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IMPACT OF HEAVY METALS IN MYCORRHIZOSPHERE: STRATEGY FOR PHYTOREMEDIATION

Anamika S and *MH Fulekar
Department of Life Sciences, University of Mumbai, Santacruz (E), Mumbai- 400 098, India

ABSTRACT

The pot culture technique has been employed for development of mycorrhizal soil in the green house. Mycorrhizal soil is a symbiotic association of bacteria, fungi and Actinomycetes which provides the effective rhizosphere for the growth of plants. The enrichment of microbial enzymes and plants exudates in mycorrhizosphere influences phytoremediation. The heavy metals (cadmium, lead and zinc) toxicity at varying concentrations, viz. 5, 10, 20, 50, 75 and 100ppm has been assessed for seed germination and growth of *Medicago sativa* plants both in mycorrhizal soil and non mycorrhizal soil. Mycorrhizosphere has found to provide suitable conditions for seed germination and growth of plants at concentrations ranging from 5-50ppm. The germination rate was found comparatively lower in NMS at the metal concentrations 5-50ppm. The seed germination *M. sativa* in cadmium amended mycorrhizal soil was found 87, 80, 70 and 55% at concentration of 5, 10, 20 and 50ppm, respectively; while for lead amended mycorrhizal soil, the percentage of seed germination was observed 80, 75, 70, and 60% at 5, 10, 20 and 50ppm, respectively. The seed germination percentage was 95, 90, 91 and 89% when zinc was amended with the concentration of 5, 10, 20 and 50ppm, respectively in mycorrhizal soil. Similarly the root/ shoot growth of *M. sativa* for each of this metal was propound in mycorrhizal soil. The higher metal concentrations i.e. 75 and 100ppm were found inhibitory for the seed germination and root/shoot growth. The enzymes studied; in particular acid & alkaline phosphatase and dehydrogenase in mycorrhizal soil have propounded the growth of plants in the mycorrhizosphere. The present research study has proved the effect of mycorrhizal soil for phytoremediation of heavy metals at concentrations ranging from 5-50 ppm using *M. sativa* as a potential candidate.

Keywords: *Mycorrhizosphere,* heavy metals, *Phytoremediation, Medicago sativa.*

INTRODUCTION

Mycorrhiza is a mutualistic symbiotic association of plant and fungi (Krik, 2001) which provide the effective rhizosphere by the action of microbial enzymes and plant enzymes along with the root zones of the plants. These symbioses are characterized by bi-directional movement of nutrients and largely based on the transfer of carbon (C) from plants to fungus, and mineral nutrients mainly phosphorus [P] and /or nitrogen [N] from fungus to host plants. In mycorrhizosphere, the assemblage of bacteria, fungi and Actinomycetes supply the effective rhizosphere for the growth and development of plants. Mycorrhizal association stimulates branching of the root and increases the absorption surface of the root, which influences uptake of heavy metals by plants (Daniell *et al*., 1999; Miller and Jastrow, 2000; Smith and Read, 1997). Sieverding (1991) has reported that the uptake of trace elements, such as zinc, copper, boron and molybdenum is thought to be enhanced by mycorrhizae. They play an important role in remediation of heavy metal contaminated soil (de Val *et al.*, 1999) and mycorrhizal fungi provide a direct link between soil and plants (Carvalho *et al.,* 2006). In addition to accelerating the nutritional state of their host plant, heavy metals can also be transported to plants via fungal hyphae.

Heavy metals traces in soil and water originate from the natural (weathering processes of the Earth's crust) and anthropogenic sources, like agriculture, mining, smelting, electroplating and other industrial activities (Fargasova, 1994; Theofanis *et al*., 2001; Xie *et al.,* 2006; Verma *et al*., 2007). Such contaminations result in high concentrations of heavy metals in environment, which clearly demonstrate pollution by these metals and contribute one of the most significant factors of degradation of the biosphere. The high concentrations of heavy metals in the environment may be deleterious for biotic lives. Therefore, effective cleanup needs their immobilization to reduce or remove toxicity. The use of phytoremediation can be a cost-effective, environmentally sound, sustainable, *in situ* technology (Salt *et al.,* 1998; Prasad, 2003; Zhou and Song, 2004; Erakhrumen, 2007) to remove or stabilize toxic chemicals. It is also a way of concentrating and harvesting valuable metals that are thinly dispersed in the ground, and offers an attractive option for the remediation of contaminated sites. This method suggests significant potential for certain application and permit a much larger site to be restored would generally be possible using more traditional remediation technologies (Fulekar, 2005). The aim of the research is to develop mycorrhizal soil for assessing the

*Corresponding author email: mhfulekar@yahoo.com

toxicity of heavy metals for phytoremediation to decontaminate the environment.

Medicago sativa has been reported as a potential plant for phytoremediation of heavy metals and grow well in contaminated soils (Baligar *et al*., 1993; Peralta-Videa *et al*., 2004). The presence of heavy metals in the contaminated environment upto the tolerable limit to the plants is of major concern for effective phytoremediation. Mycorrhizosphere influences phytoremediation, therefore the present research study investigated the impact of heavy metals at various concentrations ranging from 5-100 ppm on *M. sativa* as a potential plant in mycorrhizal soil and non mycorrhizal soil. The seed germination and growth of the plants in heavy metal contaminated mycorrhizal soil show the efficiency of plants for phytoremediation. The growth of the plants at the particular concentrations of metals (Cd, Pb and Zn) has been assessed by measuring the percentage of seed germination and morphological characteristics viz. root and shoot growth of the plants. This study will give scope for the phytoremediation of heavy metals using green plants- *M. sativa* in mycorrhizosphere.

MATERIALS AND MEHTODS

Soil sampling and characterization

Alluvial soil used for the experiment was collected from a depth of about 0-15cm along the banks of Surya River, Palghar (located 100km away from Mumbai). The soil was screened through 2mm stainless steel sieve, and stored in a plastic bag at room temperature (27-30^{o}C) until use. The physico-chemical characteristics of the soil were measured by standard methods (Table 1). The content of heavy metals (Cd, Pb and Zn) in soil was estimated by atomic absorption spectrophotometer (APHA, 1998).

Development of Mycorrhizal inoculum

Soil based mycorrhizal inoculum was developed by Pot culture technique at laboratory scale with the help of starter inoculums and using sorghum as a host plant. A starter culture of mycorrhizal fungi (VAM) was procured from Division of Microbiology, IARI, New Delhi. The mixture of 3:1, soil-sand was taken in a pot (5kg capacity with perforated base for proper aeration and drainage of water) and the starter culture of mycorrhizal was mixed thoroughly. Fifty sterilized sorghum seeds were sown in each pot to a depth of 0.5cm. The experiment was carried out in three replicates including control, i.e. without mycorrhizal starter for a period of two and half months. The pots were placed in greenhouse at 27-28^{0}C and watered daily to maintain moisture. After incubation (two and half months), pots were not watered and left for 15 days for rhizo-degradation. The dried roots were chopped and mixed with the same soil and used for the phytoremediation of heavy metals. The physico-chemical parameters and microbial characterization of soil were done during development of mycorrhizal soil at the intervals of 15 days. The mycorrhizal soil was also characterized for its root colonization by trypan blue method of Phillips and Hayman (1970) and spores counts were done by extracting spores by the Wet-sieving and decanting method (Gerdrman and Nichalson, 1963). Bacterial, fungal and Actinomycetes colony forming units (CFUs) were calculated using the standard dilution plate technique of fresh soil suspension on selective media. Bacteria were determined on tryptic soy agar (Martin, 1975). Fungi were estimated on rose bengal agar (Martin, 1950) and Actinomycetes colonies were counted on Kenknight and Munaier's medium. In graphics the total number of microorganisms (bacterial, fungal or Actinomycetes) was expressed as logarithms per g dry soil [log of CFU per gram of dry soil].

Table 1. Physico-chemical characterization of Alluvial soil and mycorrhizal soil*.

Parameters	Methods used	Alluvial Soil	Developed mycorrhizal soil
pH	APHA 1998	7.2	7.3
Electrical conductivity (mMohs)	APHA 1998	0.2	0.34
Moisture (%)	APHA 1998	35	42.2
Water holding capacity (%)	APHA 1998	65	67
Organic carbon (gm/kg)	Walkley-Black method (Jackson, 1973)	72	259
Nitrogen (gm/kg)	APHA 1998	5.8	8.4
Phosphorus (gm/kg)	APHA 1998	0.72	0.81
Sodium (mg/kg)	APHA 1998	23	32
Potassium (mg/kg)	APHA 1998	21	22
Heavy metal (ppm)			NA
Zn	APHA 1998	10.5	
Cd	APHA 1998	BDL	
Pb	APHA 1998	BDL	

*All the values are mean of three replicates. APHA= American Public Health Association; BDL= Below Detection Limit.

Enzyme assays

Acid and alkaline phosphatase [ACP & ALP] activities

Rhizospheric enzyme activities, in particular acid and alkaline phosphatase (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977) were analyzed using colorimetric methods. The enzyme activities were expressed on a dry-weight basis.

Dehydrogenase [DHA] activities

Determination of soil dehydrogenase activity in soils is based on the use of soluble tetrazolium salts [2, 3, 5-triphenyltetrazolium chloride (TTC)], as artificial electron acceptors, which are reduced to red-coloured formazans, extracted and then determined calorimetrically (Casida *et al.*, 1964). The concentration of formazan was calculated from a standard curve. Dehydrogenase activity is expressed as mg formazan formed / 10g of soil per 24h.

Mycorrhizal influence on seed germination and plant growth

The plant species *Medicago sativa* selected for the study of heavy metals toxicity on seed germination and root/ shoot growth in MS and NMS at a varying concentration of heavy metals, so as to decide the concentration of heavy metals which could be taken for the phytoremediation.

The growth medium in the pots consisted soil: sand at the ratio of 3:1 and 20% of mycorrhizal inoculum (developed in laboratory by Pot culture technique) treated as Mycorrhizal soil (MS) used for the toxicity experiment. The dried and sieved soil is treated as Non Mycorrhizal Soil (NMS). Soil was amended with heavy metals: Cd as Cd $(NO_3)_2$. $4H_2O$; Pb as Pb $(NO_3)_2$ and Zn as $ZnSO_4$. The varying concentrations applied for each heavy metal was; 0, 5, 10, 20, 50, 75 and 100 ppm. *Medicago sativa*, var Lucerene CoI seeds were obtained from Ratanshi Agro-Hortitech (Byculla, Mumbai). The healthy seeds of *M. sativa* were surface-sterilized with 0.1% mercuric chloride for 5min, thoroughly rinsed 5-6 times with distilled water to avoid fungal contamination. Ten sterilized seeds of *M. sativa* were sown in 400g all-purpose plastic pots filled with 300g of previously analyzed soil and 20% of mycorrhizal inoculum amended with respective heavy metals (Cd, Pb and Zn) at selected concentrations, viz. 0, 5, 10, 20, 50 75 and 100ppm separately. The pots were randomly placed in a green house at an average diurnal temperature of 25-27^oC for 15 days. The moisture was maintained by watering the plants during the experiment.

Determination of seed germination and root/shoot length

Germination rate was determined by counting the numbers of seeds for 7 days, at 24hour intervals. Alfalfa seeds were considered to have germinated when the radical visibly protruded from the seed coat by at least 2mm.

The percentage of seed germination was computed by following formula:

$$\text{Seed germination \%} = \frac{\text{No. of germinated seedlings}}{\text{Total no. of seeds}} \times 100$$

The experiment was carried out for 15 days for studying the effect of heavy metals on plant's growth. A sample of 3 plants from each pot was randomly selected to evaluate the length of the plants. The length of roots and shoots of all plants was measured by Vernier calipers, separately of each concentration of heavy metals and noted for MS and NMS. The length of the roots was measured from the main root apex to the crown of the plant and the shoot's length was measured from the crown of the plant to the main shoot apex.

Statistical analysis

All the experiments were conducted with three replicates and data were analyzed for mean and standard deviation (X± S.D.) using standard statistical methods (Mahajan, 1997).

RESULTS AND DISCUSSION

Phytoremediation is a recent, low cost-effective technology for remediation of heavy metals from the contaminated environment. Mycorrhizal soil provides the suitable environment wherein bacteria, fungi and Actinomycetes associates along with the root zone and plant exudates make the effective rhizosphere. In mycorrhizosphere both microbial enzymes and plant enzymes have propound effect on phytoremediation. Therefore the present research study has been carried out to assess the impact of heavy metals (cadmium, lead and zinc) on seed germination of *Medicago sativa* at varying concentrations, viz. 0, 5, 10, 20, 50, 75 and 100 ppm both in mycorrhizal and non mycorrhizal soil.

Mycorrhizal soil has been developed under controlled conditions using sorghum as a host plant for a period of two and half months. The physico-chemical and mycorrhizal characteristics were assessed during the development process at the interval of 15 days. Physico-chemical properties of alluvial and developed mycorrhizal soil are listed in table 1, which showed pH- 7.3; EC- 0.34; OC- 259; Na- 32; K-22; N-8.4; P-0.81, in developed mycorrhizal soil. pH of the soil is almost neutral and within the recommended value for proper growth and efficient uptake of nutrients and compounds from soil. The organic carbon content was observed to be low i.e. 72gm/kg in collected alluvial soil which increased upto 259g/kg in developed mycorrhizal soil (Fig. 1). However, nitrogen content of the soil was noticed to fall between

5.8 -8.4gm/ kg and phosphorus content in the soil was found varied from 0.72- 0.81gm/kg (Fig. 2). The increase in C/N (12.41 to 30.83) and N/P (8.06 to 10.73) results increase in mycorrhizal colonization. Increase in organic carbon and nitrogen contents found to contribute to the better growth and health of the plants. Sodium and potassium content was found to be constant (Fig. 3). The mycorrhizal soil is containing high nutrients which contribute proper growth and development of the plants.

Mycorrhizal characterization was done by evaluating VAM colonization and spore counts during the development process at the interval of 15 days. The microbial diversity assessed was found to be comprised of bacteria such as *Alcaligens, Bacillus, Pseudomonas, Sarciana, Serratia, Streptococcus;* Fungi -*Asergillus flavus, A. fumigatus, A. niger, Penicillium, Rhizopus, Mucor* and Actinomycetes- *Micromonospora, Nocardia.* The mycorrhizosphere was found rich in microbial assemblages and comprising of total viable counts of bacteria- $7.8x10^6$, fungi- $4.6x10^5$ and Actinomycetes- $4.4x10^5$ per gm of air-dried soil (Fig. 4). The results indicate that spore count and root colonization were enhanced with increasing period of development process (Fig. 5a and 5b). The spore counts were increased from 10 – 576 spores / 100gm mycorrhizal soil from day 0-75, whereas in control soil it was found 10-25 spores/ 100gm from day 0-75. The mycorrhizal root colonization was 0% in MS and CS during the initial stage (0 day] and increased upto 78% on 75 days in MS, while in CS root

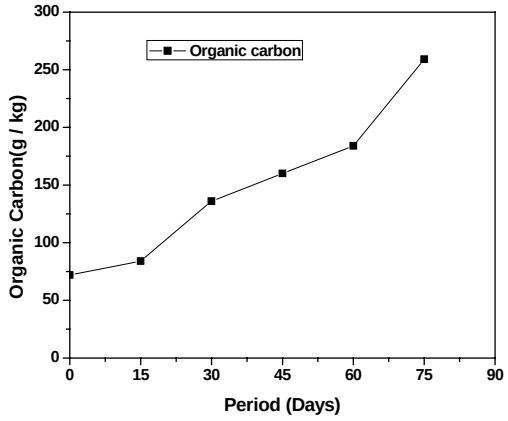

Fig. 1. Organic Carbon contents of the soil during the development of mycorrhizal soil. Organic Carbon was quantified from the soil samples taken at diffenent time intervals of 75 days of process. All the values are mean of three replicates.

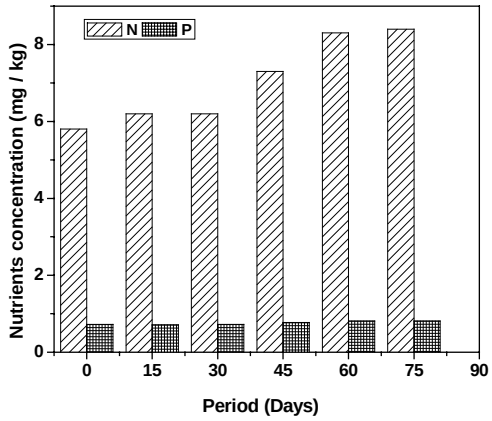

Fig. 2. Nitrogen [N] and phosphorus [P] contents of the soil during the development of mycorrhizal soil. The soil sample was analysed for N and P at the different time intervals of 75 days of process. All the values are mean of three replicates.

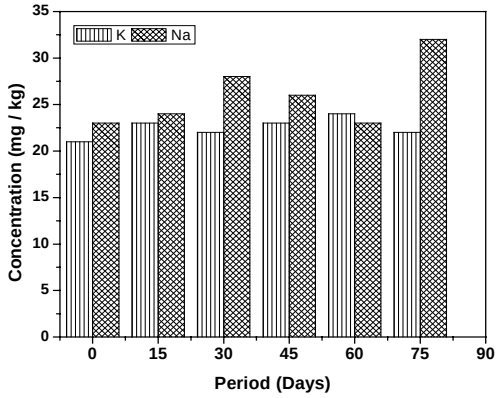

Fig. 3. Potassium [K] and sodium [Na] contents of the soil during the development of mycorrhizal soil. K and Na contents was quantified from the soil samples taken at diffenent time intervals of 75 days of process. All the values are mean of three replicates.

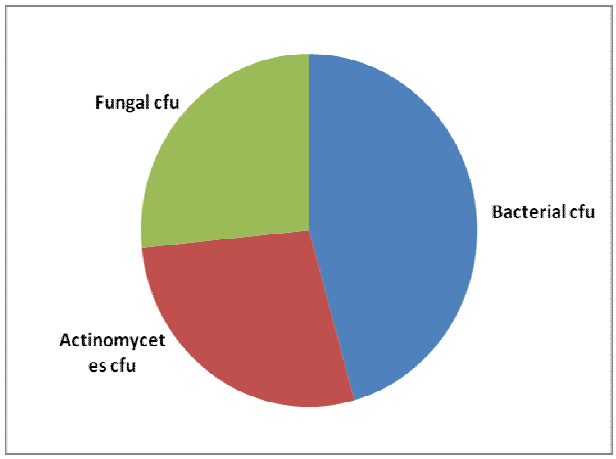

Fig. 4. Bacterial, fungal and Actinomycetes cfu present in the developed mycorrhizal soil. Log cfu/gm of soil. All the values are mean of three replicates.

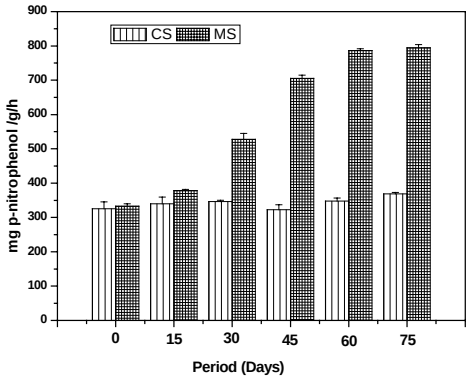

Fig. 6a. Acid phosphatase activity during Mycorrhizal development. CS- Control Soil; MS- Mycorrhizal Soil. All the values are mean of three replicates.

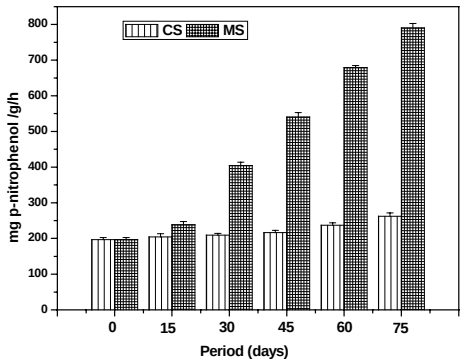

Fig. 6b. Alkaline phosphatase activity during Mycorrhizal development. CS- Control Soil; MS- Mycorrhizal Soil. All the values are mean of three replicates.

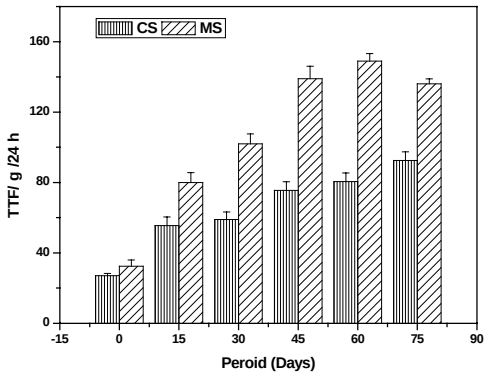

Fig. 7. Dehydrogenase activity during Mycorrhizal development. CS- Control Soil; MS- Mycorrhizal Soil. All the values are mean of three replicates.

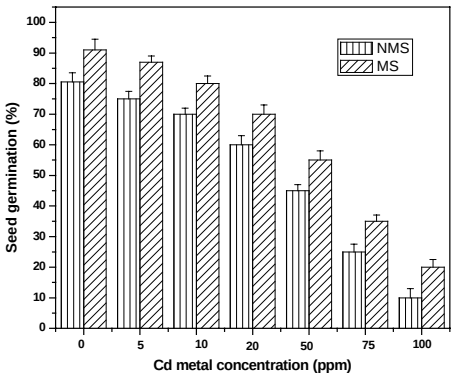

Fig. 8. Seed germination (%) of *Medicago sativa* plants in NMS and MS amended with various concentrations of cadmium metal. All the values are mean of three replicates.

colonization was reported only 6% on 75th days. The final spore count in developed mycorrhizal soil was found to be 546 spores/100gm of soil with 78% of root colonization. High levels of AM colonization observed with low nutrient diffusion rate, such as P, decreasing plant availability. Our results were in accordance and showed AM colonization was higher, when P mineralization was lower and microbial immobilization was higher.

In rhizosphere the enzyme activities released which make the suitable environment for the microbes to grow and multiply in numbers along with the root zone (Dick and Tabatai, 1992; Eivazi and Tabatabai, 1997; Dick *et al*., 2000). The enzyme activities assessed include acid and alkaline phosphatases and dehydrogenase. The results obtained indicate that the soil phosphatases (ACP and ALP) activity significantly increased in all soil samples during development of mycorrhizal soil and were higher in developed mycorrhizal soil comparatively with the control soil (Fig. 6a, 6b). Acid phosphatase activity increased 2.38 times on 75 days while alkaline phosphatase increased 4 times on 75 days. This increase, however, was proportionally higher for the first few days for acid phosphatase activity while in later stages there was increase in alkaline phosphatase activity after successful mycorrhizal colonization. The study reveals that alkaline phosphatase is generally higher when the soil is rich in organic matters. The high levels of organic matter (Fig. 3) in the soil represent sources of energy used by microorganisms that is the reason for microbial biomass and enzyme activities may increase with increasing period of experiment as there is increase in

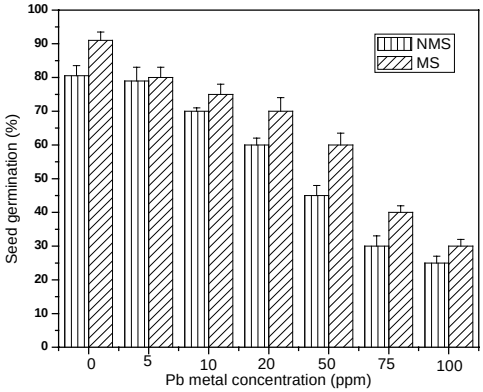

Fig. 9. Seed germination (%) of *Medicago sativa* plants in NMS and MS amended with various concentrations of lead metal. All the values are mean of three replicates.

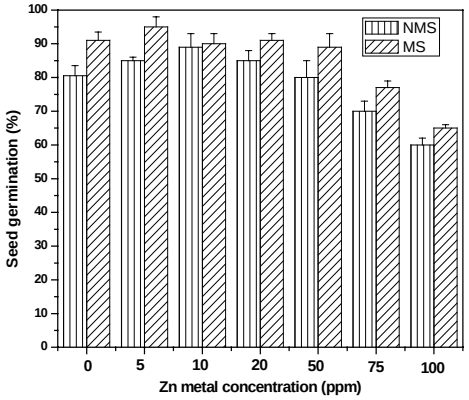

Fig. 10. Seed germination (%) of *Medicago sativa* plants in NMS and MS amended with various concentrations of zinc metal. All the values are mean of three replicates.

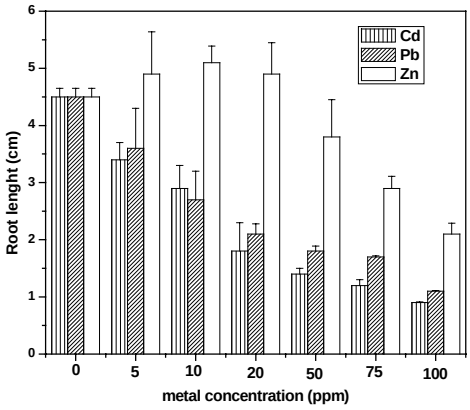

Fig. 11a. Root length of *M. sativa* grown in heavy metals (Cd, Pb and Zn) contaminated non mycorrhizal Soil (NMS). All the values are mean of three replicates.

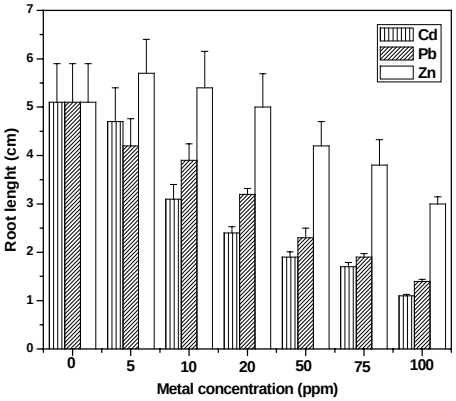

Fig. 11b. Root length of *M. sativa* grown in heavy metals (Cd, Pb and Zn) contaminated mycorrhizal Soil (MS). All the values are mean of three replicates.

organic matter contents. The acid phosphatase increases significantly in the soil with inorganic P. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. The data showed that DHA significantly increased during the development of mycorrhizal soil at different time intervals (Fig. 7). DHA activity has increased 4.18 times on 75 days. DHA can associate with changes in the microbial biomass due to long-term soil amendment (Goyal *et al*., 1993). Mycorrhizosphere soil's bacterial, fungal and Actinomycetes viable plate counts were assessed to relate DHA as indicator of soil microbial activity. The importance of soil microorganisms in these studies has been evaluated in terms of the content of nutrients in the microbial biomass. The increase in enzyme activities and nutrients in particular has been found to have suitable and favourable conditions for the plant growth (Rodrı´guez and Fraga, 1999; Oliveira *et al*., 2009). Mycorrhizosphere provide a direct link between soil and roots, and are renowned for their ability to increase plant mineral nutrients, notably P (Leyval *et al*., 1997; Gaur and Adholeya, 2004; Bush, 2008) and enhance phytoremediation.

In the laboratory setup, the toxicity of heavy metals at varying concentrations 0, 5, 10, 20, 50, 75 and 100ppm were assessed for seed germination and plant growth of *M. sativa* in mycorrhizal soil and non mycorrhizal soil separately. The percentage of seed germination and root/

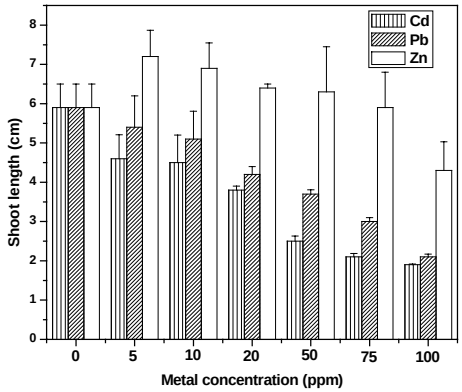

Fig. 12a. Shoot length of *M. sativa* grown in heavy metals (Cd, Pb and Zn) contaminated non mycorrhizal Soil (NMS). All the values are mean of three replicates.

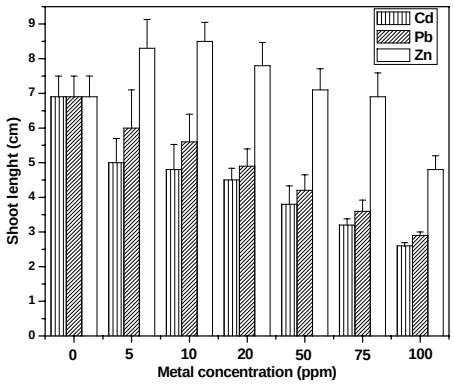

Fig. 12b. Shoot length of *M. sativa* grown in heavy metals (Cd, Pb and Zn) contaminated mycorrhizal Soil (MS). All the values are mean of three replicates.

shoot growth of plants was assessed for each heavy metal. The results showed that the seeds were found grown from a concentration of 0- 100 ppm in MS as well as NMS (Figs. 8-10) of each metal (Cd, Pb and Zn). The percentage of seed germination decreased with increasing concentrations of heavy metals. In NMS the percentage of seed germination for Cd decreased from 75-1% (5-100ppm), Pb was found 79- 25% (5-100ppm), and Zn was 85-60% (5-100 ppm). Besides, in MS the seed germination for Cd was found 87-20%, Pb was 80-30% and Zn was 95-65% from lowers to higher concentrations. Mycorrhizal soil has enhanced the seed germination by 5-20% more than non mycorrhizal soil. The results indicated that *M* .sativa tolerated Cd at 20ppm, however, levels at 50- 100ppm impacted plant growth as measured by plant root/ shoot length. The research findings show that the higher concentrations of the metal i.e. 50, 75 and 100ppm in respect of Cd, Pb and Zn are inhibitory to the growth of the plants in NMS. However in MS the growth was enhanced upto 50ppm in case Cd, Pb and Zn. MS which serve as a biofertilizers and provide condition favourable for seed germination and growth of the plants. Mycorrhizal fungi are an essential component of soil ecosystem. Mycorrhizae are ubiquitous symbiotic associations between plants and soil (Smith and Read, 1997) and their extra radical mycelium form bridges between plant roots and soil, and mediate the transfer of various elements into plants. It has been demonstrated that mycorrhizas could protect plants grown in metal-contaminated soils by enhancing metal retention in root and reducing metal partitioning to shoots (Leyval *et al*., 2002). There is direct evidence for the strong binding capacity of fungal mycelium to heavy metals, such as Zn and Cd (Chen *et al*., 2001; Joner *et al*., 2000). The findings of the present research proved that the nutrients taken up by the mycorrhizal fungi can lead to improved plant growth and reproduction. As a result, the plants grown in MS are often more competitive and better able to tolerate in heavy metals contaminated environment than the NMS.

The effect of heavy metals viz. Cd, Pb and Zn on the growth of root in non mycorrhizal and mycorrhizal soil is illustrated in figure 11a & 11b, respectively. Figure 12a and 12b report the data relative to the effect of metals (Cd, Pb and Zn) on shoot growth of *M. sativa* in non mycorrhizal soil and mycorrhizal soil. Mycorrhizal soil has promoted the roots/ shoots growth of *M. sativa* as compared to non mycorrhizal soil. The increase in metal concentrations in the germination medium leads to the reduction in roots/ shoots growth of the plants. The root length of *M. sativa* in mycorrhizal soil was found 38% - Cd, 54% -Pb and 65% -Zn more from the non mycorrhizal soil. Mycorrhizal soil has enhanced the shoot growth upto 23% (Cd), 26% (Pb) and 50% (Zn) more than non mycorrhizal soil. The increase in metal concentrations in the germination medium leads to the reduction in roots/ shoots growth of the plants. Research findings show that the growth of roots and shoots of *M. sativa* was found in order of Cd < Pb < Zn. Zn being the micro nutrient was found growth higher than the Cd and Pb. Cd was found phytotoxic for the growth as compared to Pb and Zn. Pb accumulates in the root and reduces the length of root and growth of plant. Whereas, Cd exposure to be toxic to roots and shoots proportionately. Cd is more phytotoxic to plant- *M. sativa.* The result of present research proved that seed germination and plant's growth were much better in MS compared to NMS. Mycorrhizae have enhanced the metal tolerance efficiency of alfalfa plants by providing high nutrients. Galli *et al.* (1994) suggested that mycorrhizae can play a crucial role in protecting plant roots from heavy metals.

Though Zn is an essential trace element for plants, it is highly toxic at elevated concentrations. Researchers have reported that the range of beneficial concentration of Zn is often very narrow for most plant species (Küpper *et al*., 2000; Clemens, 2006). On the other hand, Pb and Cd are widely known to be non-essential elements for plants, and both cause adverse effects on the plant's biochemical mechanisms, resulting in various symptoms of Phytotoxicity, such as chlorosis, reduction of biomass, inhibition of root elongation and finally death (Milone *et al*., 2003). Peralta *et al*. (2001) found that 20 and 40ppm of Cu, Cd and Ni inhibited ability of seeds of *Medicago sativa* to germinate and grow in the contaminated solid medium, whereas Zn did not reduce the seed germination. Compared to the control, at and above 10ppm Cr (VI) concentration, significant inhibitory effect on seedling growth of tested rice cultivars were detected by Xiong (1998). The experiment conducted by Peralta-Videa *et al*. (2004) showed that the tolerance of alfalfa plants to Cd, Cu and Zn was positively correlated to the age of the plants. They also reported that after four days germination, Cr, Cd, Ni, except Zn, had lethal effects on the alfalfa seedlings. The experiments of Gardea-Torresdey *et al*. (1999) have shown the ability of *M. sativa* to bind several metal ions under multi-contaminant conditions.

The seed germination trails have relevance in selection of plants and metal's concentrations in for their prospective use in phytoremediation. The research study shows that the plants of *M. sativa* were growing in mycorrhizal soil amended with heavy metals with a range of 5-50ppm. However the higher concentrations i.e. 75 and 100ppm were found to be toxic for plant growth and survival. The present research has proved the effectiveness of mycorrhizosphere for resistance of heavy metals which can be efficiently used for remediation of heavy metals using the recent advanced technique – phytoremediation.

CONCLUSION

The present research study deals with the development of mycorrhizal soil and the significance of enzymes released by plants and microbes in rhizosphere to tolerate the toxicants and bioaccumulate/ uptake of heavy metals. Heavy metals concentrations ranging from 5, 10, 20 and 50ppm were found tolerance by the seed of *Medicago sativa.* The heavy metals above these concentrations (17 and 100ppm) were found inhibitory for the growth of the plants. The study has proved the significance of mycorrhizal soil as compared to non mycorrhizal soil for phytoremediation of heavy metals upto a concentration of 50 ppm, which could be used for the remediation of heavy metals to clean up the environment.

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