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## Bio-remediation of Pb and Cd Polluted Soils by Switchgrass: A Case Study in India

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### ABSTRACT

**Introduction:** In the present study bioremediation potential of a high biomass yielding grass, *Panicum virgatum* (switchgrass), along with plant associated microbes (AM fungi and *Azospirillum*), was tested against lead and cadmium in pot trials.

**Methods:** A pot trial was set up in order to evaluate bioremediation efficiency of *P. virgatum* in association with PAMs (Plant Associated Microbes). Growth parameters and bioremediation potential of endomycorrhizal fungi (AMF) and *Azospirillum* against different concentrations of Pb and Cd were compared.

**Results:** AM fungi and *Azospirillum* increased the root length, branches, surface area, and root and shoot biomass. The soil pH was found towards neutral with AMF and *Azospirillum* inoculations. The bioconcentration factor (BCF) for Pb (12 mg kg<sup>-1</sup>) and Cd (10 mg kg<sup>-1</sup>) were

found to be 0.25 and 0.23 respectively and translocation index (Ti) was 17.8 and 16.7 respectively (approx 45% higher than control).

**Conclusions:** The lower values of BCF and Ti, even at highest concentration of Pb and Cd, revealed the capability of switchgrass of accumulating high concentration of Pb and Cd in the roots, while preventing the translocation of Pb and Cd to aerial biomass.

## **Keywords**

Bioremediation; Biofuels; PAMs; Endomycorrhizal fungi; *Azospirillum*

## Introduction

Toxic metal contamination, especially cadmium (Cd) and lead (Pb), generated from industrial and other human activities, possess a major environmental and human health problem which still needs an effective and affordable technological solution. Generally, polluted sites are treated through a broad range of technologies that have progressed over the last three decades.

Engineering technologies, e.g. electro-osmosis, soil fracturing, thermal decomposition, and surfactant washing are being explored, but in most of the cases will be cumbersome and expensive. Plant-based system seems to be an interesting, cost-effective alternative that possess an exciting technical challenge to the research community.

Phytoremediation may offer a cost beneficial, non-invasive, and safe alternative to standard soil cleaning technologies by utilizing potential of certain trees, shrubs, and grass species to eliminate, degrade or immobilize toxic chemicals from soil (Rajkumar *et al.* 2012).

Generally, two kinds of plants are used: 1) hyperaccumulators with a very high heavy metal accumulation potential and low biomass productivity, and 2) fast growing species (non-hyperaccumulators), which have lower phytoextraction ability than hyperaccumulators, but whose total biomass production is significantly higher. Hence, to enhance the heavy metal accumulation efficiencies of non-hyperaccumulators, the adding up of chelating agents has been proposed. However, large quantities of highly contaminated plant material require disposal. Some authors suggest that the combination of soil remediation by fast growing species with their consequent energetic utilization seems to be a suitable technological process (Rajkumar *et al.* 2012; Zhang *et al.* 2011).

Switchgrass (*Panicum virgatum* L.) is known to bioremediate Atrazine, PAHs and heavy metals such as Cu, Pb, Co, Zn, As, etc (Deng *et al.* 2010; Zhang *et al.* 2011). It is a high biomass yielding grass that can be easily cultivated on wastelands and arid lands. Areas degraded by heavy metals are often poor in organic matter and characterized by a low abundance of microorganisms; therefore, in order to accelerate the biogeochemical cycles, microorganisms (bacteria or fungi) should also be applied to soil. Soil microorganisms can help in the absorption of plant nutrients, increase the performance of plants, and, consequently, improve the physical and chemical properties of contaminated soil. The presence of soil microorganisms in the rhizosphere of plants can intensify the phytoremediation process by increasing phytostimulation or rhizodegradation (Wenzel, 2009).

Research studies are being modified to expand the range of microorganisms used for bioremediation. There is a search for naturally occurring microbes that have better pollution degradation kinetics and tolerate a wider range of growth conditions. The ability of microorganisms to uptake and accumulate heavy metals such as Co, Cd, Zn, Mn, Cu, Pb, Ni, Hg, Ag, etc. has been found in many reports. Different bacteria (*Pseudomonas*, *Azotobacter*, *Azospirillum*, PGPRs, etc), fungi such as *Glomus sp*, *Gigaspora sp.*, certain alga and diatoms have such ability and are being studied for their biotechnological potential as agents of effluent detoxification (Rajkumar *et al.* 2012).

For enhancing the effectiveness of phytoremediation process, these microbes play an important role. The microbial activities in the root/ rhizosphere soils enhance the process in metal contaminated soils by two complimentary ways: (i) directly aiming phytoremediation process in

which PAMs increased the heavy metal translocation (facilitate phytoextraction) or trapping the metal contaminants in the rhizospheric soil by reducing its mobility/availability (phytostabilization), and (ii) indirectly conferring heavy metal tolerance in plants and/or enhance the plant biomass that eventually lead to increase in the total metal uptake. In the present paper authors aim to use plant associated microbes (PAMs) *Azospirillum* and AM fungi to enhance phytoextraction of heavy metals (Pb and Cd) by switchgrass in Indian context.

## Materials and methods

### Experiment Set Up

The field experiments were conducted at Micromodel complex, IIT Delhi campus. The site was located at 77.09 °E, 20.45 °N, 228 m above sea level. The field soil was sandy loam with organic C: 0.51%, N: 123 kg ha<sup>-1</sup>, P: 9.0 kg ha<sup>-1</sup>, K: 239 kg ha<sup>-1</sup> and pH 7.5. The consortia of AM fungi (with dominating species *Glomus mossei*, *G. fasciculatum* and *Gigaspora margarita*) isolated from the normal soil of Micromodel, IIT Delhi, cultured on castor was used in the present study. *Azospirillum* biofertilizer packs were procured from Microbiology Division, Indian Agricultural Research Institute, New Delhi. Switchgrass seeds were provided by Department of Agricultural Sciences, University of Bologna, Italy.

A field experiment was set up in green net house in Micromodel, IIT Delhi. The experiments were conducted in earthen pots, each containing 1 kg soil mix (soil + FYM in 3:1). Switchgrass seeds were sown in pots. Four treatments were compared: i) switchgrass with endomycorrhizal fungi (AMF); ii) with *Azospirillum*; iii) with a combination of both; uninoculated. These sets of pots were treated with different concentrations of Pb (Pb (CH<sub>3</sub>COO)<sub>2</sub>·3H<sub>2</sub>O) (4 ppm-12 ppm) and



Cd ( $\text{CdSO}_4 \cdot 7\text{H}_2\text{O}$ ) (2 ppm-10 ppm). In this way, we had 20 pots for Cd (5AMF + 5Az. + 5 (AMF+Az.) + 5 NI), 20 pots for Pb (5AMF + 5Az. + 5 (AMF+Az.) + 5 NI) and a control which is untreated with salt solution of Cd or Pb. All the treatments were kept in triplicate and total of 132 pots were used. Five seeds were grown in each pot. The temperature during the study was 22-35 °C and relative humidity 60-80%. Pots were watered every three days with tap water. The duration of experiment was 20 weeks.

### **Harvesting of Grass Biomass**

All the biomass was harvested after 20 weeks from seeding. The shoot and root lengths of freshly harvested plants were measured separately. Plant tissues were oven dried at 70 °C for 72 h, then weighed and sampled for mineral analyses. The soil samples were collected after every 30 days for its analysis. The parameters studied were: germination, survival, total biomass yield, height, soil pH, and heavy metal (Cd and Pb) uptake.

### **Soil and Plant Mineral Analysis**

Soil samples were analyzed for pH, EC, total C, total N, and available P and K. The pH values (pH in  $\text{H}_2\text{O}$  and pH in 1M KCl) were measured by a pH-meter EUTECH con 510. Total carbon (TC) and Total N was determined after dry combustion by a Multi C/H/N 2100S Analyzer and available P and K were measured using Spectrophotometer and Flame photometer respectively.

The heavy metals were extracted and analyzed by Atomic Absorption Spectrometry (AAS).

Digestion of soil and plants (plant samples were subjected to heat to become ash) samples was performed by heating these at 75 °C for 3 h with oxi-acidic mixture of  $\text{HNO}_3:\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2$

(4:1:1, 12 ml for 2-4 g sample). After cooling it, 20 ml demineralized water was added and heated up to 150 °C for 4 h and then brought to a volume of 25 ml with demineralized water. A blank digest was also carried out in the same way (Demirbas, 2000). After that the reading pertaining to Cd and Pb were obtained by AAS.

To evaluate the mobility of heavy metals from the soil into the plants and the ability to translocate the metals to the harvestable aerial part, the following factors were used (Kacprzak *et al.* 2014):

- (i) Bioconcentration factor (BCF):

$$BCF = \frac{\text{metal concentration in plant shoots (mg / kg)}}{\text{metal concentration in soil (mg / kg)}} \quad (1)$$

The bioconcentration (BCF) is a measure of the ability of a plant to take up and transport metals to the shoots;

- (ii) Translocation index ( $Ti$ ),

$$Ti = \frac{\text{metal conc in plant shoots (mg / kg)} \times 100}{\text{metal concentration in roots (mg / kg)}} \quad (2)$$

The translocation index ( $Ti$ ) determines the ability of plants to translocate heavy metals from roots to aerial parts.

### **Recovery and quantitative estimation of microbes from soil**

Arbuscular Mycorrhizal fungi

*Spore count:* Spores were extracted from 10 g rhizospheric soil of each sample by wet sieving followed by floatation-centrifugation in 50% sucrose (Gerdemann and Nicolson, 1963). The spores were collected on a grid pattern filter paper and washed with distilled water to spread spores evenly over the entire grid. They were counted under a stereoscopic microscope (40×).

*Root colonization:* Roots were rinsed with distilled water, cleared by 10% KOH, 30-45 min at 90°C and acidified in 1% HCl for 5-10 min. Then they were stained using Trypan Blue (0.05% in lacto-glycerol) for 10 min. They were left in lacto-glycerol at 90°C for 45 min for elimination of undesired dye particles. For quantification of AMF colonization, 10 one cm sections were selected randomly and left them on slides under microscope (80×) and percentage root colonization (PRC) was calculated according to Phillips and Hayman (1970) procedure.

## *Azospirillum*

For the enumeration of the *Azospirillum*, samples of plant rhizosphere soil were collected at the end of experiment, and then 10 g root free soil was shaken for 1 h in 90 ml sterilized tap water and serial dilutions were made. The specific *Azospirillum* medium (HiMedia) was used and colony count plate method was applied for the determination of the total count.

## **Results**

### **Soil characteristics**

The initial biochemical parameters of the soil+ FYM used for plantation are: C/N: 20:1; N: 1.23%; P: 0.7%; K: 0.65%; EC: 1.2  $\mu$ S/cm and pH: 7.15. The final characteristics of the soil

depended upon the type of heavy metal and microbes inoculated. The C/N ratio was found between 15:1 to 18:1; N ranges from 1.2% to 1.10%; P and K % lies between 0.55-0.68% and 0.5 -0.65% respectively. EC has increased after heavy metal spiking and was ranging between 3.1- 1.8  $\mu\text{S}/\text{cm}$  and pH was found to be acidic (4.6 to 7.1). The AMF spore count was in the range of 25-30 / g as compared to the initial 12-15 /g and the root infection has reached upto 85%. The *Azospirillum* count was in the range of  $10^8$ .

### Growth Parameters

Figures 1-4 revealed the effects of Pb and Cd on different growth parameters, i.e. germination, survival, height of grass and biomass yield. It is clear from the figures that maximum germination and survival percentage were seen in combination of AMF and *Azospirillum* treatments. There was 13.4% decline in survival % with 12 ppm Pb concentration and 16% decline in 10 ppm Cd as compared to control. While as compared to *Azospirillum* and AMF treatment, this decline was 20.3% and 18.2%, respectively. For uninoculated treatments, germination percent was lower even at minimum concentration levels of heavy metals (i.e. 4 ppm for Pb and 2 ppm for Cd) and it got decreased down gradually as metals concentration increased. Pb and Cd showed 40% and 53% decline respectively at the concentration of 12 ppm Pb and 10 ppm Cd for uninoculated treatments over control. Survival rate for all treatments was between 80% and 90% for 4 and 2 ppm Pb, and 2 ppm Cd.

Similar trend was found for both height and biomass yield. At maximum concentrations of Pb and Cd (12 and 10 ppm, respectively) AMF + Az inoculated plants showed approx. 10% reduction in height. For uninoculated plants the height reduction was about 35%. On total

biomass yield, at 12 ppm Pb concentration, the combination of AMF and *Azospirillum* showed 10% reduction, whereas at 10 ppm Cd concentration this reduction was about 15%. In uninoculated treatments there was approx. 65% reduction in comparison with control. 12 ppm Pb and 10 ppm Cd reduced grass growth drastically.

### ***Soil pH***

Table 1 deals with the values of soil pH at different concentrations of Pb and Cd. The pH of soil having 4 ppm of Pb and 2 ppm Cd was more or less near to the pH of untreated control for both inoculated and uninoculated treatments. For uninoculated treatments, there was a decrease in pH as the concentration of Pb and Cd increased, and at maximum concentrations of both the metals soil was found to be acidic. The AMF + *Azospirillum* treatments didn't show much variation in pH, i.e. no significant change in pH was observed.

### ***Bioremediation Potential***

Bioaccumulation coefficients (concentration of the metal in dried plants divided by the initial soil content of the same element) for Cd and Pb as a function of the total metal content of the soil for the plant unit experiments are shown in Table 2 and Table 3. The addition of microbial inoculants caused decrease in the BCF value. The highest metals uptake from the soil was recorded after AMF and *Azospirillum* inoculation, whereas the lowest rate (0.05 BCF) was calculated at 10 ppm Cd. The translocation index (Ti) decreased with the addition of the soil bioinoculants (Table 2 and 3). Again, the lowest Ti (9.8), was observed for Cd at the dose of 10 ppm. Therefore, switchgrass inoculated with AMF + *Azospirillum* can be an effective

phytoremediator (phytoaccumulator/phytostabilizer), though the heavy metals were mostly concentrated in the belowground biomass

## Discussion

There has been increasing interest in heavy metal hyperaccumulators for the remediation of polluted sites. One of the most promising solution is to grow non-food energy crops that combines the soil remedy with the use of biomass for energy purposes. Moreover, exploring challenges for growing energy crops on contaminated lands will avoid ethical issues concerning food and fuel competition and land grabbing, i.e. purchases or long-term leases of farming land by either private or public investors. Our present results confirm that switchgrass can grow on polluted soils according to previous studies (Rajkumar *et al.* 2012). PAM mediated switchgrass bioremediation potential was observed in the present study. The grass was capable to tolerate 12 ppm of Pb and 10 ppm of Cd in pot studies in Indian climatic conditions.

The extracellular polymeric substances (EPS), mucopolysaccharides and proteins production by PAMs have been thought to play significant role in chelating toxic metals and in decreasing their mobility in the soils. The ability of insoluble glycoprotein, Glomalin secreted by arbuscular mycorrhizal fungi (AMF), to form complexes with heavy metals was assessed by Gonzalez-Chavez *et al.* (2004). The authors extracted up to 4.3 mg Cu, 1.1 mg Pb and 0.1 mg Cd per gram of glomalin from metal polluted soils and there was a correlation between the heavy metal binding and glomalin production, Significant glomalin producing AMF strains will be more appropriate for phytostabilization efforts. Similar kinds of results were obtained during the present study where heavy metal is chelated and concentrated in the roots of AMF inoculated

switchgrass plants. Andrade, Silveira, and Mazzafera (2010) recently observed the effect of AMF on the growth of coffee seedlings under heavy metal (Pb, Cd, Cu and Zn) stress and revealed the better nutrient uptake, growth and yield of mycorrhizal coffee seedlings as compared to non-mycorrhizal seedlings. Presence of extraradical mycelium of AMF which increase the root surface area and hence the uptake of soluble minerals mainly P might be the reason.

Further these microbes produce organic acids, siderophores, etc. which act as chelating agents, and form complexes with metals. These metal binding secretions have substantial roles in limiting the toxic effects of heavy metals and enhancing the biomass production in plants. Since the organic acids produced by rhizospheric microbes in soil may form complexes with heavy metals and inactivating and minimizing the cytological impacts of free metal ions (Gao *et al.* 2010), the heavy metal:organic acid complex is considered as less phytotoxic as compared to free form of heavy metals (Najeeb *et al.* 2009). The intercellular spaces of the AMF were found to be precipitated with metal oxalates, that leads to reduced metal availability and hence toxicity to the host plant. Additionally, the mechanisms of absorption of metal in fungal cell wall components (chitin, melanin) (Krupa and Kozdroj 2007) and accumulation of heavy metals in mycorrhizal vacuoles may have major role in limiting the detrimental effects of heavy metals in plants (Gonzalez-Chavez *et al.* 2004; Leyval, Turnau, and Haselwandter 1997). The mycorrhizal fungi may also contribute in reducing the metal phytotoxicity through ‘dilution effect’ (Chen *et al.* 2007).

Our findings pertaining to effect of dual inoculation on heavy metal uptake are corroborated with the results obtained by Arriagada *et al.* (2004) They observed that the dual inoculation of AMF *Glomus deserticola* and *Azospirillum* enhanced the tolerance of *Sorghum* towards the inoculation of 50 mg l<sup>-1</sup> of Cd, probably due to increasing AM colonization in the plant roots which enable the plant to carry out their vital activities. In the current study > 85% of root colonization was found in AMF inoculated plants, that might have increase the efficiency of metal uptake.

Similarly, the rhizospheric bacteria have also been shown to improve the host plant growth and heavy metal tolerance, especially in association with mycorrhizal fungi (Azcon *et al.* 2010).

The increase in grass growth and enhanced heavy metal (Pb and Cd) uptake might have also been due to alteration of antioxidant enzyme activities of plant defense mechanisms resulted from physiological and biochemical effects of AMF. Azcón *et al.* (2010) recently studied the effects of autochthonous microorganisms (AMF and/or plant growth promoting bacteria) on the antioxidant activities of plants growing in a multi heavy metal contaminated soil. AMF inoculation enhanced the activity of CAT, ascorbate peroxidase, or GR and decreased the levels of oxidative damage to plant biomolecules due to metal stress (Azcon *et al.* 2010). As reported by Garg and Aggarwal (2012), AMF inoculated *Cajanus cajan* have significantly higher levels of SOD, CAT, POX as well as GR and were more tolerant to high soil Cd and Pb contents than non inoculated plants . Ciatelli *et al.* (2010) mentioned the enhanced tolerance of the mycorrhizal white poplar cuttings to heavy metal stress was associated with higher expression of polyamines (membrane stabilizers and free radical scavenger) biosynthetic genes (PaADC, PaSPDS1 and PaSPDS2) and metallothioneins. This study affirms the role of polyamines and metallothioneins in the protection against heavy metal-induced stress in plants.



The improved AM root colonization is probably the response of various factors including increases in heavy metal concentration and the consequently decreasing in soil pH. Rufyikiri *et al.* (2003), who studied root – and hypha – induced substrate, pH modifications under *in vitro* conditions, depicted that roots lead to an acidification, while fungal mycelium to an alkalization of the growth medium. pH was seemed to be independent of the concentrations of the heavy metals (Pb and Cd) for AMF treatments. The phenomenon related to pH groups of soluble proteins and non-proteins thiol operating as a tolerating mechanism in root cells (Chaoui *et al.* 1997) which explains the low soil pH in non AM treatments than AM treatments at concentration higher than 4 ppm in Cd and 6 ppm in Pb. However, the differences in soil pH could also be due to the metal binding capacity of AM fungi, as well as active pH modification by the extracellular hyphal secretions (Rufyikiri *et al.* 2003). AMF and *Azospirillum* attributed towards increased plant growth and stress tolerance might be due to the synthesis of the enzyme ACC deaminase, which degrades aminocyclopropane-1-carboxylic acid (stress Ethylene), as their nitrogen source leads to reduce in the deleterious effects of stress ethylene when plants are subjected to environmental stresses such as heavy metals (Belimov *et al.* 2005).

In *Azospirillum*, the role of antiporter gene AtNHX1 is also known for pH stress control (Arora *et al.* 2014). In our study, the change in pH with dual inoculation of AMF and *Azospirillum* was non-significant, in agreement with several studies on microbial inoculations for pH tolerance (Arora *et al.* 2009; Arora *et al.* 2014).

## **Conclusion**

This study showed that switchgrass when inoculated with Plant Associated Microbes (PAM), AM fungi and *Azospirillum* showed enhanced metal absorption, enlarge root systems, improved plant biomass, resulting in approximately 2-fold phytoextraction of metals from the Pb/Cd contaminated soil. Restricted translocation of heavy metals to the plant aerial biomass makes the harvested biomass more suitable for bioenergy purposes. This report showed a new development opportunity of switchgrass as microorganism-assisted phytoextraction hyperaccumulator on highly metal contaminated sites and energy crop for fossil fuel displacement.

## **Acknowledgement**

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**Table 1** Effects over time of various concentrations of Pb and Cd on soil pH.

Microbe	Pb (mg kg <sup>-1</sup> )	Day				Cd (mg kg <sup>-1</sup> )	Day	Day			
		0	30	60	90			0	30	60	90
Control		7.3	7.3	7.2	7.2		7.3	7.26	7.2	7.18	
A	4	7.15a	7.0a	6.8b	6.5c	2	7.1a	6.5b	6.35c	6.0d	
	6	7.14a	6.8b	6.7b	6.5c	4	7.14a	6.3b	6.2c	6.1d	
	8	7.18a	6.2b	6.0c	5.55d	6	7.13a	5.7b	5.5c	5.05d	
	10	7.14a	5.98b	5.65c	5.25d	8	7.1a	5.48b	5.15c	4.75d	
	12	7.18ba	5.4b	5.25c	5.1d	10	7.13a	4.9b	4.75c	4.6d	
B	4	7.17a	7.11a	7.0a	6.95b	2	7.12a	6.6b	6.5b	6.45c	
	6	7.17ba	7.1a	7.0a	6.9b	4	7.12a	6.6b	6.5b	6.4c	
	8	7.15a	7.0a	6.9b	6.3c	6	7.1a	6.5b	6.4c	5.8d	
	10	7.18a	6.76b	6.7c	6.2d	8	7.13a	6.26b	6.2c	5.7d	
	12	7.18a	6.6b	6.5c	6.0d	10	7.1a	6.18b	6.1c	5.5d	
C	4	7.18a	7.0a	6.9b	6.8b	2	7.13a	6.5b	6.4b	6.3c	
	6	7.16a	6.9b	6.8b	6.5c	4	7.11a	6.4b	6.3c	6.23c	
	8	7.16a	6.8b	6.2c	5.9d	6	7.12a	6.35b	5.7c	5.4d	
	10	7.17a	6.8b	6.0c	5.5d	8	7.12a	6.3b	5.5c	5.0d	
	12	7.17a	6.0b	5.8c	5.3d	10	7.12a	5.56b	5.3c	4.8d	

D	4	7.14a	7.1b	7.0a	6.9b	2	7.14a	7.0a	6.85b	6.8b
	6	7.18a	7.1a	7.0a	7.0a	4	7.11a	7.0a	6.82b	6.8b
	8	7.18a	7.0a	7.0a	7.0a	6	7.13a	6.95a	6.8b	6.78b
	10	7.15a	7.02a	6.9b	6.9b	8	7.1a	6.9a	6.78b	6.75b
	12	7.14a	6.9b	6.72c	6.65d	10	7.12a	6.85b	6.77b	6.72c

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Different letters in the same column indicate significant difference between the treatments at  $P \leq 0.05$ . A to D represent, respectively

A: Control; B: AMF; C: Azospirillum; D: AMF+Azospirillum



**Table 2** Bioconcentration factor (BCF) and translocation index (*Ti*) of *P. virgatum* treated with Pb

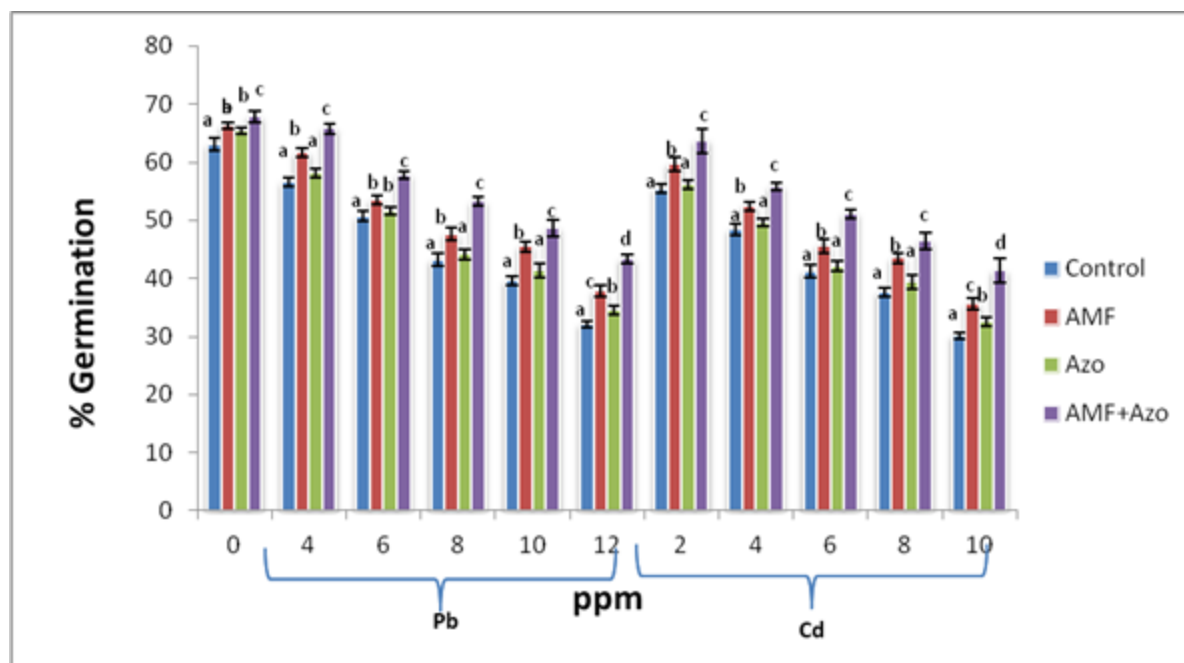
Treatment	Conc. of Pb (ppm)									
	4		6		8		10		12	
	BCF	Ti %	BCF	Ti %	BCF	Ti %	BCF	Ti %	BCF	Ti %
Control	0.19a	16	0.13a	14	0.11a	12.6	0.09a	11.2	0.06a	10.2
AMF	0.29c	21	0.25c	22	0.23c	20.2	0.20c	18	0.17c	15.4
Azo	0.23b	18	0.18b	17.5	0.15b	15.0	0.12b	13	0.1b	11.3
AMF+Azo	0.35d	24	0.33d	24	0.30d	22.4	0.27d	20.3	0.25d	17.8

The different letters followed the values in the same column indicate significant difference between the treatments at  $p < 0.05$ . The same letters followed the values in the same column indicate no significant difference between the treatments at  $p < 0.05$ .

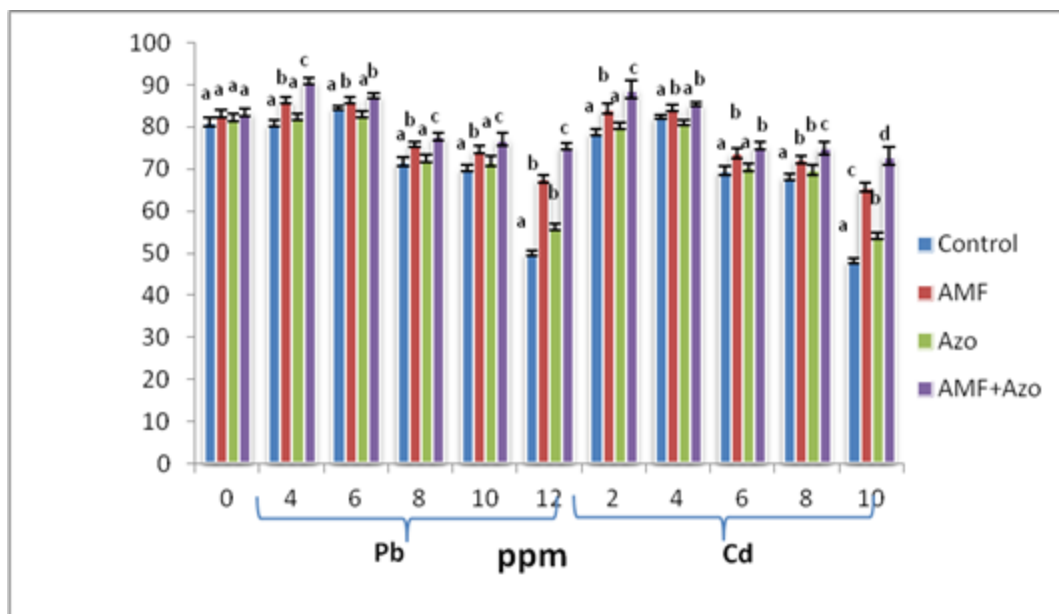
**Table 3** Bioconcentration factor (BCF) and translocation index (*Ti*) of *P. virgatum* treated with Cd

Treatment	Conc. of Cd (ppm)									
	2		4		6		8		10	
	BCF	Ti %	BCF	Ti %	BCF	Ti %	BCF	Ti %	BCF	Ti %
Control	0.17a	15	0.11a	13.1	0.09a	11	0.08a	10.7	0.05a	9.8
AMF	0.27c	19.2	0.23c	20	0.2c	18.7	0.17c	16.2	0.15c	14.3
Azo	0.21b	16.5	0.16b	15.7	0.12b	13.4	0.1b	11.5	0.09b	10
AMF+Azo	0.32d	22.6	0.3d	22.1	0.28d	20	0.24d	19	0.23d	16.7

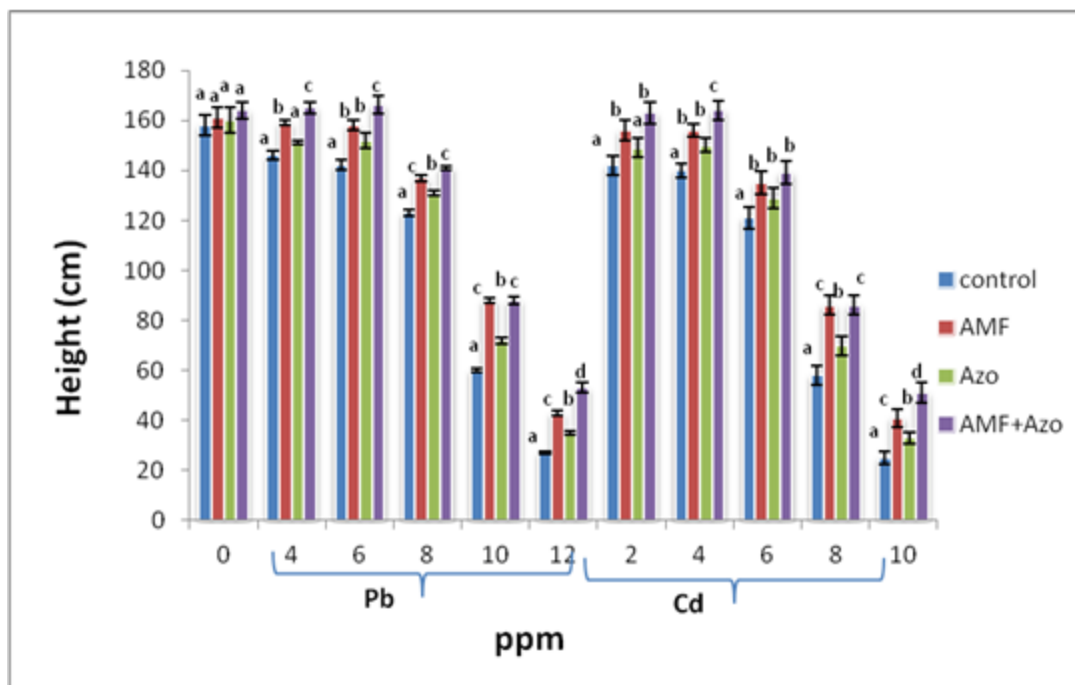
The different letters followed the values in the same column indicate significant difference between the treatments at  $p < 0.05$ . The same letters followed the values in the same column indicate no significant difference between the treatments at  $p < 0.05$ .



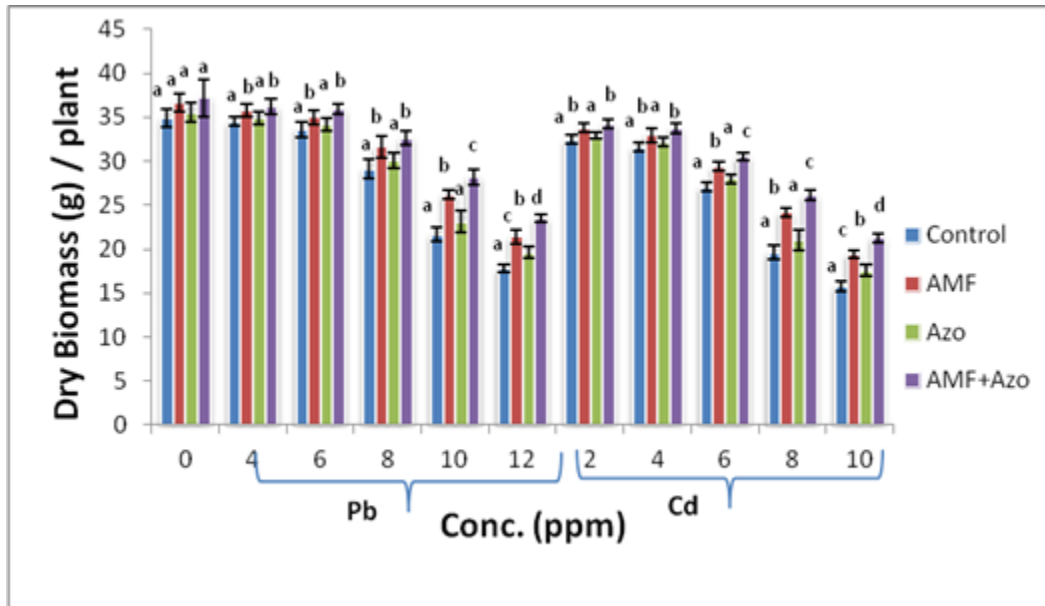
**Figure 1** Effect of various concentrations of Pb and Cd on *P. virgatum* germination (Azo: *Azospirillum*; AMF: Arbuscular Mycorrhizal Fungi)



**Figure 2** Switchgrass survival under various concentrations of Pb and Cd (Azo: *Azospirillum*; AMF: Arbuscular Mycorrhizal Fungi)



**Figure 3** Effect of various concentrations of Pb and Cd on height of *P. virgatum* (Azo: *Azospirillum*; AMF: Arbuscular Mycorrhizal Fungi)



**Figure 4** Effect of various concentrations of Pb and Cd on biomass yield of *P. virgatum* (Azo: *Azospirillum*; AMF: Arbuscular Mycorrhizal Fungi)