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Phytoremediation of Cadmium by *Ricinus communis* L. in Hydrophonic Condition

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Abstract: The potential of *Ricinus communis* L was investigated for the phytoextraction of cadmium (Cd) in hydrophonic condition. The studied plants were grown under greenhouse condition in 250 ml flasks containing a nutrient solution with increasing doses of Cd (0, 5, 10, 15, 20 and 25 mg/L) for 28 days. The growth and physiological parameters of plants were determined at the end of the experiment. Cd contents in plant tissues were found increasing with increasing dose of Cd. The maximum Cd uptake was found at T5 (25 mg/L of Cd). The pattern of Cd accumulation in different part of plant was found as: root > leaf > stem. The maximum BAF was found in T2 (10 mg/L of Cd) and for whole plant BAF value was ranged from 0.286-0.455. Translocation factor (TF) value was ranged from 0.645-0.857 and 0.626-0.837 for root-shoot and shoot-leaves respectively. All BAF and TF values were found less than 1. The plant height, root length and total plant dry-biomass was significantly reduced by Cd treatment as compared with control. Cd stress also increased proline concentration in root and leaves and maximum proline content (81.3 µg/g) was found in T3 (15 mg/L of Cd). Total phenolic content (TPC) was also increased under Cd-stress and maximum (55 and 59 mgGAE/g DW) TPC was found in T4 (20 mg/L of Cd) for root and leaves respectively. The TPC in leaves was found higher than root, while root proline was found higher than leaves.

Key words: *Ricinus communis* L • Proline • Total Phenolic Content • Phytoremediation

INTRODUCTION

Due to globalization and industrialization, heavy metal (HM) pollution is one of the most important and widespread environmental problem of the modern world due to its hazardous and toxic effects on all living organisms. Heavy metal is a serious environmental problem because HMs are non-biodegradable and persistent in nature [1-3]. Cadmium is one of the most toxic HM in the environment. Liver and kidney are the main organs where Cd is accumulated over time [4]. Due to chemical similarities of Cd with other minerals such as, Fe, Cu, Zn and Se, it may interfere the chemical processes due to struggle for binding stage. Cd is also responsible for bone metabolism problems by interfering with Ca and P in both occupational and general people exposed to Cd [5]. HMs are mutagenic, causing DNA damage and may cause cancer in humans and animals [6]. Due to the high mobility of Cd from soil to plant, causing a problem of its easy entrance into food chain and can affect animals [7, 8] and plants. On one side phytoremediation ability of plants is a great concern regarding a possible entrance of HMs into food-chain, however, on the other side, this property of plants can be used as an alternative technology, called as phytoremediation to remove HM-contaminants from soil and water. Phytoremediation can be defined as the use of living plants to absorbs and store HMs from soil and water into their parts (root, shoot and leaves), then harvested with conventional agricultural methods [9]. Phytoremediation is environmental friendly and cost-effective method for cleaning of contaminated soils and water and has gained great attention especially in the last decades. The effectiveness of phytoremediation mainly depends on the ability of plants-metal translocation and bioaccumulation factors. Unfortunately, plants which can accumulate high metal in their tissues i.e. hyperaccumulator, produced small biomass and grow very slowly [10]. The effective strategies to remove HM-contaminants are to use either hyperaccumulators plant species or to use plants having very fast growth and high
biomass [11]. Cadmium can cause oxidative stress in plants due to its interaction with the antioxidative defense and can also interfere with electron transport mechanism [12]. Lipoygenase enzymes are activated when plants are exposed to cadmium stress which intern stimulates lipid peroxidation [13]. To counter act the HM stress, plants produce phenolic compounds which can directly scavenge reactive oxygen species [12]. Proline accumulation can also occurs in plant tissues when they are subjected to HM stress [14]. It is still unclear that how proline accumulation can protect plants form HM stress, but due to some evidences we can say that proline shield the key enzymes from being deactivating by HMs and it can also act as an osmoregulator [14].

The main disadvantage come across with phytoremediation is the penetration of HMs to the food chain through the consumption of these contaminated plants by animals [15]. Due to the inedible property of *Ricinus communis*, it is not consumed by herbivores, this weed plant was utilized for this study for the purpose to avoid food chain contamination of HMs. *Ricinus communis*, the castor oil plant, is a species of flowering plant belongs to the family, Euphorbiaceae. Because of its tolerant nature, fast growing ability, tall shoot with multiple branching and deep root system, its a good candidate of phytoextraction of HM-contaminated soils.

The current study was carried out to investigate the ability of hydrophonicphytoextraction of cadmium of the selected plant

**MATERIAL AND METHODS**

**Soil and Media Preparation and Seedling Transplantation:** Soil for seeds germination was collected from the agricultural field near University of Malakand campus KPK, Pakistan; in a depth of 0-25cm. this soil is then air dried for several days, then ground and pass through a mesh to attain a uniform particle size soil. The water holding capacity and pH and conductivity of the soil was calculated (250 mL of water/ kg soil ± 4). The air dried soil was then used for seed germination. Seeds of *Ricinus communis* were obtained from herbarium of Malakand University. These seeds were then grown in this prepared soil, at appropriate distance from each other. This soil was then well watered and let the seeds to be germinated. All seeds were watered twice a day. For this research a randomized block design using a 4×1 fractional scheme with three replicate of hydroponic experiment was conducted in which MS (Murashige and Skoog) media in a 250 ml flask was used for plants growth.

Table 1: Shows treatment used in hydrophonic experiment.

<table>
<thead>
<tr>
<th>Treatments: Denoted as:</th>
</tr>
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| Control without Cd but only 5ml/L MS media | C  
| 5 mg/L Cd + 5ml/L MS media | T1  
| 10mg/L Cd + 5ml/L MS media | T2  
| 15mg/L Cd + 5ml/L MS media | T3  
| 20mg/L Cd + 5ml/L MS media | T4  
| 25mg/L Cd + 5ml/L MS media | T5  

Table 2: Average values of various morphological parameters before cadmium treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot length (cm)</td>
<td>14.4 ± 3</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>4.5 ± 1</td>
</tr>
<tr>
<td>Average Biomass per plant (gram)</td>
<td>3.6 ± 0.32</td>
</tr>
<tr>
<td>No. of leaves per plant</td>
<td>4 ± 0.21</td>
</tr>
<tr>
<td>Toxicity symptoms</td>
<td>None</td>
</tr>
</tbody>
</table>

Each flask contain MS media, distilled water and Cadmium. For each flask 0.5ml of MS median in 100ml of distilled water was used. After 30 days of seeds germination uniform seedlings of *Ricinus communis* plants were selected and transferred into flasks using one plant per flask. Three replicate were used for each treatment and control. These plants were then kept in glasshouse under normal condition of temperature (30/25°C day/night).

**Treatments Used During Experiment:** The following treatments were made during hydroponic experiments. All the treatment and the control were set up in triplicates. The treatments used in this experiment are presented in Table 1. Control (C) was compared with T1, T2, T3, T4 and T5 for heavy metal absorption and for the effect of Cd on the height, growth, biomass, root length, shoot length, proline and total phenolic content.

**Growth Parameters:** Various growth parameters of the plant were studied under cadmium stress including plant height (Cm), number of leaves per plant, dry weight, average root length and toxicity symptoms prior and after cadmium treatments.

**Physiological Parameters:** The physiological parameters of the plants included free proline and total phenolics determination.

**Bioaccumulation and Translocation Factors:** The phytoextraction ability of *Ricinus communis* plants was assessed using both the translocation factor (TF) and the Bioaccumulation factor (BF) as follows.
Translocation Factor:

\[ TF = \frac{Cd\text{concentration in shoot}}{Cd\text{concentration in root}} \] (1)

Bioaccumulation Factor:

\[ BF = \frac{Cd\text{concentration in plant tissue}}{Cd\text{concentration in solution}} \] (2)

Cadmium Analysis in Plant Tissues: Plants were harvested after 20 days of hydroponic condition. Plants were first washed with EDTA solution and then with distilled water to remove any metals from plant surface. Plant height, root and shoot length was measured with a centimeter ruler. Each plant was then divided into subsample like root, shoot and leaves and these each sample was then packed in a separate paper envelope and dried in oven at 70°C for 24 hours. Dry biomass of each sample was measured with the electronic balance. Oven dried samples of root, shoots and leaves were then ground into fine powder with the help of common kitchen blender. These powder samples were then stored in separate Petri dishes for acid digestion. Before metals analysis these powdered samples were first acid digested by using the method of Allen et al. [16]. From each sample 0.25 gram was digested in a mixture of three acids i.e. HNO₃, H₂SO₄ and HClO₄ in a 5:1:0.5 ratios. Each sample was taken in 50mL of volumetric flask and 6.5mL triacid mixture was added to each flask. These flasks were then put on electric hot plate until white fumes came out of the flask and plant tissues were completely digested. After digestion the samples were allowed to cool down at room temperature and the volume was made up to 50mL with distilled water.

Free Proline Analysis in Plant Sample: Proline extraction and biochemical quantification was done by using the method of Bates et al. [17]. Fresh samples of leaves and roots tissues (100mg each) were homogenized in 1.5mL of 3% sulfosalicylic acid in 2mL tubes. These homogenized samples were then centrifuged at 13000 rpm for 5 minutes. Supernatant was then transferred to another tube and 2mL of glacial acetic acid and acid ninhydrin (1.25g ninhydrin warmed in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid until dissolved) was added to it and kept for one hour in boiling water bath at 100°C. The tubes were then removed from water bath and quickly dipped into cooled ice water to stop the chemical reactions. To each tube 1mL of toluene was vigorously mixed. The chromophore layer containing toluene was removed from the tubes with the help of micropipette and was warmed to the room temperature. At 520nm wavelength, absorbance of each sample was measured with the help of spectrophotometer (Shimadzu, Japan). For control (blank) toluene was used. The concentration of free proline in each sample was determined from a standard curve. For every sample the test was performed in triplicate.

Total Phenolics Determination in Root and Leaves

Methanolic Extraction for Total Phenolics: For total phenolics determination 1 gram from each part (root and leaves) in a fine powder form were dissolved in 20mL of methanol using 50mL falcon tubes. The solution in falcon tubes were then shaked for 24 hours at 37°C using a shaker water bath. The extracts were then filtered through Whatman No.1 filter paper. The filtrate solution were then stored in cleaned and properly labeled tubes for further use. Total phenolics determination was done using Folin-Ciocalteu (FC) reagent according to the method of Singleton and Rossi[18] with slight modification. Standard used for total phenolics determination was Gallic acid. For this purpose, the calibration curve of Gallic acid was drawn from concentration of 1, 5, 10, 15 and 20 mg/ml of Gallic acid in methanol. Total Phenol Content was represented as mg/g of Gallic acid equivalent (GAE). Methanolic plant extracts of 0.5mL were put into tubes and 2mL of 10 fold diluted FC reagent and 2mL of 7.5% sodium carbonate were mixed with it. These tubes were then put in the dark for one hour at room temperature. During this time reducing compounds like polyphenols reach with FC reagent due to this reaction the solution turn into blue. After one hour absorbance of the blue solution was determined at 765nm using UV Spectrophotometer (Shimadzu, Japan).

RESULTS AND DISCUSSION

Effect of Treatments on Plant Height and Root Length: The plant height and root length were significantly reduced by Cd (Fig. 1A) when compared with control (without Cd). Plant height and root length are generally reduced by heavy metals [19-21]. Control (C) was compared with the treatments to investigate the effect of Cd on root length and plant height. A significant reduction in plant height was observed at T5 (25 mg/L of Cd) followed by T4 (20 mg/L of Cd), T2 (15 mg/L of Cd) and T2 (10 mg/L of Cd).
The Effects of Treatments on Dry Biomass: The effect of different treatments of Cd on dry biomass of plant is shown in fig. 1B. It is cleared from the figure that Cd treatment reduced plant dry biomass as compared with control. Dry biomass reduction is one of the common symptom of heavy metal toxicity in plants. Similar dry biomass reduction due to heavy metal stress was also reported by different researchers [19, 22]. The results showed that the lowest reduction in dry biomass was found at 5mg/L of Cd, while the highest dry biomass reduction was found at 25 mg/L of Cd followed by 20 mg/L of Cd, 15 mg/L of Cd and 10 mg/L of Cd. The dry biomass of plant of T5 (25 mg/L of Cd) significantly reduced as compared to C.

**Phytoextraction of Cd by Root, Shoot and Leaf of Ricinus communis:** Cadmium accumulation in different parts of plant grown in contaminated solution are presented in Figure 2. Accumulation of Cd in root shows a linear relationship to the increasing supplied concentration of Cd. Cadmium content in root shows significant \( P<0.05 \) difference at various treatments. The maximum Cd uptake was found at T5 (25 mg/L of Cd). Cadmium concentration in stem and leaf was also found to have a linear
relationship to the increasing supplied cadmium concentration. The maximum Cd contents in shoot and leaf was found at T5 (25 mg/L of Cd). Cadmium concentration at 25 mg/L was found significantly ($P<0.05$) different from other treatments. At T5 the accumulation of Cd in root shoot and leaf was 816 mg/kg, 587 mg/kg, 700 mg/kg, respectively. The range of Cd in root under different treatments was 138 to 816 mg/kg. The range of Cd concentration in shoot and leaf was 55 to 586 mg/kg and 90 to 700 mg/kg, respectively. Overall, the pattern of Cd accumulation in different tissues of plant was found as: root > leaf > stem.

**Effect of Cd on Proline Concentration in Root and Leaf:** A common response of plants to heavy metal stress is the accumulation of proline[23]. This accumulated proline is responsible for serving as a source of nitrogen compound during inhibited growth period [24]. Proline is one of the most important and vastly studied molecule with respect to plant responses to different abiotic stresses, like drought, salinity, temperature or heavy metal stresses [25]. Proline is assumed to increase metal tolerance [26]. Due to its chelating ability, proline bind with metal and may provide the strength against free radicals and involved in various defensive mechanism to overcome the toxic effect of metal because proline is a good osmo or redox regulator, ROS scavenger, activator of antioxidant enzyme and chelate for matter [27-29]. Under heavy metal stress plants developed their own defensive mechanism for survival and proline accumulation is one of that mechanisms. Results also have showed that proline have inverse relationship with the growth rate of plant. The effects of heavy metal (Cd) stress on proline content are presented in Fig. 3. The results shows that the amount of proline content in plant (root and leaves) were found increasing with the increased Cd supply up to T3 (15 mg/L of Cd)as compared to control (C) and a decrease in proline content was found at T4 and T5. The highest proline accumulation was found at T3, 81.3 µg/g and 71.6 µg/g for root and leaves, respectively. The results also showed higher level of proline in root as compared to leaves (Fig. 3). Similar results were also reported in previous research in which Vignaunguiculata plant was exposed to heavy metal (Cd) stress.

**Effect of Cd on Total Phenolic Content in Root and Leaf:** Phenolic compounds are known antioxidant agents which act as a scavenger of free radical and their activities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radical [30]. An increase in Total Phenolic Content (TPC) has been reported when plants were exposed to different environmental stress [31, 32]. The results from the present study also demonstrated an increase in plant (root and leaves)phenolics under Cd stress as compared to C (Fig. 4). All treatments of Cd increased the phenolics content as compared to control (C). Highest phenolics content was found at T4 (20 mg/L of Cd) and was 55mgGAE/g dry biomass and 59 mgGAE/g dry biomass, for root and leaf, respectively. Leaves phenolics content was found higher than that of roots.
Bioaccumulation and Translocation Factors of Cd in<br>\textit{Ricinus communis}: The bioaccumulation factor (BAF) of Cd in \textit{Ricinus communis} grown in hydrophonic condition is shown in Table 3. The highest BAF of Cd was noticed for T2 (10 mg/ml of Cd) in root, shoot, leaves and entire plant, followed by T3 (15 mg/l of Cd), T5 (25 mg/l) and T4 (20 mg/l of Cd) while the lowest BAF was found in T1 (5 mg/l of Cd). Bioaccumulation factor value in entire plant were ranged from 0.286 to 0.455.

The translocation factor (TF) of Cd from root to shoot and form shoot to leaves was determined and presented in Table 4. For the data presented in the table it is clear that all the treatments i.e. increased concentration of Cd also increased translocation of Cd form root to shoot and form shoot to leaves. Translocation from root to shoot was found higher as compared to that of shoot to leaves. The translocation factor values were ranged from 0.645-0.857 and 0.626-0.837 for root-shoot and shoot-leaves, respectively. All translocation factor values were found less than 1. The result of this study was found according to the finding of [33].

\textbf{CONCLUSIONS}

This experiment was conducted to determine the phytoremediation ability of \textit{Ricinus communis} to treat Cadmium contaminants in hydrophonic condition. The plant shows stability to cadmium contaminants and absorbs and accumulates it in their tissues. Both BAF and TF was found lower than 1 in hydrophonic experiment. TF was found higher than BAF. After 15 days of growing in hydrophonic condition plant shows toxic signs due to high Cd accumulation. Plant height, root length and dry biomass was reduced by Cd. Proline and Total Phenolic Content (TPC) was increased by Cd treatments. Overall, \textit{Ricinus communis} is a good choice to treat Cd-contaminants in hydrophonic condition.

\textbf{REFERENCES}


