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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

Gennaro Brunetti [a], Karam Farrag [b,*], Pedro Soler-Rovira [c], Massimo Ferrara [d], Franco Nigro [d], Nicola Senesi [a]

[a] Dipartimento di Biologia e Chimica Agroforestale e Ambientale, Università di Bari, Via Amendola, 165/A, 70126 Bari, Italy
[b] Central Lab for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), Ministry of Water Resources and Irrigation (MWRI), Egypt
[c] Instituto de Ciencias Agrarias, CSIC, Serrano 115 dpdo., 28006 Madrid, Spain
[d] Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola, 165/A, 70126 Bari, Italy

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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.

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; Zheljazkov 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)

* Corresponding author. Tel.: +20 101022229; fax: +20 222035083.
E-mail addresses: Karam_farrag@hotmail.com, karam_farrag@yahoo.com (K. Farrag).

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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 (pH_{H2O}) 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 HNO_3:H_2O_2: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 HNO_3:H_2O_2: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 brunches 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).