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Research Article

Effect of button mushroom compost on mobilization of heavy metals by sunflower

Ki-Cheon Kyeong[1], Yong-Gyun Kim[1], Chan-Jung Lee[2], Byung-Eui Lee[3], Heon-Hak Lee and Min-Ho Yoon*

Department of Bio-Environmental Chemistry, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, 305-764, Korea
[1]Crop Research Division, Chungcheongnam-do Agricultural Research & Extension Services, Yesan 340-861, Korea
[2]Mushroom Research Division, National Institute of Horticultural & Herbal Science, RDA, Eumseong 369-873, Korea
[3]Department of Chemistry, College of Natural Sciences, Soonchunghyang University, Asan 336-745, Korea

ABSTRACT: The potential ability of Button mushroom compost (BMC) to solubilize heavy metals was estimated with metal contaminated soils collected from abandoned mines of Boryeong area in South Korea. The bacterial strains in BMC were isolated for investigating the mobilization of metals in soil or plant by the strains and identified according to 16S rRNA gene sequence analysis. When metal solubilization potential of BMC was assessed in a batch experiment, the BMC was found to be capable of solubilizing metals in the presence of metals (Co, Pb and Zn) and the results showed that inoculation of BMC could increase the concentrations of water soluble Co, Pb and Cd by 35, 25 and 45% respectively, than those of non-inoculated soils. BMC-assisted growth promotion and metal uptake in sunflower (*Helianthus annuus*) was also evaluated in a pot experiment. In comparison with non-inoculated seedlings, the inoculation led to increase the growth of *H. annuus* by 27, 25 and 28% respectively in Co, Pb and Zn contaminated soils. Moreover, enhanced accumulation of Co, Pb and Zn in the shoot and root systems was observed in inoculated plants, where metal translocation from root to the above-ground tissues was also found to be enhanced by the BMC. The apparent results suggested that the BMC could effectively be employed in enhancing phytoextraction of Co, Pb and Zn from contaminated soils.

KEYWORDS: Mobilization, Button mushroom compost, Bioaugmentation, Sunflower

Introduction

Metal contamination of soils has become one of the most significant environmental problems today. Excessive metal uptake by crop plants from the contaminated agricultural lands can result in decreased crop yield due to the inhibition of plant metabolic processes (Singh and Aggarwal, 2006). Apart from the metals with unknown biological functions (Cd, Cr, Pb, Co, Ag, Se, and Hg), essential elements (Fe, Mn, Zn, Cu, Mg, Mo, and Ni) also keep accumulating in agricultural soils by means of wastewater irrigation, animal manures and sewage sludge application, use of fertilizer and agrochemicals (Thomas *et al*., 2012).

The remediation of metal contaminated soils receives increasing attention (Cao *et al*, 2007) due to the fact that metals are not easily degraded. Depending on the resource availability and nature of metal contaminated soil, different methods such as bioremediation and physical/chemical remediation have been employed in restoring the contaminated lands (Arunakumara *et al*, 2013; Luo, 2009). However, the physical and chemical methods such as physical separation, acid leaching or electrochemical processes, are considered to be ineffective because of high cost, low efficiency, and destruction of soil structure and fertility (Jing *et al*., 2007). In contrast, phytoremediation, a method which uses plants to extract, sequester and detoxify pollutants has received considerable attention (Arunakumara, 2011). However, the wider application of the technology has been restricted due to

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*Corresponding author
E-mail : mhyoon@cnu.ac.kr
Tel : +82-42-821-6733,Fax : +82-42-823-9241

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the limitations such as low soil thickness that can be treated, low translocation rate of metals from roots to shoots, and the slowness of the treatment (Lebeau *et al*., 2008).

The amount of heavy metals uptake in plants varies with the mobility and the concentration of metals in soil (Chen *et al*., 2010) and the interface between soil microbes and plant roots (rhizosphere) is displayed to have a great influence on the uptake of nutrients as well as on the decrease of metal toxicity (McNear, 2013). Since soil microbes could alter the metal status of the soil (Fazal and Bano, 2010), exploitation of such microbes to reduce the metal toxicity in plants is worth investigating (Rajkumar *et al*., 2008). It was known that some metal resistant bacterial strains were proved exceptional at enhancing the growth of the host plant through different mechanisms such as the production of plant growth promoting substances, nitrogen fixation and phosphate solubilization (Hemambika *et al*., 2013). As reported by Rajkumar *et al*. (2008), heavy metal tolerance of the microbes may be attributed to one or several mechanisms including exclusion, active removal, biosorption, and precipitation or bioaccumulation of metals both in external and intracellular spaces. Therefore, microbes having remarkable metal tolerance and plant growth-promoting abilities could play a significant role in remediation of metal-contaminated soils, because bioaugmentation with such microbes could promote phytoextraction of metals (Prapagdee *et al*., 2013).

In the present study, button mushroom(*Agaricus bisporus*) compost with bacterial strains was employed in assessing the potential of mobilization of Co, Pb and Zn in soils and it was estimated that the effect of inoculation with BMC on plant growth and uptake of Co, Pb and Zn by *Helianthus annuus* (sunflower).

Materials and Methods

Preparation of Button mushroon compost

The waste mushroom compost from *Agaricus bisporus* (button mushroom) collected in Buyeo area, South Korea was used for preparation of button mushroon compost (BMC). The waste mushroom bed was composted with pig and fowl manure as nitrogen sources and sawdust as carbon source, respectively. The BMC was prepared by mixing to the ratio of waste mushroom bed (4) : sawdust (8) : pig and fowl manure (1) and fermenting the mixture for 40 days at 30°C.

Isolation of microorganisms from BMC

Heavy metal contaminated soils were collected from the sediment tailing in abandoned mines of Boryeong area, South Korea. Aliquots of serially diluted soil samples were spread on Tryptic soy broth (TSB, 30 g L^{-1}) agar media which was adjusted to pH 6.5±0.1. The colonies on agar plate were purely isolated by repeated sub culturing at 30^{0}C for 5 days. The preculture of each colony was inoculated in 50mL TSB containing 0.2g - 0.4 g L^{-1} of $CoCl_2{\cdot}6H_2O$, $2PbCO_3{\cdot}Pb(OH)_2$, and $Cd(NO)_3$ and cultured at 30°C for 7 days on a horizontal shaker at 150 rpm. The heavy metals resistance cells were selected and used for estimating the solubilization ability of heavy metals.

Strain identification

The partial sequencing of 16S rRNA for the bacterial strain was done with the help of DNA sequencing service, SOLGENT, Daejeon, South Korea using universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The online program BLAST was used in identifying the related sequences with known taxonomic information available at the databank of NCBI (http://www.ncbi.nlm.nih.gov/BLAST). A Phylogenetic tree was constructed using CLUSTAL X program (Thompson *et al.*, 1997), which involved sequence alignment by neighbor joining method (Saitou and Nei, 1987) and maximum parsimony using the MEGA4 program (Kumar *et al.*, 2001). Grouping of sequences was based on confidence values obtained by bootstrap analysis of 1,000 replicates. Gaps were edited in the BioEdit program and evolutionary distances were calculated using Kimura two parameter model. Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

Mobility of the metals in soil

The impact of BMC inoculation on the mobility of metals in soil was investigated under laboratory conditions with 50 mL scaled polypropylene centrifuge tubes. Artificially contaminated soil (5 g) in the centrifuge tubes was inoculated with 10 ml TSB supplemented with 5 g BMC. After taking the weight of the tubes, they were wrapped with brown paper and placed on an orbital shaker at 200 rpm at 25°C. At the end of the period of 10 d, the weight of the tubes was recorded and 10 ml of sterile water were added to each tube to extract the soil water soluble heavy metals. The extracts

Table 1. Change of microbial population in the compost prepared with spent mushroom from *Agaricus bisporus*. (cfu/g)

Treatments		Aerobic bacteria	Gram negative	Bacillus species	Fluorescence bacteria	Actiono-mycetes	Fungi
PiM+SD	Before	1.3×10^7	7.6×10^7	1.2×10^6	4.8×10^3	8.0×10^3	1.6×10^5
	After	1.1×10^6	9.7×10^6	5.2×10^6	3.5×10^4	1.3×10^4	6.0×10^3
ChM+SD	Before	2.6×10^7	1.3×10^7	3.1×10^6	2.1×10^4	1.5×10^4	8.6×10^5
	After	7.5×10^7	2.7×10^7	7.0×10^6	3.5×10^4	9.6×10^3	6.0×10^3

The compost was prepared by mixing to the ratio of waste mushroom bed (4) : sawdust (8) : pig or fowl manure (1).
PiM: Pig manure, Ch: Fowl manure, SD: Sawdust, M: Spent mushroom from *Agaricus bisporus*

were centrifuged at 10000 ×g for 10 min and filtered through a 0.45 μm nylon syringe filter (Watman, England) and acidified with HNO_3 to minimize an interference by organic matters. The metal contents (Co, Pb, and Zn) in the filtrate were determined using an ICP (Perkinelmer, Aanalyst 800, USA). Artificially contaminated soil without inoculation with the strain served as the control after centrifugation.

Effect of bioagumentation on growth and metal uptake by H. annuus

A pot experiment was conducted under green house conditions at the College of Agriculture, Chungnam National University in April 2014. Several locations of soils collected from abandoned mines of Boryeong-gun as contaminated soil and a button mushroom compost in Buyeo-gun area, Chungchungnam-do, South Korea, were respectively mixed with the ratio of 1:1, air dried and sieved (2 mm). Sterilized forest soil (by steaming at 100°C for three consecutive days) was amended with aqueous solutions of different heavy metals (Co, Pb and Cd) to achieve the final concentrations of 200 mg/kg soil. They were then kept for 2 weeks in a greenhouse for metal stabilization and used in filling the plastic pots (25 cm diameter, 35 cm height). Seeds of *Helianthus annuus* were surface sterilized by immersing in alcohol (70%) for 40 s, NaClO (1.0%) for 15 min followed by rinsing several times with sterile distilled water. Seeds sown in germination trays containing sterilized non-contaminated soil were provided with 14/10 light/dark regime and kept at 25°C for germination. Three weeks old seedlings were carefully uprooted from the germination bed and were transplanted into the plastic pots (five plants/pot) containing 300 g of metal contaminated or non-contaminated soil and allowed to grow at 25°C and 14/10 light/dark regime. The average pH of soil at the time of planting was recorded as 6.8. Three weeks later, the plants were carefully uprooted and cleaned the root surface thoroughly with distilled water. As growth parameters, fresh and dry biomass was measured and accumulation of metals in plant biomass was quantified as described by Freitas *et al*. (2004). Each treatment had three replicates.

Results

Microbial diversity of BMC

The number of microorganisms in BMC were higher in the fowl manure than those in the pig manure as nitrogen source. BMC compromised diverse microorganisms ranging of aerobic bacteria 3.8×10^7 cfu g^{-1}, Gram negative bacteria 1.8×10^7 cfu g^{-1}, genus *Bacillus* 3.0×10^6 cfu g^{-1}, genus *Pseudomonas* 2.1×10^4 cfu g^{-1}, actinomycetes 7.2×10^5 cfu g^{-1}, and fungi 3.2×10^4 cfu g^{-1} (Table 1). Besides actinomycetes and fungi, the microbial population in the process of composting was increased to the level of 1×10^1~1×10^2 cfu g^{-1} after fermentation, compared with those of before fermentation.

Isolation and identification of microorganisms

The major group of bacterial strains from BMC were isolated and the strains with the potential ability to alleviate heavy metals were screened based on estimating the metal concerntration alleviated by each bacterium in medium supplemented with 3 different heavy metals (Co, Pb, Zn). The selected strains were identified by systematic analysis using phylogenetic tree according to 16S rRNA sequence analysis. The main bacteria were identified with the close promixsity to each type strains as Genus *Bacillus*, *Pseudomonas*, *Pantoea*, *Burkholderia* and *Enterobacter* (Table 2). In addition, to estimate the metal resistance potential of the bacterial strains isolated from BMC, the amount of metals remaining in Tryptic soy broth medium was

Table 2. Identification of metals resistance bacteria isolated from button mushroom compost

Genus of isolated strains	Identity with type strain (%)	*Ratios of metals resistance (%)			Most closet species (Acession number)
		Co	Pb	Zn	
Bacillus	99.7	15	11	24	*Bacillus subtilis* (AJ276351.1)
Pseudomonas	99.3	18	12	27	*Pseudomonas koreensis* (AF468452.1)
Enterobacter	98.9	14	10	24	*Enterobacter ludwigii* (AJ853891.1)
Pantoea	99.36	17	13	19	*Pantoea agglomerans* (AJ233423.1)
Burkholderia	99.71	16	14	25	*Burkholderia stabilis* (AF148554.1)

*The amount of heavy metals alleviated by the strains in TSB medium supplemented with metals (Co, Pb and Zn) at the concentration of 200 mg L^{-1}. Values are the means of three replicates.

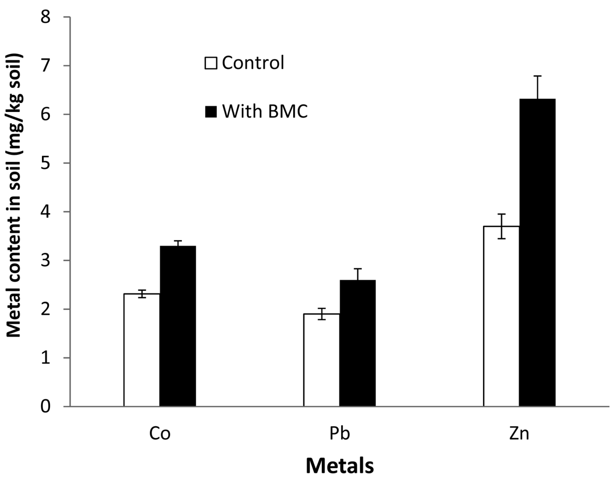

Fig. 1. Effect on mobilization of Co, Pb and Zn in soil by inoculation with button mushroom compost. Soil without inoculation of BMC served as the control. Values are the means of three replicates. Error bars represent standard deviation.

estimated 5 days after the inoculation with each strain. As was represented in Table 2, they showed the resistance of the range from 10% to 27% for heavy metals Co, Pb, and Zn, respectively. This result demonstrated that BMC with diverse microorganisms could be useful as microbial inoculant for bioremediation of metal contaminated soils.

Mobility of the metals in soil

To investigate the impact of BMC inoculation on the mobility of metals in soil, metal mobilization potential of BMC with the strains was assessed in a batch experiment with artificially contaminated soil. When alleviation effect of heavy metals was estimated by measuring the amount of metals remaining in the soil, BMC was shown to be capable of reducing the amount of metal in the order of Zn, Co and Zn. As was given in Fig. 1, the inoculation of BMC could increase the contents of water soluble metals from the soil extract, representing that heavy metals could be solubilized by BMC inoculation. The mobilization of Co, Pb and Zn was respectively 35, 25 and 45% higher than those of the control soil.

Effect of bacterial strain on growth and metal uptake by H. annuus

As showh in Table 1, inoculation with BMC into sunflower (*Helianthus annuus*) pots resulted in increased fresh and dry biomass of *H. annuus* plants compared to non-inoculated plants. In case of the non-inoculated plants exposed by heavy metal stress, the growth of plant was inhibited in a significant level with $p < 0.05$. For instance, Pb toxicity caused 30 and 24% reductions in fresh and dry weight of the plant, respectively. Inoculation however led to increase in plant fresh and dry weight in the presence of heavy metals. The fresh and dry weight of the plants grown in Co contaminated soils were respectively 26 and 31% higher than those of non-inoculated plants. Similarly, in Pb contaminated soil, the percent increments were recorded as 29 and 23% respectively, and in Zn contaminated soil, the corresponding figures were 29 and 27%.

The amounts of Co, Pb and Zn accumulated in the roots and shoots of *H. annuus* grown under inoculated and non-inoculated conditions are given in Table 2. Inoculation with BMC resulted in increased accumulation of metals both in the shoots and roots. The accumulations of Co, Pb and Zn in shoots were respectively 22, 16 and 25% higher than those of non-inoculated plants. The corresponding accumulations for Co, Pb and Zn in roots were 36, 42 and 15% higher than those of non-inoculated plants. Regardless of inoculation or non-inoculation, the accumulation of

Table 3. Effect of inoculation with button mushroom compost on shoot and root weight of *Helianthus annuus*

Metal	Treatment	Fresh weight (g/plant)		Dry weight (g/plant)	
		Shoot	Root	Shoot	Root
Metal free soil	control	1.77 (±0.044)	0.107 (±0.015)	0.088 (±0.004)	0.038 (±0.005)
	with strain	1.95 (±0.039)	0.130 (±0.009)	0.112 (±0.005)	0.059 (±0.006)
Co	control	1.33 (±0.034)	0.065 (±0.004)	0.072 (±0.003)	0.027 (±0.003)*
	with strain	1.68 (±0.045)	0.089 (±0.006)	0.094 (±0.004)	0.039 (±.004)*
Pb	control	1.25 (±0.029)	0.057 (±0.004)	0.057 (±0.002)*	0.027 (±0.004)*
	with strain	1.57 (±0.039)	0.093 (±0.006)	0.075 (±0.004)*	0.038 (±0.003)*
Zn	control	1.46 (±0.041)	0.078 (±0.003)	0.083 (±0.004)*	0.029 (±0.002)
	with strain	1.79 (±0.044)	0.108 (±0.002)	0.094 (±0.003)*	0.041 (±0.002)

Values are means ($n=3$) ± standard deviation. Within each column, the means indexed by * are not significantly different at $p > 0.05$ between inoculated and non-inoculated plants according to Duncan's multiple range test.

Table 4. Effect of inoculation with button mushroom compost on accumulation and translocation of Co, Pb and Zn in *Helianthus annuus*

Metal	Treatment	Metal content (mg/kg dry weight)		Bioconcentration	Translocation
		Shoot	Root	Factor (BCF)[a]	Factor (TF)[b]
Co	control	19.93 (±3.58)	53.82 (±4.92)	0.269	0.370
	with strain	25.46 (±4.12)	84.56 (±5.38)	0.423	0.301
Pb	control	13.76 (±0.69)*	44.68 (±7.47)	0.223	0.308
	with strain	16.42 (±0.83)*	77.49 (±6.53)	0.387	0.212
Zn	control	21.84 (±5.12)	88.47 (±5.47)*	0.442	0.247
	with strain	29.35 (±8.41)	104.53 (±6.27)*	0.522	0.281

[a]BCF=metal concentration ratio of plant roots to soil; [b]TF=metal concentration ratio of plant shoots to roots.
Values are means ($n=3$) ± standard deviation. Within each column, the means indexed by * are not significantly different at $p > 0.05$ between inoculated and non-inoculated plants according to Duncan's multiple range test.

metals in root system was found to be considerably higher than that of in shoots, which has been further confirmed by the low translocation factor (TF) for all the metals. However, TF of Co was somewhat higher than that of the other two metals. Reversely low bioconcentration factor (BCF) was also recorded from Co and Pb, compared to Zn. However, the results showed a good agreement and demonstrated that inoculation of the bacterial strain led to increase both TF and BCF of the three metals distinctly.

Discussion

Generally microorganisms isolated from heavy metals contaminated soils possess the ability to withstand against multiple pollutants as they have adapted to such environments (Pal *et al*., 2005; Abou-Shanab *et al*., 2007). Remedation of heavy metal contaminated soil have been employed with both microorganism and phytoextraction. The effectiveness of the strain for phytoextraction was assessed with *Helianthus annuus* (sunflower), was known to have an ability to accmulate biomass rapidly and take up substantial amounts of metals (Turgut *et al*., 2004; Prapagdee *et al*., 2013). As reported by Ouzounidou and Ilias (2005) and El-Tayeb *et al*. (2006), accumulation of plant biomass could be affected by excessive concentrations of heavy metals, which exert adverse impacts on growth and function of root system resulting in poor uptake of water and nutrients. As reported by Jiang *et al*. (2008), inoculation with *Burkholderia* sp. J62 led to increase shoot and root dry weights of corn and tomato plants. Inoculation with *Pseudomonas fluorescens* PsIA12 resulted in enhanced growth of *Zea mays* and its uptake of N, P and K (Egamberdiyeva et al., 2002). Most recently, Prapagdee *et al*. (2013) reported that growth of *H. annuus* could be enhanced by the inoculation of *Micrococcus* sp. MU1 and *Klebsiella* sp. BAM1 under Cd contaminated conditions. Belimov *et al*. (2005) also observed bacterial-assisted growth enhancement in *Brassica napus* grown in a soil contaminated with Cd. The plant growth-promoting potential of the present strain could be attributed at least partly to the phosphate solubilization ability of the strain under metal stress conditions.

Regardless of inoculation or non-inoculation, the accumulation of metals in root systems was found to be considerably higher than that of in shoots. This could primarily be attributed to the poor translocation of heavy metals from roots to shoots (Rajkumar *et al*., 2008). However, as shown in Table 2, translocation factor of the each metal was increased with the inoculation of the strain, which was of enormous practical significance. Furthermore, metal accumulations in both shoots and roots were found to be higher in inoculated plants than those of non-inoculated plants. Similar observations were made by Rajkumar *et al*. (2008) for Zn accumulation in *H. annuus* inoculated with *Bacillus weihenstephanensis*. However, according to Wani *et al*. (2007), inoculation of *Bradyrhizobium* sp. on surface sterilized seeds of *Vigna radiate* reduced the concentration of Ni in roots, shoots and grains by 15, 19 and 22%, respectively, compared with non-inoculated plants.

The increased accumulation of metals in the presence of bacterial strain might be due to the increased uptake of metals under acidic soil conditions created by the phosphate solubilization (Rajkumar *et al*., 2008). Inoculation of Cd-resistant bacterial strains to *Brassica napus* to a metal contaminated soil significantly increased the plant uptake of Cd when compared with the non-inoculated controls, as a result of pH reduction (Sheng and Xia, 2006). The present findings of metal mobilization are in agreement with Wu *et al*. (2006) and Prapagdee *et al*. (2012) who also reported bacteria-assisted increase in heavy metal mobilization. Generally, the low amount of metals extracted by plants from a soil is attributed mainly to the low availability of metals. As reported by several authors, the available metal content in a soil is less than 1% of the total metal content (Whiting *et al*., 2001; Braud *et al*., 2006). Metal availability is influenced by the nature of the metal and soil characteristics such as pH, CEC and organic matter (Kayser *et al*., 2001; Lebeau *et al*., 2008). Bioaugmentation could enhance metal bioavailability by increasing the concentration of the available fractions. As revealed by the present results, the release of heavy metals from the non-soluble phases to soluble phases could be facilitated by the bacterial strain. Therefore, increased accumulation of metals, in particular Co in both the shoots and roots of *H. annuus* could be attributed to the higher water soluble metal contents in soil inoculated with bacterial strain. As reported by the results of previous studies, *H. annuus* is capable of accumulating high amounts of Pb, Cd, Cu, Zn and Co, in both the shoots and the roots (Boonyapookana *et al*., 2005; Marchiol *et al*., 2007). According to Braud *et al*. (2006), inoculation of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* has resulted in 113% increment of Pb content in the exchangeable fraction of the soil. However, the Pb concentration bound to free Mn oxides, organic matter and in the residual fraction remained stable. Abou-Shanab *et al*. (2006) observed an increase of extractable Ni with *Microbacterium arabinogalactanolyticum* by a factor up to 15. As reported by Baum *et al*. (2006), the concentrations in NH_4NO_3-extractable Cd, Cu, Pb and Zn in a soil bioaugmented with ectomycorrhizal fungus *Paxillus involutus*, were 1.22-, 1.11-, 1.33- and 1.33-fold higher than those of non-bioaugmented soil, depending on the soil composition.

Conclusion

The button mushroom compost, which was prepared with waste button mushroom, fowl manure and sawdust, compromised diverse microorganisms and was found to be capable of solubilizing heavy metals (Co, Pb and Zn). Metal mobilization potential of the BMC showed that inoculation of BMC could increase the concentrations of water soluble Co, Pb and Zn in soil than those of non-inoculated soils. Inoculation with the BMC also resulted in increased shoot and root biomass and enhanced accumulation of Co, Pb and Zn in *Helianthus annuus* plants. Furthermore BMC was found to be capable of promoting metal translocation from the roots to the shoots of *H. annuus*. Therefore, BMC could be identified as an effective promoter of phytoextraction of Co, Pb and Zn from metal-contaminated soils.

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