Plant Archives Vol. 25, Supplement 1, 2025 pp. 1320-1325 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.179

EFFICACY OF HEAVY METALS-TOLERANT PLANT GROWTH PROMOTING BACTERIAL CONSORTIUM FOR ALLEVIATING CADMIUM TOXICITY IN SPINACH

A. K. Padhiyar[1], Dileep Kumar[2], K. C. Patel[2] and V. J. Patel[3]
[1]Department of Soil Science & Agricultural Chemistry, B. A. College of Agriculture, Anand Agricultural University, Anand-388110, Gujarat, India
[2]Micronutrient Research Centre (ICAR), Anand Agricultural University, Anand-388110, Gujarat, India
[3]Department of Agronomy, B. A. College of Agriculture, Anand Agricultural University, Anand-388110, Gujarat, India
*Corresponding author E-mail: arpitapadhiyar3097@gmail.com
(Date of Receiving : 15-08-2024; Date of Acceptance : 27-10-2024)

ABSTRACT

The pot experiment was undertaken at net house of the Micronutrient Research Scheme, Anand Agricultural University, B. A. College of Agriculture, Anand, during *rabi* season of the year 2021-2022. The experiment was laid out in completely randomized design (Factorial) with three replications. Cadmium is use individually in different level of concentration (Cd, 1.5, 3.0 and 6.0 mg/kg). Efficacy of bacterial inoculation (consortium of *Pseudomonas azotoformans, Bacillus infantis, Bacillus megaterium and Micrococcus terreus*) and FYM was under studied with different combination of tested heavy metals. Overall bacterial inoculation and FYM were found to promote growth and reduce the respective heavy metal toxicity in spinach plant. Since heavy metal contamination in agricultural lands is becoming serious environmental concern, the heavy metal-tolerant plant growth-promoting strains reported in this study can offer suitable economical and ecofriendly base for development of the bioremediation strategies.
***Keywords*:** Spinach, Cadmium, FYM, Bacteria, Bioremediation

Introduction

Rapid industrialization and extraction of natural resources (soil, water and air) have resulted in huge scale environmental contamination and pollution. As we know that heavy metals' long-term persistence in the ecosystem exacerbates the danger they pose to human and animal health (Mitra *et al.,* 2022). Excess concentration of metals affects crop yields, soil biomass and fertility and plant germination, growth and production. Traditional remedial methods such as ground filling and leaching, excavation and burial, or soil washing may be used to treat heavy metals polluted soils. These methods are not only expensive but detrimental to soil characteristics. These methods may also sometimes produce secondary pollutants too increasing further associated risks. The biological methods for remediation, that is bioremediation based on microorganisms, plants, or other biological systems, offer the best solution as it is simpler, cost-effective, and sustainable way to deal with these contaminants in comparison to physico-chemical detoxifying technologies (Gavrilescu, 2004; Wuana and Okieimen, 2011). Plant associated microorganisms, especially plant growth promoting rhizobacteria (PGPR), are known to play a vital role in promoting plant growth and also in remediating soils from organic and metal pollutants by various mechanisms (Rajkumar *et al.,* 2008).

Material and Methods

Heavy metals under test Cd were prepared by dissolving their respective compounds, viz. cadmium chloride ($CdCl_2$) respectively, in ultrapure water and used for the treatments. All glass wares were acid-washed prior to use to avoid metals contamination. The experiment was laid out in completely randomized design (Factorial) with three replications.

Location of experimental site

Geographically, Anand district is located between 22°.6' to 22° 43' North latitude and 72° 2' to 73° 12' East longitude

Soil characteristics

The experimental soil is alluvial in nature, light brown in colour which locally known as *Goradu* soil. The texture is loamy sand (*Typic Ustochrept*). It is very deep, well drained and fairly retentive of moisture, loose and easy to work when wet and becomes somewhat hard on drying.

Filling of pots and growing plant

Seven kilograms capacity earthen pots were selected for the pot study. Initially ten healthy seeds of spinach were sown into each pot at proper depth. Thinning was done after germination and finally 3 plants were kept for study. Before sowing soil application with FYM applied as per the treatment. The spinach crop was harvested after 42 DAS.

Table 1: Experimental details

1	Agro Climatic Sub region	:	Middle Gujarat Agro Climate Zone III
2	Year of commencement	:	2021
3	Season	:	*Rabi 2021-22*
4	Experimental design	:	CRD
5	Treatments	:	Thirty (30)
6	Repetitions	:	Three (3)
7	Total no. of pots	:	Ninety (30×3 = 90)
8	Pot Capacity	:	7 kg soil/pot
9	Test crop		Spinach
10	NPK Recommendation	:	20 kg N/ha

Note

(i) 10 seed of spinach were sown and after establishment 3 plants were

(ii) RDF was applied to each pot

(iii) One month incubation period for stabilization of heavy metals at field capacity; and labile concentration of metals was analyzed.

(A) Factor I

1. M_0: Control (without bacterial inoculation)
2. M_1: Bacterial inoculation (consortium of *Pseudomonas azotoformans*, *Bacillus infantis, Bacillus megaterium* and *Micrococcus terreus*)
3. M_2: FYM (5t/ ha)

(B) Factor II

1. HM_0 : Control (Without heavy metals)
2. HM_1 $Cd_{1.5}$: Cd @1.5 mg/kg
3. HM_1 $Cd_{3.0}$: Cd @ 3.0 mg/kg
4. HM_1 $Cd_{6.0}$: Cd @ 6.0 mg/kg
5. HM_2 Cr_{25} : Cr @ 25 mg/kg
6. HM_2 Cr_{50} : Cr @ 50 mg/kg
7. HM_2 Cr_{100} : Cr @ 100 mg/kg
8. HM_3 Pb_{25} : Pb @ 25 mg/kg
9. HM_3 Pb_{50} : Pb @ 50 mg/kg
10. HM_3 Pb_{100} : Pb @ 100 mg/kg

Observations were recorded:

Fresh weight and dry weight (g/pot)

The fresh weight and dry weight of plant was recorded after harvesting. Immediately after harvesting, all plants were washed with tap water, then distilled water and made into small pieces. Then the samples are air dried and kept in an oven at 60-65 ^{o}C for 24 h till the constant weight was obtained. The plant fresh and dry weight was recorded and expressed in g/pot.

Chemical Analysis:

Total microbial count in soil

Microbial count soil sample were carried out for serial dilution by 10 g of soil sample with sterilized distilled water and serial dilution spreading in sterilized petri-dis and put on BOD for microbial growth and colony counted as per procedure outlined by Tate (1995).

Plant Analysis:

Digestion of plant sample for determination of plant nutrients

Weighed 0.5 g of dried and processed plant in 100 ml conical flask and added 5 ml of HNO, and keep the sample for overnight as pre digestion. Next day, added 10 ml of di-acid (ratio of HNO: HCIO, is 9: 4) and kept for digestion on a hot plate. Initially the temperature of hot plate should be lower (appx. 60'C) for one hour to overcome the frothing, then gradually increase. Place the sample on a hot plate till colorless. Remove the samples from hot plate and cool; after it makes the desired volume and filter the sample and make up the volume in 50 ml volumetric flask. The digested extracts were stored in plastic bottles at 4 ºC in refrigerator for estimation of phosphorus, potassium and sulphur. The micronutrient as well as heavy metals in shoot and root of spinach plants analyzed by ICP-OES and expressed in mg/kg.

Computation of Nutrient content and Uptake

The concentration of all the nutrients in spinach leaf and root was expressed in mg/kg for micronutrients on dry weight basis. The uptake of these nutrients was computed using following formulas.

$$\text{Micronutrient and heavy metal uptake (mg/pot)} = \frac{\text{Nutrient content (mg/kg)} \times \text{Dry matter yield (g/pot)}}{1000}$$

Methods used for chemical analysis of plant samples for total content of different nutrients are presented in Table.

Statistical analysis

The statistical analyses of data of the characters were studied by the investigation through the procedure appropriate to the design of the experiment and interpretation of results was done as described by Gomez and Gomez (1984).

Result and Discussion

Table 2: Effect of cadmium on shoot & root fresh weight of spinach

Cd (mg/kg)	Shoot fresh weight (g/pot)			Mean	Root fresh weight (g/pot)			Mean
	M_0	M_1	M_2		M_0	M_1	M_2	
HM0	95.87	92.23	90.83	92.97	4.89	4.99	5.03	4.97
HM1Cd1.5	89.90	88.25	81.57	86.58	4.48	4.52	4.95	4.65
HM1Cd3.0	81.70	83.77	81.63	82.37	4.30	4.34	4.40	4.35
HM1Cd6.0	67.30	80.90	80.14	76.11	3.36	3.95	3.57	3.63
Mean	83.69	86.29	83.54		4.26	4.45	4.49	
SEm	M=0.85, HM=0.99, M×HM =1.71				M=0.02, HM=0.02, M×HM = 0.04			

The data revealed that the fresh weight of shoot and root was significantly affected by different treatments of M_1; bacterial inoculation (consortium of *Pseudomonas azotoformans, Bacillus infantis, Bacillus megaterium* and *Micrococcus terreus*) and M_2; (FYM) in spinach. The significantly highest fresh weight of shoot was observed in M_0 x HM_0 (95.87 g/pot) and lowest recorded in treatment of M_0 x HM_1 $Cd_{6.0}$ (67.30 g/pot). Inoculation of bacterial consortia led to decreased accumulation of heavy metals in the whole plant but at the same time increased the plant biomass compared with the un-inoculated control, it might be due to selected bacterial isolates were able to promote the growth of spinach as a test plant under stress condition produced by the heavy metals. All these finding attributed by Shilev *et al.* (2019). The significantly highest fresh weight of root was observed in treatment of M_2 x HM_0 $Cd_{6.0}$ (5.03 g/pot) and lowest was observed in treatment of M_0 x HM_1 $Cd_{6.0}$ (3.36 g/pot). The application of FYM was also produced significantly higher fresh and dry weight of spinach as compared to control, it might be due to Humic substances in manure exhibit behaviours similar to growth hormones in plants, increase the intake of plant nutrients, promote plant growth and have a positive effect on increasing the amount of dry matter, these are findings bare in accordance with the findings of Celick and Kunene (2020).

Table 3: Effect of cadmium content on shoot & root of spinach

Cd (mg/kg)	Shoot Cd content			Mean	Root Cd content			Mean
	M_0	M_1	M_2		M_0	M_1	M_2	
HM0	0.09	0.08	0.08	0.09	1.50	1.30	1.70	1.50
HM1Cd1.5	4.88	4.56	4.08	4.51	15.34	13.20	16.53	15.02
HM1Cd3.0	10.34	9.36	9.50	9.74	23.07	20.62	21.34	21.68
HM1Cd6.0	17.85	16.08	16.38	16.77	35.33	31.02	33.89	33.42
Mean	8.29	7.52	7.51		18.81	16.54	18.36	
SEm	M=0.122, HM=0.14, M×HM =0.24				M=0.02, HM=0.02, M×HM = 0.04			

The concentration of metals in plant tissue is a function of the metal content in the growing environment (Hseu *et al.,* 2010). The significantly highest Cd content in shoot (17.85 mg/kg) and root (35.33 g/kg) was recorded in treatment of M_0 x HM_1 $Cd_{6.0.}$ The significantly lowest Cd content (0.08 mg/kg) in shoot and root (1.30 mg/kg) was recorded in M_1 x HM_1 $Cd_{6.0.}$ Among the treatments the bacterial inoculation in soil were found lower Cd content in shoot.

Table 4: Effect of cadmium uptake on Shoot & root of spinach

Cd (mg/kg)	Shoot uptake			Mean	Root uptake			Mean
	M_0	**M_1**	**M_2**		**M_0**	**M_1**	**M_2**	
HM0	0.59	0.63	0.57	0.60	2.78	2.69	3.40	2.96
HM1Cd1.5	27.71	26.78	23.45	25.98	24.44	21.60	29.98	25.34
HM1Cd3.0	56.33	57.40	53.47	55.73	30.51	28.32	30.50	29.78
HM1Cd6.0	85.46	78.05	78.81	80.77	46.37	41.78	45.66	44.60
Mean	42.52	40.72	39.08		26.03	23.60	27.39	
SEm	M=0.76, HM=0.87, M×HM = 1.51				M=0.69, HM=0.80, M×HM = 1.38			

The presenting data to Cd uptake by spinach shoot was significantly affected by different treatments. The significantly highest Cd uptake in shoot (85.46 µg/pot) and root (46.37 µg/pot) was observed in treatment of M_0 x HM_1 $Cd_{6.0}$ and root (2.69 µg/pot) were recorded in treatment of M_1 x HM_0. Bacterial inoculated plants were less heavy metal than the un-inoculated. This may be due to bacteria may be able to reduce the heavy metals plants.

Table 5: Effect of cadmium on total microbial count

Cd (mg/kg)	Microbial count			Mean
	M0	**M1**	**M2**	
HM0	6.95	8.99	7.89	7.94
HM1Cd1.5	6.83	8.95	7.83	7.87
HM1Cd3.0	6.75	8.85	7.75	7.78
HM1Cd6.0	6.63	8.75	7.64	7.67
Mean	6.79	8.88	7.78	
SEm	M= 0.008, HM= 0.01, M×HM = 0.02			

Increasing the level of toxic metals in soil reduced the soil microbial population and diversity in soil (Aydinalpi and Marinvo 2003). Data presented on total microbial count of soil was significantly influenced due to interaction effect of different treatments. The significantly highest total microbial count (8.99 cfu/g of soil) was found with treatment combination of M_1 x HM_0. The significantly lowest total microbial count (6.63 cfu/g) of soil was recorded with treatment combination of M_0 x HM_3 $Cd_{6.0}$. Bacterial count was markedly decreased might be due to increasing concentrations of metal. These finding is accordance with Rabia and Ali (2007).

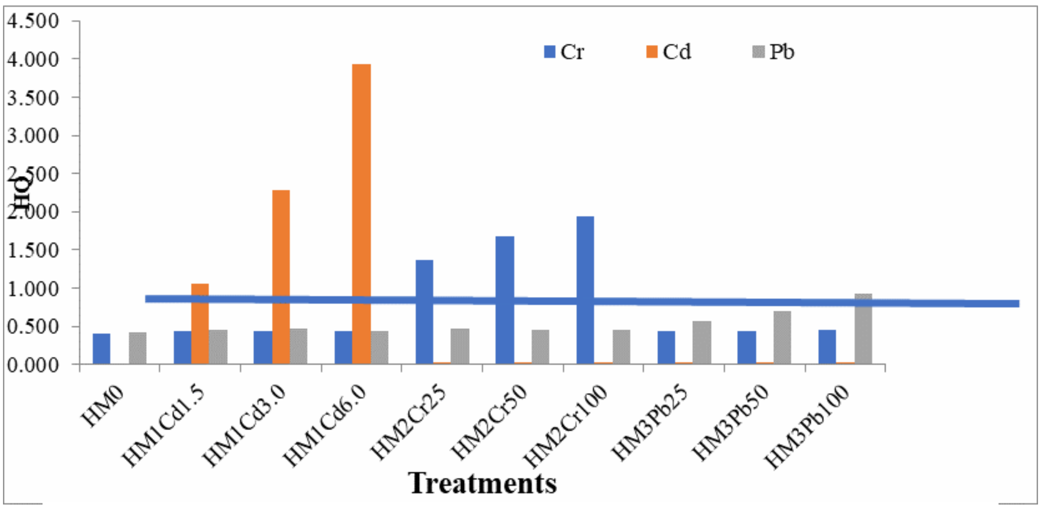

Fig. 1 : Comparison of Observed hazard quotient (HQ) of Cr, Cd and Pb for human

Hazard Index (HI): HI < 1 denotes highly unlikely significant toxic interaction & HI > 1 denotes potential non-cancer health effect

Risk Assessment using Hazard Quotient (HQ):

HQ: ADD/RfD **HQ: (M_{plant} × W × F) / RfD × 70)**

ADD= Average daily dose (mg kg^{-1} day^{-1})

RfD = Maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects (mg kg^{-1} day^{-1})

M_{plant}= Metal content (mg kg^{-1}) in spinach

W= Daily intake of green vegetable

F= Factor of conversion of fresh weight to dry weight

Conclusion

The results of this work concluded that plant exposure to heavy metal contamination resulted in toxicity in spinach was observed. Results of present study revealed that Cd treatments at high level induces a significant reduction in all growth parameters *i.e.,* shoot and root weight of in spinach crop. Increasing concentrations of Cd in soil resulted also in a corresponding increase of respective metal in the shoot and root of spinach and in soil as well as presence of bacterial inoculation (consortium of *Pseudomonas azotoformans, Bacillus infantis, Bacillus megaterium and Micrococcus terreus*) and FYM promoted growth of plants in terms of shoot and root weight in spinach plants subjected to application of different rate of Cd metals and reduced the content of respective heavy metal in spinach. Looking at the consequences of heavy metals on different components of food chain, at higher rate of heavy metals, greater accumulation of heavy metals in shoot and root is a major concern for human health due to a higher consumption rate.

Fig 2: Bird Eye View of Experiment

Fig 3: Effect of different levels of cadmium (Cd 1.5, 3.0, 6.0 mg/kg) in shoot and root length of spinach

Acknowledgement

We are highly grateful Department of Micronutrient Research Centre and Department of Microbiology for support and technical guidance and to Anand Agricultural University, Anand, Gujarat for providing all necessary facilities in conducting the experiments.

References

Adeyinka, A.K. (2017). Bioremediation of Heavy Metals in the Soil by *Pseudomonas aeruginosa* and *Trichoderma harzianum* Using *Solanum lycopersicum* as Test Plant. *Asian Journal of Soil Science and Plant Nutrition*, **2** (2), 1-13.

Ahemad, M. (2014). Bacterial mechanisms for Cr (VI) resistance and refers on, an overview and recent advances. *Folia microbiologica*, **59** (4), 321-332.

Aka, R.J.N. and Babalola, O.O. (2016). Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. *International Journal of Phytoremediation,* **8**, 200–209.

Akhtar, F.Z., Archana, K.M., Krishnaswamy, V.G. and Rajagopal, R. (2010). Remediation of heavy metals (Cr, Zn) using physical, chemical and biological methods, a novel approach. *SN Applied Sciences*, **2**,267.

Aydinalpi, C. and Marinova, S. (2003). Distribution and forms of heavy metals in some agricultural soils. *Polish Journal of Environmental Studies*, **12**, 629–634.

Baylock, M.J. and Huang, J.W. (2000). Phytoextraction of metals. In, Raskin, I. Ensley, B.D. (eds). Phytoremediation of toxic metals-using plants to clean up the environment. Wiley, New York, pp 53-70.

Celik, H. and Kunene, S.S. (2020). Assessment of heavy metal amounts of spinach plants (*Spinach Oleracea L.*) grown on Cd and chicken manure applied soil conditions. *Polish Journal of Environmental Studies*, **30**(2), 1105-1115.

Chellaiah, E.R. (2018). Cadmium (heavy metals) bioremediation by *Pseudomonas aeruginosa*. *Applied Water Science*, **8**,154.

Gavrilescu, M. (2004). Removal of Heavy Metals from the Environment by Biosorption. *Engineering in Life Sciences*, **4**, 3219-232.

Hseu, Z.Y., Su, S.W., Lai, H.Y., Guo, H.Y., Chen, T.C. and Zang, Z. (2010). Remediation techniques and heavy metal uptake by different rice varieties in metal-contaminated soils of Taiwan, New aspects for food safety regulation and sustainable agriculture. *Soil Science and Plant Nutrition,* **56**, 31–52.

Mitra, S., Chakraborty, A.J., Tareq, A.B., Emran, T.B., Nainu, F., Idris, A.M., Khandaker, M.D., Osman, H., Alhumayudhi, F.A. and Simal, J. (2022). Impact of heavy metals on the environment and human health, Novel therapeutic insights to counter the toxicity. *Journal of King Saud University – Science*, **34**(3), 101865.

Rabia, A. and Ali, T.A. (2007). Effect of heavy metals on soil microbial community and mung beans seed germination. *Pakistan Journal of Botany*, **39**(2).

Rajkumar, M. and Freitas, H. (2008). Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. *Chemosphere,* **71**, 834–842.

Shilev, S., Ivelina, B. and Todor, B. (2019). Consortium of plant growth-promoting bacteria improves spinach *(Spinacea oleracea L.)* growth under heavy metal stress conditions. *Journal of Chemical Technology and Biotechnology,* **10**.1002, 6077.

Wuana, R.A. and Okieimen, F.E. (2011). Heavy Metals in Contaminated Soils, A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *Communications in Soil Science and Plant Analysis,* **42**, 111-122.