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Improvement of Heavy Metals Removing Ability of Spinach with Microbial Enrichment

Alavala Uma Rajshekhar*, R. Subhash Reddy*, P. Chandrasekhar Rao, M. Chandini Patnaik*** and K. Damodara Chari***

*****Department of Agricultural Microbiology and Bioenergy, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad-500 030, India
******Department of Soil Science and Agricultural Chemistry, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad-500 030, India
*******AICRP on Micronutrients Soil and Plants, Telangana, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad-500 030, India

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ABSTRACT

The present study was conducted to determine the uptake and accumulation of heavy metals in different parts of vegetable crop spinach and also reduction in heavy metals in the soil due to microbial inoculation. The polybag experiment was conducted following complete randomized block design with 12 treatments and three replications in polluted soil with supply of freshwater, unpolluted soil with supply of freshwater, unpolluted soil with supply of polluted water. The heavy metal concentration of soil, shoot and leaves revels that the nickel and cobalt were more in soil in the treatments T_{10} (Soil + FYM + VAM + *Pseudomonas*) and T_2 (SF Soil + FYM + VAM + *Pseudomonas*). In the shoot, the nickel accumulated more in the treatment T_9 (Soil + FYM), cobalt accumulated more in the treatment T_8 (SF Soil + RDF + FYM + VAM + *Pseudomonas*) and cadmium accumulated more in the in treatment T_{12} (Soil + RDF + FYM + VAM + *Pseudomonas*). The heavy metal accumulation in the leaves showed that nickel accumulated more in the treatment T_{12}, cobalt accumulated more in the treatment T_9 (Soil + FYM) and cadmium accumulated more in the treatment T_8 (Soil + RDF + FYM + VAM + *Pseudomonas)*.

INTRODUCTION

Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds in the environment. Improper treatment and disposal of industrial wastewaters and solid wastes are the major causes of soil contamination by heavy metals. Heavy metals are absorbed and accumulated by plants, thus are transferred directly or indirectly to humans through food chains. Heavy metal contamination is known to have adverse effects on soil biological functions, including the size, activity and diversity of the soil microbial community (Chander et al. 2001), the activity of enzymes involved in transformations of C, N, P and S (Hemida et al. 1997), and crop growth. There is a strong evidence that soil microbes are more sensitive to heavy metal contamination than crop plants or animals (Giller et al. 1999). Significant reductions in microbial biomass and soil respiration have been found in metal contaminated soils compared to uncontaminated soils. Loss of microbial populations in metal-contaminated soils impacts elemental cycling, organic remediation efforts, plant growth, and soil structure. Phytoremediation also presents a cheap, non invasive and safe alternative to conventional clean up techniques and can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatilization and rhizofiltration (Glick 2003). Spinach is a common vegetable grown all round the year. It can take up cadmium and accumulate in stems from polluted soil significantly. It is an easily cultivable crop, with minimum cultivation practices.

The present study was conducted to determine the uptake and accumulation of heavy metals in different parts of the spinach and also reduction in heavy metals in the soil due to microbial inoculation.

MATERIAL AND METHODS

Soil samples and soil characteristics: Soil samples of polluted and unpolluted soils were collected before sowing and analysed for the physico-chemical (pH, EC, particle size, NPK and OC) parameters and microbiological properties by adopting standard procedures. Water samples were also analysed before sowing of crop in polluted and unpolluted soils.

ESTIMATION OF HEAVY METAL CONTENT AND MICROBIAL COUNT OF SPINACH BEET IN POLYBAGS TREATED WITH CHEMICAL FERTILIZERS, FARM YARD MANURE

Crop Details

The pot culture experiment was conducted during 2012-13. For this investigation, leafy vegetable crop spinach beet, Pusa Jyothi variety was sown in pots; experiments followed completely randomized block design.

Experiment Details

Treatments: The treatments for polybag experiment were fixed as 12 treatments with each treatment having three replications. All three replications were used to record observations on yield and quality parameters of spinach on 30 and 60 days after sowing.

In this context of pot culture experiment having 12 treatments with statistical design, the treatment was subdivided into three parts: polluted soil with supply of freshwater, unpolluted soil with supply of freshwater and unpolluted soil with supply of polluted water. Polluted soil with supply of freshwater has T1: SF Soil+FYM@12 t/ha, T2: SF Soil + FYM + VAM + *Pseudomonas*, T3: SF Soil + RDF, T4: SF Soil + RDF + FYM + VAM + *Pseudomonas*. Unpolluted soil with supply of freshwater has T5: Soil + FYM, T6: Soil + FYM + VAM + *Pseudomonas,* T7: Soil + RDF, T8 : Soil + RDF + FYM + VAM + *Pseudomonas*. Unpolluted soil with supply of polluted water has T9: Soil + FYM, T10: Soil+ FYM + VAM + *Pseudomonas,* T11: Soil + RDF, T12: Soil + RDF + FYM + VAM + *Pseudomonas*.

Preparation of polybags mixture: The cleaned polybags were filled with 8 kg soil and mixed with chemical fertilizer (0.14: 0.24: 0.37 g polybag^{-1} N P K), farm yard manure (78.75 g polybag^{-1}) and Vesicular Arbuscular Mycorrhizae (100 to 150 g of infected propagules polybag^{-1}) according to the treatments which were neatly arranged in the net house.

Chemical fertilizers: Phosphorus and potassium @ 0.24 g polybag^{-1} P_2O_5 and 0.37 g polybag^{-1} K_2O were applied through diammonium phosphate and muriate of potash respectively, as basal application. Nitrogen was applied in the form of urea @ 0.24 g polybag^{-1} after germination and on 30 and 60 days after sowing. Farmyard manure was applied @ 78.75 g polybag^{-1} which was mixed with soil according to the treatment's requirement. EC and pH of FYM were 0.95 dSm^{-1} and 7.59 respectively, and Ni, Co, Cd content in FYM was 0.91, 0.20, 0.01-0.02 mg/kg respectively.

Seed sowing and maintenance: The polybags were sown with Pusa Jyothi variety of spinach beet at the rate of 20 seeds per polybag. After germination, thinning was done and routine care was taken to protect the plants from pests and diseases.

Heavy metals analysis: The Ni, Co, Cd in soil before sowing crop and after harvesting of crop was determined using DTPA method by atomic absorption spectrophotometer (Lindsay & Norvell 1978).

RESULTS AND DISCUSSION

Heavy metal accumulation in soil: The nickel content in spinach pot culture experiment (Table 1) irrigated with polluted water showed highest value in treatment T_{10} 1.14 mg kg^{-1}, and the lowest values in T_5 (0.49 mg kg^{-1}) in unpolluted soils. Similar findings were reported by Srinivasarao et al. (2014) and Adriano et al. (2002) in heavy metals contaminated area. The cobalt content in spinach pot culture experiment irrigated with polluted water showed highest values in treatment T_2 and T_{10} (1.21 mg kg^{-1}) and the lowest value was observed in T_7 (0.89 mg kg^{-1}) in unpolluted soils. The values of cadmium in polluted and unpolluted soils were determined after harvesting of spinach crop. Among all the treatments, the treatment T_2 (0.30 mg/kg) showed highest values in polluted soils followed by the treatment T_1 (0.29 mg/kg), while the lowest values were found in T_4, T_5 and T_7 with the same value of 0.25 mg/kg in unpolluted soils .

Heavy metal accumulation in shoot: Nickel content in spinach shoots irrigated with different quality of water in polluted and unpolluted soils (Table 2) showed that there was more accumulation in the treatment T_9 (4.80 mg kg^{-1}) with polluted water followed by T_8 (4.62 mg kg^{-1}) and T_{12} (3.70 mg kg^{-1}). The lowest accumulation of Ni was found in T_1 (1.42 mg kg^{-1}) in polluted soil. This variation might be due to the reason that Ni content in sewage water is high compared to tube well water.

Cobalt content in spinach shoots irrigated with different quality of water in polluted and unpolluted soils showed that there was more accumulation of Co in the treatment T_8 (9.27 mg kg^{-1}) with application of freshwater in unpolluted soil, followed by T_{11} (8.65 mg kg^{-1}), T_6 (8.54 mg kg^{-1}), T_4 (8.51 mg kg^{-1}). The lowest accumulation of Co was found in treatment T_{10} (3.56 mg kg^{-1}) in application with polluted water. Similar findings were reported by Leyval et al. (2002).

Cadmium content in spinach shoots of the pot culture experiment irrigated with different quality of water in polluted and unpolluted soils showed that there was more accumulation of the Cd in the treatment T_{12} (9.66 mg kg^{-1}) with application of polluted water in unpolluted soil. The lowest accumulation of Cd was found in treatment T_{10} (4.49 mg kg^{-1}) in application with polluted water.

Table 1: Effect of microbial cultures on heavy metals Ni, Co, Cd (mg/kg) in soil at 30 and 60 DAS in polluted and unpolluted soils of spinach beet.

Treatments	Ni	Co	Cd
		Polluted Soil with supply of freshwater	
T_1- SF Soil + FYM	0.99	1.17	0.29
T_2- SF Soil + FYM+VAM+ *Psuedomonas*	1.12	1.21	0.30
T_3- SF Soil + RDF	1.03	0.93	0.27
T_4- SF Soil + RDF+FYM+VAM+*Psuedomonas*	0.81	0.99	0.25
		Unpolluted soil with supply of freshwater	
T_5- SF Soil + FYM+*Psuedomonas*	0.49	0.9	0.25
T_6- SF Soil + FYM+ VAM+*Psuedomonas*	0.90	1.15	0.27
T_7- SF Soil + RDF	0.69	0.89	0.25
T_8- SF Soil + RDF+FYM+VAM+*Psuedomonas*	0.78	1.05	0.26
		Unpolluted soil with supply of polluted water	
T_9- Soil + FYM	0.87	0.99	0.28
T_{10}- Soil + FYM+VAM+*Psuedomonas*	1.14	1.21	0.27
T_{11}- Soil + RDF	0.74	1.02	0.27
T_{12}- Soil + RDF+FYM+VAM+*Psuedomonas*	0.86	0.97	0.26
SE m±	0.014	0.031	0.006
C.D at 5%	0.083	0.056	0.018

Table 2: Effect of microbial cultures on heavy metals Ni, Co, Cd (mg/kg) in shoot at 30 and 60 DAS in polluted and unpolluted soils of spinach beet.

Treatments	Ni	Co	Cd
		Polluted Soil with supply of freshwater	
T_1- SF Soil + FYM	1.42	7.42	7.8
T_2- SF Soil + FYM + VAM + *Psuedomonas*	2.19	8.20	8.41
T_3- SF Soil + RDF	3.58	8.48	8.15
T_4- SF Soil + RDF + FYM + VAM + *Psuedomonas*	2.58	8.51	8.35
		Unpolluted soil with supply of freshwater	
T_5- SF Soil + FYM + *Psuedomonas*	2.40	6.58	6.75
T_6- SF Soil + FYM+ VAM + *Psuedomonas*	2.73	8.54	8.66
T_7- SF Soil + RDF	2.23	8.26	8.50
T_8- SF Soil + RDF + FYM + VAM + *Psuedomonas*	4.62	9.27	9.36
		Unpolluted soil with supply of polluted water	
T_9 - Soil + FYM	4.80	9.10	9.35
T_{10}- Soil + FYM+ VAM+ *Psuedomonas*	2.05	3.56	4.49
T_{11}- Soil + RDF	3.35	8.65	8.98
T_{12}- Soil + RDF+ FYM+ VAM+ *Psuedomonas*	3.70	9.04	9.66
SE m±	0.325	0.261	0.091
C.D at 5%	0.947	0.762	0.266

SF soil = Student Farm Soil, RDF = Recommended dose of fertilizers, FYM = Farm Yard Manure

Heavy metal accumulation in leaves: The data on accumulation of heavy metals in the leaves of spinach with different treatments are shown in Table 3. The data showed that the maximum Ni accumulation was in treatment T_{12} (5.88 mg kg^{-1}) in soil irrigated with polluted water, followed by T_{11} (5.00 mg kg^{-1}) and T_2 (4.34 mg kg^{-1}). The lowest Ni accumulation was found in treatment T_1(1.28 mg kg^{-1}) in polluted soil. The data showed that maximum Co accumulation occurred in treatment T_9 (4.66 mg kg^{-1}) in unpolluted soil with freshwater, which was almost at par with T_8 (4.40 mg kg^{-1}). The lowest value was found in T_{10} (1.27 mg kg^{-1}) in polluted soils.

The data on Cd accumulation showed that maximum accumulation was in treatment T_8 (8.00 mg kg^{-1}) in unpolluted soil with application of freshwater, which was at par with T_1 (7.90 mg kg^{-1}) and T_7 (7.94 mg kg^{-1}). The lowest value was found in T_{10} (2.34 mg kg^{-1}) in polluted soils.

CONCLUSION

The study indicates the improvement of heavy metals removing ability of spinach with microbial enrichment. It mainly emphasizes on microbial consortia treatment of polluted soil with supply of freshwater, unpolluted soil with supply of freshwater and unpolluted soil with supply of

Table 3: Effect of microbial cultures on heavy metals Ni, Co, Cd (mg/kg) in leaf at 30 and 60 DAS in polluted and unpolluted soils of spinach beet.

Treatments	Ni	Co	Cd
		Polluted Soil with supply of freshwater	
T_1- SF Soil + FYM	1.28	2.93	7.90
T_2- SF Soil + FYM+VAM+ *Psuedomonas*	4.34	3.48	6.51
T_3- SF Soil + RDF	2.00	3.62	6.10
T_4- SF Soil + RDF+FYM+VAM+*Psuedomonas*	2.82	4.03	6.43
		Unpolluted soil with supply of freshwater	
T_5- SF Soil + FYM+*Psuedomonas*	3.84	2.53	2.75
T_6- SF Soil + FYM+ VAM+*Psuedomonas*	2.70	3.18	7.24
T_7- SF Soil + RDF	2.67	3.52	7.94
T_8- SF Soil + RDF+FYM+VAM+*Psuedomonas*	2.20	4.40	8.00
		Unpolluted soil with supply of polluted water	
T_9- Soil + FYM	2.55	4.66	7.66
T_{10}- Soil + FYM+VAM+*Psuedomonas*	2.69	1.27	2.34
T_{11}- Soil + RDF	5.00	4.10	7.31
T_{12}- Soil + RDF+FYM+VAM+*Psuedomonas*	5.88	4.14	7.74
SE m±	0.357	0.15	0.083
C.D at 5%	1.058	0.438	0.241

polluted water, which improved the heavy metal (Ni, Co, Cd) extraction capacity of the spinach plants. This research will promote the phytoremediation and bioremediation.

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