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Molybdenum (Mo) increases endogenous phenolics, proline and photosynthetic pigments and the phytoremediation potential of the industrially important plant Ricinus communis L. for removal of cadmium from contaminated soil

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Molybdenum (Mo) increases endogenous phenolics, proline and photosynthetic pigments and the phytoremediation potential of the industrially important plant *Ricinus communis* for removal of cadmium from contaminated soil.

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Abstract

Cadmium (Cd) in agricultural soil negatively affect crops yield and compromises food safety. Remediation of polluted soil is necessary for for the re-establishment of sustainable agriculture and to prevent hazards to human health and environmental pollution. Phytoremediation is a promising technology for decontamination of polluted soil. The present study investigated the effect of molybdenum (Mo) (0.5, 1.0 and 2.0 ppm) on endogenous production of total phenolics and free proline, plant biomass and photosynthetic pigments in *Ricinus communis* plants grown in Cd (25, 50 and 100 ppm) contaminated soils and the potential for Cd phytoextraction. Mo was applied via seed soaking, soil addition and foliar spray. Foliar sprays significantly increased plant biomass, Cd accumulation and bioconcentration. Phenolic concentrations showed significantly positive correlations with Cd accumulation in roots (R^2 = 0.793, 0.807 and 0.739) and leaves (R^2 = 0.707, 721 and 0.866). Similarly proline was significantly positively correlated with Cd accumulation in roots (R^2 = 0.668, 0.694 and 0.673) and leaves (R^2 = 0.831, 0.964 and 0.930). Foliar application was found to be the most effective way to deliver Mo in terms of increase in plant growth, Cd accumulation, and production of phenolics and proline.

Key words: Phytoremediation, *Ricinus communis*, phenolics, proline, molybdenum

1. Introduction

Cadmium (Cd) is one of the hazardous heavy metals. It enters into agricultural soil mostly through industrial effluents, mining operations, municipal runoff and application of phosphate fertilizers where it can occur as a micro-contaminant [1]. Cadmium can easily be absorbed by plant roots and is translocated into aerial parts where it inhibits plant growth through reduced uptake of micro and macronutrients and a reduction in the rate of photosynthesis thus reducing crop yield and also comproming the quality of food [2, 3]. Consumption of Cd contaminated food results in serious health problems [2 - 4]. In the human body, Cd can affect gene expression, it interferes with DNA damage repair systems, inhibits apoptosis and induces oxidative stress. These cellular dysfunctions result in damage to different organs such as the kidneys, liver, lung and bone marrow [5 - 8]. Safe restoration of Cd polluted soil is of the utmost importance for sustainable agriculture, the environment and human health. Phytoremediation is an environment friendly remediation technology that uses green plants for the safe decontamination of polluted soil and water and is an economical, environment friendly and aesthetically pleasing technology [9]. Plants under heavy metal stress often showed decrease in growth and biomass which in turn reduce their phytoremediation potential [10, 11]. To combat the toxic metals in plant cells increases in concentrations of endogenous free proline and phenolic compounds have been reported in many plant species [2, 12]. Phenolic compounds protect cellular components from oxidative stress caused by reactive oxygen species while free proline has been reported to protect some important enzymes from deactivation by toxic heavy metals [13, 14].

Micronutrients are required by plants in very minute quantities for normal physiological activities. Molybdenum (Mo) is one of the micronutrients required by plants for normal growth and it's deficiency adversely affects the activities of nitrate reductase and glutamine synthetase which are enzymes catalysing the initial steps of nitrate metabolism [15]. Molybdenum has also been reported to catalyse other enzymes such as aldehyde oxidase (AO) involved in Abscisic acid biosynthesis and sulphite oxidase (SO) which catalyses the conversion of sulphite to sulphate, an essential step in the catabolism of amino acids containing sulphur [16, 17]. Molybdo-enzymes also play a role in the biosynthesis of plant growth regulators [18, 19].

Ricinus communis (Castor bean) plant belongs to family Euphorbiaceae [20] and is an industrial crop. It is used for the production of biodiesel, paints, nylone type fibre and products with insecticidal and antimicrobial purposes [21]. Castor bean is a highly suitable candidate for metal phytoremediation due to its high biomass, fast growth and non-palatable nature to herbivores which helps prevent entrance of heavy metals into the food chain. Present research was conducted with the objectives to evaluate the effect of different concentrations of molybdenum on plant growth, photosynthetic pigments, production of endogenous free proline and total phenolics and Cd phytoaccumulation, in *Ricinus communis* grown in Cd contaminated soil.

2 Materials and Methods

2.1 Preparation of soil and addition of cadmium

Fertile soil was collected from fields near the University of Malakand at Chakdara, Pakistan. The soil was air-dried in sunlight and grounded into a powdered form. Water holding capacity (300 ml water / kg soil ± 3) and pH (6.5 ± 0.3) of the soil was measured. Then 2 kg soil was poured into plastic pots (20 × 12 cm). Cadmium (Cd) in the form of cadmium acetate dihydrate $(CH_3COO)_2$ $Cd{\cdot}2H_2O$ (Merck, Germany) solution was added to the soil in pots. Cadmium was allowed to equilibrate in soil for one month. A total of four different Cd concentrations were used (0, 25, 50 and 100 ppm) (Table 1).

2.2 Transplantation of seedlings and plant growth

Each pot was watered a day before transplantation of seedlings. Seeds of *Ricinus communis* were obtained from the Herbarium of the University of Malakand, and sown in soil beds in a greenhouse. After germination, uniform sized seedlings (6 cm) were selected and transferred to the pots (single seedling per pot). Plants were maintained in the glasshouse under natural conditions of light and

temperature (35max/25min°C). Plants were watered, at three days intervals bringing the soil back to field capacity each time..

2.3 Treatments during the experiment

Table 1: The following treatments were used during the experiment. 'S.S' stands for seed soaking, 'A.S' stands for added to soil 'F.S' stands for foliar spray, 'C' stands for control and 'T' denotes treatment. C is compared with C1, C2 and C3 to find out the effect of different concentrations of Cd on growth. Treatments T1-T9 (25 ppm Cd) compared with C1, T10-T18 (50 ppm Cd) compared with C2, T19-T27 (100 ppm Cd) compared with C3.

Commented [m1]: This is an odd experimental design and not truly factorial as you do not have the Mo treatments applied in the absence of Cd!! The experiment should have 40 treatments (4 Cd x 10 Mo)!!

Treatments	Symbols	Treatments	Symbols	Treatments	Symbols
Without Cd and Mo	**C**				
25 ppm Cd	**C1**	50 ppm Cd	**C2**	100 ppm Cd	**C3**
0.50 ppm Mo [S.S]	**T1**	0.50 ppm Mo [S.S]	**T10**	0.50 ppm Mo [S.S]	**T19**
1.00 ppm Mo [S.S]	**T2**	1.00 ppm Mo [S.S]	**T11**	1.00 ppm Mo [S.S]	**T20**
2.00 ppm Mo [S.S]	**T3**	2.00 ppm Mo [S.S]	**T12**	2.00 ppm Mo [S.S]	**T21**
0.50 ppm Mo [A.S]	**T4**	0.50 ppm Mo [A.S]	**T13**	0.50 ppm Mo [A.S]	**T22**
1.00 ppm Mo [A.S]	**T5**	1.00 ppm Mo [A.S]	**T14**	1.00 ppm Mo [A.S]	**T23**
2.00 ppm Mo [A.S]	**T6**	2.00 ppm Mo [A.S]	**T15**	2.00 ppm Mo [A.S]	**T24**
0.50 ppm Mo [F.S]	**T7**	0.50 ppm Mo [F.S]	**T16**	0.50 ppm Mo [F.S]	**T25**
1.00 ppm Mo [F.S]	**T8**	1.00 ppm Mo [F.S]	**T17**	1.00 ppm Mo [F.S]	**T26**
2.00 ppm Mo [F.S]	**T9**	2.00 ppm Mo [F.S]	**T18**	2.00 ppm Mo [F.S]	**T27**

2.3.1 Molybdenum (Mo) treatments

Three concentrations (0.5, 1.0 and 2.0 ppm) of Mo were applied in three different ways i.e. seed soaking, soil addition and foliar spray (Table 1). A stock solution of Mo was prepared and then treatments solutions were obtained through serial dilution. In case of seed soaking treatments, seeds were kept in respective Mo solutions for 24 hrs before sowing. Six foliar applications were carried out at one week intervals for each of the Mo concentrations. The first foliar application was done 15 days after seed germination. During foliar sprays, the soil in the pots was covered with plastic bags to avoid entrance of Mo droplets into soil. Three replicate pots were used for each treatment.

2.4 Plant growth parameters

Plants were harvested two months after seedling transplantation. Root and shoot lengths of each plant were measured using ruler. Prior to analysis, plants were washed with a solution of 5 mM EDTA and 5

mM Tris-HCl (pH 6.0) and then with distilled water to remove any contaminating metal ions bound to the plant surface [22]. After washing each plant was cut into three fractions i.e. roots, stem and leaves and fresh weights taken. Each fraction was packed in separate paper envelopes and then kept in a drying oven for 48 h at 80^0C and dry weights taken. Dried biomass was then ground into powdered form.

Commented [m2]: Using a pestle and mortar or using a mechanical grinder?? Give details

2.5 Estimation of Free proline and total phenolics

Proline was extracted from fresh plant tissues (root and leaves) according to the method of Bates et al. [23]. The proline concentration in each sample extract were measured by spectrophotometer (250 nm wavelength). Toluene was used as a blank (control). A standard curve was obtained from the absorbance of different solutions of standard proline and used to calculate the concentration of proline in different samples. Total phenolics were extracted from dried samples (roots and stem) of each plant by using the Folin-Ciocalteau (FC) reagent method [24] and measured spectrophotometrically at an absorbance of 760 nm. Methanol (80%) was used as the blank solution (control). A standard curve was obtained from absorbance of different solutions of gallic acid in methanol (80 %). Concentration of phenolics in samples were calculated from the standard curve. Three replicates were used.

2.6 Chlorophyll and carotenoids estimation in leaves

Concentration of chlorophylls (a and b) and total carotenoids in fresh leaves were estimated using the method of Sumanta et al. [25]. Fresh leaf samples (0.5 g) were homogenized in 10 ml of 80% acetone, centrifuged at 10000 rpm for 15 minutes. The supernatants were transferred into clean test tubes containing 4.5 ml of 80% acetone. Three replicates were used for each treatment. Chlorophyll a, b and carotenoids were estimated spectrophotometrically by measuring absorbance of the samples at 663.2, 646.8 and 470 nm wavelength. The following formulas were used for calculation of photosynthetic pigments:

Chlorophyll a = $12.25\ A_{663.2} - 279\ A_{646.8}$

Chlorophyll b = $21.5\ A_{646.8} - 5.1\ A_{663.2}$

Carotenoid content = A480 x volume of extract x 10 x 100/2500 x weight of plant material (g)

2.7 Cadmium (Cd) analysis in different plant parts

Powdered dry samples were subjected to acid digestion using the method of Allen [26]. The digested samples were stored in small plastic bottles for analysis of Cd concentration. Atomic Absorption/Flame Spectrophotometer (model Hitachi Z-8000, Japan) was used for finding the concentration of Cd in each sample.

2.8 Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using software SPSS 16 and MS Excel 2007. Significant differences among the treatments for different parameters were analyzed using Tukey's Honestly Significant Difference (HSD) test.

3. Results

3.1 Length, biomass and water contents of *Ricinus communis* plant:

Plant length, biomass and water content in different parts of *Ricinus communis* under various treatments of molybdenum and Cd are shown in Table 2 (A, B, C). In Table 2A and Figure 1 the control C (without Cd and Mo) is compared with C1 (25 ppm Cd), C2 (50 ppm Cd) and C3 (100 ppm) for the effect of Cd on plant growth. In the same table, C1 is compared with treatments T1 – T9 for the effect of Mo on plant growth under Cd stress. A gradual decrease in plant growth parameters was noted with increasing concentration of Cd in soil i.e. C1 (25 ppm Cd) > C2 (50 ppm Cd) > C3 (100 ppm Cd). Treatments of Mo increased the growth and biomass of *Ricinus communis* plant as compared to C1 (Table 2A). The highest significant increase in root and stem length were found in T7 (1 ppm Mo foliar spray) as given in Table 3.2 and Figure 3.1X. It was found that 2 ppm Mo foliar treatment most significantly increased dry biomass (DBM) of the plant (Table 2A).

The Table 2B shows the effect of Mo treatments on growth parameter of *Ricinus communis* plants grown in 50 ppm Cd contaminated soil. The highest significant increase in root and stem length was demonstrated by T10 (0.5 ppm Mo seed soaking) and T18 (2.0 ppm Mo foliar spray) respectively, as compared to C2 (Table 2B and Figure 1). Dry biomass in root and stem was most significantly increased by 2 ppm Mo foliar spray (T18) while the same concentration of Mo (2 ppm) in the form of seed soaking (T12) also highly increased dry biomass in leaves.

The effect of Mo treatments on plant growth parameters in 100 ppm Cd contaminated soil is presented in Table 2C. Root and stem lengths were increased significantly by 2 ppm Mo in the form of seed soaking and foliar spray respectively as compared to C3 (Table 2C and Figure 1). Biomass (fresh and dry) in all parts of the plant were highly increased by the 2 ppm Mo foliar treatment (T27).

3.2 Biochemical variation in plants under various treatments and Cd stress:

Variation in concentrations of free proline, total phenolics and photosynthetic pigments (chlorophylls and carotenoids) in *Ricinus communis* plant under various treatments of Mo and in Cd contaminated soil

are given in Table 3 (A, B, C). In table 3A, the control C (without Cd and Mo) is compared with C1 (25 ppm Cd), C2 (50 ppm Cd) and C3 (100 ppm) for the Cd effect on free proline, total phenolics, chlorophyll and carotenoids concentration in the *Ricinus communis* plant. The treatments T1 –T9 are compared with the C1 for the effect of Mo on the biochemical parameter under Cd stress in table 3A. Increases in concentration of free proline and total phenolics were recorded with increasing Cd concentration in control soils (C3 > C2 > C1 > C). The highest significant increases in concentration of total phenolics and free proline in roots and leaves were recorded in 1.00 and 2.00 ppm Mo foliar treatments (T8 and T9) respectively, as compared to C1 (Table 3A). Photosynthetic pigments were significantly increased by the treatments T8 and T9 as compared to C1.

Table 3B presents the effect of Mo treatments on the concentration of free proline, total phenolics, chlorophyll and carotenoids in *Ricinus communis* plant in 50 ppm Cd contaminated soil. Plants treated with 2 ppm Mo as seed soaking (T12) and foliar spray (T18) most significantly increased concentration of proline and phenolics (respectively) in roots as compared to C2. Leaves demonstrated the highest concentration of proline and phenolics with the treatment T18 (Table 3B). Chlorophyll concentrations in leaves were most significantly high in the treatment T12 (2 ppm Mo foliar spray) as compared to C2, while concentration of carotenoid in leaves was highly significant in T16 (1 ppm Mo foliar spray).

The effect of Mo on free proline, total phenolics, chlorophyll and carotenoids concentrations in *Ricinus communis* plant grown in 100 ppm Cd contaminated soil is given in Table 3C. A highly significant increase in concentration of proline in roots and leaves was recorded in plants treated with 1.00 ppm Mo as seed soaking (T20) and foliar spray (T26) respectively. Foliar treatments T25 (0.50 ppm Mo) and T27 (2.00 ppm Mo) highly increased concentration of total phenolics in leaves and roots respectively (Table 3C). Carotenoid concentration within leaves was significantly increased (compared to C3) by the foliar treatments of Mo (T25, T26 and T27) and the highest significant increase in carotenoids was recorded in plants treated with foliar spray of 2.00 ppm Mo (T27).

The overall effect of Mo treatments on free proline and total phenolics under different concentrations of Cd in soil is given in Figure 2A. It was found that Mo treatments increased the concentration of free proline and total phenolics as the soil Cd concentration increased from 25 to 50 ppm and then decreased at the Cd concentration of 100 ppm.

Commented [m3]: I am not convinced that Figure shows anything about Molybdenum – it is a graph of the overall effect of Cd!!???

3.3 Cadmium concentration and bio-accumulation in *Ricinus communis*

Variation in concentration, accumulation, translocation and bioconcentration of Cd in different parts of *Ricinus communis* plant is given in Table 4 (A, B, C). Table 4A demonstrates the effect of different concentration of cadmium in soil on uptake and accumulation of cadmium in plant tissues. A gradual increase was noted in plant Cd concentration with increasing concentration of Cd in soils. Table 4A also shows the effect of molybdenum treatments (T1-T9) on plant Cd uptake from 25 ppm Cd contaminated soil as compared to C1 (25 ppm Cd, without Mo). The treatment T8 (1 ppm Mo foliar spray) most significantly increased Cd concentration in roots. Stem and leaves of the plant demonstrated the highest significant increase in Cd concentration with 2 ppm Mo foliar spray (T9) as given in table 4A. It was found that 1.00 and 2.00 ppm Mo (seed soaking and foliar spray) significantly increased Cd accumulation in the plant tissues. The treatment T9 showed the highest significant Cd accumulation in root, leaf and entire plant while the stem demonstrated the highest Cd accumulation in the treatment T8 (1 ppm Mo foliar spray) as shown in table 4A. The Mo treated plants (T1 –T9) showed an increase in Cd bioconcentration as compared to C1.

The effect of Mo treatments in combination with 50 ppm Cd in soil (T10-18) on Cd uptake in *Ricinus communis* is presented in Table 4B. Cadmium concentration in different parts of the plant increased significantly in treatments T13 (0.5 ppm Mo added to soil) and T18 (2.00 ppm Mo foliar spray) as compared to C2 (50 ppm Cd in soil, without Mo treatments). Roots accumulated Cd most significantly in plants sprayed with 0.5 ppm Mo (T16) while stem and leaves showed highly significant accumulated Cd in plants treated with 2 ppm Mo foliar spray (T18) as given in Table 4B. Cadmium translocation into leaves increased significantly with 0.5 ppm Mo as seed soaking (T10). Bioconcentration of Cd was significantly increased by the treatments T13 (0.5 ppm Mo into soil) and T18 (2 ppm Mo foliar spray) as compared to C2.

Variations in Cd uptake in plant tissues with Mo treatments (T19-T27) under 100 ppm Cd in soil are given in Table 4C. Application of 0.5 ppm Mo (seed soaking and foliar spray) significantly increased Cd concentration in roots of the plant. The same concentration (0.5 ppm) of Mo as soil addition significantly increased Cd concentration in stem (Table 4C). The foliar spray of 2.00 ppm Mo highly increased Cd concentration in leaves of the plant. The highest significant accumulation of Cd in different parts of the plant was recorded in the treatment T27 (2.00 ppm Mo foliar spray). Translocation

and bioconcentration of Cd were highly significant in plants sprayed with 2.00 ppm Mo (T27) as given in table 4C.

Figure 2B presents the overall effect of Mo treatments on Cd accumulation and bioconcentration in *Ricinus communis* plant under varied Cd concentration in soil. The Mo treatment showed an overall increase in plant Cd accumulation while a decrease was recorded in Cd bioconcentration with the increasing Cd concentration in soil.

Commented [m4]: Again, I don't think that it does!! It just shows the overall effect of Cd levels!!

Commented [m5]: This is a very odd result – so are you saying that Mo reduces bioaccumulation at higher and higher levels of soil Cd??? I does not tie in with the positive correlations presented in the next section.

3.4 Correlation among different parameters

Tables 5, 6 and 7 present correlations among different parameters in roots, stem and leaves of *Ricinus communis* plant grown in 25, 50 and 100 ppm Cd contaminated soil, under various treatments of Mo (0.5, 1.00 and 2.00 ppm). Total phenolic concentration showed significantly positive correlation with Cd accumulation in plant roots (Tables 5A, 5B and 5C) and leaves (Tables 7A, 7B and 7C). Proline concentrations in roots (Tables 5A and 5B) and leaves (Tables 7A and 7B) also demonstrated significantly positive correlations with Cd accumulation in plants grown in 25 and 50 ppm Cd contaminated soil respectively. Proline concentration showed strong positive correlation with Cd accumulation in roots in 25, 50 and 100 ppm Cd contaminated soil (Tables 5A, 5B and 5C). Photosynthetic pigments (chlorophyll and carotenoids) showed strong correlation with total phenolics concentration within leaves of the plant at all the Cd concentrations (25, 50 and 100 ppm in soil) as shown in table 7 (A, B, C). It was found that dry biomass in roots, stem and leaves demonstrated significantly positive correlation with Cd accumulation (Tables 5, 6 and 7).

4 Discussion

The effect of molybdenum on phytoextraction potential of *Ricinus communis* was evaluated in the present work. The effect of Molybdenum on the concentration of free proline, total phenolics and photosynthetic pigments in plant tissues under varying Cd stress was also studied.

It is commonly reported that th presence of toxic heavy metals in soil significantly reduces growth and biomass of plants [27, 28, 29] and in the present research, *Ricinus communis* demonstrated significant reduction in growth and biomass when subjected to various concentrations of Cd in soil. This decrease might be due to the toxic effect of Cd on the function of some key enzymes involved in plant metabolism, such as enzyme involved in nitrate metabolism and protein synthesis [27, 30]. Reduction in biomass under Cd stress have been reported in many plants, such as *Cannabis sativa* [2], *Parthenium*

hysterophorus [9], *Lycopersicon esculentum* [31], *Glycine max* [32], *Pisum sativum* [33], *Amaranthus tricolor* [34], *Brassica juncea* [27] and *Hordeum vulagare* [35]. Our results showed that Mo treatments restored growth and biomass of *Ricinus communis* plant under Cd stress. The significant effect of Mo on biomass might be due its role as a cofactor for enzymes involve in nitrate metabolism (such as nitrate reductase and glutamine synthetase), synthesis of amino acids and indole acetic [15 - 19] thus counteracting the negative effects of Cd. Deficiency of Mo has been reported for many plant species including crops, herbs and trees mostly because of a decreased bio-availability in acidic soils [36, 37] suggesting that soil application of Mo may not always be effective. Therefore we used Mo in three different ways and found that application of Mo in the form of seed soaking and as a foliar spray had more significant effects on plant growth and biomass as compared to addition of Mo into soil. This suggests higher bioavialibility of Mo in the form of foliar and seed soaking treatments as compared to the soil addition treatments.

4.2 Effect Mo treatments on free proline and total phenolics

Increases in the concentration of free proline have been reported in different plant species under abiotic stress conditions such as very low or high temperatures, heavy metal exposure and elevated salinity [2, 38]. High concentrations of proline act as environmental stress indicator in many plants [39]. Several plants such as cannabis, sunflower, tomato, cowpea and wheat have been reported with high concentrations of free proline under heavy metal stress [9, 40-43]. In our experiment, Molybdenum was found to increase free proline concentration in roots and leaves of the plant under Cd stress.

Heavy metal toxicity results in production of reactive oxygen species inside plant tissues and phenolic compounds possess antioxidant activity and thus protect cellular components from oxidative stress caused by reactive oxygen species [44]. Several investigators have reported an increase in concentration of total phenolics under Cd stresses in plant tissues [2, 13, 45]. Our results also showed increases in the concentration of total phenolics in roots and leaves of *Ricinus communis* plant under Cd stress. Treatments of Mo further increased the concentration of total phenolics in plants when subjected to Cd stress. The foliar application of Mo was the most significant in terms of stimulating total phenolic concentration in plant tissues. It was also found that the concentration of total phenolics was higher in leaves of the plant as compare to roots. High concentration of phenolic compounds under Cd stress in leaves as compared to roots of *Crotalaria juncea*, *Parthenium hysterophorus* and *Cannabis sativa* plants have also been reported by Uraguchi et al. [45], Ali and Hadi, [12] and Ahmad et al., [2] respectively.

4.3 Effect of Mo on cadmium uptake and accumulation

Molybdenum is a micronutrient that acts as cofactor for variety of enzymes promoting plant growth and biomass; one of the factors important for metal phytoextraction [9, 36]. Different treatments of molybdenum, especially in the form of foliar spray significantly increased Cd concentration in different parts of the plant as compared to the control plants. The reason for this increase in Cd concentration with Mo foliar spray might be the enhancement of plant growth and nutrient uptake along with Cd from the soil. Our results demonstrated higher concentration of Cd in roots of *Ricinus communis* followed by leaves and stem respectively which is in agreement with the work of Citterio et al., [46] and Linger et al., [47] on *Cannabis sativa* and Hadi et al., [9] on *Parthenium hysterophorus*. Increasing concentration of Cd in soil also increased Cd concentration in plant due to high bio-availability of Cd to plant at higher concentrations in the soil. Foliar spray of Mo at 2.00 ppm concentration most significantly increased accumulation of Cd in all parts of Ricinus communis, which might be due the significant effect of the foliar treatment on both biomass and Cd concentration in different parts of the plant. Plants grown in 25 ppm Cd contaminated soil showed highest percentage of Cd accumulation in roots while 50 and 100 ppm polluted soil demonstrated highest Cd was accumulation percentage in leaves of the plant. Cd bioconcentration in the plant was recorded at a very high level suggestting that *Ricinus communis* can be considered as a hyperaccumulator of Cd. The Mo treatments further increased Cd bioconcentration in the plant.

4.4 Correlation among different parameters

Strong correlations between total phenolics and Cd accumulation in plant roots and leaves were found, suggesting significant role of phenolic compounds in protection of plant cells against the toxic effects of Cd ions [2, 38, 39]. Similarly free proline also demonstrated positive correlations with Cd accumulation and dry biomass of the plant.

4.5 Conclusions and Recommendations

Ricinus communis is a good candidate for phytoextraction of toxic metals because of its fast growth, huge biomass, tolerance to heavy metals and capacity for hyperaccumulation. Molybdenum demonstrated a significantly positive effect on plant biomass and Cd phytoextraction in the presence of Cd in the soil. Foliar applications of Mo were found superior than seed soaking and soil addition treatments, in terms of increase in growth, phenolics, proline production and Cd phytoaccumulation. Correlation between total phenolics, dry biomass and Cd accumulation in different parts of the plant, under different treatments of Mo, were found to be statistically significant. It is recommended to further study various concentrations of Mo foliar spray to find the optimum concentration for growth and Cd phytoremediation in *Ricinus communis.* Further study to investigate the effect of molybdenum on the

molecular mechanism involved in Cd phytoremediation is recommended. We are further investigating the role of molybdenum in the expression of some metal tolerance target genes.

4.6 Acknowledgment

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