EVALUATION OF *TRICHODERMA HARZIANUM* AND *GLOMUS MOSSEAE* FOR BIO-REMEDIATION OF LEAD AND CADMIUM CONTAMINATION FROM AGRICULTURAL WASTE LAND SOIL

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ABSTRACT

Tonni, R. S., Chakraborty, S., Islam, M. M., Rahman, M. M. and Khokon, M. A. R. 2021. Evaluation of *Trichoderma harzianum* and *Glomus mosseae* for bio-remediation of lead and cadmium contamination from agricultural waste land soil. Bangladesh J. Plant Pathol. 37(1&2):15-22

An experiment was carried out to investigate the capability of *Trichoderma harzianum* and arbuscular mycorrhizal fungi (*Glomus mosseae*) in agricultural waste land soil to ameliorate the heavy metal toxicity caused by industrial effluents. Different combinations of *T. harzianum* and AMF (*G. mosseae*) were applied into the soil before cultivation of red amaranth. Lead (Pb) and Cadmium (Cd) were determined both in soil and red amaranth after growing period. Vegetative parameters of red amaranth were not significantly influenced by the treatments except dry weight. Both Pb and Cd concentration in soil was significantly reduced by all treatments. Soil treated with *T. harzianum*@ 0.5 g/m² had a better capacity for up taking Cd from soil. Conversely, both *T. harzianum* and AMF inoculation increased accumulation of Pb and Cd by red amaranth. *Trichoderma harzianum* had increased the accumulation of Pb and AMF inoculation had increased the Cd accumulation by red amaranth. This investigation gave an insight on the exploitation of these bio-agents for amelioration of agricultural waste land by trapping heavy metals using suitable crops.

Key words: *T. harzianum*, AMF, heavy metals, lead, cadmium, bio-remediation

INTRODUCTION

Soil is the basic element for ultimate nutrient sources of all the living organisms in the ecological system. It is the most valuable and vital resource for sustenance of life and also for any developmental activity. With so many human disturbances in nature, soil pollution is found as an abandoned environmental crime in this industrial era. As a developing country the industrial sector in Bangladesh is booming day by day that includes textile industries, leather industries, pharmaceutical industries, spinning mills, jute mills and so on.The arable land around the industrial areas remains unproductive due to discharging of untreated industrial effluents. Metal toxicity is of great environmental concern because of their bioaccumulation and non-biodegradability in nature (Aung *et al.* 2013, Gautam *et al.* 2014). Toxic heavy metals are invading into human food chain that causes serious health hazards. Potentially toxic heavy metals like As, Pb, Cd, Ni, Cr, Cu, etc. in soil has increased drastically, thus posing risk to the environment and human health (Khalid *et al.* 2017). Different alternatives have also been anticipated to widen the applications of microbiological techniques towards the remediation of heavy metals which generally known as bioremediation. Bioremediation is a green approach of reclamation, rectification or re-establishment of the natural condition of soil where microorganisms are exploited to degrade soil contaminants in the rhizosphere (Kuiper *et al.* 2001). Bioremediation is useful to some extent in developing country like Bangladesh where human intervention is not necessary and this natural attenuation results is considerable cost effective. Various kinds of bioremediation comprising phytoremediation, phytovolatilization, phytostabilization, phytoextraction, etc. are useful (Khalid *et al.* 2017). Employing microorganisms like bacteria, fungus, etc. also act as bioremediator which may incur less time and low cost.

Trichoderma is a soil-borne fungus which is genetically very diverse with a number of capabilities among different strains with agricultural and industrial significance (Azevedo*et al.* 2000). Several strains of *Trichoderma* are tolerant to a range of recalcitrant pollutants including heavy metals, pesticides, organometallic compounds, tannery effluents and polyaromatic hydrocarbons (Harman *et al.* 2004, Ezzi and Lynch 2005). Mycorrhiza is another bioremediator present naturally in soil as symbiotic association with roots of vascular plants. Arbuscular mycorrhizal fungi have been reported for bioremediation of organic and inorganic pollutants (Chibuike 2013). These fungi occur naturally in roots of plants growing on heavy metal polluted soil (Turnau 1998).

The present investigation was aimed to examine the efficacy of *Trichoderma harzianum* and AMF (*Glomus mosseae*) to reduce concentration of two heavy metals *viz*. Pb and Cd in soil and up-take by red amaranth (*Amaranthus cruentus*).

MATERIALS AND METHODS

Characterization of soil

The experiment was carried out in an agricultural land beside an industrial area located at Voaradoba (90º 24' and 90º 28' north longitude and in between 24º 28' and 28º 22' east latitude), Bhaluka, Mymensing has per Bangladesh population census 2001(Bangladesh Parisaṃkhyana Byuro 2004). The experimental area is characterized by red-brown terrace soils under Madhupur Tract (Agro-ecological zone 28). The soil of the experimental field was well to moderately well drained, red and brown, strongly acidic, clay loams and clays in texture, partly over compact, overlying and strongly red mottled substratum. Soil sample was collected two times from the experimental plot. First collection was done when the land was prepared before sowing and the second collection was done after harvesting according to the treatment with an auger from the rhizospheric zone.

Experimental treatments

The experimental treatments were T_0= Control, T_1 =*Trichoderma harzianum*@ (0.5 g/m²), T_2= Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3=*Trichoderma harzianum*@ (1.0 g/m²), T_4=Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²) and T_5= *Trichoderma harzianum*@ 0.5 g/m^2 + AMF@ 2.0 kg/m². The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The size of the unit plot was 10 m^2 (4m X 2.5m).

Selection of crop and cultivation procedure

Based on the previous reports, red amaranth was selected for this experiment as this is a short duration crop, popular and can be grown easily on heavy metal polluted soil and reported to uptake heavy metal from soil (Oluwole *et al.* 2013). Variety 'Altapeti' was collected from the Banasri Agro Seed Company Limited for the experiment. Urea(125kg/ha), TSP (75kg/ha) and MoP (100kg/ha) fertilizers were given in the entire growing period of red amaranth. Plant samples were collected during harvesting of the red amaranth plant at 25 days after sowing (DAS). From each plot ten representative plants were collected with intact root and washed thoroughly with tap water and preserved in refrigerator for heavy metal determination.

Inocula preparation

A peat-based formulated product named 'IPM biopesticide' composed of *Trichoderma harzianum* was collected from IPM lab, Bangladesh Agricultural University. The primary inoculum of AMF characterized as *Glomus mosseae* was collected from BARI, Gazipur and multiplied in Sorghum (*Sorghum bicolor*) following the trap culture method (Giovannetti and Mosse 1980). Sorghum seeds were sown in sterilized soil in pots and inoculated with 25 g of AMF inoculum by mixing them in soil. Hoagland's solution [nitrogen (N) / phosphorus (P) / potassium (K)] as 1:0.85:3.35 (25% v/v) (Hoagland and Boyer 1936) were applied for fulfilling nutrients requirement. The roots and soil were mixed and ground for use as inoculum in the next experiments (Miransari *et al.* 2007, 2008). *Trichoderma harzianum* and AMF were mixed with soil 10 days before sowing of red amaranth seed at the prescribed treatments.

Heavy metal determination

For digestion, 1g of plant or soil sample was taken in a beaker and 10 ml of nitric acid and perchloric acid was mixed with the sample at the ratio2:1. The mixture was heated at 160-180ºC temperature. The temperature was increased until the colour of the solution changed into yellowish or colourless made volume upto100 ml with distilled water. Then the final solution was filtered with filter paper (What man No. 1). Then the digested sample was analysed for heavy metal using atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Effect of *Trichoderma harzianum* and AMF (*Glomus mosseae*) on vegetative growth of red amaranth

Different application rate of *Trichoderma harzianum* and AMF (*G. mosseae*) did not show any significant differences of different vegetative parameters *viz.* no. of leaf/plant, shoot length, root length and fresh weight of red amaranth (Table 1). Significant difference was only observed in dry weight of the crop. The highest dry weight was found in untreated control (123.33 g) followed by T_3 (100.00 g) and T_2 (98.00 g). The lowest dry weight was found in T_1 (78.66 g).

Table 1. Effect of different rate of *Trichoderma harzianum* and AMF (*G. mosseae*) and combined application in soil on vegetative growth of red amaranth

Treatment	No. of leaf/ plant	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
T_0	26.20	40.53	6.63	208.66	123.33a
T_1	35.43	37.40	7.20	168.66	78.66c
T_2	39.00	39.26	5.63	185.33	98.00b
T_3	22.33	34.00	6.26	136.30	100.00b
T_4	31.00	34.13	5.56	148.00	88.00bc
T_5	30.33	35.20	5.73	144.00	82.00bc
CV (%)	31.28	8.86	16.47	20.09	10.46
Level of Significance	NS	NS	NS	NS	**

T_0 = Control, T_1 = *Trichoderma harzianum*@ (0.5 g/m²), T_2 = Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3 = *Trichoderma harzianum*@ (1.0 g/m²), T_4= Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²), T_5 = *Trichoderma harzianum*@ 0.5 g/m^2+ AMF@ 2.0 kg/m²
NS= Non significant, **= Significant at 5 %

Effect of *Trichoderma harzianum* and AMF (*Glomus mosseae*) on concentration of Pb in soil

Different treatments of *Trichoderma harzianum* and AMF (*Glomus mosseae*) significantly influenced on Pb concentration in soil (Figure 1). The concentration of Pb in soil ranged from 8.13 to 14.95 ppm. The highest Pb concentration was found in untreated control T_0 (14.95 ppm). All the treatments either alone or in combination showed significant and similar reduction of Pb concentration in soil compared to untreated control. Application of AMF inoculum @ 4.0 kg/m^2 showed the lowest Pb concentration (8.13 ppm) in soil.

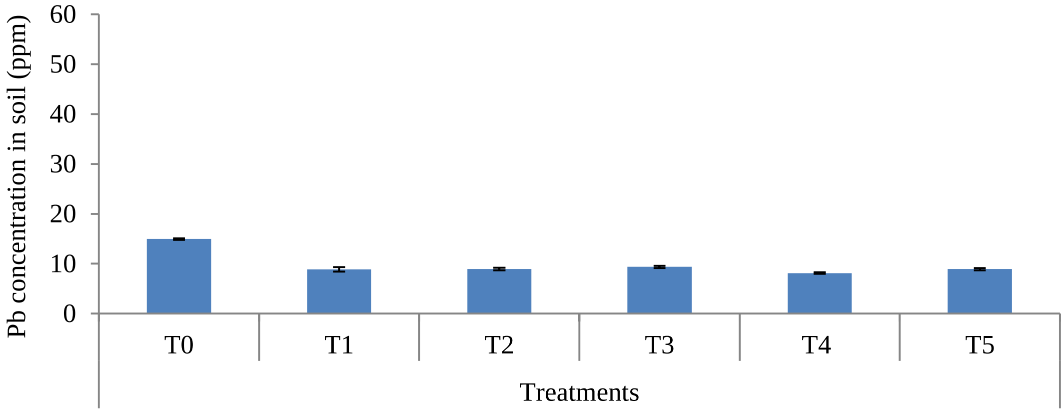

Figure 1. Effect of different treatments on Pb concentration in soil

[T_0 = Control, T_1 = *Trichoderma harzianum*@ (0.5 g/m²), T_2 = Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3 = *Trichoderma harzianum*@ (1.0 g/m²), T_4= Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²), T_5 = *Trichoderma harzianum*@ 0.5 g/m^2 + AMF@ 2.0 kg/m²]

Effect of *Trichoderma harzianum* and AMF (*Glomus mosseae*) on concentration of Cd in soil

Cadmium (Cd) concentration in soil was influenced significantly by the application of *Trichoderma harzianum* and AMF (*Glomus mosseae*) (Figure 2). All the treatments comprising *Trichoderma harzianum* and AMF (*G. mosseae*) showed statistically significant and similar reduction of Cd compared to untreated control. The Cd concentration ranged from 0.19 to 0.05 ppm in soil. The highest Cd concentration was found in untreated control T_0 (0.19 ppm). The lowest concentration was found in T_1 (0.05 ppm) where *Trichoderma harzianum* was applied @ 0.5 g/m^2 in soil.

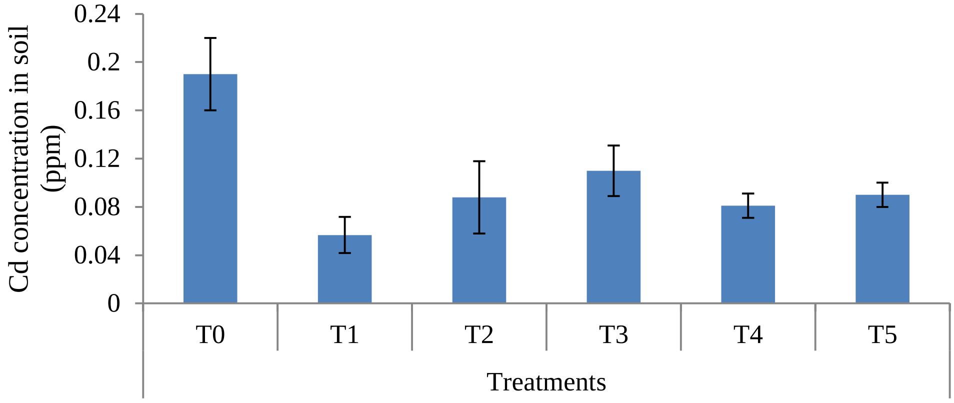

Figure 2. Effect of different treatments on Cd concentration in soil

[T_0 = Control, T_1 = *Trichoderma harzianum*@ (0.5 g/m²), T_2 = Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3 = *Trichoderma harzianum*@ (1.0 g/m²), T_4= Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²), T_5 = *Trichoderma harzianum*@ 0.5 g/m^2 + AMF@ 2.0 kg/m²]

Effect of *Trichoderma harzianum* and AMF (*Glomus mosseae*) on concentration of Pb in red amaranth

Effect of different treatments on concentration of Pb in red amaranth was significantly different (Figure 3). Accumulation of Pb by red amaranth was significantly increased by both *Trichoderma harzianum* and AMF (*Glomus mosseae*). Application of *Trichoderma harzianum* @ 0.5 g/m^2 in soil (T_1) showed the highest influenced in accumulation of Pb (50.97 ppm) in red amaranth followed by T_3 (26.92 ppm) and T_4 (25.48 ppm). The Pb accumulation was found 14.93 ppm in combined application of *Trichoderma harzianum* and AMF (*Glomus mosseae*) in T_5. The lowest accumulation of Pb in red amaranth was found in untreated control T_0 (11.20 ppm).

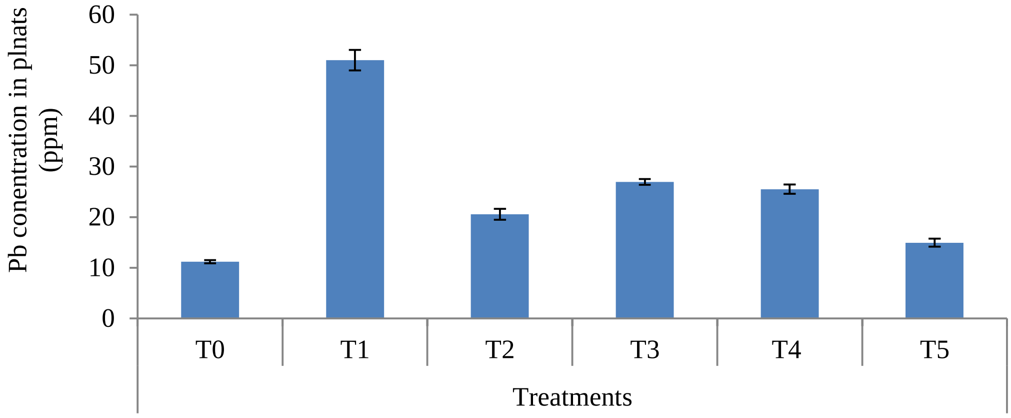

Figure 3. Effect of different treatments on Pb concentration in red amaranth

[T_0 = Control, T_1 = *Trichoderma harzianum*@ (0.5 g/m²), T_2 = Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3 = *Trichoderma harzianum*@ (1.0 g/m²), T_4= Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²), T_5 = *Trichoderma harzianum*@ 0.5 g/m^2 + AMF@ 2.0 kg/m²]

Effect of *Trichoderma harzianum* and AMF (*Glomus mosseae*) on concentration of Cd in red amaranth

Cadmium (Cd) accumulation in red amaranth was significantly varied among the treatments (Figure 4). The highest Cd accumulation was found in T_2(0.783 ppm) where AMF (*G. mosseae*) @ (2 kg/m²) were applied followed by T_4 (0.71 ppm) and T_3 (0.68 ppm). Considerably higher Cd accumulation (0.60 ppm) was also found in combined application of *Trichoderma harzianum* and AMF (*Glomus mosseae*). The lowest Cd accumulation (0.32 ppm) was found in untreated control treatment.

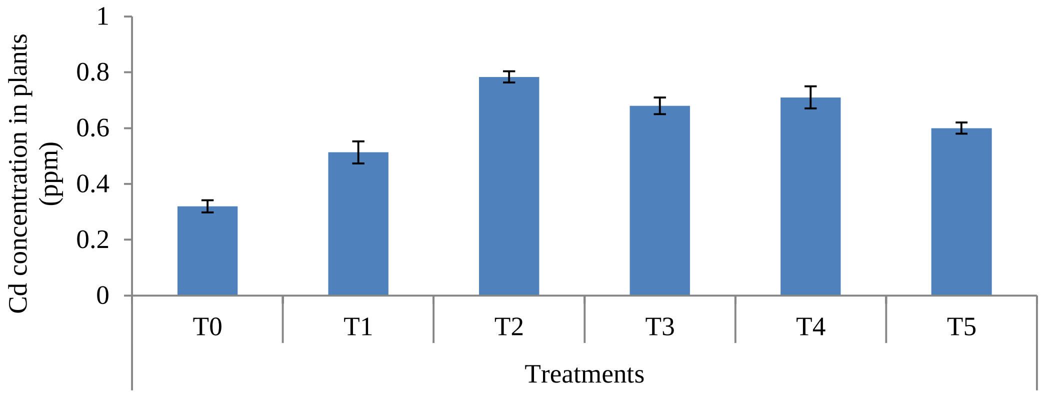

Figure 4. Effect of different treatments on Cd concentration in red amaranth

[T_0 = Control, T_1 = *Trichoderma harzianum@* (0.5 g/m²), T_2 = Arbuscular Mycorrhizal Fungi (AMF) @ (2.0 kg/m²), T_3 = *Trichoderma harzianum@* (1.0 g/m²), T_4= Arbuscular Mycorrhizal Fungi (AMF) @ (4.0 kg/m²), T_5 = *Trichoderma harzianum@* 0.5 g/m^2 + AMF@ 2.0 kg/m²]

The present investigation was conducted to examine whether the vegetative growth of red amaranth was inhibited in that particular area where some farmers cultivated red amaranths and other vegetables using waste water released from textile mills. Lavish vegetative growths of red amaranth in untreated plot indicated that red amaranth could tolerate Pd and Cd toxicity. Plants had also evolved with highly specific mechanisms to translocate and store micronutrients. These same mechanisms were also involved in the uptake, translocation and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, heavy metal accumulation mechanisms by plants were of great interest to phyto-remediation (Salido *et al.* 2003)**.** The present studies also indicated the possibility of using hyper-accumulator plants for remediation of heavy metal toxicity. Pawlowska *et al.* (2000) reported that plants could be used to remove or extract the metal pollutants from soil and the metal accumulated biomass could be harvested using standard agricultural methods and smelted to recover the metal which required cultivation of hyper-accumulating plants for several cycles that included harvest and removal of metal enriched biomass.These hyper accumulating plants naturally took up the heavy metals from soil and transport them to the shoot without exhibiting toxicity symptoms(Zhang *et al.*2018b).

The efficacy of *Trichoderma harzianum* and AMF (*Glomus mosseae*) in soil inoculation before planting on vegetative growth of red amaranth grown in industrially polluted agriculturally waste land was studied. Vegetative growth of red amaranth did not differ significantly except dry weight in different treatments of *Trichoderma harzianum* and AMF (*G. mosseae*). *Trichoderma harzianum* had diverse metabolic adaptability and showed several heavy metals resistance (Pal *et al.* 2006). Ali and Hashem (2007) reported that the mechanism of heavy metal removal by microorganisms differed depending on the species, its origin, and processing of biomass. The findings of the present research work were relevant with the findings of Al-Qurainy *et al.* (2010) to some extent where they studied the impact of *Trichoderma harzianum* and biomarker changes in *Erucasativa* plants grown in metal polluted soil. They reported that *Trichoderma harzianum* inoculation in soil increased shoot of *Eruca sativa* amended with mixture of 50 ppm Cu and Zn, but at higher concentration (100 ppm) root length was decreased. Hyper-accumulator plants like *Ipomoea reptans* were assumed to had been able to adapt to the maximum in the gripped environment so that ultimately plant growth did not appear to experience significant inhibition, resulted in the increase in biomass even though the environment was gripped by Cd (Yusuf 2014). Red amaranth in the present study showed hyper-accumulation without hampering its vegetative growth.

Application of *Trichoderma harzianum* and AMF (*G. mosseae*) significantly reduced both Pb and Cd in soil in the present experiment. It could be hypothesized that the mobility of Pb and Cd might be increased due to pH change caused by *Trichoderma harzianum* (Krantz-Rülcker *et al.* 1996) and AMF inoculation which eventually depleted concentration Pb and Cd in soil by increasing leaching. An experiment conducted by Malgorzata *et al.* (2014) reported that addition of *Trichoderma* MSO1 and MSO2 caused the mobilization of Cd, Pb and Zn resulted in increased leaching of heavy metals into the soil solution. Fariba and Farzad (2014) also demonstrated that *Trichoderma* showed the better ability of metal removing in the favourable condition regarding the initial concentration of metal ions and the suitable pH to absorption of heavy metal. Myra *et al.* (2018) conducted experiments on waste from mining industries where they reported that *Trichoderma* *virens* showed high lead removal (91-96 %) over road range of pH while at neutral pH *T. virens* had 70% to 63% reductions for Cu and Cr, respectively.

Inoculation of *Trichoderma harzianum* and AMF in soil significantly increased Pb and Cd content in red amaranth compared to untreated control in the present investigation. These finding were in accordance with Davies *et al.* (2002) who observed that mycorrhizal fungi increased chromium uptake by sunflower plants. There had been many reports that AM fungi contributed to phyto-extraction (Davies *et al.* 2002, Agely *et al.* 2005, Wang *et al.* 2005). Wei *et al.* (2003) had claimed that rhizosphere was a special ecological remediation unit to treat contaminated soils containing a great number of microorganisms such as fungi and rhizobacteria living with plant roots.

The findings of the present study conformed to the findings of Gupta and Sinha (2007), showing that the accumulation of heavy metals (Cr, Ni and Cd) in the plants was found to increase with increasing tannery sludge concentration. An earlier study showed that root colonization of maize was found to be higher in high contaminated soil compared to non-contaminated soil (Weissenhorn *et al.* 1995).

AMF enhanced heavy metal uptake and transport to shoots, which was called as phytoextraction. Tonin *et al.* (2001) reported that mycorrhizal inoculated clover roots showed higher accumulation of heavy metals. AMF had been demonstrated to alleviate heavy metal stress in plant by retaining metals in roots and reducing the translocation to shoot (Deng *et al.*2004,Janouskova*et al.* 2005).Accumulation of heavy metals in roots of sunflower was found higher than that of shoots that indicated the reduction in translocation of metal from root to shoot by AMF (Zhang *et al.* 2018a).Experimental evidence showed that mycorrhizal plants accumulated 88.1 g and non-mycorrhizal plant accumulated only 60.4 mg per kg of plant biomass grown in contaminated soils (Leung *et al.* 2006).

In conclusion it was suggested that the cultivation of vegetables in the designated area must be prohibited. Farmers should be motivated not to use the waste water from the textile mills for cultivation in spite of luxuriant growth of vegetables. Red amaranth could accumulate Pb and Cd at alarming level which could easily be incorporated in the food cycle of human being and cause serious health hazards. *Trichoderma harzianum* and AMF (*G. mosseae*) could be applied in soil to accelerate the extraction of Pb and Cd by red amaranth or other suitable plants. The present investigation shed light on the bioremediation of Pb and Cd in the industrially waste land using fast growing red amaranth. Successive cultivation of red amaranth in a waste land can effectively extract heavy metals. It is also imperative to investigate different genera of *Trichoderma* and AMF fungi as well as other heavy metal tolerant microorganisms to remediate the heavy metal toxicity.

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