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The Inhibition of Bean Plant Metabolism by Cadmium Metal: I Effect of Cd Metal on Physiological Process of Bean Plant and *Rhizobium* Species

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Abstract: Toxicity of Cadmium (Cd) metal were determined by the absorption and accumulation in bean plants. Results have been explained in relation with nitrifying bacteria and physiological process of plants. Toxicity of heavy metal Cd mainly due to the industrial wastage, medicinal pesticides, automobile exhaust which were continuously thrown in aquatic resources, causes contamination of water used for irrigation. It was observed that Cd seems to be highly toxic to the seedlings of bean plants at all concentration used and it was also harmful for the microbial life of soil. No nodule formation in the roots of bean plants were found, due to the lower population of soil bacteria, which fixes the nitrogen from the atmosphere in to the roots of plant in the form of nodules. Physiological processes of plants were effected by the absence of nitrifying bacteria, *Rhizobium* species. Protein and amino acid contents were found to be decreased at low concentration of Cd, but amino acid were absent at high percentage of Cd metal. Morphology of plants were also inflected by the presence of metal and color of plants turns yellow which in turns produce the effect on process of photosynthesis.

Key words: Cd, *Rhizobium* species, toxicity, morphology

INTRODUCTION

Among the numerous plants, Cadmium (Cd) an elements with unknown biological function is of major concern. Industrial utilization of compounds containing Cd has accelerated the immobilization and transport rates of Cd, Which far exceed the rates of natural cycling process. These rates have led to increase deposition of Cd in aquatic and terrestrial environment with subsequent increased uptake in accumulation of Cd in biota^[1].

Beneficial microorganisms, which are responsible for the biological functions of soils and thus regulate soil fertility, have been found to be highly sensitive towards toxicity of heavy metals. In particular bacteria of genus *Rhizobium* (which are able to fix atmospheric nitrogen in symbiosis with leguminous plants) died out in soil subjected to only moderate contamination with heavy metals^[2]. Smaller population exists in soil in the absence of host plants and results indicated large concentrations of metals in soil exceeding limit values may lower the population size below the threshold detectable by standard open tube isolation method^[3]. Nitrogen is a major components of DNA (Deoxy ribonucleic acid) and protein in the cell, is together with Mg also the key elemental constituent of chlorophyll the light harvesting pigment of photosynthetic plants^[4]. In addition, specific

concentration of nitrogen and other nutrient element in plants and other nutrient elements (P, S, Ca, Mg, Fe and CU) elicit the production of isoflavonoids in plants and these molecules functions as signal to mutualistic soil microbes and/or phytoalexins against infecting pathogens^[5].

The present investigations were carried out to check the toxicity of Cd metal on the growth, Physiological processes of bean plants and nitrogen fixing bacteria in the roots of plants.

MATERIALS AND METHODS

The seeds of the mung bean (*Vigna radiata* were thoroughly washed and soaked in distilled water for 4 h and washed with 0.3% calcium hypochlorite. Seeds were germinated in half strength Hoagland solution containing 0, 10, 20, 100 and 200 ppm of Cd concentration. Few drops of Tween 20 solution were used as dispersing agent^[6]. The pots were prepared and experiment was conducted in growth chamber (30 to 25°C) for 30 days in August 2004. Plants were analyzed after 15 days of germination. The contents of chlorophyll a and b in shoots and roots were determined, extract of shoot in 98% acetone spectrophotometrically by recording the optical density at 663 and 645 nm, respectively^[7]. Proteins were

estimated by Folin cicalteane phenol reagents in water extract of bean plant and optical density were recorded at 650 nm^[8]. Carbohydrate content were analyzed in protein free filtrate, optical density of extract of plants using ethanol were recorded by visible spectrophotometer at 620 nm^[9].

Microbial analysis: In order to determine the toxicity of Cd metal on nitrogen fixing bacteria, roots were analyzed after one month and following methods were adopted:

The roots of the plants were grown in different concentration of Cd were examined for the formation of root nodules; SPC (standard plate count) was performed to identify the number of bacteria found in different concentration of Cd. Serial dilution of Hoagland solution containing 0, 10, 20, 100, 200 ppm Cd were made in 9 ml water blank up to 10⁻⁵ dilution 0 ppm was taken a control. The last three dilutions were poured in sterile petri plates along with it a melted nutrient agar was also poured. After solidification the plates were incubated at 37°C for 24 h.

Bacterial colonies obtained were examined microscopically. Gram -ve were subsequently streaked on EMB agar (Eosine Methylilne Blue) Tryptone broth and TSI (Triple sugar iron agar) slants^[6]. Growth obtained in different concentrations of Cd and in control were noted and compared.

RESULTS AND DISCUSSION

Under normal circumstance the roots of mung bean plants shows the presence of nodules which were due to the nitrifying bacteria in the soil. *Rhizobium* species which were generally found in large number in soil in association with plants, responsible for fixing nitrogen from the atmosphere in the roots of leguminous plants. But the size of population lower when the concentration of heavy metal in soil exceeds its limited value. Water used for irrigation contains high percentage of toxic heavy metals^[10], which has greater tendency to accumulate and disturb the metabolic activity of plant. The presence of toxic metal Cd in the soil affects the morphology and physiology of mung bean seedling. Investigation shows that leaves of the plants turns yellow and plants were unable to stand in erect position. The roots and shoots both effected by the concentration of Cd used. Cd reduced root and shoot length (Table 1) of been plant presumably is the result of inhibition of photosynthesis due to the yellow color of leaves^[11].

Rhizobium species were not isolated from the roots of seedling at all concentration of Cd used as shown in Table 2 due to which plants were unable to synthesized its food. The contents of both chlorophyll a and b were decreased in plants treated with to 20-200 ppm

Table 1: Effect of concentration of codium metal on growth of plant

Cd conc. (ppm)	Average length of plant (cm)	
	Roots	Shoots
0	5.6±0.2	21.10±0.05
20	4.1±0.3	20.00±0.01
100	3.8±0.4	16.23±0.03
200	2.0±0.01	11.00±0.01

Table 2: Bacterial count in roots of mong bean plant

Sample Cd (ppm)	Dilutions	No. of organisms	CFU = No. of colonies × Dilution factor	Average (CFU x 10 ²)
10	10 ⁻³	200	2×10 ⁻¹	7.0200
	10 ⁻⁴	120	1.2×10 ⁻²	
	10 ⁻⁵	80	8.0×10 ⁻⁴	
20	10 ⁻³	100	1×10 ⁻¹	3.655
	10 ⁻⁴	90	9×10 ⁻³	
	10 ⁻⁵	75	7.5×10 ⁻⁴	
100	10 ⁻³	85	8.5×10 ⁻²	3.0100
	10 ⁻⁴	50	5×10 ⁻³	
	10 ⁻⁵	30	3.0×10 ⁻⁴	
200	10 ⁻³	50	5×10 ⁻²	2.6000
	10 ⁻⁴	20	2×10 ⁻³	
	10 ⁻⁵	0	0	

CFU: Colony Forming Unit

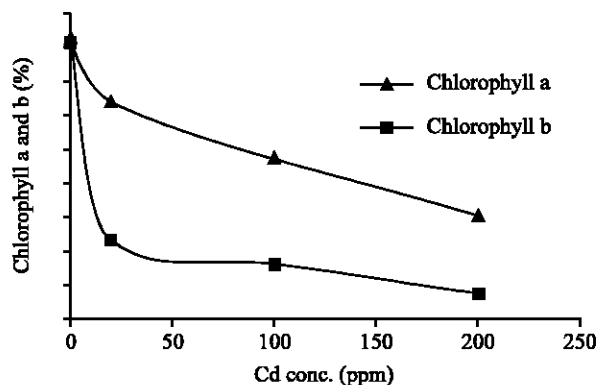


Fig. 1: Effect of conc. of Cd on chlorophyll content of bean plant

concentration of Cd (Fig. 1). This may be due to accumulation of Cd metal in the tissues of plants which inhibited the synthesis of chlorophyll a and b. Carbohydrates and protein contents declined (Fig. 2 and 3) as a results of reduced photosynthesis because of low percentage of chlorophyll a and b which may be attributed to the absence of nitrifying bacteria (*Rhizoiom* species), the nutritive value of plants were also effected and in Fig. 4 it was obvious that amino acid contents both in root and shoot were found to be absent at 100-200 ppm of Cd metal which way be related to the catalytic property of Cd metal which may convert amino acids into protein frequently^[12-14].

Bacterial count: Traditional microbiology technique I-e MPN was performed to check the microbial activity of bacteria, (Table 2) shows that in control pot, heavy

Table 3: Effect of conc. of Cd metal on soil bacteria

Sample category	Gram reaction	Arrangement	Shape	Growth on EMB	Growth on TSI	Indole	Organism
Cd 0 ppm (10^{-5})	Gram -ve	Scattered	Rods	LF	A/A Gas +ve H_2S -ve	+ve	<i>Klebsiella</i>
Cd 10 ppm (10^{-5})	Gram -ve	Scattered	Rods	LF	A/A Gas +ve H_2S -ve	-ve	<i>Enterobacter</i>
Cd 20 ppm (10^{-5})	Gram -ve	Scattered	Rods	LF	A/A Gas +ve H_2S -ve	+ve	<i>Klebsiella</i>
Cd 100 ppm (10^{-5})	Gram -ve	Scattered	Rods	LF	A/A Gas +ve H_2S -ve	+ve	<i>Klebsiella</i>
Cd 200 ppm (10^{-5})	Gram -ve	Scattered	Rods	LF	A/A Gas +ve H_2S -ve	+ve	<i>Klebsiella</i>

LF: Lactose fermentation; A/A: Acidic butt, acedie slant

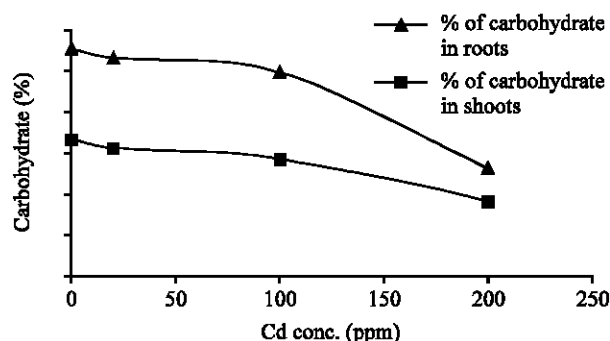


Fig. 2: Effect of conc. of Cd on the carbohydrate content of bean plant

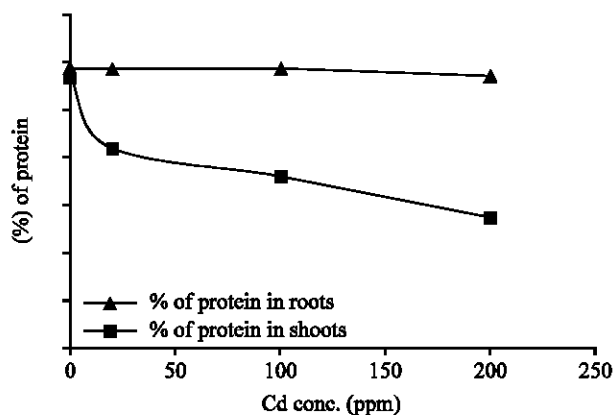


Fig. 3: Effect of conc. of Cd on the protein content of bean plant

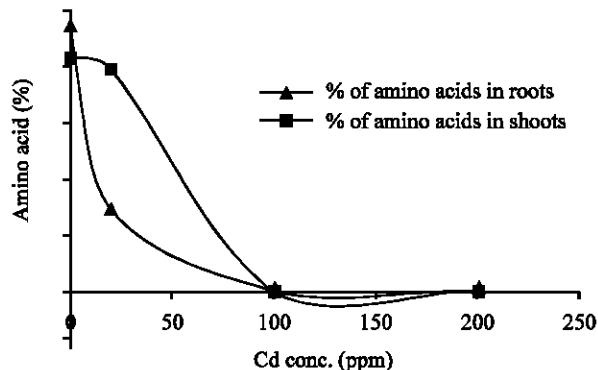


Fig. 4: Effect of conc. of Cd on the amino acids content of bean plant

growth of microorganism were obtained. But number of microorganism were found to be decreased with the increase in concentration of Cd. This indicated that heavy metal Cd was toxic not only for the growth and nutritive value of the plants but also responsible for the change/alter soil ecology. Gram negative microorganism like *Klebsiella* species and *Enterobacter* species (Table 3) were isolated and identified on EMB agar and by common biochemical tests. These species were found as a normal flora of rhizosphere which help in fixation of nitrogen non symbiotically. But microbial load were found to be decreased as the concentration of Cd were increased and organism were not able to fix the nitrogen for longer period of time which ultimately effect the growth and the nutrition of plants. Gram negative *Rhizobium* species, which were responsible for nodulation in seedlings, were found to be effected by presences of Cd metal due to which no nodule in roots of the plant were found which convert atmospheric nitrogen into ammonia and ultimately into available nitrate.

REFERENCES

- Babich, H. and G. Stotzky, 1997. Sensitivity of Various Bacteria, including Actinomycetes and Fungi to Cadmium and the influence of pH on sensitivity. J. Applied and Environ. Microbiol., 33: 681-695.
- Avskyan, Z.A., 1967. Comparative toxicity of heavy metals for certain microorganism. Mikrobiologiya, 36: 446-450.
- Leung, D., R.J. Miles and S.R. Smith, 1993. An investigation of the interaction between heavy metal and *Rhizobium leguminosarium* bv. Trifolii and also of the distribution of nitrifying bacteria in sewage sludge-treated agricultural soil. Report No. FRO342.
- Lehninger, A.L., 1970. Biochemistry. Worth Publishers, 70 Fifth Avenue, New York.
- Dakora, F.D. and D.A. Phillips, 1996. Diverse fraction of isoflavonoids in Legumes transcend anti-microbial definition of Phytoalexins. Physiol. Mol. Plant. Pathol., 49: 1-30.
- Baron, E.J., L.R. Peterson and S.M. Finegold, 1994. Bailey and Scott's Diagnostic Microbiology. 9th Edn., Mosby Publishers.

7. Chapman, S.B., 1976. *Methods in Plant Ecology*, 1st Edn., Black Well Scientific Publication, Oxford, pp: 536.
8. Lowary, O.H., N.J. Rosebragh, A.L. Farr and R.J. Randall, 1951. Protein measurements with folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
9. Ahmed, K.K., M. Younus and S.N. Singh, 1985. *Perspectives in Environmental Botany*. 1st Edn. Print House Lucknow, India, pp: 294.
10. Tandon., H.L.S., 1993. *Methods of Analysis of Soil, Plants Water and Fertilizers* Fert. Derel. Consult. Org., New Dehli, India, pp: 144.
11. Osawa, T., Ikeda and Hideo, 1979. Heavy metal toxicities in vegetable crops. *Engei Gakkai Zasshi*, 47: 435-91.
12. Felix, D.D., A. Donald and Phillips, 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, 245: 35-47.
13. Zwarun, A.A., 1973. Tolerance of *Eachericha Coli* to cadmium. *J. Environ. Qual.*, 2: 353-355.
14. Bartlett, L., F.W. Rabe and W.H. Funk, 1974. Effect of copper, zinc and cadmium on *sclanastrum capricornerim*. *Water Res.*, 8: 179-185.