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ORIGINAL ARTICLE **OPEN ACCESS**

Phytoremediation potential of Indian mustard (*Brassica juncea* L.) for Cd in contaminated soil by using FYM and EDTA

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ABSTRACT
The objective of the current study was to examine the effect of FYM and EDTA on Cd remediation by Indian mustard in contaminated soil. The pot experiment was carried out at Sheila Dhar Institute of Soil Science, Prayagraj, U.P., India under natural conditions. Plants were grown in pots having soil with different doses of Cd @ 0, 20, 40, and 60 mg kg^{-1} respectively with FYM @ 0 and 10 t ha^{-1}soils and EDTA @ 0 and 2.25 mmol kg^{-1}. Plants were also grown in pots with uncontaminated soils as control treatment (T_1). Cd is highly toxic to the plant and above 60 mg kg^{-1} of applied Cd the mortality of plant occurs. The Cd concentration increased in Indian mustard with use of EDTA.The highest concentration of Cd being found in EDTA treatment. The result indicates that the plant height, fresh and dry weight significantly decreased as level of Cd increased. Result also revealed that the use of FYM in combination with EDTA was found superior in enhancing phytoremediation potential of Cd by Indian mustard and increased these parameters as compare to their respective Cd treatment.
Keywords – *Cadmium, Contaminated soil, EDTA, FYM, Indian mustard, Phytoremediation*

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INTRODUCTION

Heavy metals naturally occur at lower concentration in soils. However, heavy metals are considered soil contaminates due to their widespread occurrence, acute and chronic toxicity. Common heavy metal contaminants include arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc, which present numerous health dangers to higher organisms and are also known to decrease plant growth, ground cover and have a negative impact on soil microflora[24,1]. Heavy metals can be classified into two categories regarding their need in a biological environment. They may be essential or non-essential metals, those heavy metals which are indispensable for the proper bio-functioning of life of organisms in very small quantities are called essential heavy metals. They include Ni, Fe, Zn, Mn, and Cu. And those heavy metals whose deficiency has not affected on proper bio-functioning of life of organisms include Cr, Cd, Hg, Pb, and As [10]. The stable performance of the living system is disturbed when the concentrations of heavy metals increase from a specific acceptable range [20].
Cadmium (Cd) is a non-essential element for plants and humans but is present in many soils in excessive amounts [5]. When it enters into the food chain, it poses a major threat to the living biota. The control of Cd accumulation in plants is complicated by the fact that most of the essential nutrient transporters, such as copper (Cu), manganese (Mn), iron (Fe), and zincs (Zn), also facilitate Cd uptake [9]. Chemical remediation is a mechanical process used for leaching the contaminated soil by using liquids enriched with solvents, freshwater, and chelating agents[11,23]. Researchers found that ethylene diamine tetra acetic acid (EDTA) is an effective chelating agent for soil washing. Recent studies have shown that biosurfactants, such as sophorolipids, saponin, and rhamnolipids, can efficiently remove Cd from contaminated soils [13].
Phytoremediation is an economically feasible method that uses plants, which have exceptional metal accumulating capabilities, to restore contaminated soils [3]. The method is less disruptive to the environment and more acceptable to surrounding communities [18].
Farm yard manure (FYM) is being used as the major source of organic manure in field's crops. Limited availability of this manure is, however, an important constraint on its use as a source of nutrients. FYM positively controls the crop production and recovers properties of soil and it can be used to decrease

heavy metal stress in plants. FYM positively influence crop production, improved soil physical, chemical and biological properties [16].
Indian mustard (*Brassica juncea* L.) typically a native herb of Indian Continent is commonly found growing in soil having high metal concentration and is effective in extracting heavy metals such as cadmium from the soil having high metal as acts as indicator species as it has a higher tolerance for these metals which gets stored n its cells [15]. Lotfy has tested five different plants for phytoremediation, he has reported sunflower plants has exhibited the higher remediation potential [12].
Keeping in view the above facts, there is a need to develop a suitable technique for soil remediation by enhancing the phytoremediation of Cd from contaminated soil.
It has been identified that Indian mustard as a high biomass, rapidly growing plant with an ability to accumulate heavy metal in its shoots and as a promising plant for phytoremediation. So the present study has been proposed to know the phytoremediation potential of Indian mustard for heavy metal (Cr, Cd, Pb and As) contaminated soil [4].

MATERIAL AND METHODS

Experimental site

A pot experiment was carried out in order to find out the phytoremediation potential of Indian mustard (*Brassica juncea* L.) for cadmium contaminated soil. The Sheila Dhar Institute Experimental Site is located at Prayagraj in Northern India at 25° 57'N latitude, 81°50 E longitude and at 120±0.4m altitude.The average annual temperature range between 22°C and 9°C respectively. Different physical and chemical characteristics are summarized in table 1.

Table1. Physico-chemical properties of soil

Parameters	Units	Value
Texture		Sandy loam
Clay	(%)	15.25
Silt	(%)	13.22
Sand	(%)	71.53
pH		7.8±0.2
EC	(dSm^{-1}) at 25°C	0.28±0.03
Organic carbon	(%)	0.56±0.15
CEC	[Cmol(P^+)/kg soil	19.6±0.6
Total nitrogen	(%)	0.07±0.02
Total phosphorus	(%)	0.03±0.01

Pot experiment

The aim of present study was to examine the phytoremediation potential of Indian mustard (*Brassica juncea* L.) for cadmium in cd-contaminated soils. The healthy and bold seeds of Indian mustard were collected from local market Chungi (Alopibagh) of Prayagraj. Indian mustard is a perennial herb which is related to the family Brassicaceae or Cruciferae. Tap root is found in Indian mustard with a depth of 90-180 cm. The uniform seeds were collected and used for the pot experiment.
For pot experiment 4 mm sieve used and 2 mm sieve is used for analysis of physico –chemical properties. Some physico- chemical properties are dipicted in Table 1. Firstly, the seeds of Indian mustard were germinated on the Whatman No. 42 filter paper in petri dishes, after that the seeds were transferred into earthen pot with untreated soil(control) and treated soil to which cadmium metal as cadmium chloride ($CdCl_2$) was applied with different concentration i.e. 0.20, 40 and 60 mg kg^{-1}.Each pot contained 3-5 kg soil. There were 2-5 seeds grown in each pot with three replicates. After 45 days, the plants were grown without Cd metal solution. This would be higher accumulation of metals in plants.

Soil and plant analysis

The organic C, N, P and K were estimated by the methods of Walkley and Black, Micro-Kjedahl, Olsen and Flame photometer, respectively, in soil as described by Rowell (1994). Cation exchange capacity was carried out using the $BaCl_2$ method [8].
The Indian mustard plant completes its life cycle within 1-2 months. When its life cycle is completed then the plants were harvested and collected separately as shoots and roots. The roots of plant were washed with normal tap water accurately followed by distilled water. The plant samples dried at 70°C for 48 hours in hot air oven till the plant sample weight become near about 0.5g, the dried weight was recorded. The dried plant materials were ground and digested with the help 15 ml of tri-acid mixture containing concentrated HNO_3 (16 M, 71%), H_2SO_4 (18 M, 96 %) and $HClO_4$ (11 M, 71 %) in (5:1:2). The cadmium

concentrations in soil and plant samples were determined with Atomic Absorption Spectrophotometer (Perkin Elmer).
Besides the accumulation concentration, bioconcentration factor (BCF), translocation factor (TF) and translocation efficiency (TE %) the three most important parameters which are used to evaluate the accumulating capacity of heavy metals by plants were calculated.
Bioconcentration factor (BCF) was calculated by using following formula given by [26].
$BCF = C_{harvested\ tissue} / C_{soil}$
Where, $C_{harvested\ tissue}$= concentration of the target metal in the plant harvested tissues (roots, shoots and leaves).
And C_{soil}= concentration of the same metal in soil, C soil represents the total Cd applied to the soil.
Translocation factor (TF) was calculated by using the following formula proposed by [17].
$TF = C_{shoots} / C_{roots}$
Translocation efficiency (TE %) was calculated as per formula proposed by Meers [14].
TE%= Cd content in the shoots $mgkg^{-1}$/ Cd content in the whole plant $mgkg^{-1} \times 100$
The % Cd removal was calculated by the following formula,
% Cd removal= Total Cd uptake by plant/ Total Cd applied to the soil ×100

Statistical analysis

Statistical analysis was done to determine the significance between the means and draw a valid conclusion. "Analysis of Variance" method was adopted as the statistical tool for the raw data observed during the whole experiment. The difference of the treatments means was verified using the critical difference (CD) at 1% level of probability following the Complete Randomized Design (CRD) to find out a valid and authentic difference among the treatments [7].

RESULT AND DISCUSSION

The phytoremediation potential of Indian mustard depends on the biomass of shoot, roots and its accumulation concentration, bioconcentration factor (BCF) translocation factor (TF) and translocation efficiency (TE %) of the applied cadmium.

Dry matter yield of plants

The dry matter yields of root and shoot of Indian mustard by the addition of FYM and EDTA significantly decreased. Plant showed a significant decrease when EDTA added more than 2.25 $mmolkg^{-1}$. The level of EDTA, dry matter yield of Indian mustard plant is given in Table 2.
The total dry weight of Indian mustard got affected by the contaminated soil. The highest biomass of Indian mustard plant found in the T_3 when Cd=0 mg kg^{-1} is applied with 2.25 mmol kg^{-1} EDTA and 0 t ha^{-1} FYM. The Indian mustard had high biomass production of root and shoot which increased from T_1-T_3 (15.47 & 19.87 g/pot and 169.31&171.02 g/pot) respectively. After the treatment (3) the dry matter yield of Indian mustard was decreased from (T_4-T_{12}). The lowest biomass was found in T_{12}.

Uptake of Cd by Indian mustard

Cadmium hyper accumulator may be defined as the plant which is capable of accumulating more than 90-100 mg Cd/kg dry matter in shoots (leaves & stems) dry matter [2]. A plant concentration of more than 90-100 mg Cd/ kg dry matter may be regarded as exceptional, even in Cd contaminated soil [22].

The accumulation of Cd by Indian mustard at different doses has been carried out in pot experiments for 45 days treatment of Cd and 60 days of growing period. It was found that by the increasing the concentration of Cd in the soils, the accumulation of Cd in plant organ also increased. Once the Cd ions are absorbd they may be accumulated in the roots and exported to the aerial part by transpiration [21].
The maximum concentration of Cd was found in roots rather than shoots and leaves of Indian mustard. Cd concentration was found highest i.e. 13.08 and 38.01 mg Cd/kg dry matter in roots and shoots respectively when Indian mustard plant incorporated with 60 mg kg^{-1} of cadmium. The same result has been found for *Amaranthus tricolor, Brassica chinensis and L. albus* [19, 11, 27].
The uptake of Cd by roots of the plant was found greater than shoot uptake of Cd. In all EDTA and FYM doses Cd concentrations in roots were about 4-6 times higher than in shoots.

Table2. Biomass (g pot^{-1} in dry weight) and Cadmium uptake(mg kg^{-1} in dry weight) by *Brassica juncea* L.

Treatments	Dry Weight(g pot^{-1})		Average metal uptake per pot(mg kg^{-1})	
Cd=mg kg^{-1},EDTA= mmol kg^{-1}, FYM=t ha^{-1}	Root	Shoot	Root	Shoot
T_1(0 Cd+ 0 EDTA)	15.47±0.53	169.31±1.01	0	0
T_2(0 Cd+ 2.25 EDTA)	17.21±1.12	170.66±1.44	0.60±0.02	0.10±0.03
T_3(0 Cd+ 2.25 EDTA+ 0 FYM)	19.87±0.97	171.02±0.99	0.82±0.01	0.17±0.04
T_4(20 Cd+ 0 EDTA)	11.21±1.79	165.34±0.46	11.35±0.20	3.19±0.04
T_5(20 Cd+ 2.25 EDTA)	12.45±0.87	166.37±1.23	13.62±0.26	5.38±0.43
T_6(20 Cd+ 2.25 EDTA+ 10 FYM)	13.34±1.21	168.03±1.02	15.38±1.23	7.12±1.06
T_7(40 Cd + 0 EDTA)	10.34±1.21	162.00±1.00	21.27±1.16	6.67±1.65
T_8(40 Cd + 2.25 EDTA)	11.51±1.33	163.26±1.15	23.30±1.23	8.21±1.11
T_9(40 Cd+ 2.25 EDTA+10 FYM)	12.34±1.20	165.37±1.22	27.61±.41	10.38±1.23
T_{10}(60 Cd + 0 EDTA)	7.20±1.20	160.73±1.50	30.06±1.03	10.13±1.07
T_{11}(60 Cd+ 2.25 EDTA)	8.44±1.44	161.51±1.33	34.32±1.19	11.11±1.05
T_{12}(60 Cd+ 2.25 EDTA +10 FYM)	9.87±1.87	159.74±0.93	38.01±1.01	13.08±1.10

Table 3. Translocation factor and Bioconcentration factor of *Brassica juncea* L.

Treatments	Translocation Factor	Bio-concentration factor
Cd=mg kg^{-1},EDTA=mmol kg^{-1}, FYM=t ha^{-1}		
T_1(0 Cd+ 0 EDTA)	0	0
T_2(0 Cd+ 2.25 EDTA)	0.16±0.02	0
T_3(0 Cd+ 2.25 EDTA+ 0 FYM)	0.20±0.01	0
T_4(20 Cd+ 0 EDTA)	0.28±0.02	0.15±0.03
T_5(20 Cd+ 2.25 EDTA)	0.39±0.02	0.26±0.03
T_6(20 Cd+ 2.25 EDTA+ 10 FYM)	0.46±0.01	0.35±0.01
T_7(40 Cd + 0 EDTA)	0.31±0.03	0.16±0.03
T_8(40 Cd + 2.25 EDTA)	0.35±0.01	0.20±0.01
T_9(40 Cd+ 2.25 EDTA+10 FYM)	0.37±0.02	0.25±0.03
T_{10}(60 Cd + 0 EDTA)	0.33±0.03	0.16±0.02
T_{11}(60 Cd+ 2.25 EDTA)	0.32±0.01	0.18±0.02
T_{12}(60 Cd+ 2.25 EDTA +10 FYM)	0.34±0.03	0.21±0.04

Values are average of three replicate ± S.D (n=3).

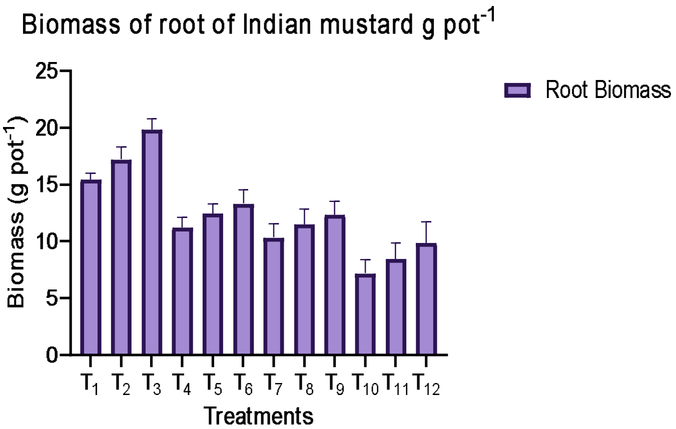

Fig.1 Biomass of root of Indian mustard

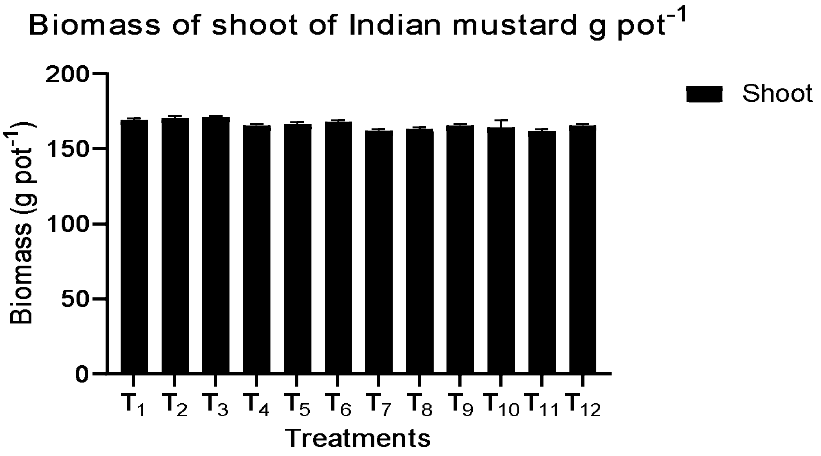

Fig.2 Biomass of shoot of Indian mustard

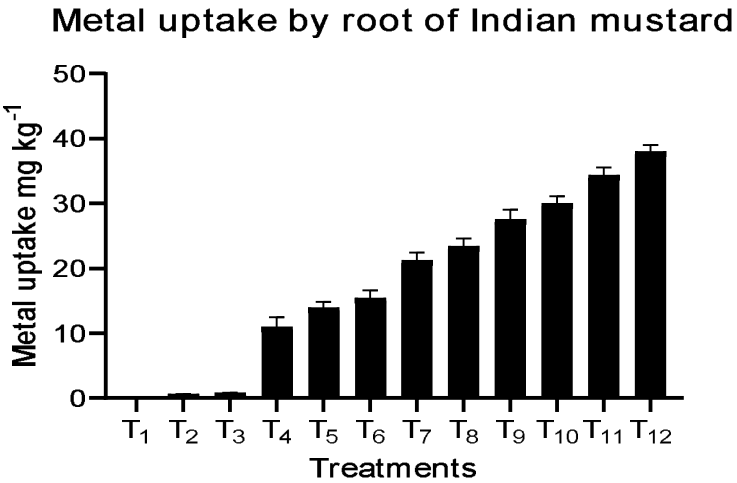

Fig.3 Cd accumulation in root of Indian mustard

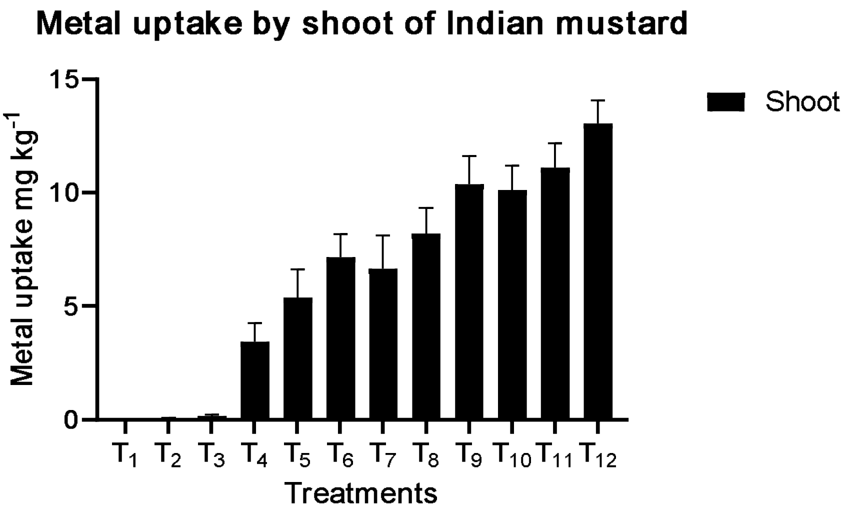

Fig.4 Cd accumulation in shoot of Indian mustard

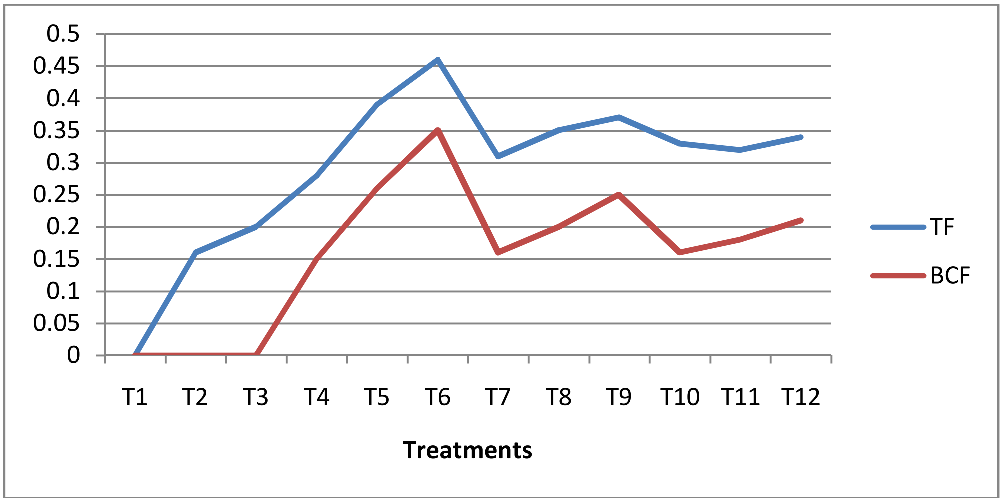

Fig.5 TF and BCF of Indian mustard

Translocation factor

Translocation factor is an effective measure for determinig the metal phytoextraction from soils. There are three categories of plant based on their TF namely accumulator (TF>1), excluder (TF<1), and indicator (TF near 1)[2,6]. More ever, the higher the TF value is, the stronger the phytoextraction ability. In this study the translocation factor was found less than 1 for Indian mustard.The present study showes that the Indian mustard plant has high accumulation of Cd in roots and low concentration in shoots, nevertheless Indian mustard plant could be used for phytoremediation of Cd in contaminated soil. Translocation factor and bioconcentration factor are dipicted in table 3.

Bio-concentration factor

Bio-concentration factor (BCF) indicates the efficiency of a plant in uptaking heavy metals from soil and accumulating them into its tissues. It is ratio of the heavy metal concentration in the plant tissues (roots stem and leaves) to that in soil [26]. For a plant to be efficient tool in the contaminated soil phytoremediation, the BCF have to be more than 1. In this present study, BCF of Cd was found to <1 suggesting Indian mustard a good plant for phytoremediation of Cd-contaminated soil. The BCF increased considerably (0.15 at 20 mg kg^{-1} Cd soil to 0.25 at 40 mg kg^{-1} Cd soil) with increasing Cd concentration of soil.

The result indicates that EDTA in combination with FYM was found superior in enhancing phytoremediation potential of Cd by Indian mustard plant. The Cd concentration increased in Indian mustard with the use of EDTA and FYM.

CONCLUSION

In this research study, it may be concluded that the Indian mustard plant has efficient phytoremediation ability to Cd. The distribution order of Cd content in the Indian mustard plant was root > shoot > leaves. According to TF and BCF the Indian mustard plant can be classified as an indicator plant to the Cd because the TF and BCF values of Indian mustard plant in this study found less than 1 or near to 1. The highest concentration of cadmium was 38.01 mg kg^{-1} and 13.08 mg kg^{-1} in roots and shoots of Indian mustard plant at 60 mg Cd/kg soil respectively with EDTA and FYM addition. The highest TF and BCF values were found 0.46 and 0.35 respectively. Therefore, the Indian mustard is an effective accumulator plant for phytoremediation of cadmium from cadmium contaminated soil.

This study provides a promising start for biomass based phytoremediation or phytoexraction. Further research in field condition is needed to phytoremediation potential of Indian mustard (*Brassica juncea* L.) for Cd in contaminated soil by using FYM and EDTA.

CONFLICT OF INTEREST

Through this, all authors declare that there is no conflict about the publication of this research paper.

REFERENCES

1. Alizadeh, S. M., Zahedi-Amiri, G., Savaghebi-Firoozabadi, G., Etemad, V., Shirvany, A. & Shirmardi, M., (2012). Assisted phytoremediation of Cd-contaminated soil using poplar rooted cuttings. International Agrophysics*,* 26, 219-224.

2. Baker A.J.M. (1981) Accumulators and excluders-Strategies in the response of plants to heavy metals. Journal of Plant Nutrition,3: 643–654.
3. Bosire G.O.(2014). Rehabilitation and Phytoremediation of Heavy Metal Polluted Riverine Wetlands using Bamboo for Phytoextraction in Kibera. [dissertation] Nairobi. Kenya. Kenyatta University.
4. Cunningham, S.D., & Lee, C.R.(1995). Phytoremediation: Plant-based remediation of contaminated soils and sediments. In: Skipper, H.D., Turco, R.F. (Eds.), Bioremediation: Science and Applications. Madison, Wisconsin, 145-156.
5. Dhaliwal, S.S.; Singh, J.; Taneja, & P.K.; Mandal (2020). A. Remediation techniques for removal of heavy metals from the soil contaminated through different sources: A review. Environmenta Science Pollution Research, 27, 1319–1333.
6. Ghosh M. & Singh S.P. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. Applied Ecology and Environmental Research ,3: 1–1
7. Gomez, A. K., & A. A. Gomez.(2007). Statistical procedures for agricultural research. 2nd edition New York: John Wile and sons, U.S.A.
8. Hendershot W.H. & Duquette M (1986) A simple barium chloride method for determining cation exchange capacity and exchangeable cations. Soil Science Society of American Journal, 50: 605–608.
9. Huang, R.; Dong, M.; Mao, P.; Zhuang, P.; Paz-Ferreiro, J.; Li, Y.; Li, Y.; Hu, X.; Netherway, P.; & Li, Z.(2020). Evaluation of phytoremediation potential of five Cd (hyper) accumulators in two Cd contaminated soils. Science Total Environment, 721, 137581.
10. Jadia C.D. & Fulekar M.H.(2020). Phytoremediation of heavy metals: recent techniques. African Journal Biotechnology, 8:6.
11. Liu,C.P., Shen, Z.G. & Li, X. D.,(2007). Accumulation and detoxification of cadmium in Brassica pekinensis and B.chinenesis.Biologia Plantarum 51, 51(1)116-120.
12. Lotfy S & Mostafa A (2013). Phytoremediation of contaminated soil with cobalt and chromium. Journal Geochemistry Explorer.
13. Maity, J.P.; Huang, Y.M.; Fan, C.-W.; Chen, C.-C.; Li, C.-Y.; Hsu, C.-M.; Chang, Y.-F.;Wu, C.-I.; Chen, C.-Y.; & Jean, J.S.(2013). Evaluation of remediation process with soapberry derived saponin for removal of heavy metalsfrom contaminated soils in Hai-Pu, Taiwan. Joural Environmental Science, 25, 1180–1185.
14. Meers E, Hopgood M, Lesage E, Vervaeke P, Tack F.M.G. & Verloo M. (2004). Enhanced Phytoextraction : In Search for EDTA Alternatives. International Journal Phytoremediation, 6(2):95–109.
15. Naser A. Anjum(2012). The plant family Brassicaceae contribution towards Phytoremediation. Dordrecht: Springer.
16. Ould Ahmed, B.A., Inoue, M. & Moritani, S.(2010). Effect of saline water irrigation and manure application on the available water content, soil salinity, and growth of wheat. Agricultural Water Management, 97: 165-170.
17. Padmavathiamma P.K. & Li LY. (2007). Phytoremediation technology: Hyperaccumulation metals in plants. Water, Air and Soil Pollution, 184:105–126.
18. Sunitha, R., S. Mahimairaja, A. Bharani, & P. Gayathri. (2014). Enhanced phytoremediation technology for chromium contaminated soils using biological amendments. International Journal of Science and Technology, 3:153–62.
19. Watanabe, T., Murata, Y. & Osaki, M., (2009). Amaranthus tricolor has the potential for phytoremediation of cadmium-contaminated soils. Communications in Soil Science and Plant Analysis, 40, 3158-3169.
20. Wuana R.A. & Okieimen F.E.(2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology.1-20. RRJBS| Volume 8 | Issue 3 | July - September 2019 15 Research & Reviews: Journal of Botanical Sciences e-ISSN: 2320-0189 p-ISSN: 2347-2308.
21. Ximenez, P., RodrÍguez, B., Madrid, B. & Camara, C., (2002). Uptake of heavy metals by lupin plants in artificially contaminated sand: preliminary results. International Journal of Environmental Analytical Chemistry, 82, 805-813.
22. Yang, X. E., Long, X. X., Ye, H. B., He, Z. L., Calvert, D.V. & Stoffella, P. J., (2004). Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (Sedum alfredii Hance). Plant and Soil, 259, 181–189.
23. Yao, Z.; Li, J.; Xie, H & Yu, C.(2016). Review on remediation technologies of soil contaminated by heavy metals. Procedia Environmental Science, 16, 722–729.
24. Zhang X et al.(2011). Identification of a new potential Cd hyperaccumulator Solanum photeinocarpum by the soil seed bank-metal concentration gradient method. Journal Hazard Material, 189:414-41.
25. Zhang, H., Dang, Z., Zheng, L. C. & Yi, X. Y., (2009). Remediation of soil co-contaminated with pyrene and cadmium by growing maize (*Zea mays* L.). International Journal of Environmental Science and Technology, 6(2), 249-258.
26. Zhuang P, Yang Q.W, Wang H.B. & Shu W.S. (2007). Phytoextraction of heavy metals by eight plant species in the field. Water, Air and Soil Pollution, 184:235–242.
27. Zoronza, P., Vázquez, S., Esteban, E., Fernández-Pascua, M. & Carpena, R., (2002). Cadmium-stress in nodulated white lupin: strategies to avoid toxicity. Plant Physiology and Biochemistry, 40, 1003-1009.

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