounts of B, Pb, and Cd in the arable layer, B, Pb, and Cd phytoextraction potential of *B. napus* plant was also very good under the given conditions, but it was too low for successful remediation in a reasonable timeframe under the used plant management.

According to Vera, Blanco Rodriguez, and Lozano (2003) the values of transfer factors are affected by such factors as characteristic of soil, humidity, kind of plant, chemical and physical properties of elements, and influence of plants competition. Confirmation of these statements is provided by the findings of Peciulytė et al. (2006). Substantial differences in the accumulation of Cd, Pb, and Zn have been observed between two plant species (maize and vetch) after 3 weeks of growth in metal-contaminated soil (Peciulytė et al. 2006).

Conclusion

The results of the study demonstrated that three doses *B. megaterium* application was more effective than the other treatments in enhancing B, Pb, and Cd desorption from soil and for increasing B, Pb, and Cd accumulation in plants by ameliorating the negative effects of the heavy metals, based on the assumption that metal pollution occurs only in the active rooting zone, the 20-cm soil layer. Thus, to have a total soil mass of 2600 t ha^{-1} (soil bulk density of 1.3 t m^{-3}) and decrease soil B, Pb, and Cd to 8 mg kg^{-1}, 4 mg kg^{-1}, and 3 mg kg^{-1}, the *B. napus* plant with 150 ml $plot^{-1}$ doses of *B. megaterium* treatments would be necessary for approximately 2, 6, and 21 years, respectively. In conclusion, inoculation with *B. megaterium* may facilitate plant growth and thus increase phytoremediation efficiency. Enhancing metal accumulation in high-yielding crop plants without diminishing their yield is fundamental to successful phytoremediation.

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