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

Rafia Azmat, Zill-e-Huma, Aliya Hayat, Tanveer Khanum and Ruksana Talat
Department of Chemistry, Department of Microbiology,
Department of Zoology, Jinnah University for Women, 5 C, Nazimabad, Pakistan

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 inflicted 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.

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. 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. Nitrogen is a major component 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. 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.

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 mug 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. 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. Proteins were
estimated by Folin cicloateane phenol reagents in water extract of bean plant and optical density were recorded at 650 nm\cite{4}. Carbohydrate content were analyzed in protein free filtrate, optical density of extract of plants using ethanol were recorded by visible spectrophotometer at 620 nm\cite{5}.

**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 concentrations 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 Methylene Blue) Tryptone broth and TSI (Triple sugar iron agar) plates\cite{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\cite{7}, 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 bean plant presumably is the result of inhibition of photosynthesis due to the yellow color of leaves\cite{8}.

*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 20-200 ppm 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 (*Rhizobium* species), the nutritive value of plants were also affected 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 may be related to the catalytic property of Cd metal which may convert amino acids into protein frequently\cite{9,10,11}.

**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

<table>
<thead>
<tr>
<th>Sample category</th>
<th>Gram reaction</th>
<th>Arrangement</th>
<th>Shape</th>
<th>Growth on EMB</th>
<th>Growth on TSI</th>
<th>Indole</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd 0 ppm (10^-5)</td>
<td>Gram -ve</td>
<td>Scattered</td>
<td>Rods</td>
<td>LF</td>
<td>A/A Gas +ve H_2S+ve</td>
<td>+ve</td>
<td>Klebsiella</td>
</tr>
<tr>
<td>Cd 10 ppm (10^-4)</td>
<td>Gram -ve</td>
<td>Scattered</td>
<td>Rods</td>
<td>LF</td>
<td>A/A Gas +ve H_2S+ve</td>
<td>-ve</td>
<td>Enterobacter</td>
</tr>
<tr>
<td>Cd 20 ppm (10^-3)</td>
<td>Gram -ve</td>
<td>Scattered</td>
<td>Rods</td>
<td>LF</td>
<td>A/A Gas +ve H_2S+ve</td>
<td>+ve</td>
<td>Klebsiella</td>
</tr>
<tr>
<td>Cd 100 ppm (10^-2)</td>
<td>Gram -ve</td>
<td>Scattered</td>
<td>Rods</td>
<td>LF</td>
<td>A/A Gas +ve H_2S+ve</td>
<td>+ve</td>
<td>Klebsiella</td>
</tr>
<tr>
<td>Cd 200 ppm (10^-1)</td>
<td>Gram -ve</td>
<td>Scattered</td>
<td>Rods</td>
<td>LF</td>
<td>A/A Gas +ve H_2S+ve</td>
<td>+ve</td>
<td>Klebsiella</td>
</tr>
</tbody>
</table>

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

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