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Article

Mobilization of Heavy Metals Induced by Button Mushroom Compost in Sunflower

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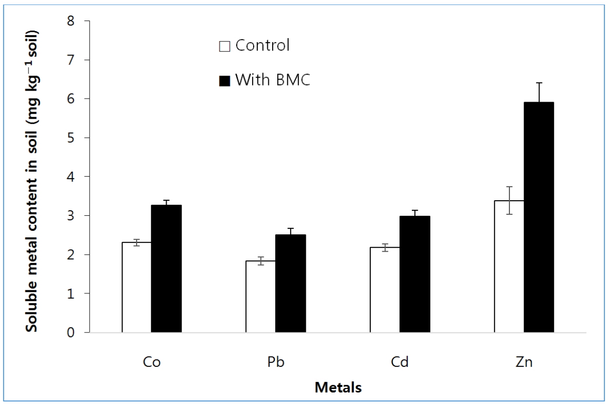
Button mushroom compost (BMC) was prepared by fermenting the mixture of waste button mushroom bed collected from Boryeong area in South Korea (4): sawdust (8) : pig and fowl manure (1) for 40 days at 30°C. The BMC compromised diverse microorganisms including aerobic bacteria 8.1 × 10^6 cfu g^{-1}, Gram negative bacteria 1.7 × 10^7 cfu g^{-1}, genus *Bacillus* 6.4 × 10^6 cfu g^{-1}, genus *Pseudomonas* 1.5 × 10^4 cfu g^{-1}, actinomycetes 1.0 × 10^4 cfu g^{-1}, and fungi 3.5 × 10^3 cfu g^{-1}. BMC was used as a microbial inoculant for estimating the mobilization of heavy metals in soil or plant. When metal solubilization potential of BMC was assessed in a batch experiment, the inoculation of BMC was shown to increase the concentrations of water soluble Co, Pb, Cd, and Zn by 29, 26, 27, and 43% 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 17, 15, 18, and 21% respectively in Co, Pb, Cd, and Zn contaminated soils. Moreover, enhanced accumulation of Co, Pb, Cd, 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 from the soils contaminated with heavy metals such as Co, Pb, Cd, and Zn.

Key words: Mobilization, button mushroom compost, heavy metals, inoculation, sunflower



Effect on mobilization of Co, Pb, Cd, 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.

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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 et al, 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 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 (Marchiol et al., 2014). 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, the button mushroom compost with diverse bacterial strains was employed in assessing the potential of mobilization of Co, Pb, Cd, and Zn in soils and the effect of inoculation with BMC on plant growth and uptake of Co, Pb, Cd, and Zn by *Helianthus annuus* (sunflower) was estimated in artificial pot soil.

Materials and Methods

Preparation of Button mushroon compost The waste mushroom bed from *Agaricus bisporus* (button mushroom) collected in Boryeong area, South Korea was used for preparation of button mushroom 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 BMC samples of 10g added in 90 mL of sterile saline solution were incubated for 1 hr on a horizontal shaker at 150 rpm and aliquots of the mixtures were collected by centrifugation at 4°C, 8000×g for 10 min. Aliquots of serially diluted BMC 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°C for 5 days. The preculture of each colonies was inoculated in 50mL TSB containing 0.2g L^{-1} of $CoCl_2 \cdot 6H_2O$, $2PbCO_3 \cdot Pb(OH)_2$, and $Cd(NO)_3$ and $ZnCl_2$ at 30°C for 7 days on a horizontal shaker at 150 rpm and the cells were identified and used for estimating the potential ability for metal solubilization.

Assay of heavy metal resistance Isolated bacterial strains were assessed for their resistance to heavy metals using the agar dilution method (Cervantes et al., 1986). Freshly prepared TSB agar plates were amended with 4 different heavy metals; $CoCl_2 \cdot 6H_2O$, $2PbCO_3 \cdot Pb(OH)_2$, and $Cd(NO)_3$ and $ZnCl_2$ at various concentrations ranging from 200-400 μg mL^{-1} (Co, Pb, Cd, and Zn). They were inoculated with isolated strains and heavy metal tolerance was determined by the appearance of the bacterial growth after 2 days of incubation at 30°C. The bacterial strain showing the highest degree of metal resistance was selected for further studies.

Bacterial 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'-AGAGTTT GATCCTGGCTCAG -3') and 1492R (5'-GGTTACCTTG TTACGACTT -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 ofthe 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 days, 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 were centrifuged at 10000 ×g for 10 min and filtered through a 0.45μm nylon syringe filter (Watman, England) and acidified with HNO_3 to minimize an interference by organic matters. The metal contents (Co, Pb, Cd, 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 bacterial strain 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 May 2015. 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,Cd, and Zn) 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 and Discussion

Microbial diversity of BMC The number of microorganisms in button mushroom compost (BMC) were higher in the fowl manure than those in the pig manure as nitrogen source, when BMC was prepared by fermenting the mixture of waste mushroom bed (4) : sawdust (8) : pig and fowl manure (1) for 40 days at 30°C. BMC compromised diverse microorganisms ranging of aerobic bacteria 8.1 × 10^6 cfu g^{-1}, Gram negative bacteria 1.7 × 10^7 cfu g^{-1}, genus *Bacillus* 6.4 × 10^6 cfu g^{-1}, genus *Pseudomonas* 1.5 × 10^4 cfu g^{-1}, actinomycetes 1.0 × 10 cfu^4 g^{-1}, and fungi 3.5 × 10 cfu^3 g^{-1} (Table 1). Besides actinomycetes and fungi, the microbial population in the process of composting was increased to the level of $1\times10^1 \sim 1\times10^2$ 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 concentration alleviated by each bacterium in medium supplemented with 4 different heavy metals (Co, Pb, Cd, and 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*, and *Streptomyces* (Table 2). In addition, to estimate the metal resistance potential of the bacterial strains isolated

Table 1. Change of microbial population in the compost prepared with spent mushroom From *Agaricus bisporus*.

(cfu g^{-1})

Treatments		Aerobic bacteria	Gram negative	*Bacillus* species	Fluorescence bacteria	Actiono-mycetes	Fungi
PiM+SD	Before	4.2×10^6	6.3×10^6	2.7×10^6	2.5×10^3	5.2×10^3	3.2×10^4
	After	6.3×10^6	4.5×10^6	4.5×10^6	8.4×10^3	8.3×10^3	5.1×10^2
ChM+SD	Before	5.7×10^6	9.1×10^6	4.1×10^6	9.3×10^3	7.9×10^3	1.2×10^5
	After	8.1×10^6	1.7×10^7	6.4×10^6	1.5×10^4	1.0×10^4	3.5×10^3

The compost was prepared by mixing to the ratio of waste mushroom bed (4) : sawdust (8) : Pig or fowl manure (1) and fermenting for 40 days.
PiM: Pig manure, ChM: Fowl manure, SD: Sawdust, M: spent mushroom from *Agaricus bisporus.*

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	Cd	Zn	
Bacillus	99.7	18	13	20	29	*Bacillus subtilis* (AJ276351.1)
Pseudomonas	99.3	16	11	13	26	*Pseudomonas koreensis* (AF468452.1)
Enterobacter	98.9	14	10	15	26	*Enterobacter ludwigii* (AJ853891.1)
Pantoea	99.36	17	10	13	24	*Pantoea agglomerans* (AJ233423.1)
Burkholderia	99.71	16	12	14	25	*Burkholderia stabilis* (AF148554.1)
Streptomyces	99.82	11	09	12	20	*Streptomyces subrutilus* (ATCC27467)

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

from BMC, the amount of metals remaining in Tryptic soy broth medium was estimated 5 days after the inoculation with each strain. As was represented in Table 2, they showed the resistance of the range from 9% to 29% for heavy metals Co, Pb, Cd, and Zn, respectively. This results appeared to be similar with those of BMC prepared with the waste mushroom bed from *Agaricus bisporus* (button mushroom) collected in Buyeo area, South Korea (Kyeonget al., 2014). Therefore, BMC with diverse microorganisms could be useful as microbial inoculant for bioremediation of metal contaminated soils.

Mobility of the metals in soil by BMC inoculation To investigate the impact of BMC inoculation on the mobility of metals in soil, metal mobilization potential of BMC with the diverse strains was assessed in a batch experiment with artificially contaminated soil by heavy metals (Zn, Co, Cd, and Zn). 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, Cd, and Pb. 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

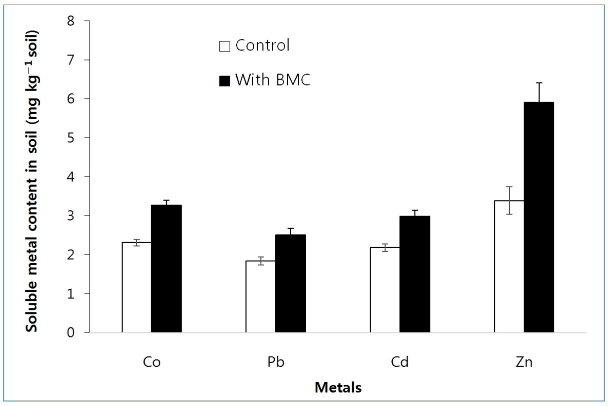

Fig. 1. Effect on mobilization of Co, Pb, Cd, and Zn in soil by inoculation with button mushroom compost. Soil without inoculation of BMC served as the control. Values are the means of three replicates. Error bars represent standard deviation.

of Co, Pb, Cd, and Zn was respectively 29, 26, 27, and 43% higher than those of the control soil.

Effect of bacterial strain on growth and metal uptake by H. annuus BMC-assisted growth promotion and metal uptake in sunflower (*Helianthus annuus*) was evaluated in a pot experiment amended with aqueous solutions of different heavy metals (Co, Pb, Cd, and Zn) to achieve the final concentrations of 200 mg kg^{-1}. The inoculation with BMC into sunflower (*Helianthus annuus*) pots resulted in the increased fresh and dry biomass of *H. annuus* plants, compared to non-inoculated plants (Table 3). 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 15 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 17 and 31% higher than those of non-inoculated plants. Similarly, in Cd contaminated soil, the percent increments were recorded as 18 and 28% respectively, and in Zn contaminated soil, the corresponding figures were 21 and 24%.

The amounts of Co, Pb, Cd, and Zn accumulated in the roots and shoots of *H. annuus* grown under inoculated and non-inoculated conditions are given in Table 4. Inoculation with BMC resulted in increased accumulation of metals both in the shoots and roots. The accumulations of Co, Pb, Cd, and Zn in shoots were respectively 22, 16, 23 and 25% higher than those of non-inoculated plants. The corresponding accumulations for Co, Pb, Cd, and Zn in roots were 36, 42, 38, and 15% higher than those of non-inoculated plants. Regardless of inoculation

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

Metal	Treatment	Fresh weight (g $plant^{-1}$)		Dry weight (g $plant^{-1}$)	
		Shoot	Root	Shoot	Root
Metal free soil	control	1.82 (± 0.032)	0.113 (± 0.017)	0.085 (± 0.003)	0.040 (± 0.004)
	with BMC	1.93 (± 0.031)	0.118 (± 0.010)	0.114 (± 0.006)	0.059 (± 0.004)
Co	control	1.37 (± 0.047)	0.069 (± 0.010)	0.070 (±0.004)	0.029 (±0.005)*
	with BMC	1.65 (± 0.038)	0.090 (± 0.007)	0.096 (±0.005)	0.038 (±0.004)*
Pb	control	1.20 (± 0.035)	0.057 (± 0.004)	0.057(±0.003)*	0.029 (±0.004)*
	with BMC	1.42 (± 0.039)	0.075 (± 0.005)	0.071 (±0.003)*	0.033 (±0.005)*
Cd	control	1.35 (± 0.032)	0.060 (± 0.004)	0.059(±0.003)	0.029(±0.005)
	with BMC	1.63 (± 0.043)	0.083 (± 0.003)	0.083 (± 0.004)	0.038(± 0.004)
Zn	control	1.43 (± 0.045)	0.080 (±0.004)	0.079 (±0.004)*	0.031 (±0.003)
	with BMC	1.81 (± 0.054)	0.105 (±0.003)	0.097 (±0.004)*	0.045 (±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, Cd, and Zn in *Helianthus annuus*.

Metal	Treatment	Metal content (mg kg^{-1} dry weight)		Bioconcentration Factor (BCF)[a]	Translocation Factor (TF)[b]
		Shoot	Root		
Co	control	18.56 (± 4.27)	49.68 (± 5.26)	0.269	0.373
	With BMC	25.07 (± 4.12)	77.56 (± 4.59)	0.387	0.323
Pb	control	13.58 (± 0.53)*	42.47 (± 6.48)	0.212	0.319
	with BMC	16.87 (± 4.75)*	69.83(± 5.45)	0.349	0.242
Cd	control	14.36 (± 4.72)	45.69 (± 5.12)	0.228	0.314
	With BMC	17.87 (± 5.62)	78.42 (± 4.38)	0.392	0.227
Zn	control	19.93 (± 4.32)	81.47 (±3.28)*	0.407	0.244
	with BMC	25.7 (± 5.23)	99.35 (±5.32)*	0.496	0.257

[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.

or non-inoculation, the accumulation of 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.

Generally metal resistant bacteria possess the ability to withstand against multiple pollutants like heavy metals contaminated soils as they have adapted to such environments (Pal et al., 2005; Abou-Shanabet al., 2007). Remedation of heavy metal contaminated soil have been employed with both microorganism and phytoextraction. The effectiveness of the microorganismsas a plant growth-promoter for phytoextraction was assessed with *Helianthus annuus* (sunflower), a species was known to have an ability to accumulate biomass rapidly and take up substantial amounts of metals (Turgutet al., 2004; Prapagdeeet al., 2013). As reported by Ouzounidou and Llias (2005) and El-Tayebet 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, Prapagdeeet 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. (2001) also observed bacterial-assisted growth enhancement in *Brassica napus* grown in a soil contaminated with Cd. 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 4, translocation factor of the each metal was increased with the inoculation of the BMC with diverse strains, 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 Rajkumaret al. (2008) for Zn accumulation in *H. annuus* inoculated with *Bacillus weihenstephanensis*. However, according to Waniet 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 BMC with bacterial strains might be due to the increased uptake of metals under acidic soil conditions created by the phosphate solubilization (Rajkumaret 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 Prapagdeeet 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; Lebeauet 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; Kyeong et al, 2014)). According to Braudet 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-Shanabet 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 the mixture of waste button mushroom, fowl manure and sawdust, compromised diverse microorganisms and was found to be capable of solubilizing heavy metals (Co, Pb, Cd, and Zn). Metal mobilization potential of the BMC showed that the inoculation of BMC could increase the concentrations of water soluble Co, Pb, Cd, and Zn in soil than those of non-inoculated soils. Inoculation with the BMC also resulted in the increased shoot and root biomass and enhanced accumulation of Co, Pb, Cd, 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, Cd, and Zn from metal-contaminated soils.

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