

Mycorrhizoremediation of Cadmium and Nickel in Peri Urban Soil of Varanasi

Barun Manjhee¹, Sumita Pal^{2,3}, Alvina Farooqui³, H.B. Singh²,
Sunita Kumari Meena¹, Indra Bahadur Maurya¹ and Amitava Rakshit¹

¹Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, India.

²Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, India.

³Department of Biosciences, Integral University, Lucknow - 226 026, India.

(Received: 06 April 2015; accepted: 09 June 2015)

The utilization of wastewater resources is essential for meeting the ever increasing demand for irrigation water, but on the other hand it may lead to adverse health implications by heavy-metal contamination in agricultural production systems. Chemical, physical and biological methods are used to remove toxic metals from soil. Among biological methods, Mycorrhizal fungi can play a role in bioremediation of heavy metal pollution in soil. A factorial CRD experiment was planned involving maize cultivars (HM4, AZAD UTTAM ,HQPM1) and mycorrhiza inoculations (M0: Uninoculated; M1: *Glomus fasciculatum* + *Gigaspora* sps.; M2: *Glomus mossae* and M3: *Glomus intraradices* + *Gigaspora* sps. + *Glomus mossae*) to evaluate the performance of different cultivar of baby corn under periurban soil with more than twenty years of irrigation history with waste water. All the mycorrhizal treated plots gave significantly higher dry matter yield than the uninoculated plots. There was also positive correlation between mycorrhizal colonization and dry matter yield. The concentrations of heavy metal in the shoots were significantly higher in no AM treatment but addition of AM fungi confers protections against toxic metals retaining them in the fungal structure. In the present experiment the mycorrhizal mixed inoculum (*Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*) was found to cater important role in metal tolerance and accumulation.

Keywords: Mycorrhizoremediation, Cadmium, Nickel, Ecosystems.

Land irrigation with waste water has a history in agriculture area in India, its capacity In India through the waste water treatment capacity is 7044 Ml day⁻¹. Only 27% of total waste water discharged is treated and some of untreated waste water from industries and communities is used for land irrigation. Although waste water irrigation may increase the agricultural production and farmer incomes, many contaminants in municipal and industrial waste water are sequestered in the soil and consequently pose environmental problem. HMs in particular cadmium, nickel, mercury and

lead are those that are the most toxic to human health (Adriano, 2003). The accumulation of these elements in the organism leads to a number of diverse and deleterious effects, both in the long and the short term, and varies according to the type of metal (Benavides et al., 2005). The classic symptoms of heavy metal intoxication include irritability, mood change, depression, headaches, tremor, and loss of memory and reduced capacity of sight. Cd and NI is the most toxic HMs, concentration of these metals in industrial sources is in the range of 0.1-100 mgL⁻¹ and 0.2- 450 ppm respectively. The retention of Cd by bacterial biomass in industrial effluent could avoid an increase in Cd contaminated of waste water sources.

* To whom all correspondence should be addressed.
E-mail: sumitapal99@gmail.com

Plants are known to accumulate Cd and as a result the Ni uptake from vegetables will be eminent (Pal et al., 2013). The uptake of too large quantities of nickel has the following consequences: lung cancer, nose cancer, larynx cancer, prostate cancer, respiratory failure, birth defects, asthma and chronic bronchitis. Food chain translocation of HMs is one of the consequences of soil polluted with such elements and excessive intake of HMs is associated with human health problems e.g. itai-itai and minimata disease. Varanasi and the adjoining cultivated areas are famous for the cultivation of world class production of vegetables (cole- crops and solanaceous crops), fruits (especially mango and guava), cereals (rice and wheat) and pulses. Moreover for the purification of the most important river, The Ganga, the Ganga Action Plan (GAP) was set up by Government of India. Thus at present two sewage treatment plant were set up through the GAP in Phase-II. The farmers of these areas are now using routinely the sewage water for irrigation purpose

and sludge's for manuring purpose. Thus, through sewage and sludge treatment in cultivated lands and the industrial wastes the fertile deep alluvial soils are now suffering from soil sickness and resulting the contamination of toxic HMs in crops particularly vegetables. Not only had the crops biota of the holy river, Ganga also contaminated with the runoff of these HMs. To overcome this chronic problem there is an urgency of need of the adoption of bioremediation technique for the soils and water (both surface and ground) in Varanasi.

The biological treatment is an innovative technology available for HMs polluted waste water. Since microorganism have developed survival strategies in HMs polluted habitats. There different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, bio-mineralization or bio-sorption can be applied either ex-situ or in-situ to design economical bioremediation processes. The choice of an adequate matrix for cell immobilization affects the performance of the process. Since the metal

Table 1. Effect of cultivars and mycorrhiza application on dry weight (g/plant) of maize at different growth stages

Treatments		Variety			Mean
		V1	V2	V3	
21 DAS	M0	0.11	0.17	0.10	0.13
	M1	0.20	0.35	0.20	0.25
	M2	0.13	0.23	0.19	0.19
	M3	0.46	0.81	0.55	0.61
	Mean	0.23	0.39	0.26	0.29
SEM±		M: 0.01	V: 0.01	M*V: 0.02	
CD(P=0.01)		M: 0.05	V: 0.03	M*V: 0.09	
42 DAS	M0	0.38	1.38	0.97	0.91
	M1	1.37	2.58	2.23	2.06
	M2	1.05	1.61	2.03	1.56
	M3	2.14	3.12	2.30	2.52
	Mean	1.23	2.17	1.88	1.76
SEM±		M: 0.10	V: 0.06	M*V: 0.18	
CD(P=0.01)		M: 0.40	V: 0.22	M*V: 0.69	
63 DAS	M0	2.09	1.92	2.02	2.01
	M1	2.66	5.18	3.18	3.67
	M2	2.60	2.35	2.41	2.45
	M3	3.35	8.89	4.71	5.65
	Mean	2.68	4.58	3.08	3.45
SEM±		M: 0.07	V: 0.04	M*V: 0.11	
CD(P=0.01)		M: 0.26	V: 0.14	M*V: 0.45	

V1: HM-4 V2: Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

biosorption efficiency can be affected by using these heterogeneous systems. Again the lack of mycorrhiza can hamper the vegetation of the HMs contaminated mine spoil or other degraded sites (Khan et al., 2000). The introduction of an AM fungal inoculums into these areas could be a strategy for enhancing the establishment of mycorrhizal crop species (Diaz et al., 1996; Guo et al., 1996; Joner et al., 2000). AM fungal isolates differ in their effect on HMs by plants.

Maize (*Zea mays* L) is the third most important cereal crop next to rice and wheat and has the highest production potential among the cereals in Indo Gangetic Plains of India. For diversification and value addition of maize as well as growth of food processing industries, an interesting recent development is of growing maize for vegetable purpose, which is known as 'baby corn'. It is a small young corn ear harvested at the stage of silk emergence. Attention is now being paid to explore its potential in India, for earning foreign exchange besides higher economic returns

to the farmers. Thus, it is essential to explore the possibilities and performance of baby corn growing in peri urban areas where it has an excellent marketing advantage, especially in light of the rapid increase in demand for organic products. Further the aim of our study is to explore the possibility of use of microorganism especially AM that are able to bio sorb HMs under soil system and can be easily immobilized in polymeric matrices for the development of a waste water bioremediation process.

MATERIALS AND METHOD

Pot experiment

A Pot experiment was conducted to investigate to mycorrhizoremediation of Cd and Ni under peri urban soils of Gangetic alluvial region with more than twenty years of irrigation history with waste water during spring season. Surface soil sample (0-18 cm) was collected from adjoining areas of Dinapur waste treatment plant, Varanasi,

Table 2. Effect of cultivars and mycorrhiza application on fresh root weight (g/plant) of maize at different growth stages

Treatments		Variety			
		V1	V2	V3	Mean
21 DAS	M0	0.85	1.00	0.68	0.84
	M1	1.25	1.23	2.27	1.58
	M2	1.25	2.51	0.92	1.56
	M3	3.96	3.36	3.91	3.74
Mean		1.83	2.03	1.95	1.93
SEM±		M: 0.04	V: 0.02	M*V: 0.07	
CD(P=0.01)		M: 0.17	V: 0.09	M*V: 0.29	
42 DAS	M0	1.20	4.24	4.89	3.44
	M1	2.66	8.46	7.99	6.37
	M2	1.81	8.54	5.71	5.35
	M3	4.03	8.57	8.22	6.94
Mean		2.42	7.45	6.70	5.53
SEM±		M: 0.12	V: 0.06	M*V: 0.20	
CD(P=0.01)		M: 0.46	V: 0.25	M*V: 0.80	
63 DAS	M0	11.56	17.24	12.93	13.91
	M1	23.47	36.45	30.75	30.22
	M2	17.20	26.37	26.51	23.36
	M3	47.23	45.70	36.75	43.23
Mean		24.86	31.44	26.74	27.68
SEM±		M: 0.48	V: 0.26	M*V: 0.84	
CD(P=0.01)		M: 1.91	V: 1.05	M*V: 3.31	

V1: HM-4 V2: Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

U.P. The mycorrhizal inoculum in different combinations was spread (@5 g kg⁻¹ containing 60 spore's g⁻¹) and mixed thoroughly into the top 2.5 cm of soil.

Maize varieties

Maize seeds (cv. HM4, Azad Uttam and HQPM1), were collected from CCS HAU, Karnal, Haryana and CSAU & T, Kanpur, UP. Maize seeds were sown on the pots of 1 kg capacity which were kept with consistent moisture at water holding capacity. Mineral fertilizations to each pot as recommended dosage (160:80:60 kg/ha). All the agronomic practices were carried out uniformly to raise the crop. A factorial CRD experiment was planned involving maize cultivars (V₁: HM4; V₂: AZAD UTTAM & V₃: HQPM1) and mycorrhiza inoculations (M₀: Uninoculated; M₁: *Glomus fasciculatum* + *Gigaspora* spp. M₂: *Glomus mossae* and M₃: *Glomus intraradices* + *Gigaspora* spp. + *Glomus mossae*). The mycorrhiza was applied to soil in respective pots at the time of sowing. Sand soil culture of *Glomus mosseae* + *Glomus*

fasciculatum + *Gigaspora* spp. brought from GKVK, UAS Bangaluru. The plants were harvested at 21, 42 and 60 days after sowing.

Plant sample analysis

The plants were collected at 21, 42 and 60 DAS and at harvesting time. The Maize plant samples were collected from the pot and washed with distilled water, after taking fresh weight were air drying; plant samples were stored in packets with pencil labelling. After hot air oven drying (65 ± 2°C) to constant weight and sample dry weight was recorded. The samples has been grinded and then was stored in desiccators (fused CaCl₂ was in the bottom) for further chemical analysis.

Plant Digestion

Cd and Ni estimation of plant sample, the dry processed plant samples were grinded and digested in di-acid mixture (HNO₃, HCl in ratio of 4:1) and analyzed for HMs content by Atomic Absorption Spectrophotometer (AAS).

Root sampling

Root systems were separated from shoots

Table 3. Effect of cultivars and mycorrhiza application root length (cm/plant) of maize at different growth stages

Treatments		Variety			Mean
		V1	V2	V3	
21 DAS	M0	178.59	167.27	149.23	165.03
	M1	281.96	545.01	456.10	427.69
	M2	265.53	246.39	171.78	227.90
	M3	747.87	649.46	817.84	738.39
	Mean	368.49	402.03	398.74	389.75
SEM±	M: 12.00	V: 6.57	M*V: 20.79		
CD(P=0.01)	M: 47.48	V: 26.00	M*V: 82.23		
42 DAS	M0	2042.66	3035.91	2338.58	2472.38
	M1	3936.45	6496.95	5341.54	5258.31
	M2	3209.04	4736.17	4381.79	4109.00
	M3	7481.76	7791.75	6527.48	7267.00
	Mean	4167.48	5515.20	4647.35	4776.67
SEM±	M: 132.65	V: 72.65	M*V: 229.75		
CD(P=0.01)	M: 524.67	V: 287.38	M*V: 908.76		
63 DAS	M0	1920.43	2974.76	2224.04	2373.08
	M1	3718.70	6415.26	5200.66	5111.54
	M2	3034.09	4623.30	4276.09	3977.83
	M3	7256.34	7666.52	6338.82	7087.23
	Mean	3982.39	5419.96	4509.90	4637.42
SEM±	M: 134.27	V: 73.54	M*V: 232.57		
CD(P=0.01)	M: 531.12	V: 290.90	M*V: 919.92		

V1: HM-4 V2: Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

and the fresh root biomass was weighed immediately. Half of each root sample was fixed in FAA (37% Formaldehyde–Glacial Acetic Acid–95 Ethanol, 9:0.5:0.5, V: V: V) for quantification of AM fungal colonization and vesicular numbers. Root samples were carefully separated from soil by washing and flooding over sieves. After cleaning of any foreign material, roots were preserved in 20 per cent ethanol for measurement of root length by line interception method of Tennant (1975), using the formula

$$RL = (11/14) \times N \times G$$

Where, N is total numbers of intercepts of root with vertical and horizontal grid lines;

G is grid square dimensions, cm; RL is root length, cm.

Analysis of microbiological parameters

Root infection was assessed on a representative root sample taken from each plot at each harvest. At harvest roots were taken from plants in fixed positions evenly distributed over

each plot. The roots from each plot sample were separated, washed free from soil and cut into 1.5 cm lengths. Root samples were stained with trypan blue (Philips and Hayman, 1970). Mycorrhiza infection of each plant was determined by estimating the percent of root segments colonised with AM as described by Bierman and Linderman (1981). Alkaline hydrolysis of root samples with 10% potassium hydroxide was done at $90 \pm 2^\circ\text{C}$ in an oven for 8–10 minutes to clear the plant cytoplasm depending upon the stiffness of the root. The roots were then washed in several changes of water and then treated with 1N hydrochloric acid for 10 minutes and ultimately stained by 0.05% trypan blue (made in lactophenol) for about 24 hours. A minimum of 50 root fragments were examined at each time. Percent root infection was obtained as follows

% Root infection = (100 × Number of root segments infected with AMF infections) / Total number of segments counted.

Table 4. Effect of cultivars and mycorrhiza application on root infection (%) of maize at different growth stages

Treatments		Variety			Mean
		V1	V2	V3	
21 DAS	M0	10.00	10.00	16.67	12.22
	M1	23.33	33.33	23.33	26.67
	M2	16.67	23.33	20.00	20.00
	M3	26.67	35.00	36.67	32.78
	Mean	19.17	25.42	24.17	22.92
SEM±	M: 2.87	V: 1.57	M*V: 4.98		
CD(P=0.01)	M: 11.37	V: 6.23	M*V: 19.69		
42 DAS	M0	13.33	13.33	10.00	12.22
	M1	50.00	60.00	53.33	54.44
	M2	40.00	46.67	45.00	43.89
	M3	63.33	70.00	80.00	71.11
	Mean	41.67	47.50	47.08	45.42
SEM±	M: 3.32	V: 1.82	M*V: 5.75		
CD(P=0.01)	M: 13.14	V: 7.20	M*V: 22.76		
63 DAS	M0	12.37	12.23	9.40	11.33
	M1	48.63	58.63	51.73	53.00
	M2	38.73	45.17	43.93	42.61
	M3	61.87	68.63	78.63	69.71
	Mean	40.40	46.17	45.93	44.16
SEM±					
CD(P=0.01)	NS				

V1: HM-4 V2: Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

RESULTS AND DISCUSSION

Dry biomass

All the mycorrhizal treated plots gave significantly higher dry matter yield than the uninoculated control plots. Results showed that among the three cultivars tested in the pot experiment Azad Uttam performed well throughout the different growth stages. At 21 DAS the dry biomass of Azad Uttam was recorded 69.5 and 50% higher compared to HM-4 and HQPM-1 respectively. However at knee height growth stage, the dry biomass was higher by 76.4 and 15.4% respectively and at harvest, Azad Uttam maintained the similar trend, with 70.8 and 48.7% higher dry biomass than HM-4 and HQPM-1 respectively. Among the different mycorrhiza combinations tested M₃ performed best followed by M₁, M₂ and M₀. Similar benefits have been reported earlier for many cereals (Habte, 2000; Garg and Chandel, 2010). These results show that the mixed inoculum of

AM can survive and improved the dry matter accumulation under HMs stress and can colonize maize roots to increase mediation in plant growth. There was also positive correlation between mycorrhizal colonization and dry weight (r=0.71). However, at 42 DAS, M₃ was significantly higher over uninoculated M₀(176%), M₁(53.9%) and M₂(61.5%) respectively, and at harvest M₃ performed in a similar note producing 181, 53.9 and 130% more dry matter yield over M₀, M₁ and M₂ respectively. These findings are in accordance with Chen *et al.* (2005).

Root weight

HM-4 showed the lowest root fresh weight, while the other two cultivars presented higher root fresh weight. The trend demonstrated by HM-4 may be indicative of a sensitive genotype to heavy metal stress. At 21 DAS, Azad Uttam was found to produce 11% higher fresh root than HM-4 and was at par with HQPM-1. At 42 DAS, among the three different cultivars of baby corn, Azad

Table 5. Effect of cultivars and mycorrhiza application on cadmium concentration (ppm) of maize at different growth stages

Treatments		Variety			Mean
		V1	V2	V3	
21 DAS	M0	0.015	0.038	0.025	0.026
	M1	0.011	0.020	0.004	0.012
	M2	0.011	0.028	0.019	0.020
	M3	0.010	0.005	0.003	0.006
	Mean	0.012	0.023	0.013	0.016
	SEM±	M: 0.0003	V: 0.0002	M*V: 0.0006	
	CD(P=0.01)	M: 0.0013	V: 0.0007	M*V: 0.0022	
42 DAS	M0	0.013	0.037	0.024	0.025
	M1	0.011	0.019	0.004	0.011
	M2	0.010	0.028	0.016	0.018
	M3	0.010	0.005	0.002	0.006
	Mean	0.011	0.022	0.012	0.015
	SEM±	M: 0.0006	V: 0.0003	M*V: 0.0011	
	CD(P=0.01)	M: 0.0002	V: 0.0001	M*V: 0.0004	
63 DAS	M0	0.002	0.027	0.012	0.014
	M1	0.008	0.014	0.010	0.011
	M2	0.008	0.023	0.010	0.013
	M3	0.002	0.012	0.009	0.008
	Mean	0.005	0.019	0.010	0.011
	SEM±				
	CD(P=0.01)				

V1: HM-4 V2: Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

Uttam was reported to produce higher root mass compared to HM-4 (20.78%) and HQPM-1(11.2%) respectively. At harvest Azad Uttam maintained the similar trend with 30.1 and 7.5% higher root growth than HM-4 and HQPM-1 respectively. When mycorrhizal fungi colonize the plant's root system, they create a network that increases the plant's capacity to absorb more water and nutrients such as phosphorus, copper and zinc (Rai et al., 2013). This process in turn enhances growth and favours rapid development of roots and plants. Among the different mycorrhiza combinations tested at different growth stages M₃ performed best followed by M₁, M₂ and M₀ at 21 DAS. M₃ produced 345.2, 136% and 139.7% more fresh root weight over M₀, M₁ and M₂. At 42 DAS, M₃ combinations of mycorrhiza performed 101.1% maximum fresh root weight than un inoculated treatment (M₀) and 8.9 29.7% over M₁ and M₂. However, at harvest M₃ produced 210, 43 and 85% more fresh root weight as compared to M₀, M₁ and M₂ respectively. Similar results were found by Chen et al. (2006).

Root length

Results of root of baby corn has increased with increasing age up to 42 DAS and then declined 63 DAS in all the treatments with reference to cultivars and mycorrhizae addition. The data presented in table revealed that the root length of baby corn was significantly increased at different growth stages in all three types of cultivars which have been conducted in pot experiment. The root length of plant was ~ 9% higher in Azad Uttam compared to HM-4 and at par with HQPM-1. Among those two cultivars one of them HQPM-1 was ~ 8% higher than HM-4. At 42 DAS, among three cultivars the mean root length of plant was reported maximum in Azad Uttam, which was almost 18.6 and 32.3% grater over HQPM-1 and HM-4 respectively. In case of at harvest, all three varieties of baby corn among them the root length of Azad Uttam was also 20.1 and 36.1% higher than HQPM-1 and HM-4 respectively. Among the different mycorrhiza combinations tested M₃ performed highest followed by M₁, M₂ and M₀. Throughout the growth stages addition of mycorrhiza improved

Table 6. Effect of cultivars and mycorrhiza application on nickel concentration (ppm) of maize at different growth stages

Treatments		Variety			
		V1	V2	V3	Mean
21 DAS	M0	0.253	0.297	0.282	0.277
	M1	0.217	0.265	0.253	0.245
	M2	0.241	0.292	0.268	0.267
	M3	0.204	0.236	0.228	0.223
	Mean	0.229	0.273	0.258	0.253
SEM±	M: 0.0038	V: 0.0021	M*V: 0.0065 NS		
CD(P=0.01)	M: 0.0149	V: 0.0081	M*V: 0.0257		
42 DAS	M0	0.250	0.279	0.244	0.258
	M1	0.215	0.253	0.217	0.228
	M2	0.241	0.263	0.239	0.248
	M3	0.195	0.219	0.201	0.205
	Mean	0.225	0.254	0.225	0.235
SEM±	M: 0.0015	V: 0.0008	M*V: 0.0026		
CD(P=0.01)	M: 0.0060	V: 0.0033	M*V: 0.0105		
63 DAS	M0	0.237	0.247	0.232	0.239
	M1	0.205	0.215	0.203	0.208
	M2	0.223	0.220	0.219	0.221
	M3	0.164	0.192	0.188	0.181
	Mean	0.207	0.218	0.210	0.212
SEM±	M: 0.0017	V: 0.0010	M*V: 0.0030		
CD(P=0.01)	M: 0.0069	V: 0.0038	M*V: 0.0120		

V1: HM-4 V2:Azad Uttam V3: HQPM-1 M0: Uninoculated M1: *Glomus fasciculatum* + *Gigaspora* species. M2: *Glomus mossae* M3: *Glomus intraradices* + *Gigaspora* species. + *Glomus mossae*

the root infections of plant in all cultivars of baby corn. Different mycorrhiza performed differently under varied heavy metal content and composition. At 21 DAS, M_3 was able to induce significantly higher root length over M_0 (347.4%), M_1 (72.6%) and M_2 (223.9%). At 42 DAS, among the different combinations of mycorrhiza M_3 produced 193, 38 and 76% higher root length compared to M_0 , M_1 and M_2 (Fortuna *et al.*, 1992; Mohammad *et al.*, 1995; Chen *et al.*, 2006).

Root infection

Higher concentrations of HMs in soil have an adverse effect on microorganisms and microbial processes, among soil microorganisms, mycorrhizal fungi are the only ones providing a direct link between soil and roots, and can therefore be of great importance in HMs availability and toxicity to plants. Results showed that the degree of mycorrhizal colonization in different treatments, the mycorrhizal colonization of roots generally increased up to middle of the growing season with maximum colonization at the second harvest (42 DAS) (Figure 1). Low percentage (10-12%) of mycorrhizal infection was observed in root of non inoculated maize plants, whereas all the plants roots inoculated with AM inoculum were mycorrhized. This is in accordance with the previous observations by that HMs having been reported to reduce AM infection at high concentration of heavy metal in soil. Percentage of mycorrhizal colonization was maximum with M_3 treatment which was statistically significant during the different growth stages of maize.

The fact that mycorrhizal colonization occurred in most of these pots suggests metal tolerance of AM fungi. At 21 DAS, The mean root infections of plant were found maximum in Azad Uttam and the trend was maintained throughout the different growth stages. Throughout the growth stages addition of mycorrhiza improved the root infections of plant in all cultivars of baby

corn. Among the different mycorrhiza combinations tested M_3 performed highest followed by M_1 , M_2 and M_0 at 21 DAS. However, at 42 DAS, different combinations of mycorrhiza performed differently, among them M_3 was reported to infect higher proportions of root followed by M_0 , M_1 and M_2 . At harvest root infections rate was decreased as compared 42 DAS (Berta *et al.*, 1995).

HMs (Cd and Ni) uptake by maize

Several studies indicate that colonization of plants by AMF confers protections against HMs toxicity. Apparently, mycorrhizae can enhance plant uptake of toxic metals, but they may also afford protection from these metals. The concentrations of Cd and Ni in the shoots were significantly higher in pots with treated no AMF treatment; results showed that the concentration of Cd and Ni was gradually decreased with advancement of growth stages. Significantly higher concentration of Cd and Ni was recorded with V_2 followed by V_3 , V_1 . Among, the different mycorrhiza combinations tested M_3 performed best throughout the growth stages followed by M_1 and M_2 . Addition of mycorrhiza improved the HMs reclamation capacity to plants (Leyval *et al.*, 2002; Davies *et al.*, 2001). Higher concentrations of HMs in soil have an adverse effect on microorganisms and microbial processes (Leyval *et al.*, 1997). The mycorrhizal plants in have been reported to have a significantly lower concentration of metal in shoots although the influence of AMF on bioavailability of HMs could not be generalized. Again under adverse conditions AMF might be more important for plant HMs resistance and under the optimized conditions of normal agricultural practice, AMF colonization even could increase plant absorption from polluted soil, and cleansed polluted sites by removing above ground parts (Gildon and Tinker 1983; Killham and Firestone 1983). The effects of AMF on plant uptake of heavy metals, such as Ni and Cd have been studied extensively in recent

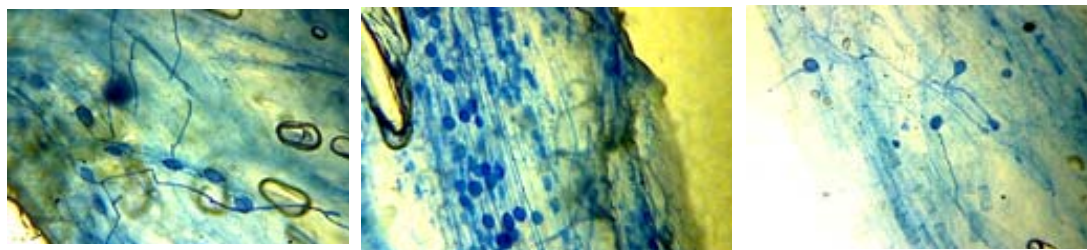


Fig. 1. Root infection in maize by different mycorrhiza combinations

years (Zhu *et al.*, 2001; Chen *et al.*, 2003). On the other hand, it was also reported that this AMF protective effect depends on the plant species and even on the plant variety and that AMF do not necessarily prevent metal uptake by the host plant; on the contrary, they may be active in the uptake process, since mycorrhizal plant species growing on mine tailings have been shown to be metal accumulators (Hayes *et al.*, 2003).

CONCLUSION

So from the above experiment it can be conferred that AM associations play an important role in protecting plants in heavy metal contaminated sites. Plants with mycorrhizal association, growing in heavy metal contaminated areas, are more tolerant as compared to the plants of clean areas. Fungal hyphae can approach the soil which is beyond the approach of plant roots; hence absorption of water and mineral nutrients is enhanced by increasing the exposed absorptive area. Healthy plants are more tolerable to different stress conditions as compared to unhealthy plants. Hence plants having mycorrhizal association show toxic metals accumulation and immobilization of excessive metals such as Cd and Ni without disturbing the mobilization of useful micro and macro-nutrients, which can further help to improve the yield of plants.

REFERENCES

- Adriano, D.C. (de): Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals (2nd Edition), Springer, New York, USA. 2003.
- Benavides, M.P., Gallego, S.M., Tomaro, M.L. Cadmium toxicity in plants. *Braz. J. Plant Physiol.*, 2005; **17**: 21-34.
- Chen, X., Wu, C., Tang, J., Hu, S. Arbuscular mycorrhizae enhance metal lead uptake and growth of host plants under a sand culture experiment. *Chemos.*, 2005; **60**: 665-671.
- Davies, J.F.T., Puryear, J.D., Newton, R.J., Egilla, J.N., Grossi, J.A.S. Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). *J. Plant Physiol.*, 2001; **158**: 777-786.
- Diaz, G., Azcón-Aguilar, C., Honrubia, M. Influence of arbuscular mycorrhiza on heavy metal (Zn and Pb) uptake and growth of *Lygедum spartum* and *Anthyllis cytisoides*. *Plant Soil*, 1996; **180**: 241-249.
- Garg, N., Chandel, S. Arbuscular mycorrhizal networks: process and functions A review. *Agron. Sustain. Dev.*, 2010; **30**: 581-599.
- Guo, Y., George, E., Marschner, H. Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean and maize plants. *Plant Soil*, 1996; **184**: 195-205.
- Habte, M. Mycorrhizal fungi and plant nutrition. In: Plant nutrient management in Hawaii's soils, approaches for tropical and subtropical agriculture, Silva JA, Uchida R (eds), College of Tropical Agriculture and Human Resources, *Univer. of Hawaii, Manoa, USA*. 2000; 127-131.
- Joner, E.J., Leyval, C. Uptake of ¹⁰⁹Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytol.*, 1997; **135**: 353-360.
- Joner, E.J., Leyval, C., Briones, R. Metal binding capacity of arbuscular mycorrhizal mycelium. *Biol. Fertil. Soils*, 2000; **226**: 227-234.
- Khan, A.G., Kuek, C., Chaudhry, T.M., Khoo, C.S., Hayes, W.J. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation, *Chemos.*, 2000; **41**: 197-207.
- Leyval, C., Joner, E. Bioavailability of heavy metals in the mycorrhizosphere. Trace elements in rhizosphere. *CRC Press*, New York, 2001; 165-185.
- Leyval, C., Joner, E.J., Delval, C., Haselwandter, K. Potential of arbuscular mycorrhizal fungi for bioremediation, in: Mycorrhizal Technology in Agriculture. *CRC Press*, New York, 2002; 175-186.
- Pal, S., Singh, H.B., Rakshit, A. Potential of different crop species for Ni and Cd phytoremediation in peri-urban areas of Varanasi district, India with more than twenty years of wastewater irrigation history. *Italian J. Agron.*, 2013; **8**(1):58-64.
- Rai, A., Rai, S., Rakshit, A. Mycorrhiza-mediated phosphorus use efficiency in plants. *Environ. Exp. Biolo.*, 2013; **11**: 107-117.
- Weissenhorn, I., Leyval, C., Berthelin, J. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant Soil*, 1993; **157**: 247-256.
- Zhu, Y.G., Christie, P., Laidlaw, A.S. Uptake of Zn by Arbuscular mycorrhizal white clover from Zn-contaminated soil. *Chemos.*, 2001; **42**: 193-199.
- Gildon, A., Tinker, P.B. Interactions of vesicular-arbuscular mycorrhizal infection and heavy

- metals in plants. I. The effect of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist.*, 1983; **95**: 247-61.
19. Phillips, J.M., Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 1970; **55**: 157-160.
 20. Tennant, D.M. A test of a modified line intersect method for estimating root length. *J. Ecol.*, 1975; **63**: 995-1001.
 21. Schüepp, H, Miller, D.D., Bodmer, M. A new technique for monitoring hyphal growth of vesicular-arbuscular fungi through soil. *Trans. Br. Mycol. Soc.* 1987; **89**: 429-435.
 22. El-Kherbawy, M., Angle, J.S., Heggo, A., Chaney, R.L. Soil pH, rhizobia, and vesicular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (*Medicago sativa* L.). *Biol. Fertil. Soils* 1989; **8**: 61-65.
 23. Weissenhorn, I., Leyval, C. Root colonization of maize by a Cd-sensitive and a Cd-tolerant *Glomus mosseae* and cadmium uptake in sand culture. *Plant Soil.* 1995; **175**: 233-238.