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Effect of Lead and Cadmium on the Growth Parameters and Protein Content of *Coleus blumei* Benth and Heavy Metal Extraction Capacity

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Abstract: ***The study was focused on the effect of heavy metals on the growth parameters of Coleus blumei benth and its remediation capacity. Even the effect of metals on the protein content was measured by Bradford method. High concentration significantly reduced protein content and plant growth. The effect of Lead and Cadmium accumulation on growth was analyzed by the measurement of various parameters like Shoot length Root length protein content. The absorption rates of heavy metal by plants were analyzed by qualitative analysis. Results of qualitative analysis showed presence of heavy metals in leaf, stem, root. A root showed highest accumulation as compare to stem and leaves. Coleus blumei benth is metal stress tolerant and has phytoextraction capacity of heavy metals from contaminated soil.***

Keywords: Heavy metals, Phytoextraction, Protein Content, *Coleus blumei* L.

1. Introduction

Development of urbanization and industrialization soil has become polluted. Hence, there is need to technique for management to reduce contaminated soil. Phytoremediation is a process to utilize the natural properties of plant to remediate soil, water and sediments. Soil contamination by inorganic and organic compounds is can be decrease by the phytoremediation (Kokyo Oh *et al.*, 2014). Soil contamination by heavy metal is one of the big environmental problems in world. The broken-down of this kind of toxicant into nontoxic form is not easy. Heavy metal like Pb, Hg, Cd, Ar, and Cr are toxic also toxic at low concentration and have not biological function. (Kuntal Shah *et al*., 2017).In comparison Cd is found to be more toxic then Pb (Sharifah Barlian Aidid *et al*.,1993). The adequate protection and restoration of soil and water ecosystem contaminated by heavy metals require their characterization and remediation. Remediation heavy metal contaminated soil and water is necessary to reduce, available for agriculture production, enhance food security and scale down land tenure problems (Elham Asari, 2014). Phytoremediation an aspect of bioremediation. That uses plants for the treatment of polluted soil and cleanup the soil environment (Kuntal Shah. *et al.*, 2017). Plants are used to degrade or detoxify the pollutants from soil, sludge, sediments, surface water and ground water (Bhargava, 2017). Phytoremediation refers to the natural ability of plants to bioaccumulation, degrade or render harmless containments in soil, water or air through the natural, biological, chemical or physical activity and process of plant (Ghoghara, 2017).

2. Materials and Method

Plant material was purchased from Anand nursery, Gandhinagar, Gujarat, India. All plants were below 10 cm height all were propagated through cuttings. The plants were transferred into pots which having 1kg garden soil. All plants were successfully transplanted in to separate pots in the Botanical garden of Gujarat University.

Treatment of Heavy metals to the plants:
All the plants were treated with different concentrations of cadmium and lead. Pb was selected in the form of nitrate and the concentrations were selected for the series were 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg. Cd was selected in the form of nitrate and the concentrations were selected for the treatment were 5 mg/kg, 10mg/kg, 15mg/kg, 20mg/kg. The plants were treated with metals up to 80 days and during this time physiological parameters were measured like leaf area, height, canopy etc.

Qualitative analysis of heavy metal

Collection and drying plant treatment:
After the 80 days of treatment plants leaves were collected separately. The leaves were dried separately with the help of oven at 100° C for 1 hour. Like a leaves root were collected separately and dried in oven dried for 100° C for 2 hours. As well as shoot were collected and cut into pieces and dried with oven for in 100° C for 4 hours. All the dry material was crushed with the help of mortar and pastel and store in different packet. The 10 g powder were measured and kept in the separate flask and solvent dilute HCl were added. This flask kept in flask shaker machine for 121 for 24 Hour. After the rotation period solution was filtered with whatman paper number 42 in Petri plates. Put the Petri plates in clean area for the evaporate the solvent. From the plant extract 1 mg extract should take for the qualitative tests.

1) Reactions for Lead ion.

- Dilute hydrochloric acid: white precipitate
  Plant Extract + 70% HCl = White precipitate
- Sodium hydroxide: white precipitate
  Plant extract + NaOH = White precipitate
- Dilute sulphuric acid: white precipitate
  Plant extract + H_2SO_4 = White precipitate

2) Reaction for Cadmium ion.

- Hydrogen sulphide: Yellow precipitate

Plant extract + H_2S= Yellow precipitate

- Potassium syanide: White precipitate

Plant extract + KCN = White precipitate

Estimation of Protein

Standard protocol of total protein by Bradford, 1976, was followed:

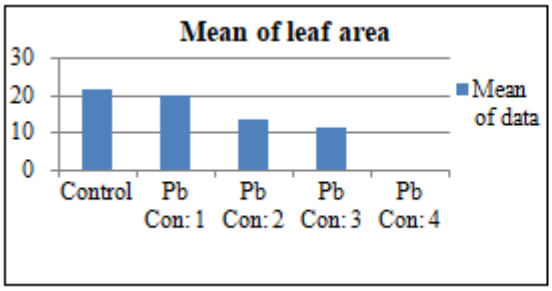
Grind 1 gm plant material in 10 ml, 0.1M phosphate buffer (pH 7.2) using mortar and pestle. Centrifuge the extract at 10,000 rpm for 15 minutes at 4°C. Use the supernatant as extract for estimation of total soluble proteins. Prepare various concentration of standard protein solution from the stock solution (i.e. 0.2, 0.4, 0.6, 0.8, and 1.0 ml) into series of test tubes and make up the volume to 1 ml by adding distilled water. Pipette out 0.2 ml of the sample in two other test tubes and make up the volume to 1 ml by adding distilled water. A tube with 1 ml of distilled water serves as blank. Add 5.0 ml of Bradford reagent to each tube and mix by vortex or inversion. Wait for 10-30 minutes and take reading of standard and sample at 595nm. Plot the absorbance of the standard verses their concentrations. Plot the graph of optical density versus concentration. From graph, find amount of protein in unknown sample.

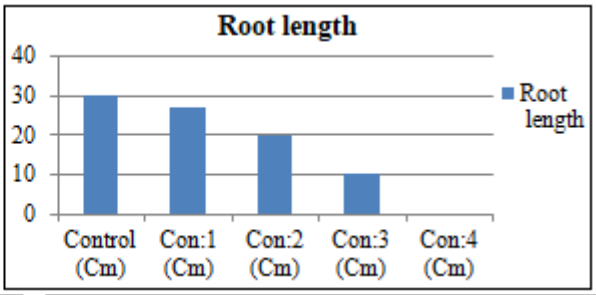
3. Results and Discussion

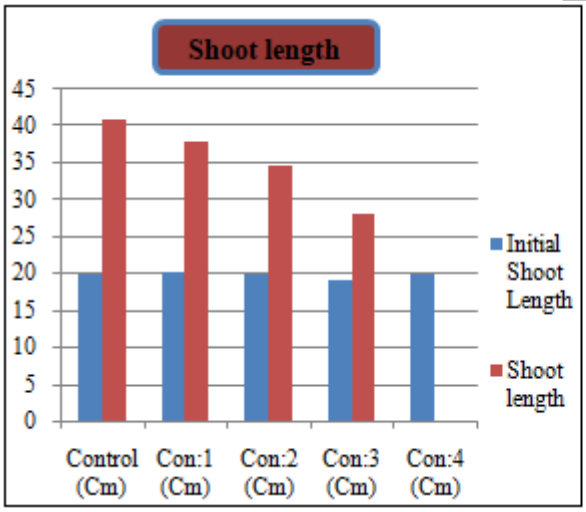
As the result table showing that as the concentrations of heavy metal increases sequentially root length, shoot length, leaf area and plant canopy decreases. All the qualitative results of heavy metals in different parts of different concentration treated plants showed presence of heavy metals in acid digested plant extracts. Roots showed maximum extraction and quantity of heavy metals as per precipitation data showed during analysis. Stem has low amount of heavy metal presence and leaf has very trace amount of heavy metals both lead and cadmium and it was found during qualitative result analysis. Protein estimation results showed that as the lead concentration increases the protein content was increases in the plant up to 400mg/kg concentration but as the concentration increases more than 400mg/kg protein content gradually decreases in the plant. Same for cadmium up to15mg/kg concentration protein content was increased and then it was decreased. So many researchers worked on phytoremediation of Coleus sp. but they worked with different pollutants here the research work was focused with lead and cadmium with nitrate salt and different concentrations.

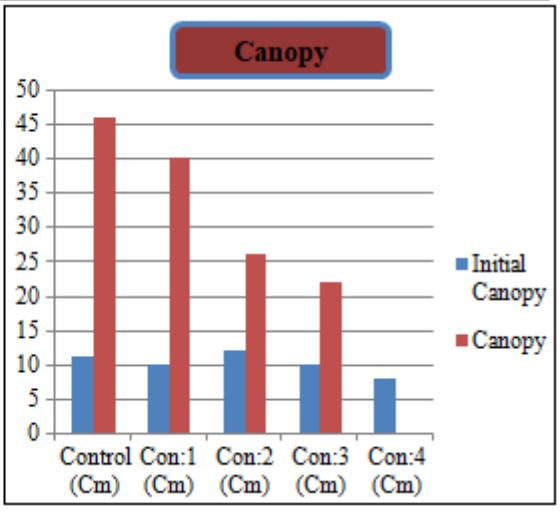
List of Different Morphological parameters

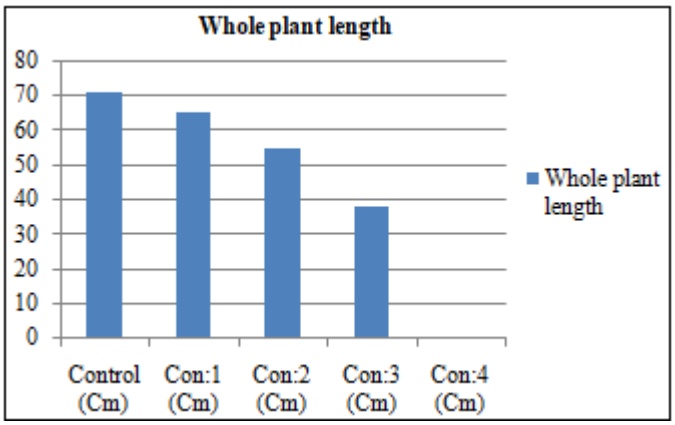
Heavy metal	Final reading	Control (Cm)	200 mg/kg (Cm)	400 mg/kg (Cm)	600 mg/kg (Cm)	800 mg/kg (Cm)
Lead (Pb)	Mean data of Leaf Area	21.2	19.8	13.4	11.2	0
	Final shoot length	41	38	34.6	28	27
	Final root length	30	24	20	10	0
	Initial canopy	11	10	12	10	8
	Final canopy	46	40	26	22	0
	Whole plant length	71	65	54.6	38	0





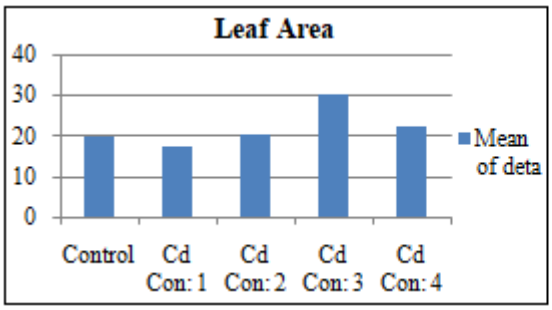


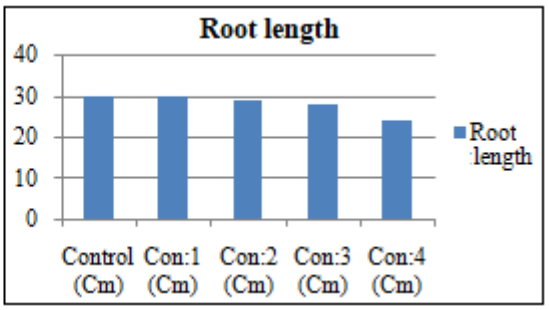


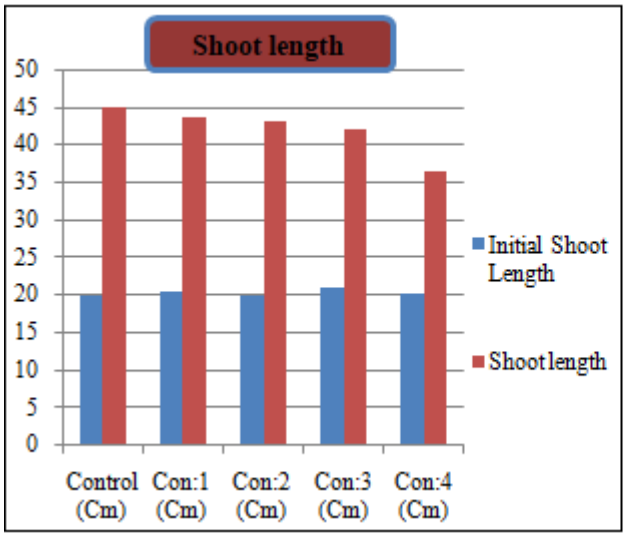


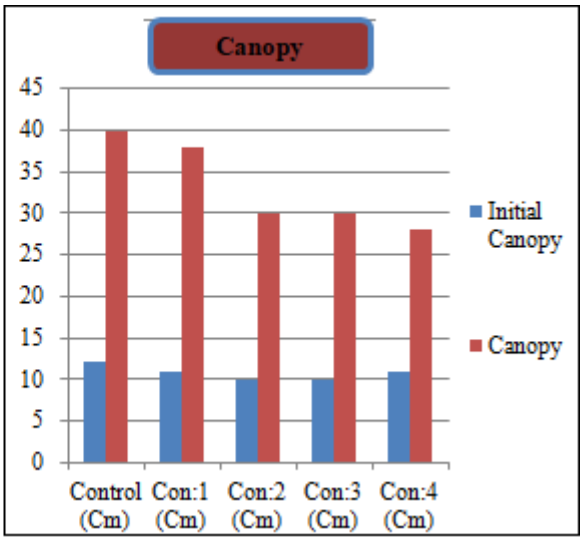
List of Different Morphological parameter

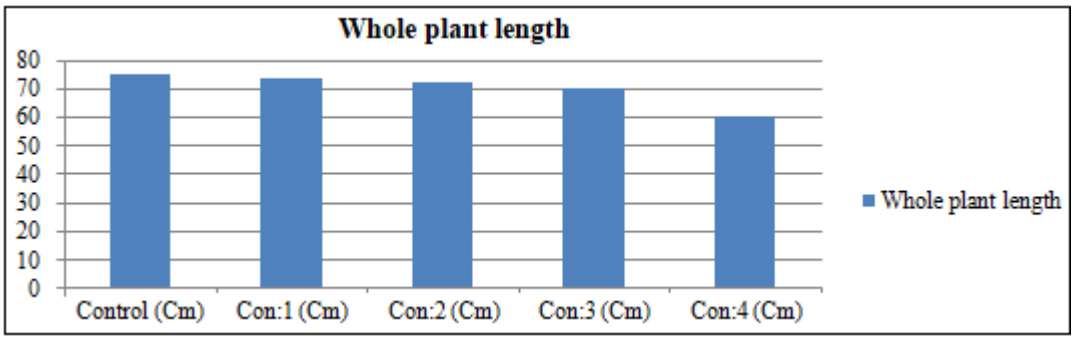
Heavy metal	Final reading	Control (Cm)	5 mg/kg (Cm)	10 mg/kg (Cm)	15 mg/kg (Cm)	20 mg/kg (Cm)
Cadmium (Cd)	Mean data of leaf area	19.8	17.2	20.2	30	22.2
	Final shoot length	45	43.5	43	42	36.4
	Final root length	30	30	29	28	24
	Initial canopy	12	11	10	10	11
	Final canopy	40	38	30	30	28
	Whole plant length	75	73.5	72	70	60.4









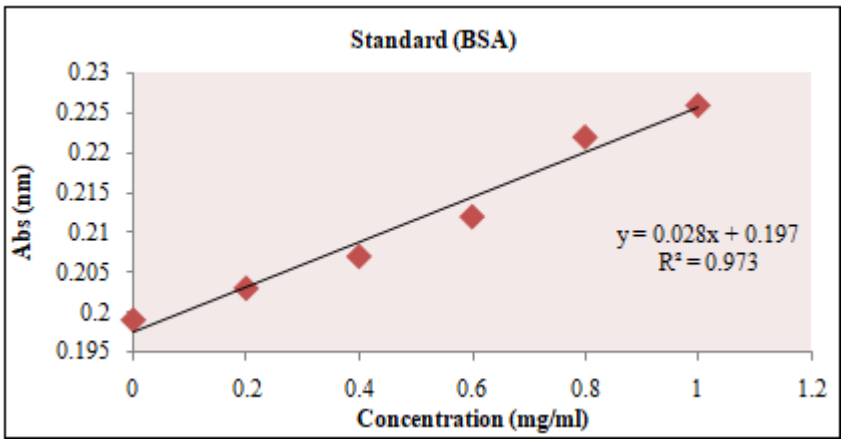


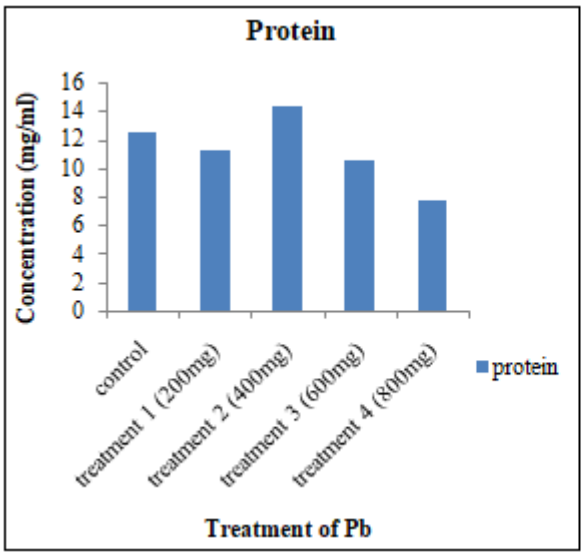
(2) Qualitative analysis of heavy metal:

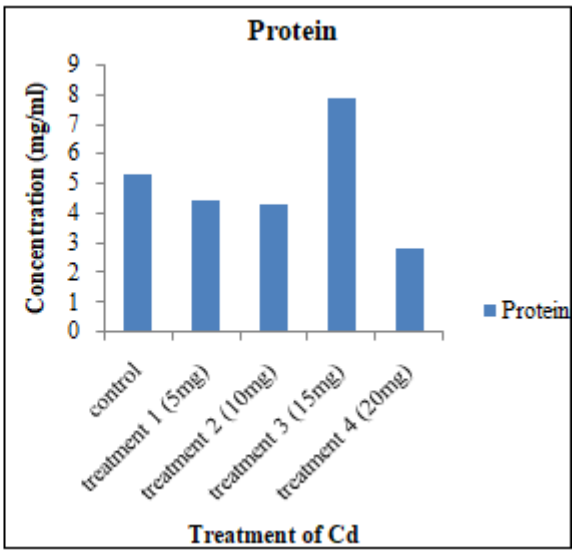
	1 mg Plant extract with different chemical	Concentration	Precipitate	Result		
				Leaf	Shoot	Root
Heavy metal Pb	Plant Extract + 70% HCl	Control	White precipitate	-	-	-
		200 mg/kg	White precipitate	+	++	+++
		400 mg/kg	White precipitate	+	++	+++
		600 mg/kg	White precipitate	+	++	+++
	Plant extract + NaOH	Control	White precipitate	-	-	-
		200 mg/kg	White precipitate	+	++	++
		400 mg/kg	White precipitate	+	++	++
		600 mg/kg	White precipitate	+	++	+++
	Plant extract + H_2SO_4	Control	White precipitate	-	-	-
		200 mg/kg	White precipitate	+	++	+++
		400 mg/kg	White precipitate	+	++	+++
		600 mg/kg	White precipitate	+	++	+++

	1 mg Plant extract with different chemical	Concentration	precipitate	Result		
				Leaf	Shoot	Root
Heavy metal Cd	Plant Extract + H_2s	Control	Yellow precipitate	-	-	-
		5 mg/kg	Yellow precipitate	+	+	++
		10 mg/kg	Yellow precipitate	+	++	+++
		15 mg/kg	Yellow precipitate	+	++	+++
	Plant extract + KCN	Control	White precipitate	-	-	-
		5 mg/kg	White precipitate	+	++	++
		10 mg/kg	White precipitate	+	++	++
		15 mg/kg	White precipitate	+	++	++

Estimation of Protein







4. Conclusion

Coleus blumei is one of the heavy metal stress tolerant ornamental species. It has capacity to even uptake or extract heavy metals from the soil. So, it can be planted to decrease theheavy metal contaminants from the soil and can be implemented and nearby industrial zones where metal concentration is very high in the soil. In future with the help of *in-silico* analysis the proteins or the phytochemicals can be identified where the heavy metal is binding. *Coleus blumei* is one of the good biological sources to remove heavy metal from contaminated soil.

References

[1] Aidid, S. B., & Okamoto, H. (1993). Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc. *Biometals*, *6*(4), 245-249.
[2] Elham Asari, (2014). Heavy metal contamination of water and soil analysis, assessment, remediation strategies Page No:1.
[3] Jaimisha Ghoghara, (2017). Effect of selected heavy metals on the growth of *Hydrilla verticillota* (L.f.) Royal. [M. Sc. Botany]. Department of botany, School ofScience.
[4] Kotagiri, D., & Kolluru, V. C. (2017). Effect of salinity stress on the morphology and physiology of five different Coleus species. *Biomedical and Pharmacology Journal*, *10*(4), 1639-1649.
[5] Oh, K., Cao, T., Li, T., & Cheng, H. (2014). Study on application of phytoremediation technology in management and remediation of contaminated soils. *Journal of Clean Energy Technologies*, *2*(3), 216-220.
[6] Ram Naresh Bhargava (2017). Environmental Pollutants and Their Bioremediation Approaches.
[7] Shah, K., Mankad, A. U., & Reddy, M. N. (2017). Cadmium accumulation and its effects on growth and biochemical parameters in *Tagetes erecta* L. *J Pharmacogn Phytochem*, *6*(3), 111-115.
[8] Shah, K., Mankad, A. U., & Reddy, M. N. (2017). Lead Accumulation and its Effects on Growth and Biochemical Parameters in *Tagetes erecta* L. *Int. J. Life. Sci. Scienti. Res*, *3*(4), 1142-1147.