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Myco-phytoremediation of arsenic- and lead-contaminated soils by *Helianthus annuus* and wood rot fungi, *Trichoderma* sp. isolated from decayed wood

M. Govarthanan[a,b,*,1], R. Mythili[b,1], T. Selvankumar[b], S. Kamala-Kannan[c], H. Kim[a,**]

[a] Department of Energy and Environmental System Engineering, University of Seoul, Seoul 02504, Republic of Korea
[b] PG & Research Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Kalippatti, Namakkal 637501, Tamil Nadu, India
[c] Division of Biotechnology, Advanced Institute of Environment and Bioscience, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan 570 752, South Korea

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ABSTRACT

In the present study, *Helianthus annuus* grown in arsenic- (As) and lead- (Pb) contaminated soil were treated with plant-growth promoting fungi *Trichoderma* sp. MG isolated from decayed wood and assessed for their phytoremediation efficiency. The isolate MG exhibited a high tolerance to As (650 mg/L) and Pb (500 mg/L), and could remove > 70% of metals in aqueous solution with an initial concentration of 100 mg/L each. In addition, the isolate MG was screened for plant-growth-promoting factors such as siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA) synthesis, and phosphate solubilisation. Phytoremediation studies indicated that treatment of *H. annuus* with the isolate MG had the maximum metal-accumulation in shoots (As; 67%, Pb; 59%). Furthermore, a significant increase in the soil extracellular enzyme-activities was observed in myco-phytoremediated soils. The activities of phosphatase (35 U/g dry soil), dehydrogenase (41 mg TPF/g soil), cellulase (37.2 mg glucose/g/2 h), urease (55.4 mg N/g soil/2 h), amylase (49.3 mg glucose/g/2 h) and invertase (45.3 mg glucose/g/2 h) significantly increased by 12%, 14%, 12%, 22%, 19% and 14% in As contaminated soil, respectively. Similarly, the activities of phosphatase (31.4 U/g dry soil), dehydrogenase (39.3 mg TPF/g soil), cellulase (37.1 mg glucose/g/2 h), urease (49.8 mg N/g soil/2 h), amylase (46.3 mg glucose/g/2 h), and invertase (42.1 mg glucose/g/2 h) significantly increased by 11%, 15%, 11%, 18%, 20% and 14% in Pb contaminated soil, respectively. Obtained results indicate that the isolate MG could be a potential strain for myco-phytoremediation of As and Pb contaminated soil.

1. Introduction

Metals, especially arsenic (As) and lead (Pb), accumulating in soil and/or water via various natural routes as well as anthropogenic activities (e.g., mining and smelting) exerts a significant impact on human health and other living organisms in the ecosystem (Pan et al., 2009). As and Pb are the metal elements without any known biological function and one of the most toxic heavy metals. Soil and/or water contamination with these metals are widespread and pose a substantial threat to the environment. As and Pb contaminated soils are not suitable for feed-crop cultivation and require remediation to reduce risk associated with them (Marques et al., 2013). Most physicochemical methods to remove As and Pb are expensive, inefficient, and labour-intensive (Xiao et al., 2010).

The application of biological remediation techniques, such as phytoremediation appears as an excellent cost-effective alternative, which uses metal tolerant or hyperaccumulating energy-crops. This process may become a promising alternative for renewable energy source (Mlezeck et al., 2010; Antonkiewicz et al., 2016). However, most of the plants produce very less biomass and exhibit stunted growth in metal-contaminated soils. Hence, it is important to propose effective phytoremediation strategy for heavy-metal-contaminated soils (Rajkumar et al., 2009; Weyens et al., 2009). Recently, interaction between plants, microbes, and metals has attracted much attention because of the physiological potential of microorganisms to remove metals directly from contaminated soil and the possible role of microorganisms in promoting plant-growth in metal-contaminated soils (Deng et al., 2011).

* Corresponding author at: Department of Energy and Environmental System Engineering, University of Seoul, Seoul 02504, Republic of Korea.
** Corresponding author.
E-mail addresses: gova.muthu@gmail.com (M. Govarthanan), h_kim@uos.ac.kr (H. Kim).
[1] The first two authors equally contributed to this work.

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It has been reported that soil-extracellular enzme activities are likely to be affected by As, Pb as well as microbial biomass (Bhattacharyya et al., 2008). Koo et al. (2012) identified that As exerts a strong inhibitory effect on soil enzyme activities. Boshoff et al. (2014) observed that the microbial biomass is the major source of enzymes in soil and is highly susceptible to disruption by As and Pb contamination. The bioaugmentation of specific metal resistant microorganisms capable of metals speciation may enhance the microbial biomass in the soil, which subsequently influences soil enzyme activities (Tripathi et al., 2015; Wang et al., 2015). Accordingly, analysis of soil-extracellular enzyme activity will be useful in assessing the quality of contaminated soils after bioaugmentation with specific metal-resistant microorganisms.

The anamorphic *Trichoderma* sp. is imperfect filamentous fungi belonging to the ascomycete division. *Trichoderma* sp. are among the most frequently isolated soil-fungi and are well known for their biocontrol activity and plant-growth enhancement (Harman et al., 2004; Hoyos-Carvajal et al., 2009a, 2009b). *Trichoderma* sp. can influence the plant growth by producing siderophores, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole acetic acid (IAA), acid phosphatase under biotic or abiotic stresses (Babu et al., 2014a, 2014b). *Trichoderma* plays an important role in the ecosystem by taking part in decomposition of plant residues, as well as in biodegradation of anthropogenic chemicals. *Trichoderma* can also uptake a variety of metals from soil and/or water even under an extreme pH, temperature or nutrient shortage (Anand et al., 2006). Recently, *Trichoderma* sp. has been reported for its abilities of heavy-metal resistance, speciation and transformation (Zeng et al., 2010; Su et al., 2009). Thus, the present study describes the influence of plant growth promoting *Trichoderma* sp. on enhanced phytoremediation of artificially As- and Pb- contaminated soil.

Helianthus annuus is an annual economic plant with food and energy values. Several studies have been reported the phytoremediation potential of *H. annuus* (Cindy et al., 2006; Niu et al., 2007; Fassler et al., 2010; Rojas-Tapias et al., 2012) and its growth on contaminated soil for simultaneous remediation and energy production (Madejon et al., 2003). It has been suggested that the application of integrative capability of phytoremediation along with fungal remediation would result in enhanced As and Pb removal and further improve soil fertility. Hence, the present study deals (i) isolation and identification of As- and Pb- resistant *Trichoderma* sp. MG from decayed wood collected from soil, (ii) assessment of the As- and Pb- removal efficiency of *Trichoderma* sp. MG in batch experiments, (iii) screen of the isolate MG for plant-growth promoting traits, (iii) assessment of the efficiency of the isolate MG in enhancing *H. annuus* growth and metal-accumulation, and (iv) evaluation of the extracellular enzyme activities in the myco-phytoremediated soil.

2. Materials and methods

2.1. Collection of decayed wood sample and isolation of fungi

A sample of decayed wood was collected from Pallipalayam, a municipality located in Namakkal District in the State of Tamil Nadu, India, where soil and water were contaminated with dyes and auxiliary chemicals associated with textile industrial wastes (Thangaraj et al., 2017). The wood sample was transported to the a laboratory and processed within 18 h. Fungi were isolated from decayed logs of wood using the pour-plate technique on potato-dextrose agar (PDA) supplemented with 30 µg/mL of chloramphenicol. The plates were incubated at 26 ± 2 °C for 8–16 d and observed for the fungal growth. The pure cultures of the isolates were transferred to PDA slants and maintained by sub-culturing every three weeks.

2.2. Identification of the isolate MG

The isolate MG was cultured in the potato-dextrose broth at 26 °C for 6–8 d. After incubation, mycelia mats were separated from the medium and the genomic DNA was extracted using Qiagen DNA extraction kit (QIAGEN, CA, USA), according to the manufacturer's protocol. The ITS region of the isolate was amplified using the primers, ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The PCR product was purified using a PCR purification kit (QIAGEN, CA, USA) and the amplicons were sequenced using an automated ABI PRISM 3700 sequencer (Foster City, USA). The sequences were compared using the BLAST program (http://www.ncbi.nlmnih.gov/BLAST) for identification of the isolate MG.

2.3. Metal tolerance and removal

The isolate MG was screened for metal-tolerance assay according to the agar dilution method. In brief, the fungal plug was aseptically inoculated onto the PDA medium supplemented with increasing concentration of metals (50–750 mg/L of As ($NaAsO_2$), Cu ($CuCl_2{\cdot}2H_2O$), Cr (Cr (VI), Ni (Ni^{2+}) and Pb (Pb ($NO_3)_2$). The plates were incubated at 26 ± 2 °C and observed for the fungal growth. PDA plates without metals were used as controls.

Metal removal experiments were performed according to Babu et al. (2014a, 2014b) with a minor modification. In brief, 10 mL of *Trichoderma*-spore suspension (10^8 cfu/mL) was inoculated into 100 mL of the potato-dextrose broth amended with different concentrations (100–500 mg/L) of As and Pb individually. The flasks were incubated in a shaking incubator (Thermo Scientific, Massachusetts, USA) (200 rpm) at 26 ± 2 °C for 7 d. After incubation, samples were collected and centrifuged (Kubota, Japan) at 10,000 rpm for 5 min. One mL of supernatant was immediately filtered through a 0.2 µm membrane, As and Pb concentrations were determined by inductively coupled plasma mass spectrometry (ICP, Leemans Labs, USA) and the metal removal by the fungus was determined by evaluating the difference from the initial and final metal concentrations.

2.4. Plant-growth promoting factors

Indole acetic acid production was determined according to the method described by Gordon and Weber (1951). Siderophore production was estimated using a modified chrome azurol S (CAS) agar medium (Milagres et al., 1999). Phosphate-solubilisation activity was estimated using the Pikoskoskaya medium (Pikovskaya, 1948). The ACC deaminase activity was measured according to Gravel et al. (2007).

2.5. Pot experiment

The natural soil was spiked with As and Pb solutions, generating a final concentration of 250 mg/kg, which was chosen according to the occurrence of As- and Pb-contaminated soils with the average concentration of a nearby site contaminated with heavy metals. The physico-chemical characteristics of the study soil are presented in Table 1. Soil samples were air-dried, sieved to < 2 mm, sterilized at 120 °C for 70 min for four consecutive days and dried in an oven at 40 °C for a week. The contaminated soil was incubated for 1 month, being irrigated with deionized water to 60% of the water holding capacity, to obtain a

Table 1
Physico-chemical characteristics of the study soil.

Soil properties	Values
Organic matter (%)	9.5 ± 0.14
pH	6.48 ± 0.03
EC ($m^{-s}\,m^{-1}$)	0.39 ± 0.023
N ($\mu g\,g^{-1}$)	24.3 ± 1.19
P ($\mu g\,g^{-1}$)	6.42 ± 0.85
K ($\mu g\,g^{-1}$)	23.9 ± 1.25

stable state (Yan and Lo, 2013).

Seeds of *H. annuus* were surface-sterilized with 70% ethanol and washed with sterile water. After surface sterilization, the seeds were placed in petri-plates filled with sterile double distilled water and were allowed to germinate at room temperature for a week. The seedlings (5 seedling/pot) were transferred into the plastic pots containing 250 mg/kg of As- and Pb-contaminated soil. The pots were inoculated with 20 mL of spore suspension (10^8 CFU/mL), whereas 20 mL of sterile distilled water was added in the control pots. Pots were maintained to have 60% of water-holding capacity by the addition of sterile distilled water. Each treatment (four to five plants/pot) was replicated three times and the plants were harvested after 2–3 weeks. After harvesting the shoot and root lengths and biomass of *H. annuus* were measured. The roots and shoots of the plants were washed with tap water and rinsed with distilled water, and dried at 60–70 °C followed by HNO_3 digestion (5:1 (v/v) $HNO_3/HClO_4$) (Govarthanan et al., 2016). The digested plant samples were used to analyze Pb and As accumulation using ICP.

2.6. Soil enzyme activities

Soil alkaline phosphatase and dehydrogenase activity were estimated according to Tabatabai (1994) with a slight modification in incubation time and the temperature. Briefly, 5 g of the soil samples were mixed with 1 mL of 3% 2,3,5-triphenyltetrazolium and 5 mL of sterile water. Later, the samples were vortexed and incubated in dark at 37 °C for 48 h. After incubation, 10 mL of methanol was added, and the samples were shaken for 5 min and filtered. The filtrate was analyzed for triphenyl formazan by spectrophotometric method at 485 nm (Govarthanan et al., 2014; Govarthanan et al., 2015). Soil cellulase activity was estimated according to Kelley and Rodriguez-Kabana (Kelley and Rodriguez-Kabana, 1975). Urease activity was estimated according to Kandeler (1996). Amylase activity was measured according to Galstyan, (1965). Soil invertase-activity was estimated according to Ill et al. (1989). All the experiments were repeated three times.

3. Results and discussion

3.1. Isolation and identification of isolate MG

In the present study, three morphologically different fungal isolates have been isolated from the decayed wood. All the three isolates were obtained in pure cultures using standard microbial pure culture techniques. Among these three fungal isolates, isolate designated MG showed maximum As- and Pb-resistance along with plant-growth promoting activity. Thus, the strain MG was used for the myco-phytoremediation studies.

Fungal colony on the surface of the PDA plates was white colour and later developed into yellowish tints. The microscopic observation showed one-cell ovoid conidia structure. The conidia are produced from tips of phialides. Conidiophores are erect and produce side branches bearing whorls of short phialides, the branches are not swollen at the apex and bear terminal conidial heads. The results are consistent with previous study reporting a similar growth pattern for *Trichoderma atroviride* (Cao et al., 2008). The result of the BLAST homology search for ITS region nucleotide sequences obtained from strain MG showed 99% homology with the nucleotide sequences of *Trichoderma* sp. CC-2016 (KX344995). The partial ITS region of the isolate MG was deposited in GenBank (Accession Number: KX856353). A phylogenetic tree was derived from the partial ITS sequences of the isolate MG with existing sequences in the NCBI database, and the results are shown in Fig. 1.

3.2. Metal tolerance test of Trichoderma sp. MG

The isolate MG has a high tolerance to Pb and As, whereas the isolate did not grow in the PDA medium amended with other tested metals (Cu, Cr and Ni) even at a low concentration (50 mg/L). The minimal inhibitory concentration (MIC), a lowest concentration of a metal that inhibits the visible growth of the fungi, for As and Pb was 650 and 500 mg/L. MIC of the isolate MG was found to be Pb > As > Cu > Cr > Ni. The differences in metal tolerance by fungal species might be due to the existence of more than one type of metal-resistance strategy providing an active resistance for the organisms (Mohammadian et al., 2017; Baldrian and Gabriel, 2002).

3.3. As and Pb removal by isolate MG

As- and Pb- removal ability of the isolate MG was evaluated in batch studies and the results are presented in Fig. 2. The results showed that the removal of both As and Pb by the isolate was relatively less and slow at a higher concentration (400 and 500 mg/L of As and Pb). However, 77% of As and 70% of Pb could be removed at a lower concentration (100 mg/L). The high As and Pb removal in lower concentrations could be due to the differences in affinity and electro negativity of the metals (AjayKumar et al., 2009), and the intracellular sequestration, and ATP dependent efflux of the isolate (Deng et al., 2012). However, the moderate removal rate was observed at 200 and 300 mg/L of metals. It has been reported that the metal-resistant plant-growth promoting fungi play an important role in enhanced phytoremediation (Deng et al., 2011). Thus, the isolate MG was applied for myco-phytoremediation. It is expected that the isolate may reduce the metal toxicity to plants by intracellular and/or extracellular localization of heavy metals (Babu and Reddy, 2011; Bareen et al., 2012).

3.4. Plant-growth promoting characteristics of Trichoderma sp. MG

Generally, *Trichoderma* sp. has the ability to improve plant growth by producing plant-growth-promoting factors (Qi and Zhao, 2013). Expectedly, the isolate *Trichoderma* sp. MG had the ability to synthesize siderophore, ACC deaminase, IAA and, phosphate- solubilisation activity. The isolate MG produced 49.8 ± 1.5 mg/L of IAA. Siderophores are high-affinity iron-chelating proteins that play a vital role in making iron available to the microbial system. Thus, the isolate MG was screened for siderophore production. Simultaneously, the isolate MG utilized ACC as the sole nitrogen source and showed relatively high levels of ACC deaminase activity (25.4 ± 2.4 μM α KB/mg/h). A clear zone around *Trichoderma* sp., MG in the Pikoskoskaya medium confirmed the phosphate- solubilisation potential of the isolate. The results are in agreement with the previous study reported that the *Trichoderma* sp., have plant-growth promoting activity (Qi and Zhao, 2013). Hoyos-Carvajal et al. (2009b) reported that *Trichoderma* sp., extensively induce the plant growth through the modification of plant metabolism by synthesizing of plant growth promoting traits.

3.5. Effects of Trichoderma sp. MG on H. annuus growth

In the control soil group (without any metal amendment), the shoot and root lengths of *H. annuus* were measured 16.54 and 7.21 cm, respectively. However, decreased plant growth was observed in As-contaminated soils (shoot: 11.2; root length: 4.87 cm) and Pb-contaminated ones (shoot: 11.14; root length: 4.68 cm) (Table 2). It was observed that the treatment of *H. annuus* with the isolate MG significantly stimulated the growth of the plant in metal amended soils; shoot and root lengths of the plant were 14.75 and 6.10 cm for As-treatment and 14.35 and 6.12 cm for Pb-treatment. Moreover, the *Trichoderma* sp., MG treatment significantly improved the biomass of *H. annuus* under the As (135 ± 3.35 mg) and Pb stress (137 ± 5.24 mg) (Table 2). Su et al. (2017) reported that the *Trichoderma asperellum*

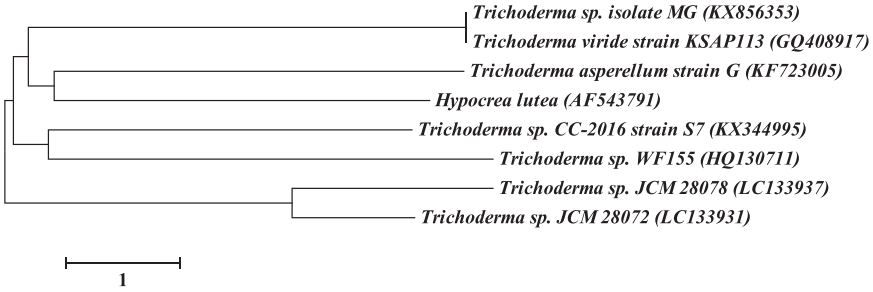

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship of the ITS region of *Trichoderma* sp. MG with the related organisms. Accession numbers at the GenBank of National Centre for Biotechnology Information (NCBI) are shown in parenthesis.

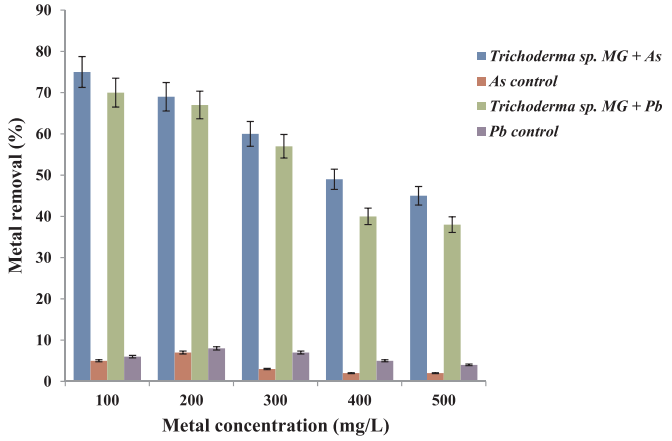

Fig. 2. As and Pb removal by the isolate *Trichoderma* sp. MG in liquid medium containing different concentrations of As and Pb (100–500 mg/L).

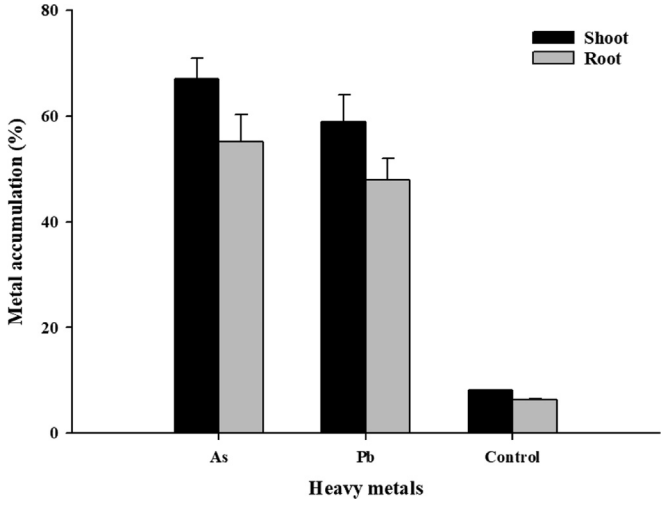

Fig. 3. As and Pb accumulation in shoots and roots of *H. annuus* grown in As and Pb contaminated soil inoculated with *Trichoderma* sp. MG.

improved the growth of water-spinach by producing plant-growth hormones under metal stress.

3.6. Plant growth and metal uptake

The choice of plants is a key characteristic for the application of phytoremediation because it has been established that all species are not efficient in accumulating and storing the metals in their tissues (Tu and Ma, 2005; Kacalkova et al., 2015; Kumar et al., 2017). *H. annuus* was chosen for this study since it was one of the plants that could survive in metal contaminated soil. The pot experiment results (Fig. 3) showed that the *H. annuus* grown in the As- and Pb-contaminated soil inoculated with *Trichoderma* sp. MG had higher shoot (As; 67%, Pb; 59%) and root (As; 55%, Pb; 48%) accumulation than plants grown in the control soil. The increased shoot and root accumulation in the *Trichoderma* sp. MG-treated plants might be due to the reduced phytotoxicity and, the growth-promoting property of the isolate MG. A higher concentration of Pb was toxic for the plants, and it may inhibit many of the essential enzymatic processes which resulted in less accumulation of As (Sytar et al., 2013). Adams et al. (2007) reported the increased biomass and metal uptake in *Trichoderma harzianum* Rifai 1295-22 treated *Salix fragilis* grown in metal-contaminated soil. The uptake and accumulation of metals depend on the solubility of metals (Zaurov et al., 1999) and specific behaviour of the plants such as, complexing agents (Römheld, 1991) and transporters (Sharma et al., 2016).

3.7. Soil enzymes after inoculation of Trichoderma sp. MG

The soil samples inoculated with the isolate MG considerably increased the extracellular enzyme-activities, which play a vital role in soil fertility (Fig. 4a, b). However, the increase in the soil enzyme-activities was varied according to the type of metal. The activities of phosphatase (35 U/g dry soil), dehydrogenase (41 mg TPF/g soil), cellulase (37.2 mg glucose/g/2 h), urease (55.4 mg N/g soil/2 h), amylase (49.3 mg glucose/g/2 h) and invertase (45.3 mg glucose/g/2 h) significantly increased by 12%, 14%, 12%, 22%, 19% and 14% in As contaminated soils. In Pb-contaminated soils, the activities of phosphatase (31.4 U/g dry soil), dehydrogenase (39.3 mg TPF/g soil), cellulase (37.1 mg glucose/g/2 h), urease (49.8 mg N/g soil/2 h), amylase (46.3 mg glucose/g/2 h) and invertase (42.1 mg glucose/g/2 h) also significantly increased by 11%, 15%, 11%, 18%, 20% and 14%. It has been reported that, the response of soil enzymes to heavy-metal stress is not always the same and sometimes even varies due to their different characteristics in metals (Hu et al., 2014). Soil enzyme-activity is sensitive to metal stress, hence regarded as an indicator of

Table 2
Effect of *Trichoderma* sp. MG inoculation with As and Pb shoot and root length and total dry biomass of *H. annuus*.

Treatments	Shoot length (cm)	Root length(cm)	Dry biomass (mg)
Control soil (without any treatment)	16.54 ± 0.35	7.21 ± 0.12	142.34 ± 4.3
As control (Soil contaminated with As)	11.20 ± 0.35	4.87 ± 0.20	110 ± 4.41
As amended soil + *Trichoderma* sp. MG	14.75 ± 0.89	6.10 ± 0.35	135 ± 3.35
Pb control (Soil contaminated with Pb)	11.14 ± 0.34	4.68 ± 0.31	112 ± 3.49
Pb amended soil + *Trichoderma* sp. MG	14.35 ± 0.65	6.12 ± 0.44	137 ± 5.24

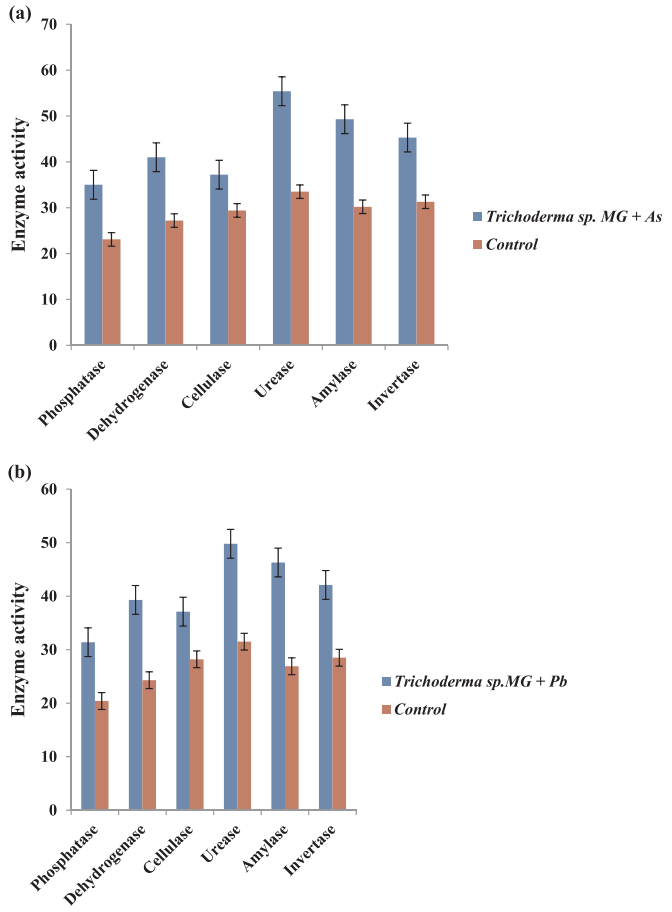

Fig. 4. Soil extracellular enzyme activities after phytoremediation of As-(a) and Pb-(b) contaminated soil.

soil quality (Chaperon and Sauve, 2007). Su et al. (2017) reported that the addition of *Trichoderma* to As- contaminated soils increased the soil enzyme-activity. The results indicated that the isolate not only promote the growth of the plant also increase the soil enzyme activity. Therefore, it is suggested that isolate *Trichoderma* sp. MG can be used as an eco-indicator of the soils contaminated by heavy metals.

4. Conclusion

The present study revealed that As- and Pb- contaminated soil can be effectively treated by combinational and sequential myco-phytoremediation using *Trichoderma* and *H. annuus*. Inoculating As- and Pb-contaminated soil with *Trichoderma* sp. MG resulted significant As- and Pb-accumulation in shoots (As, 67%; Pb, 59%) and roots of *H. annuus* (As, 55%, Pb, 48%). *Trichoderma* sp. MG enhances the growth of *H. annuus* by producing IAA, ACC deaminase, siderophores and phosphate solubilisation. Inoculating *Trichoderma* into the contaminated soil significantly improves the soil enzymes activity. These observations provide new insight into the plant –fungi partnership for enhanced bioremediation of As- and Pb- contaminated soils.

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References

Adams, P., De-Leij, F.A., Lynch, J.M., 2007. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. Microb. Ecol. 54, 306–313.

Anand, P., Isar, J., Savan, S., Saxena, P.K., 2006. Bioaccumulation of copper by *Trichoderma viride*. Bioresour. Technol. 97, 1018–1025.

Antonkiewicz, J., Kołodziej, B., Bielińska, E., 2016. The use of reed canary grass and giant miscanthus in the phytoremediation of municipal sewage sludge. Environ. Sci. Pollut. Res. 23, 9505–9517.

Babu, A.G., Shim, J., Bang, K.S., Shea, P.J., Oh, B.T., 2014a. *Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth promoting fungus for remediation and bioenergy crop production on mine tailing soil. J. Environ. Manag. 132, 129–134.

Babu, A.G., Shim, J., Shea, P.J., Oh, B.T., 2014b. *Penicillium aculeatum* PDR-4 and *Trichoderma* sp. PDR-16 promote phytoremediation of mine tailing soil and bioenergy production with sorghum-sudan grass. Ecol. Eng. 69, 186–191.

Babu, G., Reddy, M.S., 2011. Dual inoculation of arbuscular mycorrhizal and phosphate solubilizing fungi contributes in sustainable maintenance of plant health in fly ash ponds. Water Air Soil Pollut. 219, 3–10.

Baldrian, P., Gabriel, J., 2002. Intra specific variability in growth response to cadmium of the wood-rotting fungus *Piptoporus betulinus*. Mycologia 94, 428–436.

Bareen, F., Shafiq, M., Jamil, S., 2012. Role of plant growth regulators and a saprobic fungus in enhancement of metal phytoextraction potential and stress alleviation in pearl millet. J. Hazard. Mater. 237–238, 186–193.

Bhattacharyya, P., Tripathy, S., Kim, K., Kim, S.H., 2008. Arsenic fractions and enzyme activities in arsenic-contaminated soils by groundwater irrigation in West Bengal. Ecotoxicol. Environ. Saf. 71, 149–156.

Boshoff, M., Jonge, D.M., Dardenne, F., Blust, R., Bervoets, L., 2014. The impact of metal pollution on soil faunal and microbial activity in two grassland ecosystems. Environ. Res. 134, 169–180.

Chaperon, S., Sauve, S., 2007. Toxicity interaction of metals (Ag, Cu, Hg, Zn) to urease and dehydrogenase activities in soils. Soil Biol. Biochem. 39 (9), 2329–2338.

Cindy, H.W., Thomas, K.W., Ashok, M., Chen, W., 2006. Engineering plant microbe symbiosis for rhizoremediation of heavy metals. J. Appl. Environ. Microbiol. 72, 1129–1134.

Cao, L.X., Jiang, M., Zeng, Z.R., Du, A.X., Tan, H.M., Liu, Y.H., 2008. Trichoderma atroviride F6 improves phytoextraction efficiency of mustard (Brassica juncea (L.) Coss. var. foliosa Bailey) in Cd Ni contaminated soils. Chemosphere 71, 1769–1773.

Deng, Z., Cao, L., Huang, H., Jiang, X., Wang, W., Shi, Y., Zhang, R., 2011. Characterization of Cd- and Pb-resistant fungal endophyte *Mucor* sp. CBRF59 isolated from rapes (*Brassica chinensis*) in a metal-contaminated soil. J. Hazard. Mater. 185, 717–724.

AjayKumar, A.V., Darwish, N.A., Hilal, N., 2009. Study of various parameters in the biosorption on heavy metals on activated sludge (special issue for environment). World Appl. Sci. J. 5, 32–40.

Deng, X., Chai, L., Yang, Z., Tang, C., Tong, H., Yuan, P., 2012. Bioleaching of heavy metals from a contaminated soil using indigenous Penicillium chrysogenum strain F1. J. Hazard. Mater. 233, 25–32.

Fassler, E., Robinson, B.H., Stauffer, W., Gupta, S.K., Papritz, A., Schulin, R., 2010. Phytomanagement of metal contaminated agricultural land using sunflower, maize and tobacco. Agric. Ecosyst. Environ. 136, 49–58.

Galstyan, A.S., 1965. A method of determining the activity of the hydrolytic enzymes in soil. Sov. Soil Sci. 2, 170–175.

Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indole acetic acid. Plant Physiol. 26, 192–195.

Govarthanan, M., Kamala-Kannan, S., Kim, S.A., Seo, Y.S., Park, J.H., Oh, B.T., 2016. Synergistic effect of chelators and Herbaspirillum sp. GW103 on lead phytoextraction and its induced oxidative stress in Zea mays. Arch. Microbiol. 198, 737–742.

Govarthanan, M., Lee, G.W., Park, J.H., Kim, J.S., Lim, S.S., Seo, S.K., Cho, M., Myung, H., Kamala-Kannan, S., Oh, B.T., 2014. Bioleaching characteristics, influencing factors of Cu solubilization and survival of *Herbaspirillum* sp. GW103 in Cu contaminated mine soil. Chemosphere 109, 42–48.

Govarthanan, M., Park, S.H., Park, Y.J., Myung, H., Krishnamurthy, R.R., Lee, S.H., Lovanh, N., Kamala-Kannan, S., Oh, B.T., 2015. Lead biotransformation potential of allochthonous *Bacillus* sp. SKK11 with sesame oil cake extract in mine soil. RSC Adv. 5, 54564–54570.

Gravel, V., Antoun, H., Tweddell, R.J., 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol. Biochem. 39, 1968–1977.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56.

Hoyos-Carvajal, L., Orduz, S., Bissett, J., 2009a. Genetic and metabolic biodiversity of *Trichoderma* from Colombia and adjacent neotropic regions. Fungal Genet. Biol. 46, 615–631.

Hoyos-Carvajal, L., Orduz, S., Bissett, J., 2009b. Growth stimulation in bean (*Phaseolus vulgaris* L.) by *Trichoderma*. Biol. Control 51, 409–416.

Ill, F.G., Clausen, C.A., Highley, T.A., 1989. Adaptation of the Nelson–Somogyi reducing sugar assay to a microassay using microtiter plates. Anal. Biochem. 182, 197–199.

Kacalkova, L., Tlustoa, P., Szakova, J., 2015. Phytoextraction of risk elements by willow and poplar trees. Int. J. Phytorem. 17 (5), 414–421.

Kandeler, E., 1996. Urease activity by colorimetric technique. In: Schinner, F., Ohlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer-Verlag, New York, pp. 171–174.

Kelley, W.D., Rodriguez-Kabana, R., 1975. Effects of potassium azide on soil microbial populations and soil enzymatic activities. Can. J. Microbiol. 21, 565–570.

Koo, N., Lee, S.H., Kim, J.G., 2012. Arsenic mobility in the amended mine tailings and its impact on soil enzyme activity. Environ. Geochem. Health 34, 337–348.

Kumar, D., Tripathi, D.K., Liu, S., Singh, V.K., Sharma, S., Dubey, N.K., Prasad, S.M.,

Chauhan, D.K., 2017. *Pongamia pinnata* (L.) Pierre tree seedlings offer a model species for arsenic phytoremediation. Plant Gene. http://dx.doi.org/10.1016/j.plgene.2017.06.002.

Madejon, P., Murillo, J.M., Maranon, T., Cabrera, F., Soriano, M.A., 2003. Trace element and nutrient accumulation in sunflower plants 2 years after the Aznacollar mine spill. Sci. Total. Environ. 307, 239–257.

Marques, G.C., Moreira, H., Franco, A.R., Rangel, A.O.S.S., Castro, P.M.L., 2013. Inoculating *Helianthus annuus* (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria – Effects on phytoremediation strategies. Chemosphere 92, 74–83.

Milagres, A.M.F., Machuca, A., Napoleao, D., 1999. Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. J. Microbiol. Methods 37, 1–6.

Mlezeck, M., Rutkowski, P., Rissmann, I., Kaczmarek, Z., Golinski, P., Szentner, K., Strazynska, K., Stachowiak, A., 2010. Biomass productivity and phytoremediation potential of *Salix alba* and *Salix viminalis*. Biomass-. Bioenergy 34, 1410–1418.

Mohammadian, E., Ahari, A.B., Arzanlou, M., Oustan, S., Khazaei, S.H., 2017. Tolerance to heavy metals in filamentous fungi isolated from contaminated mining soils in the Zanjan Province, Iran. Chemosphere 185, 290–296.

Niu, Z., Sun, L., Sun, T., Li, Y., Wang, H., 2007. Evaluation of phytoextracting cadmium and lead by sunflower, ricinus, alfalfa and mustard in hydroponic culture. J. Environ. Sci. 19, 961–967.

Pan, R., Cao, L., Zhang, R., 2009. Combined effects of Cu, Cd, Pb, and Zn on the growth and uptake of consortium of Cu-resistant *Penicillium* sp. A1 and Cd-resistant *Fusarium* sp. A19. J. Hazard. Mater. 171, 761–766.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17, 363–370.

Qi, W., Zhao, L., 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. J. Basic Microbiol. 53, 355–364.

Rajkumar, M., Ae, N., Freitas, H., 2009. Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77, 153–160.

Rojas-Tapias, D.F., Bonilla, R.B., Dussan, J., 2012. Effect of inoculation with plant growth promoting bacteria on growth and copper uptake by sunflowers. Water Air Soil. Pollut. 223, 643–654.

Römheld, V., 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach. Plant Soil. 130, 127–134.

Sharma, S.S., Dietz, K.J., Mimura, T., 2016. Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant Cell Environ. 39 (5), 1112–1126.

Su, S., Zeng, X., Bai, L., Williams, P.N., Wang, Y., Zhang, L., Wu, C., 2017. Inoculating chlamydospores of *Trichoderma asperellum* SM-12F1 changes arsenic availability and enzyme activity in soils and improves water spinach growth. Chemosphere 175, 497–504.

Su, S.M., Zeng, X.B., Li, L.F., Duan, R., Bai, L.Y., 2009. Arsenate reduction and methylation in the cells of *Trichoderma asperellum* SM-12F1, *Penicillium janthinellum* SM-12F4, and *Fusarium oxysporum* CZ-8F1 investigated with X-ray absorption near edge structure. J. Hazard. Mater. 243, 364–367.

Sytar, O., Kumar, A., Latowski, D., 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol. Plant. 35 (4), 985–999.

Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angel, J.S., Bottomley, P.S. (Eds.), Methods of Soil Analysis, Part 2—Microbiological and Biochemical Properties. SSSA Book Series No. 5. Soil Science Society of America, Madison, WI, pp. 775–833.

Thangaraj, A., Muralidharan, S., Senthilkumar, A., Moorthi1, M., 2017. The physico-chemical characteristics of different textile dyeing effluents and their influence on the total protein levels of dragonfly larvae *Bradinopyga Geminata*. Int. J. Sci. Res. Publ. 7, 534–538.

Tripathi, P., Singh, P.C., Mishra, A., Tripathi, R.D., Nautiyal, C.S., 2015. *Trichoderma* inoculation augments grain amino acids and mineral nutrients by modulating arsenic speciation and accumulation in chickpea (*Cicer arietinum* L.). Ecotoxicol. Environ. Saf. 117, 72–80.

Tu, C., Ma, L.Q., 2005. Effects of arsenic on concentration and distribution of nutrients in the fronds of the arsenic hyperaccumulator *Pteris vittata* L. Environ. Pollut. 135, 333–340.

Wang, X.R., Su, S.M., Zeng, X.B., Bai, L.Y., Li, L.F., Duan, R., Wang, Y.N., Wu, C.X., 2015. Inoculation with chlamydospores of *Trichoderma asperellum* SM-12F1 accelerated arsenic volatilization and influenced arsenic availability in soils. J. Integr. Agr. 14, 389–397.

Weyens, N., van der Lelie, D., Taghavi, S., Vangronsveld, J., 2009. Phytoremediation: plant–endophyte partnerships take the challenge. Curr. Opin. Biotechnol. 20, 248–254.

Xiao, X., Luo, S., Zeng, G., Wei, W., Wan, Y., Chen, L., Guo, H., Cao, Z., Yang, L., Chen, J., Xi, Q., 2010. Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsis* sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. Bioresour. Technol. 101, 1668–1674.

Yan, D.Y.S., Lo, I.M.C., 2013. Removal effectiveness and mechanisms of naphthalene and heavy metals from artificially contaminated soil by iron chelate-activated persulfate. Environ. Pollut. 178, 15–22.

Zaurov, D.E., Perdomo, P., Raskin, I., 1999. Optimizing soil fertility and pH to maximize cadmium removed by Indian mustard from contaminated soils. J. Plant Nutr. 22 (6), 977–986.

Zeng, X.B., Su, S.M., Jiang, X.L., Li, L.F., Bai, L.Y., Zhang, Y.R., 2010. Capability of pentavalent arsenic bioaccumulation and biovolatilization of three fungal strains under laboratory conditions. Clean-Soil Air Water 38, 238–241.