Asian J. Exp. Sci., Vol. 23, No. 1, 2009; 341-348 **Rapid Communication**

Toxic Metal (Cd) Removal from Soil by AM Fungi Inoculated *Sorghum*

Kalpana Arora* and Satyawati Sharma
Centre for Rural Development and Technology,
Indian Institute of Technology,
Hauz Khas, New Delhi-110 016; India.

Abstract : Health hazards posed by the accumulation of toxic metals in the environment accompanied by the high cost of removal and replacement of metal polluted soil have prompted efforts to develop bioremediation strategies. This green house study was carried out with 'Jowar' (*Sorghum vulgare*) aimed to determine the effect of Arbuscular Mycorrhizal (AM) colonization on the uptake of cadmium (Cd) from artificially contaminated soil. Plants of *S. vulgare* were grown in AM and non AM inoculated substrate and subjected five soil- [Cd] concentrations (0.1%, 0.2%, 1.0%, 2.0% and 5.0%). The inoculation of AM Fungi resulted in significantly better absorption and accumulation of Cd by *Sorghum.* Compared to non-AM inculated treatments the percent increase in Cd accumulation was 47.1%, 45.2%, 35.7%, 33.9% and 23.5% for 0.1%, 0.2%, 1.0%, 2.0% and 5.0% respectively after 80 days of treatment. As for growth parameters were studied, there were significant differences between treatments. Soil pH was significantly lower in non-AM than AM treatments at the highest soil- [Cd]. The results indicated possible exploitation of AM colonization for better metal accumulation in plant for phytoremediaation purpose.

Key words : AM fungi, Bioremediation, Phytoremediation, *Sorghum vulgare* Heavy metals, Cadmium.

Introduction

Cadmium is one of the components of the earth's crust and present everywhere in the environment. The natural occurrence of cadmium in the environment results mainly from gradual phenomenon such as rock erosion and abrasion that estimate for 15,000 mt per annum (WHO, 1992). Naturally existing concentration of Cd in atmosphere is 0.1-0.5 ng/m^3, in earth crust is 100-500 mg/gm but much higher levels may accumulate in sedimentary rocks and marine phosphates. The widespread use of Cd is based on its unique physical and chemical properties. It is highly resistant to chemicals, high temperature and ultraviolet light (Morrow and Keatings, 1997). Cd is widely used in special alloys, pigments coatings stabilizers above all (almost 70% of its use) in Ni-Cd batteries (Morrow, 1996). It can enter air from the burning of coal, household waste, and metal mining as well as refining process which may increase the level of Cd in the soil varying from 100-600 mg/Kg dry weight (Ernest and Neilson, 2000; Lombi et al, 2000) or more (Meaghler, 2000). The general trend of metal enrichment appears to urban> rural> remote location. Presently, it's rapidly increasing concentration in the environment becoming a threat.

Some countries have set tolerance limits on heavy metal addition to soil because their long-term effects are unknown. These limits

*** Corresponding author :** Kalpana Arora, Centre for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi-110 016; India; E-mail : kalps.arora@gmaill.com

are usually set for plough layer of soil where most of the root activity occurs. The value of potentially toxic elements (PTE) proposed by council of European Economic Committee (Smith, 1996) for Cd concentration in soil is 1.0-3.0 mg/Kg of dry soil and maximum annual addition of total cadmium to soils is 150 gm ha^{-1} (Palanaippan, 2002).

Phytoremediation of metal contaminated sites offers a low cost method for soil remediation and some extracted metals may be recycled for value. Because cost of growing a crop is minimal compared to those of soil removal and replacement, so the use of plants to remediate the hazardous soils is seen as having great promise. Other recent reviews on many aspects of soil phytoremediation are available (Raskin et al., 1996; Moffat A. 1999; Salt D E et al., 1995; Cunningham S. D., 1995). Phytoremediation is the use of plants to make soil contaminants non-toxic and is also often referred to as bioremediation, botanical-bioremediation, or Green Remediation. The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced in 1983 (Chaney RL 1997), gained public exposure in 1990 (Anonymous 1990), and has increasingly been examined as a potential practice and more cost effective technology than soil replacement, solidification, or washing strategies recently used (Salt D.E.. et al, 1995; Cunningham et al,1996).

Recently various studies are carried out 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, fungi such as *Glomus sp*, *Gigaspora sp.*, *c*ertain alga and diatoms have such ability and are being studied for their biotechnological potential as agents of effluent detoxification.

Objectives

The main objective of the present study was to evaluate the efficacy of *Sorghum vulgare* as phytoramediator for heavy metal (cadmium). The role of AM fungus phytoremediation process was also considered an important study. The detailed objectives of the study are as follows;

1. To assess the potential of AMF associated with *Sorghum vulgare* in uptake of heavy metals i.e. cadmium from the soil.

2. To study the effect of various concentrations of cadmium on different plant growth parameters viz. percent survival, yield and total biomass yield and biochemical parameters (Chl *a* and b)

3. To evaluate to effect of metal concentrations and role of AMF on soil pH.

Materials and Methods

To evaluate the potential of AM in bioremediation, the work was accomplished in the following steps with different sets of experiments:

Experimental Design

A number of native AM fungi (*G. microcarpusum, G. macrocarpum, G. mossei, G. intraradices* and *G. fasciculatum* and two species of *Gigaspora* namely *G. margarita* and *G. heterogama*) isolated from the normal soil of Micromodel, IIT Delhi, cultured on castor was used in the present study.

A field experiment was set up in green house in Micromodel, IIT Delhi. The experiments were conducted in earthen pots, each of capacity 2 kg, with a small hole at the bottom. Pots containing soil mix (soil + FYM in 3:1) are inoculated with seeds of *Sorghum vulgare*. Two sets of experiments were conducted, one with endomycorrhizal fungi (AMF) and another without AMF. Now these sets of pots were treated with different concentrations of Cd ($CdSO_4.7H_2O$) i.e. 0.1%, 0.2%, 1.0%, 2.0% and 5.0% of concentrations. In this way we had 10 pots for Cd (5AMF + 5NAMF), and one control in which is untreated with salt solution of Cd. All the treatments were kept in triplicate, total of 33 pots were used. Seeds were grown in a green house, with temperature ranging from 22-30'C and relative humidity ranging from 60-80%. Pots were watered every three days with tap water. The duration of experiment was 80 days.

Harvesting of plant biomass

All the plants were harvested after 80 days of seeding. The fresh plant tissues were measured separately as shoots and roots. All the roots were thoroughly cleaned with tap water. Plants from replicates were randomly selected for the determination of percent AM root colonization. Plant tissues were oven dried at 70° C for 72 hr, then later weighed and sampled for mineral analyses. The soil pH measurements were done after every 20 days.

Assessment of plant growth parameters

Various parameters studied are: % germination, % survival, total biomass yield, soil pH, Physiological parameters: Photosynthetic pigments (Bruinsma, 1963), (Chl a, Chl b, total chlorophyll), Cd uptake, and enumeration mycorrhizal aspects.

AMF spore extraction and identification

AMF spores were isolated from of soil by the wet sieving and decanting method. The precipitate in the 50 µm pore size was quickly rinsed with tap water. Spores were counted using stereozoom microscope and grouped according to morphological characteristics (Gedermann et al., 1963).

Assessment of AM colonization in plants

Root samples were rinsed in tap water. After clearing at 90% in 10% KOH (wt/vol) for 60 min, roots were washed with H_2O_2 and acidified in 1% HCl for 7-10 mins and then keep them in stain Trypan blue. Stained roots were kept in destaining solution (water-lactic acid- glycerine) so that excess stain was removed (Phillips and Hayman, 1970). Mycorrhizal colonization was estimated as the percentage of total root segments containing vesicles, arbuscles and hyphae individually, as well as the percentage of roots containing at least one of these AM fungal structure.

Soil and plant mineral analysis

Soil minerals 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 75^0C for 3 hours with oxi-acidic mixture of HNO_3:H_2SO_4:H_2O_2 (4:1:1, 12 ml for 2-4 g sample). After cooling it, 20 ml demineralized water was added and heated up to 150^0C for 4 hours and then brought to a volume of 25 ml with deminaralized water. A blank digest was also carried out in the same way (Demirbas, 2000). After that the reading pertaining to Cd were obtained by AAS.

Chlorophyll analysis

One gm of fresh leaf tissue from each replicate (Bruinsma, 1963) was submerged into 100 mL of ethanol and kept in dark at room temperature until the tissue was discolored. The readings were taken at 649 and 665 nm by spectrophotometer.

Results

Figure 1 reveals the effects of Cd on different growth parameters as % survival and figure 2 deals with the biomass yield. It is clear from the table that maximum survival percentage is seen in AMF treatments with concentration of heavy metals up to 0.2 %. 26 % decline was observed for survival percent in 5 % Cd concentration as compared to control. For non-AMF treatments germination

Table 1 : Physico Chemical characterization of soil used in the experiments

Parameters	Values
TOC	1.10%
TKN	0.32%
Potassium	0.22%
Phosphorus	0.12%
EC	1.48
pH	7.4

percent was less even at 0.1% decline at 5 % concentration. At 0.1 % concentration survival rate for AMF treated plants as well as non-AMF treatments was between 80-90%.

Similar trend was seen for biomass. At 5 % concentration of Cd AMF plants showed approx 10 % reduction in height and for non AMF plants it was about 35 %. For total biomass, at 5 % concentration AMF treatments showed about 35 % in Cd, and in non-AMF treatments there was approximately 65% reduction in comparison with control. Hence, the lower concentration of 0.1 % and 0.2 % of both Cd seemed to enhance the growth of plants. 1 % of Cd reduced the growth and 2 % and 5 % concentrations proved to be toxic affecting the plant growth severely (Riffat John, 2006).

Table 2 deals with the values of soil pH at different concentrations of Cd. The pH of soil having 0.1 % concentration of Cd was more or less near to the pH of control for both AMF and non-AMF treatments. For non-AMF treatments, there was a decrease in pH as the concentration of Cd was increased. At 0.2 % and 1 % of Cd, pH was slightly acidic but at 5 % concentration soil was found to be acidic. The AMF treatments didn't show much variation in pH i.e. no significant change in pH was observed. For AMF treatments pH was seemed to be independent of the concentrations of the metal Cd (Pawloska et al, 2000)

Figure 3 refers to the uptake of heavy metals (Cd) in plants from the various soils treated with different concentrations of Cd. It has been reported that mycorrhizal fungi can colonize plant roots even in metal contaminated sites (Diaz and Honrublia, 1994; Pawlowska et al., 1996; Sambandan et al., 1992). In our experiment, also the metal uptake is more in AM than in non AM plants. Compared to non-AM inculated treatments the percent increase in Cd accumulation was 47.1%, 45.2%, 35.7%, 33.9% and 23.5% for 0.1%, 0.2%, 1.0%, 2.0% and 5.0% respectively after 80 days of treatment.

The effect of various concentrations of Cd on chlorophyll content is listed in Figure 4.

1. Chlorophyll 'a' content

The variation in chlorophyll 'a' was found to be insignificant in control plants over 30 days period. Cadmium treated plants showed a dose

Table 2 : Effect of Cd at different concentrations on soil pH

Days	Control	Concentration of Cd (%)									
		0.1		0.2		1		2		5	
		AMF	NAMF	AMF	NAMF	AMF	NAMF	AMF	NAMF	AMF	NAMF
0	7.3	7.08	6.8	7.02	6.36	6.97	6.16	6.95	6.69	6.94	5.2
20	7.36	7.1	6.75	7.06	6.21	7.01	6.11	7	5.79	6.98	5.16
40	7.26	7.09	6.65	7.04	6.18	6.99	6.07	6.96	5.75	6.95	5.15
60	7.2	7.07	6.6	7.03	6.08	6.94	6.02	6.91	5.69	6.93	5.1
80	7.18	7.05	6.45	7	6	6.9	5.98	6.9	5.6	6.89	5.06

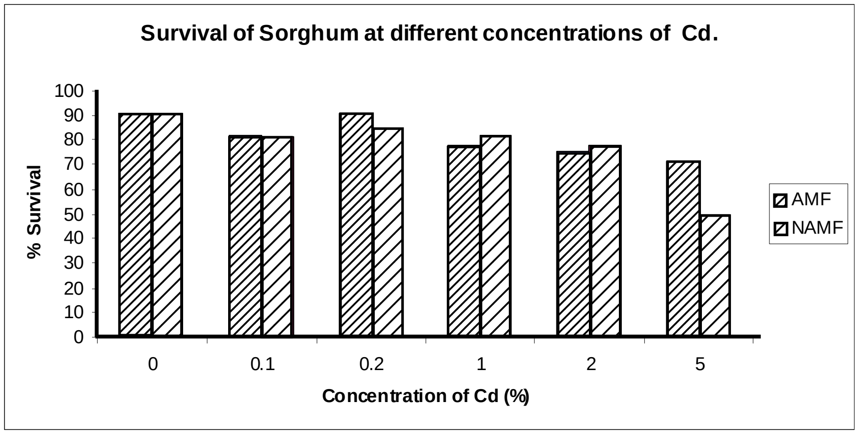

Fig. 1 : Survival of Sorghum at different concentrations of Cd (with and without AMF)

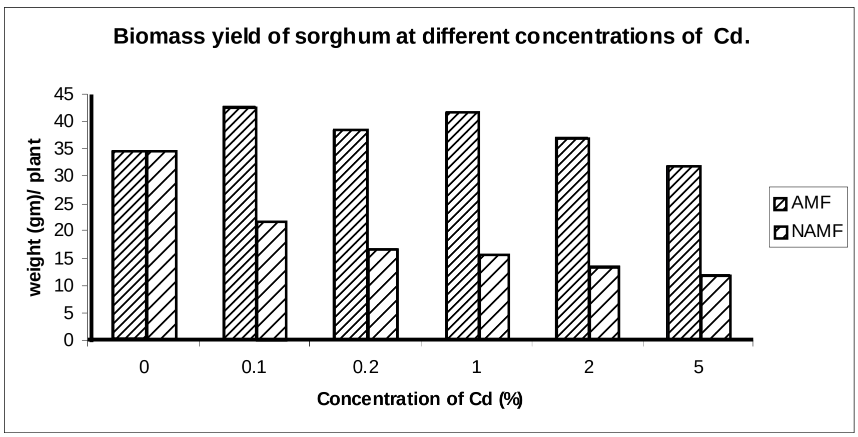

Fig. 2 : Biomass yield of Sorghum at different concentrations of Cd (with and without AMF)

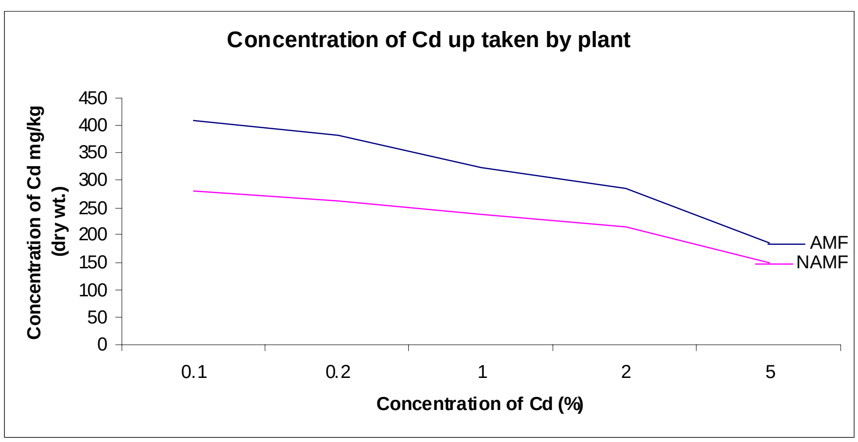

Fig. 3 : Cd uptake by Sorghum vulgare

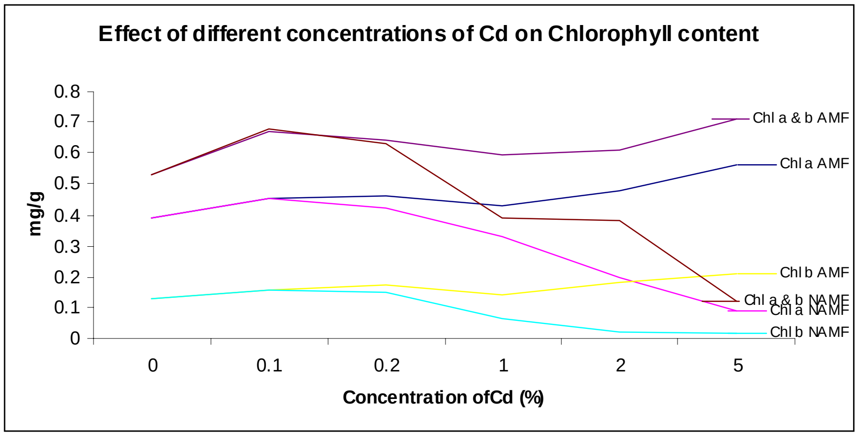

Fig. 4 : Effect of different concentrations of Cd on cholorophyll content (with and without AMF)

dependent and time dependent decrease in chl 'a' content. 0.1% of Cd showed increase in chlorophyll. In 5 % Cd showed 82 % decline comparing control, at the end of the fourth week (Riffat john et al 2007).

2. Chlorophyll 'b' content

The levels of chlorophyll 'b' content in control were recorded maximum at 30 days. Cd treated plants showed a dose dependent decrease in chl 'b' content. The plants treated with 0.1 % Cd led to 23% increase of chlorophyll over control after 30 days. Treatment with 1 % and 5 % led to 62 % and 97 % decline respectively after 30 days (Riffat john et al 2007).

3. Total chlorophyll (Chl 'a' + Chl 'b') content

The levels of total chlorophyll (chl 'a' + chl 'b') content was found to vary by 27% and 28% with the duration over 30 days at higher concentrations of Cd. Cd showed dose-dependent and time-dependent decrease in total chlorophyll content (Riffat john et al 2007).

Discussion

Our results in this study showed that the accumulation of cadmium in plants was maximum at low concentration because as the concentration of the metal increases it becomes toxic to the plant and thus retard its growth. With the inoculation of the AM fungi, the potential of *Sorghum vulgare* to accumulate Cd from the soil increases significantly. The efficiency of phytoremediation of heavy metal contaminated site increases with the presence of higher proportion of metal resistant microbial population in the soil, which may likely, conferred a better nutritional assimilation and protective effect on plants (Doelman; 1985). As far as growth parameters are concerned Growth inhibition of plants was concentration and time dependent (Riffat John, 2006). Chlorosis was evident at concentrations of 2 % and 5 % of Cd. Under relatively high rates of Cd in our studies, the plants showed very poor growth compared with plants growing in control (no heavy metal). There have been reports of significant inhibition of mycorrhizal colonization by heavy metals like Cd (Griffioen et al., 1994; Leyval et al., 1995). For AMF treatments pH was seemed to be independent of the concentrations of the metals Cd. The increase in AM root colonization is likely the result of numerous factors including increases in soil-metal (Cd) concentration and the subsequent decrease in soil pH (Rufyikiri et al. 2003), who assessed root – and hypha–induced substrate. pH groups of soluble proteins and non-proteins thiol operating as a tolerating mechanism in root cells (Chaui et al, 1997).

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AM colonization percentage was observed to increase as soil-metal (Cd) concentration increases (Janouskova and Vosatka, 2005). In our experiment, also the metal uptake is more in AM than in non-AM plants Studies showed that in addition to the metal immobilization in the mycorrhizosphere, mycorrhizal fungi may also act as an effective barrier controlling excessive metal uptake into the root cells (Li and Christie, 2001).

Conclusion

Present work demonstrates that inoculation with AM fungi can facilitates the plant growth and thus increase phytoremediation sfficiency. Study also suggests that plant – mycorrhizal relationship in relevance to toxic metal removal from soil can be an effective tool to enhance plant efficiency.

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