



The effect of compost and *Bacillus licheniformis* on the phytoextraction of Cr, Cu, Pb and Zn by three brassicaceae species from contaminated soils in the Apulia region, Southern Italy

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ARTICLE INFO

Article history:

Received 23 December 2010

Received in revised form 5 November 2011

Accepted 25 November 2011

Available online 27 December 2011

Keywords:

Contaminated soil

Heavy metals

Phytoextraction

Bacillus licheniformis

Compost

ABSTRACT

The selection of appropriate plant species is critical in the successful application of phytoremediation techniques. The present study is an attempt to assess the capability of three brassicaceae, *Brassica alba* (L.) Rabenh, *Brassica carinata* A. Braun and *Brassica nigra* (L.) Koch, for the phytoextraction of Cr, Cu, Pb and Zn from an unpolluted and polluted silty loamy soil added with either *Bacillus licheniformis* BLMB1 or compost or both. Experiments were conducted in a greenhouse in pots filled with the soils. In all experiments metals were shown to accumulate in shoots and roots of plants grown on polluted soils, and both compost and *B. licheniformis* BLMB1 strain were able to enhance the accumulation of metals, especially Cr. In particular, Cr accumulation in *B. alba* resulted higher than the Cr threshold for hyperaccumulator plants (1000 mg kg⁻¹). This result provides a new plant resource that may have a potential use for phytoextraction of Cr from contaminated soil. However, because of the low bioconcentration factors (<1) for all studied metals, these species cannot be regarded as suitable for the phytoextraction of excessive Cr, Cu, Pb and Zn from polluted soils. Thus, these species may be used with success only for low metal polluted soils.

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1. Introduction

Phytoremediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from polluted sites or decrease their toxicity (Salt et al., 1998). One of the main mechanisms for heavy metal phytoremediation of soil is phytoextraction, in which plants are used to concentrate metals from the soil into roots and shoots (Jing et al., 2007).

The uptake and accumulation of pollutants vary from plant to plant and also from species to species within a genus (Singh et al., 2003). A number of plant species, the so-called “metal hyperaccumulators”, are able to uptake metals from soil, transport them to the aboveground parts, and accumulate them at concentrations up to 100-fold greater than those normally found in non-accumulator species (Baker and Brooks, 1989; Baker et al., 2000; McGrath and Zhao, 2003). The threshold values of metal concentrations used to define metal hyperaccumulation are: 10,000 mg kg⁻¹ dry weight of shoots for Zn and Mn, 1000 mg kg⁻¹ for Co, Cr, Cu, Ni, As and Se, and 100 mg kg⁻¹ for Cd. However, hyperaccumulator plants often

accumulate only a specific metal and have a small biomass and a slow growth rate, which implies long times for completion of remediation (Baker and Brooks, 1989; Baker et al., 2000; Cunningham et al., 1995; McGrath and Zhao, 2003).

Many crops and weed species have been screened for metal uptake, translocation and tolerance. Much effort has received the *Brassicaceae* with 87 hyperaccumulators found (Bogs et al., 2003; Broadley et al., 2001; Milner and Kochian, 2008). Regardless of the plant used, efficient metal extraction by plants is often limited by the availability of metals for root uptake, in particular in neutral and alkaline soils (Quartacci et al., 2006).

A promising strategy has been shown to be the application of soil amendments which are able to enhance phytoextraction by increasing metal availability in the soil. In particular, different kinds of composts, including those derived from municipal solid wastes (MSW), have been shown to increase metal availability in soil through the formation of soluble metal-organic complexes, thus increasing plant uptake efficiency (Maftoun et al., 2004; Murillo and Cabrera, 1997; Murphy and Warman, 2001; Ozores-Hampton and Hanlon, 1997; Pinamonti et al., 1999; Sebastiao et al., 2000; Warman and Rodd, 1998; Zheljaskov and Warman, 2004a,b; Zhou and Wong, 2001).

Another strategy used to improve metal phytoextraction is based on the application of plant growth promoting rhizobacteria (PGPR)

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that are able to increase plant biomass and/or promote metal uptake by production of enzymes, siderophores, organic acids and/or biosurfactants (Abou-Shanab et al., 2003; Glick, 2003; Glick et al., 1998,1999). In particular, *Bacillus licheniformis*, a Gram-positive, spore-forming soil bacterium, classified as GRAS (*generally recognized as safe*), was shown to be an efficient plant growth promoting rhizobacterium. Previous studies have demonstrated that various microbial strains of *B. licheniformis* are able to improve the growth and development of the host plant in heavy metal contaminated soils by mitigating the toxic effects of heavy metals on the plants (McLean et al., 1990, 1992; Ramos et al., 2003; Yakimov et al., 1995).

Thus, combining the use of *B. licheniformis* and MSW compost may be expected to be a good means for increasing phytoremediation efficiency. In this context, this study had the objectives of assessing metal phytoextraction by three brassicaceae species, *Brassica alba* (L.) Rabenh., *Brassica carinata* A. Braun and *Brassica nigra* (L.) Koch, and evaluating the effects of the use of MSW compost and *B. licheniformis* BLMB1 on the availability, accumulation, uptake, and removal efficiency of Cr, Cu, Pb and Zn from polluted soils.

2. Materials and methods

2.1. Experimental procedure

Seeds of *B. alba* and *B. nigra* (L.) Koch were purchased from a commercial supplier in Egypt, whereas *B. carinata* seeds were provided by the Department of Plant Production, University of Bari, Italy. Plants were grown in a greenhouse covered with a screen without supplementary light or heat. The average temperature of the greenhouse ranged from 29.6 ± 5.6 °C (day) to 14.5 ± 4.3 °C (night), and the relative humidity was $65.5 \pm 10.9\%$, with an average photoperiod of 12 h per day. To prevent emergence failures, twenty seeds were sown in each pot. Then, when the first pair of true leaves appeared, seedlings were thinned out and 4 uniform ones per pot were allowed to grow.

The set-up consisted of 45 pots (15 pots for each species) made of polyvinyl chloride (PVC) having a diameter of 20 cm and a height of 20 cm. The design included T1 (uncontaminated soil), T2 (contaminated soil), T3 (T2 + 10% compost), T4 (T2 + 10% *B. licheniformis* BLMB1), and T5 (T2 + 10% compost + 10% *B. licheniformis* BLMB1). All treatments were triplicated and seeds planted in all five treatments. All pots were watered and kept at the field capacity moisture throughout the growing season.

The contaminated soil (T2) used in this experiment is a Typic Haploxeralfs, fine-loamy, mixed, thermic (Soil Taxonomy, 2003) and featured total concentrations of Cr, Cu, Pb and Zn largely exceeding the maximum levels permitted by the Italian legislation for agricultural soils (Italy, 2006). The soil was collected from the National Park of Alta Murgia (Apulia region, Southern Italy), where a number of sites are contaminated by various heavy metals originated from the disposal of wastes of different sources of origin (Brunetti et al., 2009). An uncontaminated agricultural soil (T1) was collected from the same area and used as a control. Soils were collected after grass cover removal from the top 20 cm, air-dried, gently ground to pass through a 2-mm sieve, homogenized and used to fill the pots (3 kg soil per pot).

A MSW compost (10% w/w) and a cell suspension (10^8 cells ml^{-1}) of *B. licheniformis* BLMB1, isolated from a semi-commercial formulate (10% v/w), were used as amendments by thorough mixing with the soil. The heavy metal content of the compost used was within the Italian legislation limits (Iwegbue et al., 2007).

2.2. Analytical procedures

2.2.1. Soil

Soil analyses were carried out following internationally recommended procedures and the Italian official methods (Italy, 1999). Soil

pH was determined by a glass electrode in distilled water ($\text{pH}_{\text{H}_2\text{O}}$) suspensions at 1:2.5 soil to liquid ratio. Electrical conductivity (EC) was measured using a conductimeter in filtrates from suspensions of 1:2 soil to water ratio. Texture was determined by the pipette method after dispersing the soil sample in a solution of sodium hexametaphosphate and sodium carbonate (Gee and Bauder, 1986). Total organic carbon (TOC) was measured by the Walkley–Black method (Walkley and Black, 1934). Total nitrogen (N_{tot}) was measured by the Kjeldhal method (Jones, 2001). Available phosphorus (P_{ava}) was determined according to the Olsen method (Olsen et al., 1954) on sodium bicarbonate and sodium hydroxide soil extracts using a spectrophotometer UV/VIS.

The total contents of heavy metals were determined in microwave assisted digests (Multiwave Perkin Elmer 3000) of soil samples added with a suprapure $\text{HNO}_3\text{:H}_2\text{O}_2\text{:HCl}$ mixture (5:1:1 v/v). The metal extractable fraction in soil was estimated on soil extracts by diethylenetriamine pentaacetic acid (DTPA)– CaCl_2 –triethanolamine (TEA) buffered at pH 7.3 (Lindsay and Norvell, 1978). This protocol is generally recommended for alkaline calcareous soils and excludes the effects of carbonate dissolution. The contents of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in both acid-digested and DTPA extracts were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES ICAP 6300 Thermo Electron).

All chemicals were of analytical reagent grade and distilled water Milli-Q was used for solution preparation and dilution. Reagent blanks and laboratory NIST certified standards were used and routinely checked during ICP-OES determinations.

2.2.2. Plant

To evaluate heavy metal concentrations, the examined plants were harvested (90 days after sowing), and separated into roots and shoots (all the above ground parts). Successively the separated fractions were thoroughly washed with tap water to remove all visible fine soil particles, rinsed with deionised water, oven dried at 60 °C for 3 days, and ground to a powder using a Retsch MM200 mixer mill and a kitchen miller. The powdered fractions were then subjected to microwave assisted digestion with a suprapure $\text{HNO}_3\text{:H}_2\text{O}_2\text{:HCl}$ mixture (5:1:1 v/v), and finally to spectroscopic measurement as described for soil samples.

The Bioconcentration Factor (BF) of each metal in plants was calculated by dividing the total content in shoots by the total content in soil (Brooks, 1998). Further, the Translocation Factor (TF) was calculated by dividing the total metal content in shoots by the total metal content in roots (Brooks, 1998). Both factors were calculated on a dry weight basis.

2.2.3. Compost

The MSW compost used in this study originated from Progeva SRL Company, Laterza, Taranto. Mainly the organic fractions composted at this company included branches and trimmings, food wastes, fats and greases, grass clippings, napkins, paper towels, coffee filter, leaves and vegetable scrap. The compost was analyzed using standard methods (CCME, 1996; Collana Ambiente, 1998; TMECC, 2002) after air-drying, grinding with a mixer mill, and passing through a 1-mm sieve. The pH was measured in distilled water at a 1:10 sample to water ratio using a pH meter. The EC was determined on the filtrate at 1:10 sample to water ratio by a conductimeter. The TOC, N_{tot} , P_{ava} , and heavy metal contents were determined as described above for soil samples.

2.2.4. Bacterium

B. licheniformis BLMB1, isolated from the rhizosphere of olive trees, was grown in Nutrient Agar plates (NA, Oxoid Limited, UK) for 48 h at 30 °C. The pH value of the medium was adjusted to 7.2 with 10% (w/v) NaOH and 10% (w/v) HCl. A loop of the bacterial culture was then used to inoculate a starter culture (300 ml) of *B. licheniformis* BLMB1 in Nutrient Broth (NB, Oxoid Limited, UK).

Table 1

Main properties and contents of total and DTPA-extractable heavy metals in soils and compost studied.

Parameters	Unit	soils		Compost
		T1	T2	
Texture	–	SL	SL	–
pH (H ₂ O)	–	8.7 ± 0.03	8.5 ± 0.02	8.7 ± 0.02
EC	ds m ⁻¹	0.22 ± 0.02	0.32 ± 0.02	1.3 ± 0.03
CO _{3tot}	g kg ⁻¹	85 ± 1.40	89 ± 1.09	–
CO _{3act}	g kg ⁻¹	30 ± 0.06	83 ± 0.04	–
TOC	g kg ⁻¹	41.7 ± 1.10	65.9 ± 0.08	232 ± 3.75
N _{tot}	mg kg ⁻¹	6.1 ± 0.08	8.4 ± 0.06	14.8 ± 1.30
P _{ava}	mg kg ⁻¹	71 ± 0.06	83 ± 0.04	83 ± 0.02
Total metal concentrations				Limits
				soils ^a compost ^b
Cd	mg kg ⁻¹	0.91 ± 0.01	1.62 ± 0.03	<0.001 2 105
Cr	mg kg ⁻¹	66.26 ± 0.44	1977.8 ± 6.34	48 ± 0.01 150 100
Cu	mg kg ⁻¹	28.7 ± 0.24	188.8 ± 2.62	105 ± 0.02 120 300
Ni	mg kg ⁻¹	39.15 ± 0.08	74.75 ± 1.26	39 ± 0.02 120 50
Pb	mg kg ⁻¹	58.46 ± 0.38	202.31 ± 2.42	67 ± 0.01 100 140
Zn	mg kg ⁻¹	114.65 ± 0.82	679.5 ± 3.52	230 ± 0.03 150 500
DTPA-extractable metal contents				
Cd	mg kg ⁻¹	0.09 ± 0.01	0.18 ± 0.02	
Cr	mg kg ⁻¹	0.002 ± 0.01	0.06 ± 0.01	
Cu	mg kg ⁻¹	0.67 ± 0.08	6.93 ± 0.38	
Ni	mg kg ⁻¹	0.18 ± 0.01	1.37 ± 0.08	
Pb	mg kg ⁻¹	0.60 ± 0.03	4.57 ± 0.24	
Zn	mg kg ⁻¹	0.44 ± 0.04	60.45 ± 0.02	

SL, silty loam; EC, electrical conductivity; CO_{3tot}, total carbonates; CO_{3act}, active carbonates; TOC, total organic carbon; N_{tot}, total nitrogen; P_{ava}, available phosphorus.^a Values are the maximum total metal contents allowed in soils for public and private green areas and residential sites (Italy, 2006).^b Values are the maximum metal total contents in compost allowed by the Italian legislation (Iwegbue et al., 2007).

The culture was grown for 48 h, at 30 °C, 150 rpm; then, bacterial cells were harvested by centrifugation (6000 rpm, 10 min, 4 °C), washed three times with sterile phosphate buffer (NaCl 0.14 M, KCl 2.7 mM, Na₂HPO₄·2H₂O 8.1 mM, KH₂PO₄ 1.8 mM, pH 7.4), and resuspended in the same buffer, to a final concentration of 10⁸ cells ml⁻¹. Three hundred milliliters of BLMB1 cells suspension was inoculated in 10 l of a commercial medium (Biomedium, Elep Biotechnologies SpA, Milan, IT) according to the manufacturer's instructions and incubated for 8 h at room temperature (RT) to produce vegetative cells. Before seeding, the bacterial suspension (about 10⁸ cell ml⁻¹) was added to the pots (10% v/w), according to the experimental plan. Control plots were treated with sterile Biomedium at the same rate (10% v/w).

The total population of sporogenous bacillaceae in the soil was determined either before the application of the BLMB1 strain and after, i.e. at the end of the brassicae vegetative cycle. To this purpose, the protocol of Chilcott and Wigley (1993) was used, with some modification. In particular, soil samples (250 g) were collected from each plot, crushed, and sieved at 2 mm. Twenty-five grams of sieved soil was suspended in 225 ml NB enriched with 0.05% glucose and 30 ppm of MnSO₄·H₂O (Finley and Fields, 1962), homogenized for 30 min at 150 rpm, and incubated (30 °C, 150 rev min⁻¹) for 5 days to allow formation of mature spores (Földes et al., 2000). Five milliliters of soil suspension was then incubated at 70 °C for 60 min h in a water bath, to separate endospores from vegetative cells (Garbeva et al., 2003; Logan and De Vos, 2009; Sneath, 1986; Walker et al., 1998). Aliquots (100 µl) from 10-fold serial dilutions to 10⁻³ of the heat-treated samples were spread onto NA plates in ten replicates for each dilution, and incubated in the dark at 28 °C. After 24 h incubation, the colonies of *Bacillus* spp. were counted and the population expressed as colony forming unit (CFU) per gram of dry soil. Pure colonies were obtained by triple streaking on NA and identified based on morphological characters (Logan and De Vos, 2009; Sneath, 1986). To confirm the presence of the applied *B. licheniformis* strain BLMB1 in the population, selected colonies were tested by

Southern blot analysis by using a specific DNA probe, and according to the procedures reported by Ferrara et al. (2009).

2.2.5. Statistical analyses

Statistical analysis of data was made by Statgraphics Plus 5.1. software. One-way analysis of variance (ANOVA) and Tukey's test were applied. Data on the population of sporogenous bacillaceae were transformed as Log (CFU + 1) g⁻¹, before the analysis. Means and standard deviations were calculated using Microsoft Excel.

3. Results and discussion

3.1. Total and available metal contents in soils

The main properties and total and DTPA-extractable heavy metal contents of the soils and compost used are shown in Table 1. The

Table 2Roots and shoots dry matter (g plant⁻¹) of brassicaceae at the end of treatments.

	<i>B. alba</i>	<i>B. carinata</i>	<i>B. nigra</i>
Roots dry matter (g plant ⁻¹)			
T1	3.50 a	3.22 a	3.54 a
T2	3.62 b	3.41 b	3.67 b
T3	3.67 c	3.52 c	3.79 c
T4	3.78 c	3.53 c	3.83 c
T5	3.79 c	3.54 c	3.83 c
Shoots dry matter (g plant ⁻¹)			
T1	10.47 c	9.97 b	10.84 c
T2	10.21 a	9.76 a	10.63 c
T3	10.51 d	10.10 c	10.96 d
T4	10.40 b	9.80 a	10.76 b
T5	10.52 d	10.12 c	10.97 d

Analysis of variance (ANOVA): Tukey's test with 95% of significance (values in a column followed by the same letter for the same plant part are not significantly different at p<0.05).

Table 3

Soil population [$\log (CFU + 1) g^{-1}$, d.w] of sporogenous *Bacillus* spp. as affected by treatment (T1, T2, T3, T4 and T5), plant species (*B. alba*, *B. carinata* and *B. nigra*), and their interaction. Values in columns followed by the same letter are not significantly different according to Tukey's Test ($p < 0.05$); *: significant at 5%; **: significant at 1%.

	Probability	Means [$\log (CFU + 1) g^{-1}$, d.w]
Treatment	<0.001**	
T1		6.84 a
T2		7.10 b
T3		7.36 c
T4		7.35 c
T5		7.58 d
Plant species	<0.001**	
<i>B. alba</i>		7.24 a
<i>B. carinata</i>		7.22 a
<i>B. nigra</i>		7.26 a
Treatment $\times$ plant species	0.0472*	

slightly alkaline pH values, the high percentages of TOC and the presence of carbonates suggest that an important retention of heavy metals is to be expected in these soils (Greger, 2003; Kabata-Pendias and Pendias, 2001; Larson et al., 2000; Shuman, 1999).

The total contents of Cr, Cu, Pb and Zn in the polluted soils were higher and that of Ni and Cd lower than the maximum admissible levels for agricultural soils in Italy (Italy, 2006). In particular, the value recorded for Cr ($1977.8 \text{ mg kg}^{-1}$) was 13 times higher than the maximum admissible limit in Italy. Based on their content in soils, the metals studied can be classified, from the higher to the lower pollution level, as follows: $\text{Cr} > \text{Zn} > \text{Pb} > \text{Cu}$. However, metal availability in these soils appears to be low, as shown by the DTPA-extractable contents (Table 1), and in the order: $\text{Zn} > \text{Cu} > \text{Pb} > \text{Cr}$.

3.2. Plant growth

In general, no evident symptoms of metal toxicity were observed during the experiments, except for *B. carinata* that showed pale leaves in the treatments T2 and T4, where the compost was not applied. The three plant species showed satisfactory growth parameters in all experiments, even though the growth pattern was different among the species. *B. nigra* showed the fastest growth, *B. alba* grew more slowly, and *B. carinata* showed the slowest growth. Generally, the amendment with compost and/or bacillus increased significantly the dry matter of brassicaceae, roots in particular, with respect to treatments T1 and T2 (Table 2).

3.3. Bacterial population

The population level of sporogenous *Bacillus* spp. [expressed as $\log (CFU + 1) g^{-1}$, CFU = Colony Forming Unit] in the soils of the various treatments, before seeding and after harvesting, is shown in

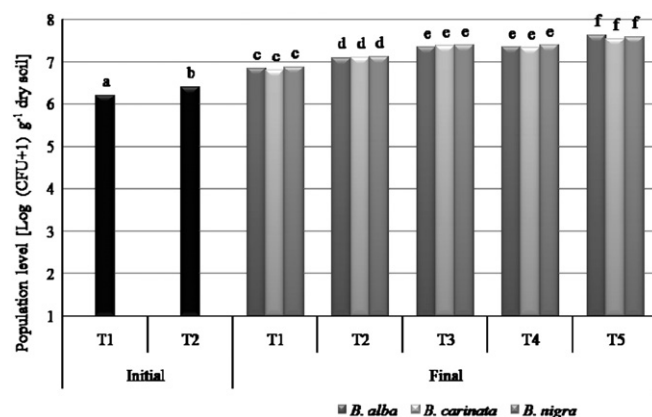


Fig. 1. Population level of sporogenous *Bacillus* spp. in the control (T1) and polluted soil (T2) before seeding the brassicas species, and in T1, T2, polluted soil + compost (T3), polluted soil + *B. licheniformis* BLMB1 (T4), and polluted soil + compost 10% + *B. licheniformis* BLMB1 (T5) after harvesting. Columns marked with the same letter are not significantly different, according to one-way analysis of variance and Tukey test ($p \leq 0.05$).

Fig. 1. Results obtained suggest that this bacterial population can tolerate high metal concentrations in the soil and can be augmented by adding exogenous strain, under the tested experimental conditions and in the presence of the three brassicaceae planted.

Managing the microbial populations in the rhizosphere by using exogenous microbial inoculum able to tolerate high metal concentration, could provide plants with benefits crucial for ecosystem restoration (Khan, 2005). Statistical analyses of data (Fig. 1) show that the population level of the sporogenous *Bacillus* spp. is higher: (a) after harvesting than before seeding; (b) in treated polluted soil (T3, T4 and T5) than in the untreated ones (T1 and T2); (c) in the presence of both *B. licheniformis* BLMB1, and compost (T5) than in the presence of compost (T3) or strain BLMB1 (T4) alone. In general, the population level appears to be significantly related to the different treatments considered (Table 3). However, more specific studies are needed for a better evaluation of this strain and its effects on metal availability in soil and plant growth parameters. Thus, advances in understanding the role of microorganisms in such processes, together with the ability to modulate their activities using the tools of molecular biology, will improve the metal bioremediation processes.

3.4. Heavy metal contents in plant shoots and roots

The contents of heavy metals studied in plant shoots and roots are shown in Fig. 2. The total content of heavy metals in plants grown on the polluted soil (T2) is high, which suggests metal accumulation and high tolerance of tested brassicaceae. Metal accumulation is greatest

Table 4

Two-way ANOVA results for metals concentrations in roots and shoots as a function of treatment (T1, T2, T3, T4 and T5) and plant (*B. alba*, *B. carinata* and *B. nigra*), and their interaction. Values in columns followed by the same letter are not significantly different according to Tukey's multiple Range Test.

		Shoots						Roots					
		Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cr	Cu	Ni	Pb	Zn
Treatment (T)	p	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
	T1	0.66 a	5.4 a	7.2 a	2.2 a	0.8 a	37.3 a	0.34 a	10.8 a	10.4 a	2.1 a	1.5 a	26.9 a
	T2	0.79 c	599.0 b	59.6 b	20.7 b	62.4 b	297.8 b	0.34 a	77.6 b	12.8 a	3.9 b	5.6 b	49.4 b
	T3	0.77 bc	776.5 c	65.5 c	23.9 d	72.3 c	341.0 d	0.42 b	196.2 c	18.8 b	6.8 c	13.1 c	87.8 c
	T4	0.74 b	748.2 c	62.9 c	22.5 c	69.1 c	324.4 c	0.45 c	296.3 d	25.6 c	9.8 d	20.5 d	126.2 d
	T5	0.76 bc	634.5 b	63.5 c	21.4 b	68.3 c	309.8 bc	0.47 c	331.5 e	25.4 c	10.6 d	22.5 d	136.8 d
Plant (P)	p	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001
	<i>B. alba</i>	0.84 c	777.0 c	73.6 c	21.4 b	79.8 c	343.4 c	0.32 a	147.1 a	16.2 a	5.9 b	10.3 a	73.7 a
	<i>B. carinata</i>	0.79 b	403.2 a	34.7 a	16.7 a	34.6 a	209.9 a	0.59 b	250.0 b	24.5 b	8.6 c	17.3 b	110.5 b
	<i>B. nigra</i>	0.59 a	477.9 b	46.9 b	16.3 a	49.4 b	232.9 b	0.31 a	150.3 a	15.2 a	5.4 a	10.4 a	72.1 a
Interaction T $\times$ P		≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.001	≤ 0.05	≤ 0.001	≤ 0.001	≤ 0.001

P: is the level of the statistical significance.

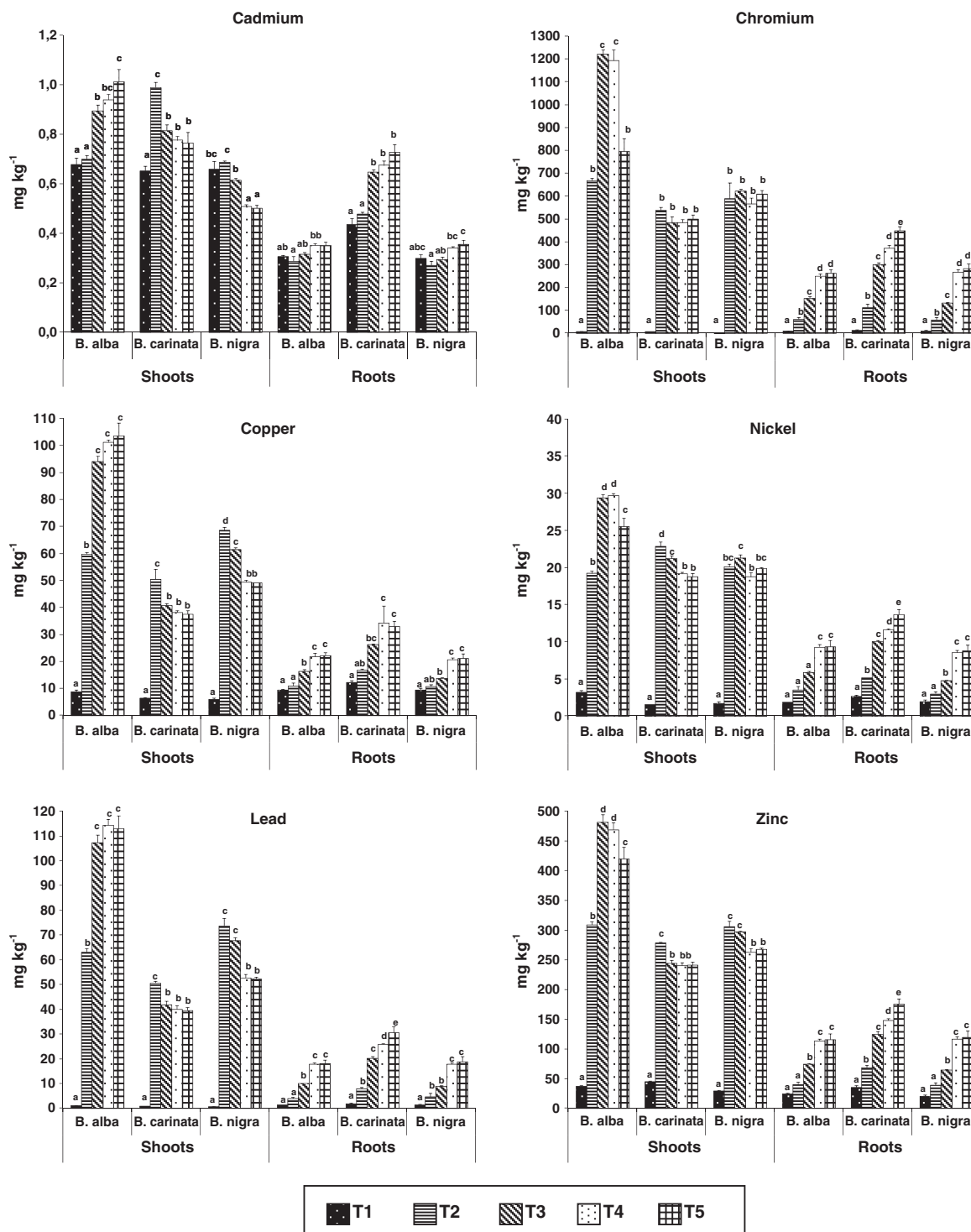


Fig. 2. Content of heavy metals ($\pm$ standard error, three replicates) in shoots and roots in the tested brassica species (values of the same plant and same tissue followed by the same letter are not significantly different according to ANOVA analysis by Tukey's test at $p \leq 0.05$).

in shoots and lowest in roots, which can be attributed to the efficient translocation from the root to the shoot system, that is, a typical behavior of accumulator species (Baker, 1981). According to the statistical analysis (Table 4) significantly higher metal concentrations in shoots of brassica species follow the order: *B. alba* > *B. nigra* > *B. carinata* for Cr, Cu, Pb and Zn, *B. alba* > *B. carinata* > *B. nigra* for Cd and *B. alba* > *B. carinata* = *B. nigra* for Ni. While in roots the order is *B. carinata* > *B. alba* = *B. nigra* for Cd, Cr, Cu, Pb and Zn and *B. carinata* > *B. alba* > *B. nigra* for Ni. The data from statistical analysis

(Table 4) also indicate that addition of soil amendment to polluted soil significantly affects the accumulation of heavy metals in plants tissues, except for Cd in shoots. Similar concentrations of Cu and Pb exist in the shoots of the three amended treatments (T3, T4 and T5) while accumulation effectiveness of Cr, Ni and Zn is only observed with T3 and T4 treatments, especially in the former (compost addition). The addition of the three amendments to polluted soil (T3, T4 and T5 treatments) significantly increases the concentrations in roots of the six studied metals (Table 4). The highest effectiveness

for Cd, Cu, Ni, Pb and Zn accumulation can be observed in the treatments T4 (bacillus) and T5 (bacillus + compost), while the maximum Cr concentration is achieved with T5 treatment.

The greatest Cr accumulation occurs in shoots of *B. alba*, with values of 1222.4 and 1193.8 mg kg⁻¹, respectively for compost (T3) and *B. licheniformis* BLMB1 (T4) treatments. These values (more than 1000 mg kg⁻¹) suggest that *B. alba* behaves as a Cr hyperaccumulator. To date few Cr hyperaccumulator species have been identified (Baker and Brooks, 1989). In particular, *Brassica juncea* L. has been shown to be an excellent accumulator for Cr, Cd, Cu, Ni and Zn (Kumar et al., 1995; Salt et al., 1995). Roots of *B. carinata* exhibit the highest Cr value (450 mg kg⁻¹) under the mixed treatment (T5). Generally, soil amendment enhances Cr accumulation in roots of brassica species by 2–5 times. In agreement with the results of Han et al. (2004), and despite the low bioavailability of Cr, a high concentration is recorded in both shoots and roots of brassica species. These authors suggest that high Cr concentrations (2000 mg kg⁻¹) in soils may lead to a marked increase of Cr uptake by roots (3000–3300 mg kg⁻¹) and accumulation in leaves (2000–3000 mg kg⁻¹) of *B. juncea*. They interpret their data by suggesting the passive uptake of large amounts of Cr at a phytotoxic level occurring through broken cell membranes.

The Cu concentration in shoots of *B. alba* grown on treated polluted soils (T3, T4 and T5) is significantly ($p \leq 0.05$) higher than that found in the untreated polluted soil (T2). The highest Cu content (103.7 mg kg⁻¹) is recorded in the treatments with compost and bacillus together (T5), with no significant difference ($p \leq 0.05$) among the three species. On the contrary, Cu concentration in shoots of *B. carinata* and *B. nigra* grown in any treated polluted soil appears to be inhibited by amendment application. Further, the mean concentrations of Cu in plant roots for treatments T3, T4 and T5 (18.8, 25.6, and 25.4 mg kg⁻¹, respectively) are higher than the corresponding values for treatment T2 (12.9 mg kg⁻¹). The highest Cu concentration in roots (34.4 mg kg⁻¹) was recorded for *B. carinata* in the bacillus treatment (T4).

Similar to Cu, soil amendment increases Pb concentration in shoots of *B. alba* by 70.5%, 81.6%, and 79.5% for treatments T3, T4, and T5, respectively. On the contrary, a reduction in Pb accumulation, respectively 19.9% and 21.8%, is measured in shoots of *B. carinata* and *B. nigra* in the treatments with amendments. However, no statistical difference ($p \leq 0.05$) is observed between treatments T3 and T2 for *B. nigra*. The Pb concentration in plant roots varies from 4 to 30.7 mg kg⁻¹. In particular, the highest values are measured for treatments T4 and T5. These results indicate the efficiency of the bacillus used in increasing Pb availability to plant roots.

Similar to Cu and Pb, the highest Zn accumulation is measured in plant shoots of *B. alba* in treatments T3 (481.7 mg kg⁻¹) and T4 (469.4 mg kg⁻¹), with no statistically significant variation ($p \leq 0.05$) between the two values. In contrast, when amendments are used accumulation of Zn is inhibited in shoots of *B. carinata* and *B. nigra*. In particular, the mean Zn values recorded for the three treatments are 242.1 and 276.1 mg kg⁻¹, respectively in *B. carinata* and *B. nigra*, while the same plants grown in the absence of amendments show a mean Zn concentration of 278.2 and 306.3 mg kg⁻¹, respectively. Further, the mean values recorded in roots are 27, 49.4 and 117 mg kg⁻¹, respectively for T1, T2, and T3, T4 and T5. The greatest Zn accumulation (175.7 mg kg⁻¹) occurs in the roots of *B. carinata* for treatment T5.

B. carinata shows the highest values of metals studied in the roots, whereas lowest values of Cr, Cu, Pb, and Zn are measured in the shoots. In contrast, the highest values in the shoots are recorded for *B. alba* that exhibits intermediate values in the roots. These results may refer to the different tolerance of these plants to heavy metals toxicity. According to many authors (Baker, 1981; Küpper et al., 1999; Marschner, 1995; Rauser, 1995; Vogel-Mikus et al., 2005), plant can use two different strategies to deal with high metal concentrations in soil. These include: (a) avoidance mechanisms, by which

the uptake and/or root-to-shoot transport of metals are restricted; and (b) internal tolerance mechanisms, by which metals are immobilized, compartmentalized or detoxified in the symplast by chemical binding.

As mentioned above Cd and Ni concentrations in soils examined are lower than the maximum admissible levels. A significant increase ($p \leq 0.05$) of Cd and Ni concentration is recorded in the roots of Brassica species in treatments T3, T4 and T5, whereas shoots show a different response. In particular, treatment with any amendment enhances Cd and Ni accumulation in *B. alba*, whereas no effect is observed in *B. carinata*, and only the presence of compost increases significantly the accumulation of Ni in *B. nigra*.

In general, these results suggest that *B. licheniformis* BLMB1 has the capacity to tolerate high concentrations of metals, Cr in particular, and enhance plant metal accumulation from multi metal-contaminated soils. Significant activities on the capacity of bacillaceae strains to stimulate the growth of different brassica species, protect plant from metal toxicity, increase Cd and Ni accumulation and decrease Cr toxicity have been demonstrated in several studies (Faisal and Hasnain, 2006; Rajkumar et al., 2006; Sheng and Xia, 2006; Sheng et al., 2008; Wu et al., 2006; Zaidi and Khan, 2006). These effects can be achieved by various mechanisms including: increased mobilization of metals (Chen et al., 2005; Gadd, 1990; Idris et al., 2004), reduced toxicity of metals transformed into less toxic forms, immobilization of metals on the cell surface and/or in intracellular polymers, and metal precipitation and/or biomethylation (Silver, 1996).

Similar to the bacillus, compost addition shows also able to induce significant metal accumulation in shoots and/or roots of tested Brassicaceae, as previously reported by various authors (Maftoun et al., 2004; Murphy and Warman, 2001; Pinamonti et al., 1999; Warman and Rodd, 1998; Zheljzkov and Warman, 2004a,b). This effect is probably due to the capacity of compost to increase metal bioavailability. Heyes et al. (1998) reported that the composting process may release metals from organic combinations by degradation of organometallic complexes, thus leading to increased metal bioavailability.

3.5. Phytoextraction potential

The TF and BF values of metals are shown in Fig. 3. The BF values of species tested are lower than 1 for all treatments. In particular, the ranges of values for treatments T2, T3, T4 and T5 are: 0.26–0.71 for *B. alba*, 0.20–0.61 for *B. carinata*, and 0.25–0.45 for *B. nigra*. The BF is reported to decrease with increasing metal concentration in soil (Zhao et al., 2003), and low values are considered normal when plants are grown on polluted soils (McGrath and Zhao, 2003).

On the contrary, the TF values are higher than 1 for treatments T2, T3, T4 and T5. In particular, the TF value ranges are: 2.45–16.13 for *B. alba*, 1.05–6.28 for *B. carinata* and 1.41–16.96 for *B. nigra*. As tolerant plants have TF values less than 1 and hyperaccumulators higher than 1 (Baker, 1981), the TF values measured here indicate that the three species behave as highly metal accumulator plants in greenhouse conditions.

According to McGrath and Zhao (2003), the efficiency of phytoextraction is determined by two key factors: the biomass production and metal BF. These authors conclude that phytoextraction is not feasible using plants having a low BF, regardless of how large is the biomass produced. In particular, the BF should be 20 or greater to achieve a halving of soil metal contents in less than 10 crop harvests, assuming that the metal is taken up by plants from the top 20-cm soil. Further, with a biomass of 20 t ha⁻¹ per crop, a BF greater than 10 would be required (McGrath and Zhao, 2003). On these bases, phytoextraction by the three species cannot be considered the appropriate choice for removal of these metals from polluted soils in the studied area. Thus, a realistic hypothesis is that brassica species can perform better in the case of light metal pollution of soil.

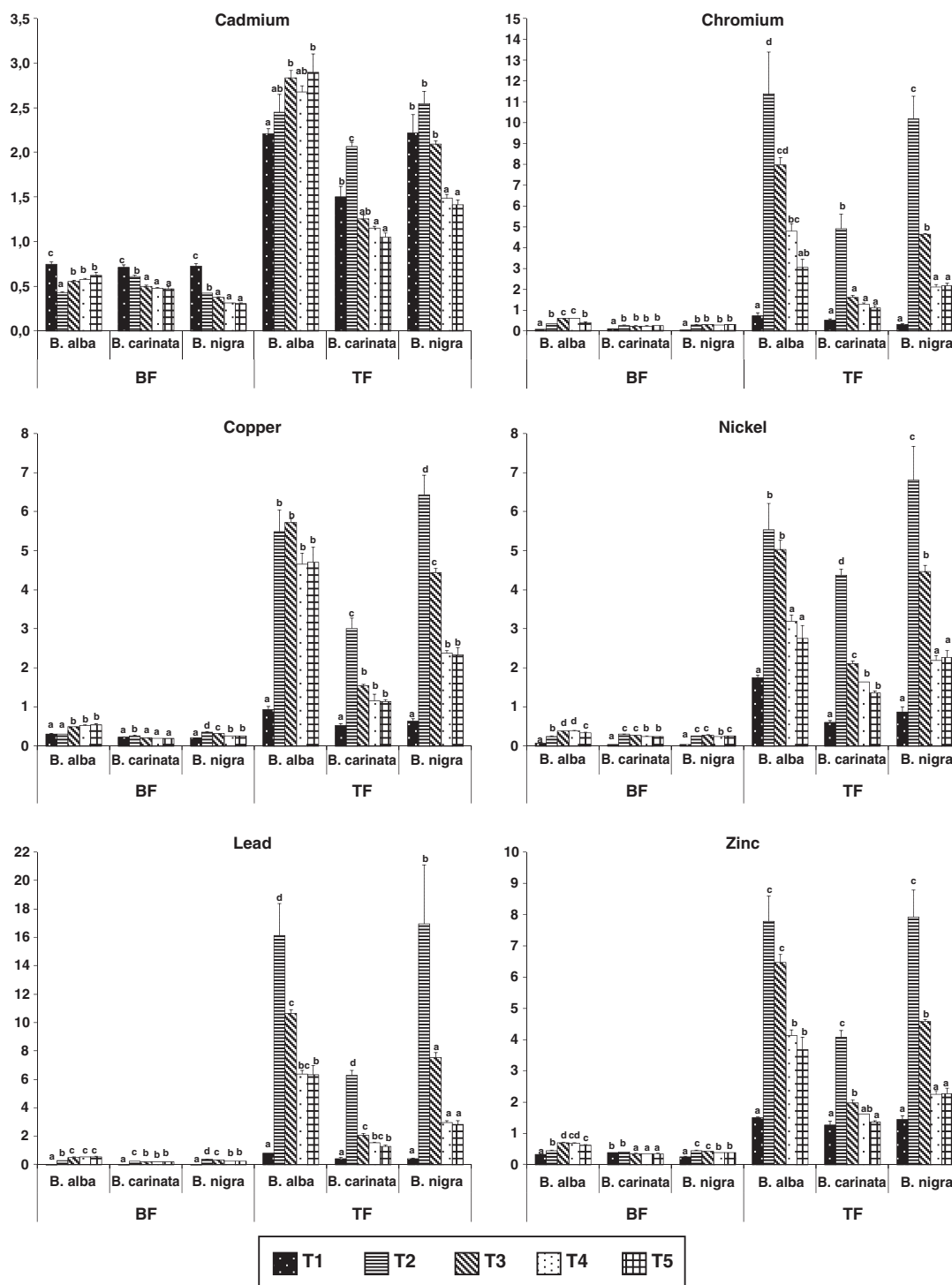


Fig. 3. Bioconcentration factor (BF) and translocation factor (TF) values ($\pm$ standard error, three replicates) of heavy metals in the tested brassica species (values of the same plant and same tissue followed by the same letter are not significantly different according to ANOVA analysis by Tukey's test at $p \leq 0.05$).

4. Conclusions

In the experimental conditions used in this work, three *Brassicaceae* examined behave as good accumulators, and *B. alba* can be considered as a Cr hyperaccumulator plant, based on Cr concentration recorded in its shoots which exceeds the standard values of hyperaccumulators plants (1000 mg kg^{-1}). The three brassica species show to be promising for phytoextraction use on the basis of their efficiency in accumulating one or more metal contaminant and their ability to

tolerate high metal contamination. Data obtained also show that the type of soil amendments used affects significantly the concentration of heavy metals found in plants. In particular, both compost and *B. licheniformis* MBBL1 strain are able to induce a significant metal accumulation in shoots and/or roots of tested brassicaceae.

Due to the low bioconcentration factors of tested species (less than 1), these cannot be considered the appropriate choice for metal phytoextraction from the polluted soils examined. Thus, these species may be used with success only for low metal polluted soils.

Acknowledgments

This work has been funded by Regione Puglia (Italy) through the research project POR Puglia 2000–2006, Misura 1.8-Azione 4: “Monitoraggio siti inquinati”. Supporto scientifico alle attività di recupero funzionale ed il ripristino ambientale del sito inquinato dell'Alta Murgia. P. Soler-Rovira is a recipient of a contract from JAE-Doc program of CSIC.

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