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Enhancement of Heavy Metals Eliminating Ability of Spinach With Microbial Enrichment

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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 poly bag experiment was conducted following complete randomized block design with 12 treatments and three replications.Polluted Soil with supply of fresh water ,Unpolluted soil with supply of fresh water, Unpolluted soil with supply of polluted water.The heavy metal concentrations of soil, shoot and leaves revels that the Ni and Cobalt were more in the treatments T_{10}(Soil+FYM+VAM+Psuedomonas),T_{10}(Soil+FYM+VAM+Psuedomonas),T_2(SFSoil+FYM+VAM+ Psuedomonas) respectively, where as in the shoot the Ni accumulated more in the treat mentT_9(Soil+FYM),Co accumulated more in the shoots of treatment T_8(SF Soil+ RDF+ FYM+ VAM+ Psuedomonas),Cd was accumulated in the shoots of treatment T_{12}(Soil +RDF+ FYM+ VAM+ Psuedomonas),Where as in the heavy metal accumulation content in the leaves,Ni content more in the treatment T_{12},Co was accumulated more in the treatment T_9(Soil+FYM),Cd was more accumulated in the treatment T_8.
Keywords: *Microbial cultures, polluted and unpolluted soils, spinach, water, heavy metal concentration*

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INTRODUCTION
Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Improper treatment and disposal of industrial waste waters and solid wastes are the major causes of soil contamination by heavy metals. Heavy metals are absorbed and accumulated by plants thus are absorbed directly or indirectly by human through the food chain.Heavy metal contamination is known to have adverse effects on soil biological functions, including the size, activity and diversity of the soil microbial community [2], the activity of enzymes involved in transformations of C, N, P and S [5] and crop growth. There is strong evidence that soil microbes are more sensitive to heavy metal contamination than crop plants or animals [3]. 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 [4]. 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 vegetable crop 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 physical(pH, EC, and particle size and chemical characters like NPK and OC parameters) and microbiological properties by adopting standard procedures at Department of Agricultural Microbiology and Bio-energy and Department of Soil Science and Agricultural Chemistry, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad.Water samples were also analyzed before sowing of crop in polluted and unpolluted soils.

Estimation of heavy metal content and microbial count of spinach beet in poly bags treated with chemical fertilizers, farm yard manure

Crop details

The pot culture experiment was conducted at Department of Agricultural Microbiology and Bioenergy during 2012-13. For this investigation leafy vegetable crop, spinach beet, PusaJyothi variety was sown in pot experiments followed completely randomized block design with four treatments and three replications.

Experiment details

Treatments

The treatments forpoly bag experiment were fixed as twelve treatments each treatment with three replications were designed. All three replications were used to record observations on yield, quality parameters of spinach around 30 and 60 days after sowing.

In this context of pot culture experiment having twelve treatments and followed statistical design .in this treatment subdivided into three parts: polluted soil with supply of fresh water, unpolluted soil with supply of fresh water and unpolluted soil with supply of polluted water. Polluted soil with supply of fresh water have 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 fresh water, have T5: Soil + FYM,T6: Soil + FYM + VAM + *Pseudomonas,*T7: Soil +RDF, T8 :Soil+ RDF+ FYM+ VAM + *Pseudomonas* .Unpolluted soil with supply of polluted water, have T9:Soil + FYM, T10: Soil+ FYM + VAM+ *Pseudomonas*, T11:Soil + RDF, T12:Soil + RDF + FYM + VAM + *Pseudomonas*.

Preparation of poly bags mixture

The cleaned poly bags were filled with 8 kg soil and this soil was mixed with chemical fertilizer (0.14: 0.24: 0.37 g poly bag^{-1} NPK), farm yard manure (78.75 g poly bag^{-1}) and Vesicular Arbuscular Mycorrhizae (100 to 150 g of infected propagules poly bag^{-1}) according to the treatments which were neatly arranged in the net house.

Chemical fertilizers

Phosphorus and potassium @ 0.24 g poly bag^{-1} P_2O_5 and 0.37 g poly bag^{-1} K_2O were applied through Di Ammonium Phosphate and Muriate of Potash respectively as basal application. Nitrogen was applied in the form of Urea @ 0.24 g poly bag^{-1} after germination and after 30 and 60 days after sowing.Farmyard manure was applied @ 78.75 g poly bag^{-1} which was mixed with soil according to the treatments requirement. EC and pH of FYM were 0.95 dS/m and 7.59 respectively and Ni, Co, Cd content in FYM was 0.91, 0.20, 0.01-0.02 respectively.

Seed Sowing and maintenance

The poly bags were sown with PusaJyothi variety of spinach beet at the rate of 20 seeds per poly bag. After germination, thinning was done and routine care was taken to protect the plants from pest and diseases.

Heavy metals analysis

The Ni, Co, Cd in soil before sowing crop and after harvesting of crop determined using DTPA method by Atomic absorption spectrophotometer [7].

RESULTS AND DISCUSSION

Heavy metal accumulation in soil

The data showing the Nickel content in spinach pot culture experiment irrigated with polluted water showed highest value in treatment T_{10} 1.14mg/kg and the lowest values were observed in T_5 (0.49mg/kg) in unpolluted soils. Similar findings were reported by Srinivasrao *et al*. [8] and Adriano *et al.* [1] in heavy metals contaminated area.

The data showing the Cobalt content in spinach pot culture experiment irrigated with polluted water showed highest values in treatment T_2 and T_{10} (1.21mg/kg) and the lowest values was observed in T_7 (0.89mg/kg) in unpolluted soils.

The values of heavy metal Cadmium in polluted and unpolluted soils collected after harvesting of spinach crop. Among all the treatments, the treatment T_2 0.30 mg/kg showed highest values in polluted soils as followed by treatment T_1 (0.29) and the lowest values were found in T_4, T_5 and T_7 as same value 0.25mg/kg in unpolluted soils (Table 1).

Heavy metal accumulation in shoot

Nickel content in spinach shoots of the pot culture experiment irrigated with different quality of water in polluted and unpolluted soils.The data showed that there was more accumulation of the Ni in the treatment T_9 (4.80mg/kg) with irrigation with polluted water and followed by T_8 (4.62mg/kg) and T_{12} (3.70mg/kg). The lowest accumulation by Ni was found in T_1 (1.42mg/kg) 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 of the pot culture experiment irrigated with different quality of water in polluted and unpolluted soils. The data showed that there was more accumulation of the Co in the treatment T_8 (9.27mg/kg) with application of fresh water in unpolluted soil ,followed by ,T_{11} (8.65 mg/kg), T_6 (8.54 mg/kg), T_4 (8.51 mg/kg) and the lowest accumulation of Co was found in treatment T10 (3.56mg/kg) in application with polluted water. Similar findings were reported by Leyval*et al.* [6].

Cadmium content in spinach shoots of the pot culture experiment irrigated with different quality of water in polluted and unpolluted soils. The data showed that there was more accumulation of the Cd in the treatment T_{12} (9.66mg/kg) with application of polluted water in unpolluted soil. The lowest accumulation of Cd was found in treatment T10(4.49mg/kg) in application with polluted water .

Heavy metal accumulation in leaves

Nickel content in spinach leaves grown in pot culture experiment irrigated with different quality water, is presented in the Table 2. The data showed that maximum Ni accumulation T_{12} (5.88mg/kg) was observed soil in irrigated with polluted water, followed by T_{11} (5.00mg/kg) and T_2 (4.34mg/kg) and the lowest Ni accumulation was found in treatment T_1(1.28mg/kg) in polluted soil.

Cobalt content in spinach leaves grown in pot culture experiment irrigated with different quality of water is presented in the Table 2. The data showed that maximum Co accumulation showed in treatment T_9 (4.66mg/kg) was observed in unpolluted soil application with fresh water treatments which was on par with T_8 (4.40mg/kg) .The lowest value was found in T_{10} (1.27mg/kg) in polluted soils.

Cadmium content in spinach leaves grown in pot culture experiment irrigated with different quality of water is presented in the Table 2. The data showed that maximum Co accumulation showed in treatment T_8 (8.00 mg/kg) was observed in unpolluted soil application with fresh water treatments which was on par with T_1 (7.90mg/kg) and T_7 (7.94mg/kg). The lowest value was found in T_{10} (2.34 mg/kg) in polluted soils.

Table 1.Effect of microbial cultures on heavymetals 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 fresh water			
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 fresh water			
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 2Effect 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 fresh water			
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 fresh water			
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

Table 3 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 fresh water			
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 fresh water			
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

CONCLUSION

In this present study the improvement of heavy metals removing ability of Spinach with microbial enrichment by the mainly with the comparison of heavy metals in the soil with accumulation of heavy metals in the leaves and shoots. Mainly emphasizes on microbial consortia treatment of Polluted Soil with supply of fresh water,Unpolluted soil with supply of fresh water, Unpolluted soil with supply of polluted water were 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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