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The Inhibition of Bean (*Vigna radiata*) Plant Metabolism by Cadmium Metal II: The Inhibition of Mineral Acquisition in Heavy Metal Contaminated Environment

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Abstract: In this study the effect of (Cadmium) Cd metal on essential mineral ions nutrients of *Vigna radiata* (bean) plants has been examined. Results have been explained in relation with soil microorganism and acquisition of mineral nutrients like Na, K, Ca, Mg, Mn, PO_4 and Fe. Thus environments, which are polluted by toxic metal can pose a challenge to nutrients acquisition by plant roots and threaten to survival of many beneficial microbes including the roots of plants themselves. Analysis of roots showed that *Rhizobium* species were effected by heavy metal Cd which results in the inhibition of nodulation of leguminous plant and decreases in the acquisition of mineral ions concentration at all concentration of cadmium.

Key words: Cadmium, *Vigna radiata,* mineral nutrient, nodulation, microorganism

INTRODUCTION

Plant developmental processes are controlled by internal signals that depends on the adequate supply of mineral nutrients by soil to roots. But if the soils were contaminated with high percentage of heavy metals then growth of plants can be markedly effected by pollution. Increase industrialization and human activities have impacted on the environment through the disposal of waste containing heavy metals. Mine drainage, metal industries, refining, electroplating, dye and leather industries, domestic effluents, landfill, leachate agricultural runoff and acid rain contribute such a kind of waste[1]. Microorganism play a unique role in the eco system, because of their contribution to soil fertility. The bioavailability of Cd and associated toxicity to soil biota vary with time, soil type, speciation, aging, Cd source, organism and environmental factors[2]. Plant uptake of nutrients from soil is more marked in the rhizosphere, surrounding the root than outside this zone[3]. Micronutrients are important components of major enzymes which regulate all biological processes in plants. It is clear from these considerations that low nutrients availability can constrain plant growth in many environment of the world, ultimately effect the nutrient acquisition by plants.

Fytianos *et al.*[4] proposed that the correlation of metal concentration between soil and vegetables was poor for majority of cases and shows inhibitory effect in growth of vegetables. It has been proposed by Farooq *et al.*[5] that the analysis of heavy metal accumaltation on a variety of fruit and vegetable samples grown on the dry river bed results. The use of dry river bed for growing summer vegetables fruit constitutes a high-risk area due to large quantity of metal accumulation specially near industrial areas[5]. Vegetables and crops growing in these areas should be wasted and monitored periodically for toxic contamination.

In this study, the effect of heavy metal Cd on the mineral ion concentration of bean plant in relation with bacterial community was analyzed.

MATERIALS AND METHODS

The growth of the bean plants with varying concentration of cadmium chloride viz., 0, 10, 20, 50, 100 and 150 ppm were observed in growth chamber in July 2004 in six pots containing half strength Hoagland solution. Ten to fifteen seeds of bean, soaked in water for 4-6 h were surface sterilized with 0.3% calcium hypochlorite for five minutes and rinsed with deionised water. They were introduced into different pots containing 0-200 ppm Cd based Hoagland solution. Plants were analyzed after two weeks. Na, K, Mn, Mg, Ca and Fe were estimated through dry ash method by flame photometer and atomic absorption spectrophotometer. For phosphate content the extracts, prepared by dry ashing method, were shaken with ammonium molybdate and stannous chloride. A blue colored complex with phosphate was obtained. Absorbance was measured at 660 nm by visible spectrophotometer[6].

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Analysis of microbes in roots: Roots of bean plants have been removed from mature plants. Roots were rinsed with sterile saline, crushed and suspended in saline. Serial dilution of saline were made and plated on nutrient agar plate. Next day Colony Forming Units (CFU) were counted. The isolated colonies were taken and identified by gram negative staining. Further identification was performed for gram-negative bacteria on TSI agar slant, trypton broth nitrate reduction test and growth on EMB agar plate.

RESULTS AND DISCUSSION

Plant root exudates contain many complex components and serve not only as a source of carbon substrate for microbial growth but also promote chemotaxis of soil microbes to the rhizosphere[7]. In addition living cells are subjected to the toxic effect of heavy metals reaching a certain level, resulting in cell death. Vegetable and fruit cultivated in heavy metal contaminated soil, suffered by accumulation of heavy metal, which results in growth inhibition and low uptakes of mineral ion concentration from soil due to the absence of nodules in roots of bean plant. Investigation shows that seed germination inhibited due to toxicity of cadmium metal and absence of *Rhizobium* species from the roots of plant.

Morphology of plants was also affected due to Cd toxicity and plant turns yellow and weeken, unable to stand in errect position. The roots and shoots of plants were effected due to ionic toxicity or due to osmotic effect. It was found that mineral ions concentrations were decreased with increased in concentration of Cd metal in roots and shoots of bean plants.

The decrease in concentration of minerals results in the weekness of plants as shown in Table 1. Low P concentration in tissues elicits the synthesis and exudation in to the rhizosphere of novel acid phosphate associated with cluster roots of minus-P plants[8]. In germinating seedling phytase hydrolyze phytic acid to release inorganic P for plant utilization[9]. A strong correlation between P-deficiency and release of phytase in several plant species is reported[10], which results in growth inhibitory effect in seedlings (Table 2).

Fe-deficiency can increase micronutrients concentration in the rhizosphere. Calcium is involved in the closure of guards cells and also mediates the response of plants to ethylene, a gaseous plant hormone that controls important physiological processes including seedlings development, flowering, fruit ripening and seneocence[11]. Beside their involvement in signaling, some element also plays crucial physiological roles in plant life. The less concentration of calcium in roots and

Table 1: Effect of different concentration of Cd metal on growth of *Vigna radiata*

Conc. of Cd (ppm)	Average root length (cm)	Average shoot length (cm)
0	15.60±0.01	20.72±0.01
10	10.10±0.01	15.15±0.01
20	8.51±0.01	14.12±0.01
50	7.51±0.02	11.00±0.2
100	6.10±0.02	9.10±0.01
150	4.01±0.01	7.10±0.01

Table 2: Effect of various concentration of Cd on phosphate ion contents of *Vigna radiata*

Conc. of Cd (ppm)	% age PO_4 in root	% age PO_4 in shoot
0	4.13±0.1	4.47±0.01
10	4.00±0.01	4.36±0.01
20	3.39±0.01	3.20±0.01
50	2.90±0.01	2.80±0.01
100	2.10±0.01	1.90±0.01
150	1.90±0.01	0.89±0.01

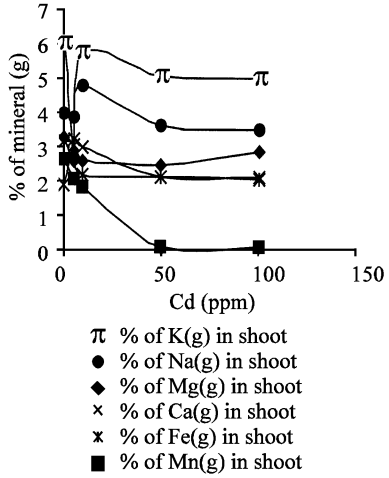

Fig. 1: Effect of various concentration of cadmium on mineral ions in shoots of bean plant

shoots of bean plants leads in the decrease in the concentration of chlorophyll a and b and carbohydrates contents. Low concentration of nutrients such as K^+, Na^+ and Mg^{++} also readily stimulates the activity of major enzymes of the glycotylic pathway, namely phospho fructokinase and pyruvate kinase, which together regulates glycolysis in plant cell, osmo regulation, the opening and closure by K^+ mobility in plant, cells. Individual micronutrients are similarly important components of major enzymes, which regulates all biological process in plant. Low concentration of these important micronutrients suppress all important biological processes and also cause the drop in the growths and uptakes of metal ion in the roots and shoots of bean plants.

Figure 1 represents that Cd is toxic at all experimental condition used. At 5 ppm there were slight changes in the metal ion concentration in the plants.

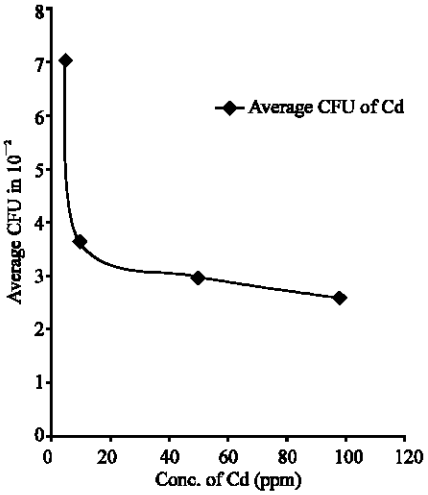

Fig. 2: Effect of Cadmium on soil microorganism. Average CFU in presence of Cd

In order to avoid metal toxicity, following mechanism was adopted by plants[12].

Biochemical detoxification; compartmentalization of the metal with in the cell; limited uptake of ions; less heavy metal are taken up by tolerant than non tolerant plants; restricted transport from the roots to the shoots; formation of metal binding polypeptides; chelating by organic acid and the plasma lemma have a role in the heavy metal tolerance.

Bacterial community: The presences of heavy metals in sewage sludge is often the main factor restricting the application of sewage sludge to agriculture soil[13]. After detailed assistant of the uptake and transfer of heavy metals into the food chain Via crops, the commission of the European communities has set limits on the amount of selected heavy metal that can be added to agricultural soil receiving sewage sludge[14]. However, even heavy metal contamination that is below the upper limit set the European commission can have no effect on microbial community structure. Figure 2 showed that bacterial community were effected by the increase in concentration. of Cd. *Rhizobium* species, which fixes nitrogen in roots of leguminous plants in form of nitrate but nodulation was inhibited in presence of Cd metal in the roots[15] of bean plants.

REFERENCES

1. Guangyu, Y. and T. Viraraghavan, 2000. Effect of pretreatment on the bio adsorption of heavy metals on *Mucor rounii*. J. Water SA., 26: 119-125.
2. Vig, K., M. Megharaj, N. Sethunathan and R. Naidul, 2003. Bioavailability and toxicity of Cd to microorgainism and their activities in soil. Adv. Environ. Res., 8: 121-135.
3. Gilbert, G.A., J.D. Knight, D.L. Allan and C.P. Vance, 1999. Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant Cell Environ., 22: 801-810.
4. Fytianos, K., G. Katsianis, P. Triantafyllou, G. Zachariadis, 2001. Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. Bull. Environ., 67: 423-430.
5. Farooq, M., R.K. Hans, P.N. Viswanathan and P.C. Joshi, 1999. Health hazards from dry river bed agriculture. Bull. Environ. Contain. Toxicity, 62: 555-562.
6. Chapman, S.B., 1976. Methods in Plant Ecology. 1st Edn., Black Well Scientific Publication, Oxford, pp: 536.
7. Felix, Dakora., A. Donald and Phillips, 2002. Root exudates as mediators of mineral acquisition in low-nutrient environment. Plant and Soil, 245: 35-47.
8. Duff, S.M., G. Sarath and W.C. Plaxton, 1994. The role of acid phosphotase in plant phosphorus metabolism. Physiol. Plant, 90: 791-800.
9. Li, M., M. Osaki, I.M. Rao and T. Tadano, 1997. Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. Plant Soil, 195: 161-169.
10. Moorby, J. and R.T. Besford, 1983. Mineral Nutrition and Growth. In: Encyclopedia of Plant Physiology. New Series 15 B: Inorganic Plant Nutrition. Eds. Lauchli, A. and R.L. Bieleski, Springer-Verlag, Berlin, pp: 481-515.
11. Raz, V. and R. Fluhr, 1992. Calcium requirement for ethylene dependent responses. Plant Cell, 4: 1123-1130.
12. Hsiao, T.C. and Lanchi, 1986. Role of Potassium in Plant Water Relations in Advance in Plant Nutrition. New York, USA., 2: 281-312.
13. Ruth-Anne, S., O. Enger and V. Torsvik, 1999. Abundance and diversity of archaea in heavy metal contaminated soils. J. Applied Environ. Microbiol., 65: 3293-3297.
14. Commision of the Eurpean Communities, 1986. Council directive of 12 June 1986 on the protection of the environment and in particular of the soil. When sewage sludge is used in agricultural J. European Communites No. L 181/6 (86/278/EFC)
15. Baath, E., M. Diaz-Ravina, A.A. Frostegand and C.D. Campball, 1998. Effect of Metal-rich sludge amendments on the soil microbioal community. Applied Environ. Microbial., 64: 238-245.