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Effect of Arbuscular Mycorrhizal Fungus on the Accumulation of Ni and Cd in Rice (*Oryza sativa* L.) Crop

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Abstract

Use of wastewater for irrigation has been associated with a number of advantages, but it may lead to adverse health implications by heavy-metal contamination in agricultural production systems. Keeping this in view the following aspect was designed in Kamauli (80.71 meter MSL and lies between 25°18′ N latitude and at 80°36′ E longitude) in farmers field to evaluate the impact of treated wastewater irrigation on soil properties including heavy metal concentrations and bioaccumulation in a cereal crop rice (*Oryza sativa* L. var Sonam). The highest shoot biomass was found in plants inoculated with mixed arbuscular mycorrhiza (AM) inoculum and half recommended phosphorus fertilizer application. All the mycorrhizal treated plots gave significantly higher dry matter yield than the uninoculated and uninoculated control plots. The concentrations of Cd and Ni in the shoots were significantly higher in plots irrigated with poor quality water and no AM treatment but addition of AM fungi confers protections against toxic metals retaining them in the fungal structure.
Key words : Arbuscular mycorrhiza, Nickel, Cadmium, Inceptisol, Rice.

Industrial or municipal waste water is mostly used for the irrigation of crops mainly in periurban ecosystem due to easy availability disposal problem and scarcity of fresh water and an increases in the total nitrogen, total phosphorus and total viable count was also recorded. Irrigation with waste water is known to contribute significantly to the heavy metal content of soil (1). The total amount of heavy metal in a soil can be used to quickly assess the condition of a soil through comparison with regulatory guide line values. How ever increasingly the bioavailable fraction of heavy metal in soil is deemed essential for risk assessment purposes. Heavy metal mobility and availability in soil is frequently studied with sequential extraction procedure which allow partitioning the total metal content in to different fractions. Heavy metal in particular cadmium and nickel are those that are the most toxic to man. 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 it varies according to the type of metal. They can cause damage to the kidney, to the nervous and immune system, and in some cases they can have cancerous effects (2). The classic symptoms of heavy metal intoxication include irritability, mood change, depression, headaches, tremor, loss of memory and reduced capacity of sight. Cadmium and nickel are the most toxic heavy metals whose environmental appearance is a consequence of human activities fundamentally from industrial source. Cadmium and nickel concentration in the range 0.1—100 mg/liter are typical in waste water from several industries. For these elements daily maximum recommendation lies between 0.5—0.73 mg/liter and monthly average of 0.20 mg/liter, when best practicable control technology is applied. Because of its high mobility, both of them can be found either in water or soil of any polluted ecosystem. Mycorrhizal fungi provide a direct link between soil and roots therefore be of great importance in heavy metals tolerance uptake and accumulation by mycorrhizal hyphae. This open up the possible use of mycorrhizal fungi as bioremediation agents in polluted soils or as bioindicators of pollution particularly in the context of in the context of phytoaccumulation and phytoremediation (3, 4). The earlier results indicated a potential adaption of AM fungi (*G. mosseae*) to elevated metal concentration in soil (0—100 mg/liter) (5, 6).

Table 1. Dry weight (g / m^2) of rice at different growth stages. T_1 : Control with AM, T_2 : 120 : 60 : 60 + AM, T_3 : 120 : 30 : 60 + AM, T_4 : Control without AM, T_5 : 120 : 60 : 60—AM, T_6 : 120 : 30 : 60—AM ; AM : Arbuscular mycorrhiza ; DAT : Day after transplanting.

Treat- ment	30 DAT	60 DAT	At harvest (120 DAT)
T_1	86.2	341.8	1883.5
T_2	122.6	412.8	2498.4
T_3	157.7	455.5	2714.4
T_4	78	315.4	1564.1
T_5	137.8	433.9	2606.4
T_6	120	373.4	2204.2
CD	0.09	0.45	1.20
SE ±	0.04	0.20	0.53

Table 2. Grain yield (g/m^2) and yield parameters of rice. T_1 : Control with AM, T_2 : 120 : 60 : 60 + AM, T_3 : 120 : 30 : 60 + AM, T_4 : Control without AM, T_5 : 120 : 60 : 60 – AM, T_6 : 120 : 30 : 60 – AM ; AM : Arbuscular mycorrhiza ; DAT : Day after tnsplanting.

Treat- ment	No. of panicles/ plant	Length of panicles (cm)	Total grain/ plant	Grain yield (g/m^2)
T_1	14	11.7	138	248.2
T_2	17	12.3	173	311.4
T_3	21	13.3	178	320.4
T_4	12	11.4	127	228.6
T_5	19	12.7	162	291.6
T_6	16	12.5	157	282.6
CD	2.29	1.04	28.78	58.19
SE ±	1.03	0.47	12.92	26.12

Some small scale industries and large scale industries (DLW, Textile industries) and thermal power station were developed in Varanasi and its adjoining areas in last 50—75 years. 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 plants 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 sludges for manurial 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 heavy metals in crops particularly vegetables and fruit. Not only the crops, biota of the holy river, Ganga also contaminated with the runoff of heavy metals. To over come this chronic problem there is an urgency of need of the adoption of bioremediation technique for the soils and water in Varanasi. Keeping in view the following aspects the aim of our study is to explore the possibility of use of microorganism especially VAM. The present work was conducted to test the effects of inoculation with mixed AM inoculum on the growth and heavy metal content of rice (*Oryza sativa* L. var Sonam) grown in natural field conditions in the peri-urban areas of Varanasi following the standardized practices by local farmers.

Methods

The experimental soil developed on Gangetic alluvial have predominance of illite, quartz and feldspar minerals. Illitic minerals are predominant in sand and silt fractions. Quartz and feldspar are supposed to be inherited from the parent material. The soil which had a pH 7.3, Electrical conductivity (dS/m) 0.254, free $CaCO_3$ (%) 0.37, Organic carbon (g/kg) 4.21, CEC {Cmol (p+) kg-1} 11.8 , Available nitrogen (kg/ha) 210, Avail-

Table 3. Ni, Cd, Zn concentration (ppm) in rice plant at different growth stages. T_1 : Control with AM, T_2 : 120 : 60 : 60 + AM, T_3 : 120 : 30 : 60 + AM, T_4 : Control without AM, T_5 : 120 : 60 : 60 – AM, T_6 : 120 : 30 : 60 – AM ; AM : Arbuscular mycorrhiza ; DAT : Day after transplanting.

Treat- ment		Cd			Ni			Zn	
	30 DAT	60 DAT	120 DAT	30 DAT	60 DAT	120 DAT	30 DAT	60 DAT	120 DAT
T_1	2.36	2.15	1.83	3.19	2.91	2.13	34.1	36.0	38.1
T_2	2.41	2.24	1.96	3.83	3.48	3.09	33.8	35.3	37.9
T_3	1.60	1.47	1.28	2.71	1.49	0.93	37.3	39.26	42.6
T_4	3.05	2.97	2.84	3.97	3.63	3.14	31.7	33.9	36.8
T_5	1.77	1.67	1.53	2.87	1.68	1.01	35.8	37.9	41.2
T_6	1.90	1.81	1.78	3.03	2.78	2.03	35.0	36.3	39.8
CD	0.47	0.39	0.34	0.6	1.28	0.44	6.58	7.04	7.15
SE ±	0.17	0.16	0.12	0.22	0.47	0.16	2.35	2.76	2.79

Table 4. Soil properties after harvest of rice crop. T_1 : Control with AM, T_2 : 120 : 60 : 60 + AM, T_3 : 120 : 30 : 60 + AM, T_4 : Control without AM, T_5 : 120 : 60 : 60 –AM, T_6 : 120 : 30 : 60 – AM ; AM : Arbuscular mycorrhiza ; DAT: Day after transplanting.

Treatments	Cd (ppm)	Ni (ppm)	OC (g/kg Soil)
T_1	3.33	17.0	3.6
T_2	3.47	17.2	3.87
T_3	2.52	16.0	3.74
T_4	3.63	17.6	3.86
T_5	2.81	16.4	3.70
T_6	2.96	16.6	3.62
CD	0.59	2.95	0.7
SE ±	0.22	1.02	0.31

able phosphorus (ppm) 18.3, Cd (ppm) 8.5, Ni (ppm) 27.3 and texture is sandy loam nature.

A field experiment was conducted on Rice crop (cv Sonam) with respect to heavy metal accumulation with reference to AM (arbuscular mycorrhiza) during rainy season in the year 2009. A dry nursery bed was prepared. Recommended levels N and K (1 kg N as ammonium sulfate and 0.23 kg K as muriate of potash per 100 m^2) were applied. The mycorrhizal mixed inoculum (*Glomus mosseae* + *Gigaspora* sp) was spread (at 1.25 kg/m^2 containing 60 spores / g) over the puddle, water-drained, unsterile soil which served as the nursery bed and mixed thoroughly into the top 2.5 cm of soil pre-germinated rice seeds were sown on the drained nursery bed in the rate of 50 kg per ha. The beds were kept moist until the seedlings were about 25 cm high (15 days) and then a shallow layer (5 cm) of water was allowed to stand on the soil in the nursery bed. The 25 days old seedling were transplanted in the experimental field, were one seedling per hill. The mycorrhizal and non-mycorrhizal seedlings were removed on the 28th day and transplanted to the main field. Water were maintain throughout the growth period of rice as per required along with necessary plant protection measures. Weeding were done 20 and 45 days after transplanting. The required data were collected 30, 60 days after transplanting and at final harvest. The plants were harvested after 120 days.

Six treatments (T_1 : Control with AM (arbuscular mycorrhiza), T_2 : 120 : 60 : 60 + AM, T_3 : 120 : 30 : 60 + AM, T_4 : Control without Am, T_5 : 120 : 60 : 60 – AM, T_6 : 120 : 30 : 60 – AM) and one local rice cultivar (cv Sonam) were replicated three times. Experiment was laid out in a completely randomized design. The fertilizer dose of 325 g DAP, 652 g urea [Co $(NH_2)_2$] and 62.5 g zinc sulfate ($ZnSO_4 7H_2O$) was applied to soil in respective plots 25 days after transplanting. Sand soil culture of *Glomus mosseae* + *Gigaspora* sp. brought from GKVK, UAS, Bangaluru and was directly applied in seed bed.

Results and Discussion

The experimental soil was sandy loam in texture. Soil was alkaline in reaction (pH 7.1), low organic carbon (3.9 g/kg) and available P was 18.3 mg/kg and DTPA extractable Cadmium and Nickel was 8.5 and 27.3 mg/kg respectively.

Total Shoot and Grain Yield

Table 1 shows the growth of rice during the growing season and the increase in shoot biomass (dry matter yield) in successive growth stages starting from tillering to harvest have been presented. The highest shoot biomass (weight) was found in plants inoculated with mixed AM innoculum and half recommended phosphorus fertilizer application. All the mycorrhizal treated plots gave significantly higher dry matter yield than the uninoculated and uninoculated control plots. Similar benefits have been reported earlier for many cereals including wheat, maize and rice (7, 8). Though wet land rice has previously been considered non mycorrhizal a positive response to AM inoculated have been also observed (9).

The present study has shown significant increase in number of panicles, length of panicles, total grain/ panicles and grain yield fallowing inoculation with the mixed inoculum of AM (Table 2). The increase in grain yield was maximum with T_3 treatment and was nearly one and half time compared to uninoculated control (T_4). These results show that the mixed inoculum of AM can survive under flood condition and can colonize rice roots to increase mediation in plant growth. There was also positive correlation between mycorrhizal colonization and grain yield ($r = 0.71$).

The experiment by Medina et al. (6) suggested that in Cd polluted conditions, inoculation of AM stimulated the growth, mineral nutrition and soil enzymatic activities of soil as inoculants *G. mosseae* enhanced plant establishment due to the interactive effect which increase the potential fertility and its ability to decrease the transfer of Cd from soil to plant. The amount of Cd transferred from soil solution to biomass of AM colonized plants ranged from 0.09 to 0.6 µg Cd/g.

The lower grain yield after irrigation with treated water and no AM treatment in T_4 could be due to the disturbed mineral nutrition or to relatively higher heavy metal toxicity. High concentrations of heavy metals 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 heavy metal availability and toxicity to plants (3).

Ni, Cd, Zn Content of Shoot in Rice Plant

Several studies indicate that colonization of plants by AM fungi confers protections against toxic metals. Apparently, mycorrhizae can enhance plant uptake of toxic metals, but they may also afford protection from these metals.

The concentrations of heavy metal in the shoots were significantly higher in plots irrigated with treated water and no AM treatment. The mycorrhizal plants in T_1, T_2 and T_3treatments have been reported to have a significantly lower concentration of metal in shoots (Table 3) although the influence of AM on bioavailability of heavy metals could not be generalized. Again under adverse conditions AM might be more important for plant metal resistance and under the optimized conditions of normal agricultural practice, AM colonization even could increase plant absorption from polluted soil, and cleansed polluted sites by removing above ground parts (10, 11).

This disagrees with the present experimental result and that part of the literature data that suggests that AM attenuate the toxic effect of metals, retaining them in the fungal structure with the subsequent restriction of metal transfer to the plant (12, 13). On the other hand, it was also reported that this AM protective effect depends on the plant species and even on the plant variety and that AM fungi 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 (14).

Soil Properties after Harvest of Rice Crop

After harvesting of rice, soil samples were collected from different plots and were analyzed for Cd, N and Organic carbon. Among the different treatments the concentrations of DTPA extractable heavy metal were in the order of : $T_3 > T_5 > T_6 > T_1 > T_2 > T_4$. Metals in soil are present as free metal ions, soluble metal complexes (sequestered to ligands), exchangeable metal ions, organically bound metals, precipitated or insoluble compounds such as oxides, carbonates and hydroxides, or they may form part of the structure of silicate minerals (indigenous soil content). The toxicity of metals in soil depends on their bioavailability, defined as their ability to be transferred from a soil compartment to a living organism. Metal bioavailability is a function not only of their total concentration but also of physico-chemical (e.g. pH, Eh, organic matter, clay content) and biological (e.g. biosorption, bioaccumulation and solubilization) factors. From the Table 4 it is pertinent that variations in concentrations of heavy metals in soil were significant due to different treatment, except for Ni, which did not show significant treatment wise variation (Table 4). The concentration (ppm) of heavy metals in the experimental soil ranged between 2.51 to 3.63 ppm for Cd, and 16 to 17.6 ppm for Ni for different treatments. The comparison of mean heavy metal concentrations in treated and untreated wastewater from the data of other countries suggests that the values of the present study were manyfold lower than the levels observed at Harare, Zimbabwe (15, 16), but higher than those recorded at Wodonga and Canberra, Australia (17). The range of concentrations of Cd and Ni in soil observed during the present study was below the official Indian standard (18). The comparison of the data from the present study with earlier findings (19, 20) at Dinapur, Varanasi suggested that the range of concentrations of Cd in soil was higher ; however Ni were lower than the previously reported range. This variation may be ascribed to the differences in sites, frequency of soil collection, and crops under cultivation between the two studies and also may be correlated with the soil properties that influence heavy metal availability at different sites. From the data it is evident that non-significant positive relationship was observed between heavy metal content (Cd and Ni) and soil organic carbon content.

The results of Vivas et al. (21) revealed that coinoculation of native microorganisms with arbuscular mycorrhizae increased plant growth and colonization of AM. This was due to enhancement of Cd tolerance in plant after co inoculation with native microorganisms and seemed to decrease the concentration of Cd, Cr, Mn, Mo, Fe, Ni in plant tissue. Dehydrogenase and phosphatase activities are indicative parameters of microbial metabolism and soil fertility which were maximized by the co inoculation in Cd polluted soil and prove to be efficient for bioremediation.

The study concludes that irrigation by treated wastewater has increased the heavy metal concentrations in soil and plants of receiving area. An important issue is that the contamination levels were frequently higher than permissible limits in the plant tissue, at the same sites as water and soil samples that comply with established safe standards. This has important implications for policy in that programmes aimed at monitoring and controlling heavy metal concentrations in irrigation water sources will not necessarily result in acceptable levels in crops. Policies and programs need to be adapted so that local edaphic conditions and agricultural practices are taken into account, and appropriate local measures developed for ameliorating heavy metal uptake by crops for a given set of local conditions. Microbiological means through arbuscular mycorrhizal fungi are of significance in determining metal mobility and have actual and potential application in bioremediation of heavy metal pollution. In the present experiment the mycorrhizal mixed inoculum (*Glomus mosseae* + *Gigaspora* sp.) was found to cater important role in metal tolerance and accumulation.

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