

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

Fazal Hadi¹ · Nasir Ali^{1,2} · Michael Paul Fuller³

Received: 29 March 2016 / Accepted: 11 July 2016 / Published online: 25 July 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Cadmium (Cd) in agricultural soil negatively affects crops yield and compromises food safety. Remediation of polluted soil is necessary 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 ($R^2 = 0.707, 0.721$ 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.

Keywords Heavy metal · Phytoextraction · Foliar application of Mo · Bioconcentration factor

Introduction

Cadmium (Cd) is one of the hazardous heavy metals. It enters into the agricultural soil mostly through industrial effluents, mining operations, municipal runoff and application of phosphate fertilizers where it can occur as a microcontaminant (Rogers et al. 2007). 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 compromising the quality of food (Ahmad et al. 2015; Zadeh et al. 2008). Consumption of Cd-contaminated food results in serious health problems (Ahmad et al. 2015; Clemens 2006). In the human body, Cd can affect gene expression, 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 (Joseph 2009; Huang et al. 2008; Takiguchi et al. 2003; Krocova et al. 2000). 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 (Hadi et al. 2014). Plants under heavy metal stress often showed decrease in growth and biomass which in turn reduce their phytoremediation potential (Falkowska et al. 2011; Tassi et al. 2008). To combat the toxic metals

Responsible editor: Elena Maestri

✉ Fazal Hadi
dr.fhadi@uom.edu.pk

- ¹ Department of Biotechnology, University of Malakand, Chakdara, Dir Lower, Khyber Pakhtunkhwa 18800, Pakistan
- ² Department of Biotechnology and Microbiology, Sarhad University of Science and Information Technology, Peshawar, Khyber Pakhtunkhwa, Pakistan
- ³ School of Biological Science, Plymouth University, Devon PL4 8AA, UK

in plant cells, increases in concentrations of endogenous free proline and phenolic compounds have been reported in many plant species (Ahmad et al. 2015; Ali and Hadi 2015). 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 (Handique and Handique 2009; Michalak 2006).

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 its deficiency adversely affects the activities of nitrate reductase and glutamine synthetase which are enzymes catalyzing the initial steps of nitrate metabolism (Hristozkova et al. 2006). Molybdenum has also been reported to catalyze other enzymes such as aldehyde oxidase (AO) involved in abscisic acid biosynthesis and sulfite oxidase (SO) which catalyses the conversion of sulfite to sulfate, an essential step in the catabolism of amino acids containing sulfur (Williams and Frausto da Silva 2002; Mendel and Haensch 2002). Molybdo-enzymes also play a role in the biosynthesis of plant growth regulators (Hesberg et al. 2004; Sagi et al. 2002).

Ricinus communis (castor bean) plant belongs to family Euphorbiaceae (Rana et al. 2012) and is an industrial crop. It is used for the production of biodiesel, paints, nylon-type fibre and products with insecticidal and antimicrobial purposes (Rix 1999). 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 *R. communis* grown in Cd-contaminated soil.

Materials and methods

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), pH (6.5 ± 0.3) and Cd (0.046 ppm) content of the soil was measured. Then 2 kg soil was poured into plastic pots (20 × 12 cm). Cd in the form of cadmium acetate dihydrate (CH₃COO)₂ Cd · 2H₂O (Merck, Germany) solution was added to the soil in pots. Cadmium was allowed to equilibrate in soil for 1 month. A total of four different Cd concentrations were used (0, 25, 50 and 100 ppm) (Table 1).

Transplantation of seedlings and plant growth

Each pot was watered a day before transplantation of seedlings. Seeds of *R. 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 (35 max/25 min °C). Plants were watered, at 3 days intervals, bringing the soil back to field capacity each time.

Table 1 The following treatments were used during the experiment. SS stands for seed soaking, AS stands for added to soil, FS 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

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 [SS]	T1	0.50 ppm Mo [SS]	T10	0.50 ppm Mo [SS]	T19
1.00 ppm Mo [SS]	T2	1.00 ppm Mo [SS]	T11	1.00 ppm Mo [SS]	T20
2.00 ppm Mo [SS]	T3	2.00 ppm Mo [SS]	T12	2.00 ppm Mo [SS]	T21
0.50 ppm Mo [AS]	T4	0.50 ppm Mo [AS]	T13	0.50 ppm Mo [AS]	T22
1.00 ppm Mo [AS]	T5	1.00 ppm Mo [AS]	T14	1.00 ppm Mo [AS]	T23
2.00 ppm Mo [AS]	T6	2.00 ppm Mo [AS]	T15	2.00 ppm Mo [AS]	T24
0.50 ppm Mo [FS]	T7	0.50 ppm Mo [FS]	T16	0.50 ppm Mo [FS]	T25
1.00 ppm Mo [FS]	T8	1.00 ppm Mo [FS]	T17	1.00 ppm Mo [FS]	T26
2.00 ppm Mo [FS]	T9	2.00 ppm Mo [FS]	T18	2.00 ppm Mo [FS]	T27

Table 2 Effect of different treatments of molybdenum (Mo) on plant length, biomass and water content in different parts of *R. communis* plant grown in 25 ppm Cd-contaminated soil. *SD* denotes standard deviation and the *different letters in superscript* present the significant difference among the values within a column

Treatments	Length (cm) ± SD			Fresh biomass (g) ± SD			Dry biomass (g) ± SD		
	Roots	Stem	Roots	Roots	Stem	Leaves	Entire plant	Roots	
C	Control (without Cd and Mo)	31.25 ± 3.13 ^{abc}	3.75 ± 0.30 ^{cde}	7.81 ± 0.70 ^{cde}	21.33 ± 1.71 ^{abc}	9.77 ± 0.78 ^{abcd}	1.13 ± 0.09 ^{de}		
C1	Control (with Cd 25 ppm)	24.32 ± 2.43 ^{cde}	2.92 ± 0.26 ^{ef}	6.08 ± 0.49 ^{ef}	15.99 ± 1.44 ^{cde}	6.99 ± 0.63 ^{efg}	0.79 ± 0.07 ^{fg}		
C2	Control (with Cd 50 ppm)	20.30 ± 2.03 ^{de}	2.78 ± 0.22 ^{ef}	5.08 ± 0.51 ^{ef}	14.40 ± 1.15 ^{de}	6.54 ± 0.65 ^{fg}	0.66 ± 0.05 ^{fg}		
C3	Control (with Cd 100 ppm)	17.02 ± 1.70 ^e	2.04 ± 0.14 ^f	4.26 ± 0.34 ^f	11.19 ± 1.12 ^e	4.89 ± 0.39 ^g	0.55 ± 0.06 ^g		
T1	Cd 25 ppm + Mo 0.5 ppm (seed soaking)	15.00 ± 1.50 ^a	3.68 ± 0.40 ^{cde}	6.81 ± 0.75 ^{cde}	19.20 ± 1.54 ^{bcd}	8.71 ± 0.78 ^{cddef}	1.25 ± 0.13 ^{cde}		
T2	Cd 25 ppm + Mo 1.0 ppm (seed soaking)	14.21 ± 1.42 ^{ab}	5.25 ± 0.47 ^{ab}	8.75 ± 0.70 ^{ab}	24.97 ± 3.00 ^a	10.97 ± 0.77 ^{abcd}	1.52 ± 0.11 ^{bc}		
T3	Cd 25 ppm + Mo 2 ppm (seed soaking)	13.00 ± 1.30 ^{abc}	5.46 ± 0.55 ^a	8.45 ± 0.85 ^a	25.03 ± 2.25 ^a	11.12 ± 1.22 ^{abc}	1.64 ± 0.13 ^{ab}		
T4	Cd 25 ppm + Mo 0.5 ppm (soil addition)	12.00 ± 1.20 ^{abcd}	3.16 ± 0.25 ^{de}	6.58 ± 0.59 ^{de}	18.97 ± 1.33 ^{cd}	9.24 ± 0.83 ^{bcde}	0.98 ± 0.09 ^{ef}		
T5	Cd 25 ppm + Mo 1.0 ppm (soil addition)	11.00 ± 0.99 ^{bcd}	3.98 ± 0.32 ^{cd}	7.36 ± 0.44 ^{cd}	20.28 ± 1.62 ^{abc}	8.94 ± 0.72 ^{cddef}	1.23 ± 0.10 ^{cde}		
T6	Cd 25 ppm + Mo 2 ppm (soil addition)	10.45 ± 0.94 ^{cde}	3.99 ± 0.36 ^{cd}	7.02 ± 0.77 ^{cd}	19.55 ± 1.56 ^{bcd}	8.54 ± 0.68 ^{def}	1.47 ± 0.12 ^{bc}		
T7	Cd 25 ppm + Mo 0.5 ppm (foliar spray)	10.90 ± 0.98 ^{cde}	3.31 ± 0.23 ^{de}	6.68 ± 0.53 ^{de}	18.96 ± 1.71 ^{cd}	8.97 ± 0.90 ^{cddef}	1.28 ± 0.12 ^{cde}		
T8	Cd 25 ppm + Mo 1.0 ppm (foliar spray)	15.76 ± 1.33 ^a	4.43 ± 0.40 ^{bc}	9.00 ± 0.81 ^a	25.58 ± 2.30 ^a	12.15 ± 1.09 ^a	1.45 ± 0.13 ^{bcd}		
T9	Cd 25 ppm + Mo 2.0 ppm (foliar spray)	12.71 ± 1.14 ^{abcd}	4.68 ± 0.37 ^{abc}	8.42 ± 0.67 ^{abc}	24.55 ± 1.96 ^{ab}	11.45 ± 0.92 ^{ab}	1.91 ± 0.19 ^a		
Total water contents (g) ± SD									
Dry biomass (g) ± SD	Entire plant			Roots			Leaves		
	Stem	Leaves	Entire plant	Roots	Stem	Leaves	Entire plant	Roots	
2.34 ± 0.19 ^{bcd}	2.93 ± 0.23 ^{abc}	6.40 ± 0.51 ^{cde}	2.63 ± 0.21 ^{bcd}	5.47 ± 0.44 ^{abcd}	6.84 ± 0.55 ^{bcd}	14.93 ± 1.19 ^{abc}	2.77 ± 0.22 ^b	3.41 ± 0.34 ^a	
1.64 ± 0.15 ^{ef}	1.89 ± 0.15 ^{ef}	4.32 ± 0.35 ^{fg}	2.13 ± 0.17 ^{cde}	4.44 ± 0.36 ^{cddef}	5.10 ± 0.41 ^{ef}	11.67 ± 0.93 ^{cde}	2.77 ± 0.22 ^b	3.41 ± 0.34 ^a	
1.19 ± 0.11 ^{fg}	1.58 ± 0.13 ^{fg}	3.42 ± 0.27 ^{gh}	2.12 ± 0.17 ^{cde}	3.89 ± 0.31 ^{ef}	4.96 ± 0.40 ^{ef}	10.97 ± 0.88 ^{de}	2.14 ± 0.15 ^{def}	2.39 ± 0.22 ^{cde}	
1.02 ± 0.08 ^g	1.05 ± 0.08 ^g	2.62 ± 0.21 ^h	1.49 ± 0.12 ^e	3.24 ± 0.26 ^f	3.84 ± 0.31 ^f	8.57 ± 0.69 ^e	1.98 ± 0.16 ^{de}	2.87 ± 0.23 ^{abc}	
2.11 ± 0.17 ^{cde}	2.70 ± 0.22 ^{bcd}	6.06 ± 0.48 ^{de}	2.43 ± 0.19 ^{bcd}	4.70 ± 0.42 ^{bcd}	6.01 ± 0.54 ^{cde}	13.14 ± 1.05 ^{bcd}	2.42 ± 0.22 ^{bcd}	2.96 ± 0.27 ^{abc}	
2.71 ± 0.30 ^{bc}	3.47 ± 0.28 ^a	7.70 ± 0.62 ^{abc}	3.73 ± 0.30 ^a	6.04 ± 0.48 ^a	7.50 ± 0.60 ^{abc}	17.27 ± 1.38 ^a	3.42 ± 0.27 ^{ab}	3.34 ± 0.27 ^{ab}	
2.77 ± 0.22 ^b	3.24 ± 0.26 ^{ab}	7.65 ± 0.61 ^{abc}	3.82 ± 0.31 ^a	5.68 ± 0.45 ^{ab}	7.88 ± 0.63 ^{ab}	17.38 ± 1.39 ^a	3.95 ± 0.29 ^a	3.41 ± 0.34 ^a	
1.91 ± 0.15 ^{de}	2.14 ± 0.17 ^{def}	5.02 ± 0.40 ^{ef}	2.18 ± 0.17 ^{cd}	4.67 ± 0.37 ^{bcd}	7.10 ± 0.57 ^{bcd}	13.95 ± 1.12 ^{abc}	2.14 ± 0.15 ^{def}	2.14 ± 0.17 ^{def}	
2.14 ± 0.19 ^{cde}	2.39 ± 0.22 ^{cde}	5.76 ± 0.52 ^{def}	2.74 ± 0.25 ^{bc}	5.23 ± 0.42 ^{abcd}	6.55 ± 0.65 ^{bcd}	14.52 ± 1.16 ^{abc}	2.14 ± 0.19 ^{cde}	2.39 ± 0.22 ^{cde}	
1.98 ± 0.16 ^{de}	2.87 ± 0.23 ^{abc}	6.32 ± 0.51 ^{cde}	2.52 ± 0.20 ^{bcd}	5.04 ± 0.40 ^{abcde}	5.67 ± 0.45 ^{de}	13.23 ± 1.06 ^{bcd}	1.98 ± 0.16 ^{de}	2.87 ± 0.23 ^{abc}	
2.42 ± 0.22 ^{bcd}	2.96 ± 0.27 ^{abc}	6.66 ± 0.60 ^{bcd}	2.03 ± 0.18 ^{de}	4.26 ± 0.38 ^{def}	6.01 ± 0.54 ^{cde}	12.30 ± 1.11 ^{cd}	2.42 ± 0.22 ^{bcd}	2.96 ± 0.27 ^{abc}	
3.42 ± 0.27 ^{ab}	3.34 ± 0.27 ^{ab}	8.21 ± 0.66 ^{ab}	2.98 ± 0.24 ^b	5.58 ± 0.45 ^{abc}	8.81 ± 0.70 ^a	17.37 ± 1.39 ^a	3.42 ± 0.27 ^{ab}	3.34 ± 0.27 ^{ab}	
3.95 ± 0.29 ^a	3.41 ± 0.34 ^a	8.27 ± 0.83 ^a	2.77 ± 0.28 ^{bc}	4.48 ± 0.55 ^{cd}	8.04 ± 0.80 ^{ab}	16.29 ± 1.63 ^{ab}	3.95 ± 0.29 ^a	3.41 ± 0.34 ^a	

Table 3 Role of Mo treatments on length, biomass and water content of *R. communis* in 50 ppm Cd-polluted soil. *SD* denotes standard deviation and the *different letters in superscript* present significant difference among the values within a column

Treatments	Length (cm) ± SD			Fresh biomass (g) ± SD			Dry biomass (g) ± SD		
	Roots	Stem	Entire plant	Roots	Stem	Entire plant	Roots	Leaves	Entire plant
C2	8.12 ± 0.97 ^c	20.30 ± 2.44 ^{ab}	2.78 ± 0.33 ^d	5.08 ± 0.61 ^d	6.54 ± 0.78 ^b	14.40 ± 1.73 ^c	0.66 ± 0.08 ^c		
Cd 50 ppm + Mo 0.5 ppm (seed soaking)	13.46 ± 1.21 ^a	26.05 ± 2.34 ^{ab}	4.68 ± 0.42	6.11 ± 0.55 ^c	7.82 ± 0.70 ^{ab}	18.61 ± 1.68 ^{bc}	1.12 ± 0.10 ^{ab}		
T10	12.75 ± 1.53 ^{ab}	25.32 ± 3.04 ^{ab}	6.20 ± 0.74 ^a	7.85 ± 0.94 ^{ab}	9.84 ± 1.18 ^a	23.90 ± 2.87 ^a	1.24 ± 0.15 ^{ab}		
T11	11.67 ± 1.63 ^{abc}	18.00 ± 2.52 ^b	6.14 ± 0.86 ^{ab}	7.29 ± 1.02 ^{bc}	9.98 ± 1.40 ^a	23.41 ± 3.28 ^a	1.38 ± 0.19 ^a		
T12	8.98 ± 1.08 ^{bc}	20.14 ± 2.42 ^{ab}	4.02 ± 0.48 ^{cd}	5.45 ± 0.65 ^{cd}	8.29 ± 0.99 ^{ab}	17.76 ± 2.13 ^{bc}	0.87 ± 0.10 ^{bc}		
T13	9.87 ± 1.28 ^{abc}	24.02 ± 3.12 ^{ab}	5.07 ± 0.66 ^b	6.61 ± 0.86 ^{bc}	7.45 ± 0.97 ^{ab}	19.12 ± 2.49 ^b	1.11 ± 0.14 ^{ab}		
T14	10.88 ± 1.31 ^{abc}	21.41 ± 2.57 ^{ab}	5.33 ± 0.64 ^b	7.85 ± 0.94 ^{ab}	7.40 ± 0.89 ^{ab}	20.58 ± 2.47 ^{ab}	1.21 ± 0.15 ^{ab}		
T15	9.79 ± 1.47 ^{abc}	25.00 ± 3.75 ^{ab}	4.21 ± 0.63 ^c	6.54 ± 0.98 ^{bc}	8.02 ± 1.20 ^{ab}	18.77 ± 2.82 ^{bc}	1.42 ± 0.21 ^a		
T16	12.56 ± 2.01 ^{ab}	25.23 ± 4.04 ^{ab}	4.87 ± 0.78 ^{bc}	8.08 ± 1.29 ^a	7.89 ± 1.26 ^{ab}	20.84 ± 3.33 ^{ab}	1.30 ± 0.21 ^{ab}		
T17	12.02 ± 1.44 ^{abc}	28.14 ± 3.38 ^a	6.32 ± 0.61 ^a	7.56 ± 0.91 ^{ab}	6.23 ± 0.75 ^{ab}	20.11 ± 2.27 ^{ab}	1.49 ± 0.13 ^a		
T18									
Total water contents (g) ± SD									
Treatments	Length (cm) ± SD			Fresh biomass (g) ± SD			Dry biomass (g) ± SD		
	Roots	Stem	Entire plant	Roots	Stem	Entire plant	Roots	Leaves	Entire plant
C2	3.42 ± 0.41 ^c	2.12 ± 0.25 ^c	3.89 ± 0.47 ^b	2.12 ± 0.25 ^c	4.96 ± 0.60 ^{ab}	10.97 ± 1.32 ^b			
Cd 50 ppm + Mo 0.5 ppm (seed soaking)	5.14 ± 0.46 ^{abc}	3.56 ± 0.32 ^{ab}	4.21 ± 0.38 ^{ab}	3.56 ± 0.32 ^{ab}	5.70 ± 0.51 ^{ab}	13.48 ± 1.21 ^{ab}			
T10	6.62 ± 0.79 ^a	4.96 ± 0.60 ^a	5.33 ± 0.64 ^{ab}	4.96 ± 0.60 ^a	6.98 ± 0.84 ^a	17.28 ± 2.07 ^a			
T11	6.84 ± 0.96 ^a	4.76 ± 0.67 ^a	4.97 ± 0.70 ^{ab}	4.76 ± 0.67 ^a	6.84 ± 0.96 ^a	16.57 ± 2.32 ^a			
T12	3.76 ± 0.45 ^{bc}	3.15 ± 0.38 ^{bc}	4.25 ± 0.51 ^{ab}	3.15 ± 0.38 ^{bc}	6.60 ± 0.79 ^a	14.00 ± 1.68 ^{ab}			
T13	5.05 ± 0.66 ^{abc}	3.96 ± 0.51 ^{ab}	4.69 ± 0.61 ^{ab}	3.96 ± 0.51 ^{ab}	5.42 ± 0.70 ^{ab}	14.07 ± 1.83 ^{ab}			
T14	6.41 ± 0.77 ^a	4.12 ± 0.49 ^{ab}	5.77 ± 0.69 ^a	4.12 ± 0.49 ^{ab}	4.28 ± 0.51 ^b	14.17 ± 1.70 ^{ab}			
T15	6.25 ± 0.94 ^a	2.79 ± 0.42 ^{bc}	4.37 ± 0.66 ^{ab}	2.79 ± 0.42 ^{bc}	5.37 ± 0.80 ^{ab}	12.53 ± 1.88 ^{ab}			
T16	6.19 ± 0.99 ^a	3.57 ± 0.57 ^{ab}	5.63 ± 0.90 ^{ab}	3.57 ± 0.57 ^{ab}	5.45 ± 0.87 ^{ab}	14.64 ± 2.34 ^{ab}			
T17	6.02 ± 0.67 ^a	4.98 ± 0.48 ^a	5.05 ± 0.61 ^{ab}	4.98 ± 0.48 ^a	4.25 ± 0.51 ^{ab}	14.19 ± 1.59 ^{ab}			

Table 4 Effect of Mo treatments on growth parameter of *R. communis* plant grown in 100 ppm Cd-contaminated soil. SD denotes standard deviation and the different letters in superscript present significant difference among the values within a column

Treatments	Length (cm) ± SD			Fresh biomass (g) ± SD			Dry biomass (g) ± SD		
	Roots	Stem	Entire plant	Roots	Stem	Leaves	Roots	Stem	Entire plant
C3	6.81 ± 0.82 ^b	17.02 ± 2.04 ^{bc}	11.19 ± 1.34 ^b	2.04 ± 0.25 ^e	4.26 ± 0.51 ^e	4.89 ± 0.59 ^b	0.55 ± 0.07 ^e		
T19	7.68 ± 0.69 ^b	21.77 ± 1.96 ^{ab}	15.09 ± 1.36 ^{ab}	3.45 ± 0.31 ^{cde}	5.11 ± 0.46 ^{cde}	6.54 ± 0.59 ^{ab}	0.80 ± 0.07 ^{cde}		
T20	8.48 ± 1.02 ^{ab}	21.16 ± 2.54 ^{ab}	18.86 ± 2.26 ^a	5.18 ± 0.62 ^{ab}	5.55 ± 0.65 ^{bc}	8.23 ± 0.99 ^a	1.00 ± 0.12 ^{abcd}		
T21	11.35 ± 1.59 ^a	19.21 ± 2.69 ^{ab}	18.97 ± 2.80 ^a	5.54 ± 0.78 ^a	6.09 ± 0.85 ^{bc}	7.34 ± 1.17 ^a	1.13 ± 0.18 ^{ab}		
T22	7.51 ± 0.90 ^b	16.83 ± 2.02 ^c	14.58 ± 1.75 ^{ab}	3.10 ± 0.37 ^{de}	4.56 ± 0.55 ^{de}	6.93 ± 0.83 ^{ab}	0.73 ± 0.09 ^{de}		
T23	8.25 ± 1.07 ^{ab}	16.98 ± 2.21 ^c	16.22 ± 2.11 ^{ab}	4.54 ± 0.59 ^{abcd}	5.35 ± 0.71 ^{cd}	6.23 ± 0.81 ^{ab}	0.92 ± 0.12 ^{bcd}		
T24	10.23 ± 1.23 ^{ab}	17.89 ± 2.15 ^{bc}	17.76 ± 2.13 ^a	5.01 ± 0.60 ^{abc}	6.36 ± 0.79 ^{ab}	6.18 ± 0.74 ^{ab}	1.10 ± 0.13 ^{abc}		
T25	8.18 ± 1.23 ^{ab}	20.90 ± 3.13 ^{ab}	17.04 ± 2.41 ^{ab}	3.87 ± 0.58 ^{abcd}	5.47 ± 0.82 ^{cd}	6.70 ± 1.01 ^{ab}	1.19 ± 0.18 ^{ab}		
T26	9.47 ± 1.52 ^{ab}	22.45 ± 3.59 ^a	17.35 ± 2.78 ^a	4.01 ± 0.64 ^{abcd}	6.75 ± 1.08 ^a	6.59 ± 1.06 ^{ab}	1.09 ± 0.17 ^{ab}		
T27	11.55 ± 1.21 ^a	23.52 ± 2.82 ^a	19.52 ± 1.91 ^a	5.96 ± 0.51 ^a	7.38 ± 0.79 ^a	6.18 ± 0.62 ^{ab}	1.44 ± 0.11 ^a		
Total water contents (g) ± SD									
Length (cm) ± SD									
Dry biomass (g) ± SD									
Roots	Stem	Leaves	Entire plant	Roots	Stem	Leaves	Entire plant	Roots	Entire plant
6.81 ± 0.82 ^b	1.02 ± 0.12 ^c	3.84 ± 0.46 ^b	8.37 ± 1.03 ^b	3.24 ± 0.39 ^c	3.52 ± 0.32 ^{bc}	4.76 ± 0.43 ^{ab}	10.94 ± 0.98 ^{ab}		
7.68 ± 0.69 ^b	1.58 ± 0.14 ^{abc}	4.76 ± 0.43 ^{ab}	10.94 ± 0.98 ^{ab}	3.52 ± 0.32 ^{bc}	3.34 ± 0.40 ^c	5.69 ± 0.68 ^a	13.21 ± 1.59 ^a		
8.48 ± 1.02 ^{ab}	2.11 ± 0.25 ^a	5.69 ± 0.68 ^a	13.21 ± 1.59 ^a	3.34 ± 0.40 ^c	4.15 ± 0.58 ^{bc}	4.79 ± 0.78 ^a	13.35 ± 1.96 ^a		
11.35 ± 1.59 ^a	1.94 ± 0.27 ^{ab}	4.79 ± 0.78 ^a	13.35 ± 1.96 ^a	4.15 ± 0.58 ^{bc}	3.45 ± 0.41 ^{bc}	5.61 ± 0.67 ^a	11.42 ± 1.37 ^{ab}		
7.51 ± 0.90 ^b	1.11 ± 0.13 ^{bc}	5.61 ± 0.67 ^a	11.42 ± 1.37 ^{ab}	3.45 ± 0.41 ^{bc}	3.85 ± 0.50 ^{abc}	4.53 ± 0.59 ^{ab}	11.99 ± 1.56 ^{ab}		
8.25 ± 1.07 ^{ab}	1.60 ± 0.21 ^{abc}	4.53 ± 0.59 ^{ab}	11.99 ± 1.56 ^{ab}	3.85 ± 0.50 ^{abc}	4.82 ± 0.58 ^a	3.53 ± 0.42 ^b	12.27 ± 1.47 ^{ab}		
10.23 ± 1.23 ^{ab}	1.74 ± 0.21 ^{ab}	3.53 ± 0.42 ^b	12.27 ± 1.47 ^{ab}	4.82 ± 0.58 ^a	3.65 ± 0.55 ^{bc}	4.48 ± 0.67 ^{ab}	10.82 ± 1.62 ^{ab}		
8.18 ± 1.23 ^{ab}	1.82 ± 0.27 ^a	4.48 ± 0.67 ^{ab}	10.82 ± 1.62 ^{ab}	3.65 ± 0.55 ^{bc}	4.70 ± 0.75 ^a	4.61 ± 0.74 ^{ab}	12.24 ± 1.96 ^a		
9.47 ± 1.52 ^{ab}	2.05 ± 0.33 ^a	4.61 ± 0.74 ^{ab}	12.24 ± 1.96 ^a	4.70 ± 0.75 ^a	4.85 ± 0.52 ^a	4.26 ± 0.44 ^b	13.63 ± 1.36 ^a		
11.55 ± 1.21 ^a	2.53 ± 0.27 ^a	4.26 ± 0.44 ^b	13.63 ± 1.36 ^a	4.85 ± 0.52 ^a					



Fig. 1 Effect of different treatments of Mo on growth of *Ricinus communis* plant grown in soil contaminated with 25 ppm (a), 50 ppm (b) and 100 ppm (c) cadmium

Treatments during the experiment

Molybdenum 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 h before sowing. Six foliar applications were carried out at 1 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.

Plant growth parameters

Plants were harvested 2 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 (Genrich et al. 2000). After washing, each plant was cut into three fractions i.e. roots, stem and leaves and fresh weights were taken. Each fraction was packed in separate paper envelopes and then kept in a drying oven for

48 h at 80 °C and dry weights were taken. Dried biomass was then ground into powdered form through mechanical grinder.

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. (1973). The proline concentration in each sample extract was 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-Ciocalteu (FC) reagent method (Singleton and Rossi 1965) 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 was calculated from the standard curve. Three replicates were used.

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. (2014). Fresh leaf samples (0.5 g)

Table 5 Effect of various Mo treatments on concentrations of free proline, total phenolics and photosynthetic pigments in *R. communis* plant grown in 25 ppm Cd-contaminated soil. *SD* denotes standard deviation and the *different letters in superscript* present significant difference among the values within a column

Treatments	Free proline ($\mu\text{g/g}$) \pm SD		Total phenolics ($\mu\text{g/g}$) \pm SD		Chlorophyll contents ($\mu\text{g/g}$) \pm SD			Carotenoids ($\mu\text{g/g}$) \pm SD	
	Leaves		Roots		leaves		a + b		
	Roots	Leaves	Roots	Leaves	a	b			
C	Control (without Cd and Mo)	28.80 \pm 2.30 ^e	20.70 \pm 1.66 ^e	26.45 \pm 2.12 ^f	36.80 \pm 2.94 ^e	36.61 \pm 2.93 ^a	19.37 \pm 1.55 ^a	55.98 \pm 4.48 ^a	40.23 \pm 3.22 ^{ab}
C1	Control (with Cd 25 ppm)	40.20 \pm 4.82 ^{de}	37.08 \pm 4.45 ^d	35.00 \pm 4.20 ^{ef}	58.54 \pm 7.02 ^d	6.99 \pm 0.84 ^{de}	5.76 \pm 0.69 ^{cde}	12.75 \pm 1.53 ^{de}	29.00 \pm 3.48 ^{cde}
C2	Control (with Cd 50 ppm)	43.54 \pm 3.48 ^{de}	40.86 \pm 3.27 ^d	39.00 \pm 3.12 ^{de}	67.74 \pm 5.42 ^{cd}	6.62 \pm 0.53 ^{de}	5.32 \pm 0.43 ^{de}	11.94 \pm 0.96 ^{de}	21.28 \pm 1.70 ^{ef}
C3	Control (with Cd 100 ppm)	48.98 \pm 4.41 ^{cd}	54.00 \pm 4.86 ^c	44.32 \pm 3.99 ^{bcd}	69.12 \pm 6.22 ^{bcd}	4.58 \pm 0.41 ^e	3.91 \pm 0.35 ^e	8.49 \pm 0.76 ^e	15.63 \pm 1.41 ^f
T1	Cd 25 ppm + Mo 0.5 ppm (seed soaking)	48.00 \pm 3.84 ^{cd}	39.00 \pm 3.12 ^d	44.97 \pm 3.60 ^{bcd}	62.00 \pm 4.96 ^d	7.89 \pm 0.63 ^d	6.00 \pm 0.48 ^{cde}	13.89 \pm 1.11 ^d	28.45 \pm 2.28 ^{cde}
T2	Cd 25 ppm + Mo 1.0 ppm (seed soaking)	52.00 \pm 5.72 ^{bcd}	48.00 \pm 5.28 ^{cd}	52.00 \pm 4.40 ^{ab}	68.23 \pm 7.51 ^{bcd}	8.18 \pm 0.90 ^d	7.06 \pm 0.78 ^{cd}	15.24 \pm 1.68 ^d	30.20 \pm 3.32 ^{cd}
T3	Cd 25 ppm + Mo 2 ppm (seed soaking)	64.07 \pm 5.12 ^{ab}	49.23 \pm 3.94 ^{cd}	55.00 \pm 4.40 ^{ab}	76.35 \pm 6.11 ^{abcd}	8.52 \pm 0.68 ^{cd}	7.19 \pm 0.57 ^{cd}	15.71 \pm 1.26 ^d	34.20 \pm 2.74 ^{bc}
T4	Cd 25 ppm + Mo 0.5 ppm (soil addition)	54.00 \pm 4.32 ^{abcd}	45.23 \pm 3.62 ^{cd}	38.41 \pm 3.07 ^{def}	62.33 \pm 4.99 ^d	7.12 \pm 0.57 ^{de}	6.08 \pm 0.49 ^{cd}	13.20 \pm 1.06 ^{de}	24.30 \pm 1.94 ^{de}
T5	Cd 25 ppm + Mo 1.0 ppm (soil addition)	65.00 \pm 7.80 ^{ab}	43.00 \pm 5.16 ^{cd}	40.71 \pm 4.89 ^{cde}	63.71 \pm 7.65 ^{cd}	7.45 \pm 0.67 ^{de}	6.51 \pm 0.59 ^{cd}	13.96 \pm 1.26 ^d	26.03 \pm 2.34 ^{de}
T6	Cd 25 ppm + Mo 2 ppm (soil addition)	67.00 \pm 6.03 ^a	40.21 \pm 3.22 ^d	34.73 \pm 2.78 ^{ef}	61.41 \pm 4.91 ^d	7.38 \pm 0.59 ^{de}	6.15 \pm 0.49 ^{cd}	13.53 \pm 1.08 ^{de}	24.60 \pm 1.97 ^{de}
T7	Cd 25 ppm + Mo 0.5 ppm (foliar spray)	47.00 \pm 3.76 ^{cd}	55.00 \pm 4.40 ^{bc}	48.24 \pm 3.86 ^{abcd}	81.88 \pm 6.55 ^{abc}	8.97 \pm 0.72 ^{cd}	7.56 \pm 0.60 ^c	16.53 \pm 1.32 ^{cd}	36.25 \pm 2.90 ^{abc}
T8	Cd 25 ppm + Mo 1.0 ppm (foliar spray)	60.00 \pm 6.00 ^{abc}	67.00 \pm 5.36 ^{ab}	59.92 \pm 4.79 ^a	91.77 \pm 7.34 ^a	12.15 \pm 0.97 ^b	10.37 \pm 0.83 ^b	22.52 \pm 1.80 ^b	41.48 \pm 3.32 ^{ab}
T9	Cd 25 ppm + Mo 2.0 ppm (foliar spray)	67.70 \pm 5.20 ^{ab}	67.98 \pm 5.44 ^a	57.00 \pm 4.56 ^a	86.23 \pm 6.90 ^{ab}	11.45 \pm 0.92 ^{bc}	10.00 \pm 0.80 ^b	21.45 \pm 1.72 ^{bc}	44.00 \pm 3.52 ^a

Table 6 Role of different Mo treatments on free proline, total phenolic compounds, and photosynthetic pigments in *R. communis* plant grown in 50 ppm Cd-contaminated soil. *SD* denotes standard deviation, and the *different letters in superscript* present significant difference among the values within a column

Treatments	Free proline ($\mu\text{g/g}$)		Total phenolics ($\mu\text{g/g}$)		Chlorophyll contents ($\mu\text{g/g}$)			Carotenoids ($\mu\text{g/g}$)	
	Leaves		Roots		Leaves		a + b		
	Roots	Leaves	Roots	Leaves	a	b			
C2	Control (with Cd 50 ppm)	43.54 \pm 4.79 ^{ab}	40.86 \pm 4.49 ^c	39.00 \pm 4.29 ^b	67.74 \pm 7.45 ^d	6.62 \pm 0.53 ^b	5.32 \pm 0.43 ^d	11.94 \pm 0.96 ^b	21.28 \pm 1.70 ^d
T10	Cd 50 ppm + Mo 0.5 ppm (seed soaking)	45.23 \pm 4.07 ^{ab}	48.65 \pm 4.38 ^{de}	41.23 \pm 3.71 ^b	84.15 \pm 7.57 ^{cd}	7.08 \pm 0.64 ^{ab}	5.68 \pm 0.51 ^{bcd}	12.76 \pm 1.15 ^{ab}	23.98 \pm 1.92 ^{cd}
T11	Cd 50 ppm + Mo 1.0 ppm (seed soaking)	54.32 \pm 5.98 ^{ab}	59.87 \pm 6.59 ^{abcd}	46.54 \pm 5.12 ^{ab}	92.61 \pm 10.19 ^{abcd}	8.00 \pm 0.64 ^{ab}	6.88 \pm 0.55 ^{abc}	14.88 \pm 1.19 ^{ab}	28.02 \pm 2.24 ^{bc}
T12	Cd 50 ppm + Mo 2.0 ppm (seed soaking)	57.43 \pm 4.59 ^a	61.41 \pm 4.91 ^{abcd}	51.01 \pm 4.08 ^{ab}	112.00 \pm 8.96 ^{abc}	8.42 \pm 0.67 ^a	7.33 \pm 0.59 ^a	15.75 \pm 1.26 ^a	33.10 \pm 2.65 ^{ab}
T13	Cd 50 ppm + Mo 0.5 ppm (soil addition)	55.00 \pm 6.05 ^{ab}	56.42 \pm 6.21 ^{bcd}	50.12 \pm 5.51 ^{ab}	92.00 \pm 10.12 ^{abcd}	6.39 \pm 0.51 ^b	5.49 \pm 0.44 ^{bcd}	11.88 \pm 0.95 ^b	22.05 \pm 1.54 ^d
T14	Cd 50 ppm + Mo 1.0 ppm (soil addition)	52.00 \pm 4.16 ^{ab}	53.63 \pm 4.29 ^{bcd}	44.54 \pm 3.56 ^{ab}	88.23 \pm 7.06 ^{bcd}	6.69 \pm 0.53 ^{ab}	5.75 \pm 0.46 ^{bcd}	12.43 \pm 0.99 ^b	22.35 \pm 1.34 ^{cd}
T15	Cd 50 ppm + Mo 2.0 ppm (soil addition)	47.00 \pm 5.17 ^{ab}	52.00 \pm 5.72 ^{cde}	41.24 \pm 4.54 ^b	83.35 \pm 9.17 ^{cd}	7.36 \pm 0.59 ^{ab}	6.40 \pm 0.51 ^{abcd}	13.76 \pm 1.10 ^{ab}	24.00 \pm 1.92 ^{cd}
T16	Cd 50 ppm + Mo 0.5 ppm (foliar spray)	42.17 \pm 3.37 ^b	68.60 \pm 5.49 ^{abc}	45.38 \pm 3.63 ^{ab}	111.14 \pm 8.89 ^{abc}	7.49 \pm 0.60 ^{ab}	7.00 \pm 0.56 ^{ab}	14.49 \pm 1.16 ^{ab}	35.00 \pm 2.45 ^a
T17	Cd 50 ppm + Mo 1.0 ppm (foliar spray)	46.23 \pm 5.09 ^{ab}	70.00 \pm 7.70 ^{ab}	49.35 \pm 5.43 ^{ab}	116.00 \pm 12.76 ^{ab}	7.29 \pm 0.58 ^{ab}	6.42 \pm 0.51 ^{abcd}	13.71 \pm 1.10 ^{ab}	28.00 \pm 2.24 ^{bc}
T18	Cd 50 ppm + Mo 2.0 ppm (foliar spray)	49.23 \pm 5.91 ^{ab}	75.00 \pm 9.00 ^a	55.68 \pm 6.68 ^a	120.00 \pm 14.40 ^a	6.73 \pm 0.81 ^{ab}	5.45 \pm 0.65 ^{cd}	12.18 \pm 1.46 ^b	24.32 \pm 1.70 ^{cd}

were homogenized in 10 ml of 80 % acetone, centrifuged at 10000 rpm for 15 min. 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:

$$\begin{aligned} \text{Chlorophyll a} &= 12.25 A_{663.2} - 2.79 A_{646.8} \\ \text{Chlorophyll b} &= 21.5 A_{646.8} - 5.1 A_{663.2} \\ \text{Carotenoid content} &= A_{480} \times \text{volume of extract} \times 10 \times 100 / 2500 \times \text{weight of plant material (g)} \end{aligned}$$

Cadmium analysis in different plant parts

Powdered dry samples were subjected to acid digestion using the method of Allen (1974). 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.

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 honest significant difference (HSD) test.

Results

Length, biomass and water contents of *R. communis* plant

Plant length, biomass and water content in different parts of *R. communis* under various treatments of molybdenum and Cd are shown in Tables 2, 3 and 4. In Table 2 and Fig. 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 *R. communis* plant as compared to C1 (Table 2). It was found that 2 ppm Mo foliar treatment most significantly increased dry biomass (DBM) of the plant (Table 2).

Table 3 shows the effect of Mo treatments on growth parameter of *R. 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 3 and Fig. 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 4. 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 4 and Fig. 1). Biomass (fresh and dry) in all parts of the plant was highly increased by the 2 ppm Mo foliar treatment (T27).

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 *R. communis* plant under various treatments of Mo- and in Cd-contaminated soil are given in Tables 5, 6 and 7. In Table 5, 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 *R. 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 5. 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 5). Photosynthetic pigments were significantly increased by the treatments T8 and T9 as compared to C1.

Table 6 presents the effect of Mo treatments on the concentration of free proline, total phenolics, chlorophyll and carotenoids in *R. 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

Table 7 Effect of Mo treatments on free proline, total phenolics and photosynthetic pigments in *R. communis* plant grown in 100 ppm Cd-contaminated soil. SD denotes standard deviation, and the different letters in superscript present significant difference among the values within a column

Treatments	Free proline ($\mu\text{g/g}$)		Total phenolics ($\mu\text{g/g}$)		Chlorophyll contents ($\mu\text{g/g}$)			Carotenoids ($\mu\text{g/g}$)
	Roots	Leaves	Roots	Leaves	a	b	a + b	
C3	35.00 \pm 3.85 c	30.25 \pm 3.33 d	33.25 \pm 3.66 c	67.74 \pm 7.45 cd	27.30 \pm 2.18 abc	20.22 \pm 1.62 ab	47.52 \pm 3.80 abc	42.20 \pm 3.38 d
T19	54.94 \pm 4.94 abc	34.16 \pm 3.07 cd	57.00 \pm 5.13 ab	85.00 \pm 7.65 abcd	28.46 \pm 2.56 ab	23.22 \pm 2.09 a	51.68 \pm 4.65 ab	45.12 \pm 3.61 cd
T20	71.25 \pm 7.84 a	49.43 \pm 5.44 ab	63.00 \pm 6.93 ab	91.05 \pm 10.02 ab	25.37 \pm 2.03 bc	21.20 \pm 1.70 ab	46.57 \pm 3.73 abc	47.02 \pm 3.76 cd
T21	67.00 \pm 5.36 ab	36.24 \pm 2.90 cd	61.25 \pm 4.90 ab	75.00 \pm 6.00 bcd	20.84 \pm 1.67 c	16.82 \pm 1.35 b	37.66 \pm 3.01 c	50.23 \pm 4.02 cd
T22	47.49 \pm 5.22 bc	43.59 \pm 4.79 abc	47.57 \pm 5.23 bc	70.35 \pm 7.74 bcd	24.75 \pm 1.98 bc	18.06 \pm 1.44 ab	42.81 \pm 3.42 bc	46.31 \pm 3.24 cd
T23	57.08 \pm 4.57 ab	44.53 \pm 3.56 abc	51.73 \pm 4.14 ab	73.00 \pm 5.84 bcd	30.11 \pm 2.41 ab	21.17 \pm 1.69 ab	51.28 \pm 4.10 ab	53.02 \pm 3.18 bcd
T24	67.00 \pm 7.37 ab	39.97 \pm 4.40 bcd	65.00 \pm 7.15 a	64.48 \pm 7.09 d	30.88 \pm 2.47 ab	21.71 \pm 1.74 ab	52.59 \pm 4.21 ab	67.42 \pm 5.39 bc
T25	51.22 \pm 4.10 abc	45.14 \pm 3.61 abc	57.00 \pm 4.56 ab	101.85 \pm 8.15 a	28.09 \pm 2.25 abc	19.88 \pm 1.59 ab	47.97 \pm 3.84 abc	58.03 \pm 4.06 b
T26	57.99 \pm 6.38 ab	51.24 \pm 5.64 a	64.00 \pm 7.04 a	96.36 \pm 10.60 a	31.71 \pm 2.54 a	22.30 \pm 1.78 a	54.01 \pm 4.32 a	81.89 \pm 6.55 a
T27	69.00 \pm 8.28 ab	40.56 \pm 4.87 abc	66.00 \pm 7.92 a	85.00 \pm 10.20 abc	33.52 \pm 4.02 a	23.57 \pm 2.83 a	57.09 \pm 6.85 a	88.61 \pm 6.20 a

(respectively) in roots as compared to C2. Leaves demonstrated the highest concentration of proline and phenolics with the treatment T18 (Table 6). 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 carotenoid concentrations in *R. communis* plant grown in 100 ppm Cd-contaminated soil is given in Table 7. A highly significant increase in the 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 7). 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 Fig. 2. 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.

Cadmium concentration and bioaccumulation in *R. communis*

Variation in concentration, accumulation, translocation and bioconcentration of Cd in different parts of *R. communis* plant is given in Tables 8, 9 and 10. Table 8 demonstrates the effect of different concentrations 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 8 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. The 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 8. 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 8. 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 *R. communis* is presented

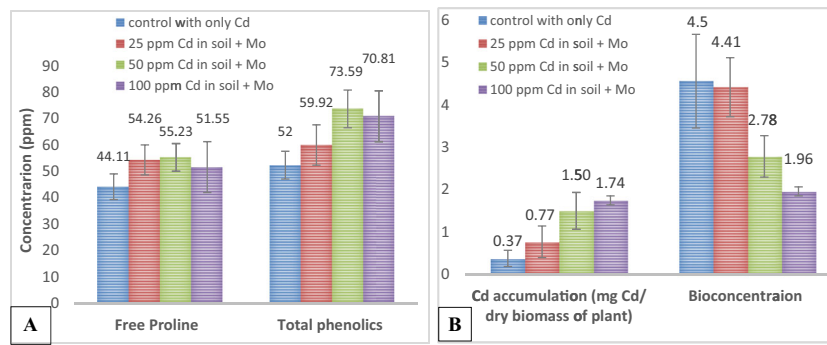


Fig. 2 Overall effect of the molybdenum treatments on concentration of total phenolic and free proline (a) and Cd accumulation and bioconcentration (b) in *R. communis* plant grown in soil containing different concentrations of Cd (25, 50 and 100 ppm)

in Table 9. 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 9. 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 10. The 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 10). 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 10.

Figure 2 presents the overall effect of Mo treatments on Cd accumulation and bioconcentration in *R. communis* plant under varied Cd concentrations 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.

Correlation among different parameters

Tables 11, 12, 13, 14, 15, 16, 17, 18 and 19 present correlations among different parameters in roots, stem and leaves of *R. 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). The total phenolic concentration showed significantly positive correlation with Cd accumulation in plant roots (Tables 11, 12 and 13) and leaves (Tables 17, 18 and 19).

Proline concentrations in roots (Tables 11 and 12) and leaves (Tables 17 and 18) 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 11, 12 and 13). 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 Tables 17, 18 and 19. It was found that dry biomass in roots, stem and leaves demonstrated significantly positive correlation with Cd accumulation (Tables 11, 12, 13, 14, 15, 16, 17, 18 and 19).

Discussion

The effect of molybdenum on phytoextraction potential of *R. 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 the presence of toxic heavy metals in soil significantly reduces growth and biomass of plants (Hadi et al. 2010; John et al. 2009; Hadi and Bano 2009) and in the present research, *R. 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 (John et al. 2009; Gouia et al. 2000). Reduction in biomass under Cd stress has been reported in many plants, such as *Cannabis sativa* (Ahmad et al. 2015), *Parthenium hysterophorus* (Hadi et al. 2014), *Lycopersicon esculentum* (Haouari et al. 2012), *Glycine max* (Sheirdil et al. 2012), *Pisum sativum* (Bavi et al. 2011), *Amaranthus tricolor* (Varalakshmi and Ganeshamurthy 2009), *Brassica juncea* (John et al. 2009) and *Hordeum vulgare* (Kaznina et al. 2006). Our results

Table 8 Role of different treatments of Mo on Cd contents in *R. communis* plant grown in 25 ppm Cd-contaminated soil. *R-S* denotes 'roots into stem,' *R-L* denotes 'roots into leaves,' *SD* represents standard deviation, while *different letters in superscript* represent significant difference among the values in a column

Treatments	Cd concentration (ppm) ± SD						Cd accumulation (mg/DBM) ± SD					
	Roots		Stem		Leaves		Roots		Stem		Leaves	
	Roots	Stem	Roots	Stem	Leaves	Roots	Stem	Roots	Stem	Leaves		
C1 Control (with Cd 25 ppm)	137.00 ± 16.44 ^b	58.07 ± 6.38 ^c	78.00 ± 10.14 ^b	0.11 ± 0.02 ^e	0.10 ± 0.02 ^d	0.15 ± 0.03 ^c						
C2 Control (with Cd 50 ppm)	219.05 ± 26.28 ^a	79.00 ± 8.69 ^b	87.00 ± 11.31 ^b	0.14 ± 0.03 ^{de}	0.09 ± 0.02 ^d	0.14 ± 0.05 ^c						
C3 Control (with Cd 100 ppm)	268.70 ± 32.16 ^a	114.00 ± 12.54 ^a	125.00 ± 16.25 ^a	0.15 ± 0.05 ^{de}	0.12 ± 0.02 ^d	0.13 ± 0.01 ^c						
T1 Cd 25 ppm + Mo 0.5 ppm (seed soaking)	195.60 ± 23.40 ^{ab}	65.23 ± 7.18 ^b	85.23 ± 8.52 ^b	0.25 ± 0.02 ^{bcd}	0.14 ± 0.03 ^{cd}	0.23 ± 0.04 ^{abc}						
T2 Cd 25 ppm + Mo 1.0 ppm (seed soaking)	204.0 ± 24.48 ^{ab}	69.00 ± 7.59 ^b	89.28 ± 8.92 ^b	0.31 ± 0.06 ^{bcd}	0.19 ± 0.04 ^{abcd}	0.31 ± 0.06 ^{ab}						
T3 Cd 25 ppm + Mo 2 ppm (seed soaking)	214.54 ± 25.68 ^{ab}	81.10 ± 9.03 ^b	98.87 ± 9.89 ^{ab}	0.35 ± 0.07 ^{abc}	0.23 ± 0.04 ^{abc}	0.32 ± 0.05 ^{ab}						
T4 Cd 25 ppm + Mo 0.5 ppm (soil addition)	208.68 ± 24.96 ^{ab}	70.00 ± 7.70 ^b	91.00 ± 8.19 ^b	0.21 ± 0.04 ^{cde}	0.13 ± 0.03 ^{cd}	0.20 ± 0.03 ^{bc}						
T5 Cd 25 ppm + Mo 1.0 ppm (soil addition)	200.35 ± 24.00 ^{ab}	67.00 ± 7.37 ^b	84.56 ± 7.61 ^b	0.25 ± 0.05 ^{bcd}	0.14 ± 0.03 ^{bcd}	0.21 ± 0.02 ^{bc}						
T6 Cd 25 ppm + Mo 2 ppm (soil addition)	196.63 ± 23.52 ^{ab}	69.42 ± 7.64 ^b	83.24 ± 7.49 ^b	0.29 ± 0.06 ^{bcd}	0.14 ± 0.03 ^{cd}	0.24 ± 0.04 ^{abc}						
T7 Cd 25 ppm + Mo 0.5 ppm (foliar spray)	245.00 ± 29.40 ^a	67.89 ± 7.47 ^b	82.43 ± 10.03 ^b	0.32 ± 0.08 ^{abcd}	0.17 ± 0.03 ^{abcd}	0.25 ± 0.05 ^{abc}						
T8 Cd 25 ppm + Mo 1.0 ppm (foliar spray)	267.86 ± 32.04 ^a	70.45 ± 7.75 ^b	100.20 ± 12.22 ^{ab}	0.39 ± 0.08 ^{ab}	0.24 ± 0.05 ^a	0.34 ± 0.07 ^{ab}						
T9 Cd 25 ppm + Mo 2.0 ppm (foliar spray)	256.00 ± 30.72 ^a	82.23 ± 8.94 ^b	102.12 ± 12.46 ^a	0.49 ± 0.11 ^a	0.24 ± 0.05 ^{ab}	0.35 ± 0.08 ^a						
Cd accumulation (mg/DBM) ± SD		Percentage of Cd accumulation		Cd translocation ± SD		Cd bioconcentration ± SD						
Entire plant	Roots	Stem	Leaves	R-S	R-L							
0.35 ± 0.07 ^e	30.80	27.19	42.01	0.42 ± 0.05 ^a	0.57 ± 0.01 ^a	3.25 ± 0.41 ^c						
0.38 ± 0.08 ^e	38.41	25.07	36.52	0.36 ± 0.03 ^c	0.40 ± 0.03 ^e	2.19 ± 0.25 ^c						
0.40 ± 0.08 ^{de}	37.36	29.45	33.19	0.43 ± 0.02 ^a	0.47 ± 0.02 ^b	1.51 ± 0.11 ^d						
0.62 ± 0.12 ^{bcd}	39.80	22.52	37.69	0.35 ± 0.04 ^d	0.44 ± 0.01 ^d	4.04 ± 0.48 ^{bc}						
0.81 ^{abcd}	38.42	23.16	38.42	0.34 ± 0.05 ^d	0.44 ± 0.01 ^d	4.19 ± 0.47 ^{bc}						
0.16 ^{abcd}												
0.90	39.02	25.31	35.67	0.38 ± 0.03 ^b	0.46 ± 0.01 ^{bc}	4.70 ± 0.52 ^{abc}						
0.17 ^{abc}												
0.53	38.24	25.11	36.64	0.34 ± 0.02 ^d	0.44 ± 0.03 ^{cd}	4.23 ± 0.47 ^{bc}						
0.10 ^{cde}												
0.60	41.62	24.16	34.22	0.33 ± 0.03 ^d	0.42 ± 0.02 ^d	4.11 ± 0.42 ^{bc}						
0.11 ^{bcd}												
0.67	43.32	20.69	36.00	0.35 ± 0.06 ^c	0.43 ± 0.01 ^d	4.21 ± 0.45 ^{bc}						
0.12 ^{abcde}												
0.73	43.48	22.80	33.72	0.28 ± 0.04 ^f	0.34 ± 0.02 ^f	4.33 ± 0.51 ^{bc}						
0.15 ^{abcde}												
0.97	40.19	25.05	34.76	0.26 ± 0.03 ^g	0.38 ± 0.03 ^e	4.69 ± 0.57 ^{abc}						
0.19 ^{ab}												
1.08	45.42	22.24	32.34	0.32 ± 0.02 ^e	0.40 ± 0.02 ^e	5.21 ± 0.62 ^{ab}						
0.24 ^a												

Table 9 Effect of Mo treatments on Cd contents in *R. communis* plant grown in 50 ppm Cd-contaminated soil. *R-S* denotes roots into stem, *R-L* denotes roots into leaves, *SD* represents standard deviation, while *different letters in superscript* represent significant difference among the values in a column

Treatments	Cd concentration (ppm) ± SD				Cd accumulation (mg/DBM) ± SD			
	Roots	Stem	Leaves		Roots	Stem	Leaves	
C2	219.07 ± 19.71 ^b	79.00 ± 9.48 ^b	87.00 ± 11.31 ^d		0.14 ± 0.03 ^b	0.09 ± 0.02 ^b	0.14 ± 0.03 ^b	
T10	228.47 ± 20.52 ^{ab}	88.35 ± 10.60 ^b	137.07 ± 17.81 ^{abc}		0.26 ± 0.05 ^{ab}	0.17 ± 0.04 ^{ab}	0.29 ± 0.06 ^{ab}	
T11	232.00 ± 20.88 ^{ab}	89.23 ± 10.71 ^{ab}	125.25 ± 16.25 ^{abcd}		0.29 ± 0.06 ^{ab}	0.23 ± 0.05 ^{ab}	0.36 ± 0.09 ^a	
T12	238.75 ± 21.42 ^{ab}	95.00 ± 11.40 ^{ab}	98.45 ± 12.74 ^{bcd}		0.33 ± 0.08 ^a	0.22 ± 0.06 ^{ab}	0.31 ± 0.08 ^{ab}	
T13	289.05 ± 26.01 ^a	121.00 ± 14.52 ^a	142.35 ± 18.46 ^{ab}		0.25 ± 0.05 ^{ab}	0.15 ± 0.03 ^{ab}	0.24 ± 0.06 ^{ab}	
T14	246.63 ± 22.14 ^{ab}	86.92 ± 10.43 ^b	128.12 ± 16.64 ^{abcd}		0.27 ± 0.06 ^{ab}	0.17 ± 0.04 ^{ab}	0.26 ± 0.07 ^{ab}	
T15	229.08 ± 20.61 ^{ab}	83.25 ± 9.99 ^b	92.20 ± 11.99 ^{cd}		0.28 ± 0.06 ^{ab}	0.18 ± 0.04 ^{ab}	0.29 ± 0.07 ^{ab}	
T16	248.12 ± 22.32 ^{ab}	79.23 ± 9.51 ^b	112.00 ± 14.56 ^{bcd}		0.36 ± 0.08 ^a	0.19 ± 0.05 ^{ab}	0.30 ± 0.08 ^{ab}	
T17	257.58 ± 23.13 ^{ab}	85.00 ± 10.20 ^b	123.98 ± 16.12 ^{abcd}		0.34 ± 0.08 ^a	0.21 ± 0.06 ^{ab}	0.31 ± 0.09 ^{ab}	
T18	287.21 ± 25.83 ^a	108.00 ± 12.96 ^{ab}	168.00 ± 21.84 ^a		0.32 ± 0.07 ^{ab}	0.27 ± 0.07 ^a	0.36 ± 0.08 ^a	

Cd accumulation (mg/DBM) ± SD	% Cd accumulation		Cd translocation ± SD		Cd bioconcentration ± SD
	Roots	Stem	R-S	R-L	
0.38 ± 0.09 ^b	38.46	25.04	0.36 ± 0.05 ^{cde}	0.40 ± 0.02 ^e	2.19 ± 0.25 ^b
0.72 ± 0.15 ^{ab}	35.91	23.44	0.39 ± 0.03 ^{abc}	0.60 ± 0.03 ^a	2.78 ± 0.31 ^{ab}
0.88 ± 0.20 ^{ab}	33.13	25.83	0.38 ± 0.04 ^{bcd}	0.54 ± 0.04 ^{bc}	2.63 ± 0.30 ^{ab}
0.87 ± 0.22 ^{ab}	38.41	25.72	0.40 ± 0.06 ^{ab}	0.41 ± 0.05 ^e	2.50 ± 0.28 ^{ab}
0.64 ± 0.15 ^{ab}	39.56	22.79	0.42 ± 0.03 ^a	0.49 ± 0.02 ^{cd}	3.39 ± 0.38 ^a
0.71 ± 0.17 ^{ab}	39.02	23.83	0.35 ± 0.02 ^{de}	0.52 ± 0.02 ^c	2.77 ± 0.31 ^{ab}
0.75 ± 0.17 ^{ab}	37.62	23.45	0.36 ± 0.05 ^{cd}	0.40 ± 0.03 ^e	2.30 ± 0.26 ^b
0.84 ± 0.21 ^{ab}	42.93	20.94	0.32 ± 0.06 ^f	0.45 ± 0.04 ^{de}	2.63 ± 0.29 ^{ab}
0.86 ± 0.23 ^{ab}	39.63	24.63	0.33 ± 0.03 ^{ef}	0.48 ± 0.03 ^{cd}	2.73 ± 0.30 ^{ab}
0.93 ± 0.22 ^a	34.81	29.29	0.38 ± 0.01 ^{bcd}	0.58 ± 0.02 ^{ab}	3.30 ± 0.37 ^a

Table 10 Effect of Mo treatments on Cd contents in *R. communis* plant grown in 100 ppm Cd-contaminated soil. R-S denotes roots into stem, R-L denotes roots into leaves, SD represents standard deviation, while different letters in *superscript* represent significant difference among the values in a column

Treatments	Cd concentration (ppm) ± SD				Cd accumulation (mg/DBM) ± SD			
	Roots	Stem	Leaves	Stem	Roots	Stem	Leaves	Stem
C3	268.70 ± 32.16 ^c	114.00 ± 12.54 ^c	125.00 ± 16.25 ^e	114.00 ± 12.54 ^c	0.15 ± 0.05 ^b	0.12 ± 0.02 ^c	0.13 ± 0.01 ^b	0.12 ± 0.02 ^c
T19	331.05 ± 19.86 ^a	142.90 ± 10.00 ^{ab}	190.75 ± 16.21 ^{bcd}	142.90 ± 10.00 ^{ab}	0.27 ± 0.04 ^{ab}	0.23 ± 0.04 ^{abc}	0.34 ± 0.06 ^{ab}	0.23 ± 0.04 ^{abc}
T20	302.45 ± 18.12 ^{abc}	144.33 ± 10.10 ^{ab}	172.89 ± 14.70 ^{bcd}	144.33 ± 10.10 ^{ab}	0.30 ± 0.05 ^{ab}	0.31 ± 0.06 ^{ab}	0.44 ± 0.09 ^a	0.30 ± 0.06 ^{ab}
T21	274.51 ± 16.44 ^c	153.66 ± 10.76 ^{ab}	167.86 ± 14.27 ^{cde}	153.66 ± 10.76 ^{ab}	0.36 ± 0.07 ^a	0.30 ± 0.06 ^{ab}	0.47 ± 0.10 ^a	0.30 ± 0.06 ^{ab}
T22	326.36 ± 19.56 ^{ab}	164.98 ± 11.55 ^a	215.63 ± 18.33 ^{ab}	164.98 ± 11.55 ^a	0.24 ± 0.04 ^{ab}	0.18 ± 0.03 ^{bc}	0.29 ± 0.06 ^{ab}	0.18 ± 0.03 ^{bc}
T23	295.28 ± 17.70 ^{abc}	152.50 ± 10.68 ^{ab}	210.29 ± 17.87 ^{abc}	152.50 ± 10.68 ^{ab}	0.27 ± 0.05 ^{ab}	0.25 ± 0.05 ^{abc}	0.36 ± 0.08 ^{ab}	0.25 ± 0.05 ^{abc}
T24	287.58 ± 17.25 ^{abc}	134.65 ± 9.43 ^{bc}	152.93 ± 13.00 ^{de}	134.65 ± 9.43 ^{bc}	0.32 ± 0.06 ^a	0.24 ± 0.04 ^{abc}	0.41 ± 0.08 ^a	0.24 ± 0.04 ^{abc}
T25	275.00 ± 16.50 ^c	128.15 ± 8.97 ^{bc}	185.77 ± 15.79 ^{bcd}	128.15 ± 8.97 ^{bc}	0.33 ± 0.07 ^a	0.23 ± 0.05 ^{abc}	0.42 ± 0.10 ^a	0.23 ± 0.05 ^{abc}
T26	279.72 ± 16.74 ^{bc}	133.26 ± 9.33 ^{bc}	205.64 ± 17.48 ^{abc}	133.26 ± 9.33 ^{bc}	0.31 ± 0.07 ^{ab}	0.27 ± 0.06 ^{ab}	0.41 ± 0.10 ^a	0.31 ± 0.07 ^{ab}
T27	290.43 ± 17.40 ^{abc}	162.81 ± 10.42 ^a	242.17 ± 20.58 ^a	162.81 ± 10.42 ^a	0.32 ± 0.05 ^a	0.37 ± 0.06 ^a	0.51 ± 0.07 ^a	0.32 ± 0.05 ^a

Cd accumulation (mg/DBM) ± SD	Cd translocation ± SD			Cd bioconcentration ± SD		
	Roots	Stem	Leaves	R-S	R-L	R-L
0.40 ± 0.08 ^b	37.36	29.45	33.19	0.43 ± 0.02 ^d	0.47 ± 0.02 ^g	1.51 ± 0.11 ^c
0.83 ± 0.14 ^{ab}	31.96	27.29	40.75	0.43 ± 0.02 ^d	0.58 ± 0.02 ^{ef}	2.00 ± 0.15 ^{ab}
1.05 ± 0.20 ^a	28.92	29.09	41.99	0.48 ± 0.01 ^c	0.57 ± 0.01 ^{ef}	1.85 ± 0.14 ^{abc}
1.12 ± 0.24 ^a	31.94	26.71	41.35	0.56 ± 0.03 ^a	0.61 ± 0.02 ^e	1.86 ± 0.14 ^{abc}
0.71 ± 0.14 ^{ab}	33.65	25.99	40.36	0.51 ± 0.01 ^b	0.66 ± 0.04 ^d	2.23 ± 0.16 ^a
0.88 ± 0.18 ^{ab}	31.23	27.95	40.81	0.52 ± 0.02 ^b	0.71 ± 0.02 ^{bc}	2.07 ± 0.15 ^{ab}
0.94 ± 0.11 ^a	33.12	24.50	42.38	0.47 ± 0.01 ^c	0.53 ± 0.03 ^f	1.74 ± 0.13 ^{bc}
0.98 ± 0.22 ^a	33.63	23.96	42.41	0.47 ± 0.03 ^c	0.67 ± 0.02 ^{cd}	1.86 ± 0.14 ^{abc}
0.99 ± 0.23 ^a	30.87	27.75	41.38	0.48 ± 0.03 ^c	0.74 ± 0.04 ^b	1.92 ± 0.14 ^{ab}
1.20 ± 0.18 ^a	26.67	30.08	42.50	0.51 ± 0.02 ^b	0.83 ± 0.03 ^a	2.25 ± 0.15 ^a

Table 11 Correlations among different parameters in roots of *R. communis* plant grown in 25 ppm Cd-contaminated soil

	Pearson correlation	Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation	Free proline	Total phenolics
Length (cm)	1	0.758 ^{***}	0.002	0.731 ^{***}	0.699 ^{**}	-0.117	0.646 [*]	0.340	0.596 [*]
Fresh biomass (g)	Pearson correlation Sig. (1-tailed)	0.758 ^{***} 1	0.002	0.003	0.006	0.358	0.012	0.140	0.021
Dry biomass (g)	Pearson correlation Sig. (1-tailed)	0.002 0.731 ^{***}	0.896 ^{***} 0.896 ^{***}	0.000	0.964 ^{***}	-0.114	0.777 ^{***}	0.637 [*]	0.640 [*]
Total water content (g)	Pearson correlation Sig. (1-tailed)	0.731 ^{***} 0.003	0.896 ^{***} 0.000	1	0.744 ^{***}	0.056	0.939 ^{**}	0.013	0.013
Cd concentration (ppm)	Pearson correlation Sig. (1-tailed)	0.003 0.699 ^{***}	0.000 0.964 ^{***}	0.744 ^{***}	0.003	0.431	0.000	0.004	0.012
Cd accumulation (mg/DBM)	Pearson correlation Sig. (1-tailed)	0.699 ^{***} -0.117	0.964 ^{***} -0.114	0.003	1	-0.205	0.602 [*]	0.624 [*]	0.694 [*]
Free proline (ppm)	Pearson correlation Sig. (1-tailed)	0.006 0.358	0.000 0.362	0.056	-0.205	0.262	0.019	0.020	0.016
Total phenolics (ppm)	Pearson correlation Sig. (1-tailed)	-0.117 0.646 [*]	-0.114 0.777 ^{***}	0.431	0.602 [*]	1	0.377	0.668 ^{**}	0.793 ^{***}
	Pearson correlation Sig. (1-tailed)	0.646 [*] 0.012	0.777 ^{***} 0.001	0.939 ^{**}	0.602 [*]	0.377	0.113	0.009	0.001
	Pearson correlation Sig. (1-tailed)	0.012 0.340	0.001 0.637 [*]	0.000	0.019	0.113	0.668 ^{**}	0.009	0.001
	Pearson correlation Sig. (1-tailed)	0.340 0.140	0.637 [*] 0.013	0.721 ^{**}	0.624 [*]	0.093	1	1	0.342
	Pearson correlation Sig. (1-tailed)	0.140 0.596 [*]	0.013 0.640 [*]	0.004	0.020	0.387	0.009	0.342	0.138
	Pearson correlation Sig. (1-tailed)	0.596 [*] 0.021	0.640 [*] 0.013	0.644 [*]	0.574 [*]	0.619 [*]	0.793 ^{***}	0.138	1
	Pearson correlation Sig. (1-tailed)	0.021	0.013	0.012	0.026	0.016	0.001	0.138	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 12 Correlations among different parameters in roots of *R. communis* plant grown in 50 ppm Cd-contaminated soil

	Pearson correlation	Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation	Free proline	Total phenolics
Length	1	0.581*	0.695*	0.581*	0.661*	-0.064	0.508	0.124	0.271
Fresh biomass	Sig. (1-tailed)	0.013	1	0.039	0.019	0.430	0.067	0.367	0.224
Dry biomass	Pearson correlation	0.695*	0.013	0.717**	0.982**	-0.021	0.632*	0.610*	0.410
Total water content	Sig. (1-tailed)	0.013	0.013	0.010	0.000	0.477	0.025	0.030	0.119
Cd concentration	Pearson correlation	0.581*	0.717**	1	0.573*	-0.026	0.915**	0.094	0.322
Cd accumulation	Sig. (1-tailed)	0.039	0.010	0.042	0.042	0.472	0.000	0.398	0.182
Free proline	Pearson correlation	0.661*	0.982**	0.573*	1	-0.018	0.497	0.692*	0.396
Total phenolics	Sig. (1-tailed)	0.019	0.000	0.042	-0.018	0.480	0.072	0.013	0.129
	Pearson correlation	-0.064	-0.021	-0.026	0.480	1	0.373	0.289	0.809**
	Sig. (1-tailed)	0.430	0.477	0.472	0.480	0.497	0.144	0.694*	0.002
	Pearson correlation	0.508	0.632*	0.915**	0.497	0.373	1	0.013	0.807**
	Sig. (1-tailed)	0.067	0.025	0.000	0.072	0.144	0.694*	0.328	0.026
	Pearson correlation	0.124	0.610*	0.094	0.692*	0.289	0.013	1	0.522
	Sig. (1-tailed)	0.367	0.030	0.398	0.013	0.209	0.807**	0.522	0.061
	Pearson correlation	0.271	0.410	0.322	0.396	0.809**	0.026	0.061	1
	Sig. (1-tailed)	0.224	0.119	0.182	0.129	0.002	0.026	0.061	0.061

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 13 Correlations among different parameter in roots of *R. communis* plant grown in 100 ppm Cd-contaminated soil

	Pearson correlation	Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation	Free proline	Total phenolics
Length	1	1	0.799**	0.794**	0.756**	-0.354	0.751**	0.444	0.246
Fresh biomass	Sig. (1-tailed)	0.799**	1	0.824**	0.989**	-0.113	0.665*	0.634*	0.201
Dry biomass	Sig. (1-tailed)	0.003	0.824**	1	0.000	0.378	0.019	0.025	0.289
Total water content	Pearson correlation	0.794**	0.824**	0.733**	1	-0.375	0.763*	0.325	0.203
Cd concentration	Sig. (1-tailed)	0.003	0.002	0.008	0.008	0.143	0.012	0.180	0.287
Cd accumulation	Pearson correlation	0.756**	0.989**	0.733**	1	-0.040	0.792**	0.677*	0.189
Free proline	Sig. (1-tailed)	0.006	0.000	0.008	-0.040	0.457	0.003	0.016	0.300
Total phenolics	Pearson correlation	-0.354	-0.113	-0.375	1	1	-0.115	0.315	0.450
	Sig. (1-tailed)	0.158	0.378	0.143	0.457	0.376	0.376	0.188	0.096
	Pearson correlation	0.751**	0.665*	0.763*	0.792**	-0.115	1	0.673*	0.339
	Sig. (1-tailed)	0.006	0.019	0.012	0.003	0.376	0.021	0.021	0.739*
	Pearson correlation	0.444	0.634*	0.325	0.677*	0.315	0.673*	1	0.010
	Sig. (1-tailed)	0.099	0.025	0.180	0.016	0.188	0.021	0.346	0.164
	Pearson correlation	0.246	0.201	0.203	0.189	0.650*	0.739**	0.346	1
	Sig. (1-tailed)	0.356	0.289	0.287	0.300	0.016	0.010	0.164	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 14 Correlations among different parameters in stem of *R. communis* plant grown in 25 ppm Cd-contaminated soil

		Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation
Length (cm)	Pearson correlation	1	0.954**	0.920**	0.916**	-0.527*	0.753**
	Sig. (1-tailed)		0.000	0.000	0.000	0.039	0.002
Fresh biomass (g)	Pearson correlation	0.954**	1	0.957**	0.967**	-0.418	0.862**
	Sig. (1-tailed)	0.000		0.000	0.001	0.088	0.000
Dry biomass (g)	Pearson correlation	0.920**	0.957**	1	0.851**	-0.342	0.926**
	Sig. (1-tailed)	0.000	0.000		0.000	0.138	0.000
Total water content (g)	Pearson correlation	0.916**	0.967**	0.851**	1	-0.454	0.747**
	Sig. (1-tailed)	0.000	0.000	0.000		0.069	0.003
Cd concentration (ppm)	Pearson correlation	-0.527*	-0.418	-0.342	-0.454	1	0.018
	Sig. (1-tailed)	0.039	0.088	0.138	0.069		0.478
Cd accumulation (mg/DBM)	Pearson correlation	0.753**	0.862**	0.926**	0.747**	0.018	1
	Sig. (1-tailed)	0.002	0.001	0.001	0.003	0.478	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

showed that Mo treatments restored growth and biomass of *R. communis* plant under Cd stress. The significant effect of Mo on biomass might be due its role as a cofactor for enzymes involved in nitrate metabolism (such as nitrate reductase and glutamine synthetase) and synthesis of amino acids and indole acetic (Hristozkova et al. 2006; Hesberg et al. 2004; Williams and Frausto da Silva 2002; Mendel and Haensch 2002; Sagi et al. 2002) 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 bioavailability in acidic soils (Kaiser et al. 2005; Gupta 1997) 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 bioavailability of Mo in the form of foliar and seed soaking treatments as compared to the soil addition treatments.

Effect of 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 (Sun et al. 2007; Ahmad et al. 2015).

Table 15 Correlations among different parameters in stem of *R. communis* plant grown in 50 ppm Cd-contaminated soil

		Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation
Length (cm)	Pearson correlation	1	0.348	0.522	0.172	-0.215	0.442
	Sig. (1-tailed)		0.163	0.061	0.318	0.276	0.101
Fresh	Pearson correlation	0.348	1	0.912**	0.949**	-0.033	0.879**
	Sig. (1-tailed)	0.163		0.000	0.000	0.464	0.000
Dry biomass	Pearson correlation	0.522	0.912**	1	0.735**	-0.069	0.964**
	Sig. (1-tailed)	0.061	0.000		0.008	0.425	0.000
Total water content	Pearson correlation	0.172	0.949**	0.735**	1	0.000	0.708*
	Sig. (1-tailed)	0.318	0.000	0.008		0.499	0.011
Cd concentration	Pearson correlation	-0.215	-0.033	-0.069	0.000	1	0.187
	Sig. (1-tailed)	0.276	0.464	0.425	0.499		0.303
Cd accumulation	Pearson correlation	0.442	0.879**	0.964**	0.708*	0.187	1
	Sig. (1-tailed)	0.101	0.000	0.000	0.011	0.303	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 16 Correlations among different parameters in stem of *R. communis* plant grown in 100 ppm Cd-contaminated soil

		Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation
Length	Pearson correlation	1	0.541	0.773**	0.270	−0.361	0.686*
	Sig. (1-tailed)		0.053	0.004	0.225	0.153	0.014
Fresh biomass	Pearson correlation	0.541	1	0.829**	0.920**	−0.304	0.759**
	Sig. (1-tailed)	0.053		0.001	0.000	0.197	0.005
Dry biomass	Pearson correlation	0.773**	0.829**	1	0.543	−0.270	0.953**
	Sig. (1-tailed)	0.004	0.001		0.052	0.225	0.000
Total water content	Pearson correlation	0.270	0.920**	0.543	1	−0.266	0.471
	Sig. (1-tailed)	0.225	0.000	0.052		0.229	0.085
Cd concentration	Pearson correlation	−0.361	−0.304	−0.270	−0.266	1	0.027
	Sig. (1-tailed)	0.153	0.197	0.225	0.229		0.471
Cd accumulation (mg/DBM)	Pearson correlation	0.686*	0.759**	0.953**	0.471	0.027	1
	Sig. (1-tailed)	0.014	0.005	0.000	0.085	0.471	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

High concentrations of proline act as environmental stress indicator in many plants (Khatamipour et al. 2011). Several plants such as cannabis, sunflower, tomato, cowpea and wheat have been reported with high concentrations of free proline under heavy metal stress (Zengin and Munzuroglu 2006; Sagi et al. 2002; De and Mukherjee 1998; Bhattacharjee and Mukherjee 1994; Lalk and Dorfling 1985). 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 the 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 (Sakishima and Yamasaki 2002). Several investigators have reported an increase in concentration of total phenolics under Cd stresses in plant tissues (Ahmad et al. 2015; Michalak 2006; Uraguchi et al. 2006). Our results also showed increases in the concentration of total phenolics in roots and leaves of *R. 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. The high concentration of phenolic compounds under Cd stress in leaves as compared to roots of *Crotalaria juncea*, *P. hysterophorus* and *C. sativa* plants have also been reported by Uraguchi et al. (2006); Ali and Hadi (2015) and Ahmad et al. (2015), respectively.

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 (Hadi et al. 2014; Kaiser et al. 2005). 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 *R. communis* followed by leaves and stem respectively which is in agreement with the work of Citterio et al. (2003) and Linger et al. (2005) on *C. sativa* and Hadi et al. (2014) on *P. hysterophorus*. Increasing concentration of Cd in soil also increased Cd concentration in plant due to high bioavailability 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 accumulation percentage in leaves of the plant. Cd bioconcentration in the plant was recorded at a very high level, suggesting that *R. communis* can be considered as a hyperaccumulator of Cd. The Mo treatments further increased Cd bioconcentration in the plant.

Table 17 Correlations among different parameters in leaves of *R. communis* plant grown in 25 ppm Cd-contaminated soil

	Pearson correlation	Length	Fresh biomass	Dry biomass	Total water content	Cd concentration	Cd accumulation	Free proline	Total phenolics	Chlorophyll a	Chlorophyll b	Carotenoids
Length (cm)	1	0.932**	0.981**	-0.095	0.947**	0.532*	0.600*	0.870**	0.877**	0.875**	0.836**	
Fresh biomass (g)	Sig. (1-tailed)	0.000	0.000	0.385	0.000	0.037	0.020	0.000	0.000	0.000	0.000	
	Pearson correlation	0.932**	0.843**	-0.219	0.949**	0.433	0.531*	0.817**	0.810**	0.816**	0.823**	
Dry biomass (g)	Sig. (1-tailed)	0.000	0.000	0.247	0.000	0.080	0.038	0.001	0.001	0.001	0.001	
	Pearson correlation	0.981**	0.843**	-0.023	0.893**	0.556*	0.603*	0.849**	0.864**	0.858**	0.796**	
Total water content (g)	Sig. (1-tailed)	0.000	0.000	0.472	0.000	0.030	0.019	0.000	0.000	0.000	0.001	
	Pearson correlation	-0.095	-0.219	1	0.077	0.582*	0.393	-0.064	-0.013	-0.040	-0.103	
Cd concentration (ppm)	Sig. (1-tailed)	0.385	0.247	0.472	0.406	0.023	0.103	0.422	0.484	0.451	0.375	
	Pearson correlation	0.947**	0.949**	0.077	1	0.637*	0.687**	0.855**	0.863**	0.861**	0.845**	
Cd accumulation (mg/DBM)	Sig. (1-tailed)	0.000	0.000	0.406	0.582*	0.013	0.007	0.000	0.000	0.000	0.000	
	Pearson correlation	0.532*	0.556*	0.406	0.637*	1	0.831**	0.707**	0.753**	0.730**	0.670**	
Free proline (ppm)	Sig. (1-tailed)	0.037	0.080	0.023	0.013	0.001	0.001	0.005	0.002	0.004	0.009	
	Pearson correlation	0.600*	0.531*	0.393	0.687**	0.831**	1	0.794**	0.813**	0.805**	0.768**	
Total phenolics (ppm)	Sig. (1-tailed)	0.020	0.038	0.103	0.007	0.001	0.794**	0.001	0.001	0.001	0.002	
	Pearson correlation	0.870**	0.817**	-0.064	0.855**	0.707**	0.794**	1	0.991**	0.998**	0.955**	
Chlorophyll a (ppm)	Sig. (1-tailed)	0.000	0.001	0.422	0.000	0.005	0.001	0.000	0.000	0.000	0.000	
	Pearson correlation	0.877**	0.810**	-0.013	0.863**	0.753**	0.813**	0.991**	1	0.998**	0.949**	
Chlorophyll b (ppm)	Sig. (1-tailed)	0.000	0.001	0.484	0.000	0.002	0.001	0.000	0.000	0.000	0.000	
	Pearson correlation	0.875**	0.816**	-0.040	0.861**	0.730**	0.805**	0.998**	0.998**	1	0.954**	
Carotenoids (ppm)	Sig. (1-tailed)	0.000	0.001	0.451	0.000	0.004	0.001	0.000	0.000	0.000	0.000	
	Pearson correlation	0.836**	0.823**	-0.103	0.845**	0.670**	0.768**	0.955**	0.949**	0.954**	1	
	Sig. (1-tailed)	0.000	0.001	0.375	0.000	0.009	0.002	0.000	0.000	0.000	0.000	

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 18 Correlations among different parameters in leaves of *R. communis* plant grown in 50 ppm Cd-contaminated soil

		Length (cm)	Fresh biomass (g)	Dry biomass (g)	Total water content (g)	Cd concentration (ppm)	Cd accumulation (mg/DBM)	Free proline (ppm)	Total phenolics (ppm)	Chlorophyll a (ppm)	Chlorophyll b (ppm)	Carotenoids (ppm)
Length (cm)		1	0.606*	0.848**	-0.027	0.620*	0.350	0.421	0.783**	0.722**	0.764**	0.603*
Fresh biomass (g)	Pearson correlation Sig. (1-tailed)	0.606*	1	0.001	0.471	0.028	0.161	0.113	0.004	0.009	0.005	0.032
	Pearson correlation Sig. (1-tailed)	0.092	0.092	0.400	-0.400	0.783**	0.276	0.324	0.892**	0.900**	0.912**	0.683*
Dry biomass (g)	Pearson correlation Sig. (1-tailed)	0.092	0.092	1	0.233	0.255	0.254	0.311	0.386	0.304	0.348	0.300
	Pearson correlation Sig. (1-tailed)	0.400	0.400	0.400	0.126	0.004	0.220	0.181	0.000	0.000	0.000	0.015
Total water content (g)	Pearson correlation Sig. (1-tailed)	-0.027	-0.400	0.233	1	0.247	0.627*	0.578*	-0.430	-0.358	-0.398	-0.132
	Pearson correlation Sig. (1-tailed)	0.471	0.126	0.259	0.246	0.246	0.026	0.040	0.107	0.155	0.127	0.358
Cd concentration (ppm)	Pearson correlation Sig. (1-tailed)	0.620*	0.783**	0.255	0.247	1	0.743**	0.763**	0.680*	0.738**	0.723**	0.684*
	Pearson correlation Sig. (1-tailed)	0.028	0.004	0.239	0.627*	0.743**	0.007	0.005	0.015	0.007	0.009	0.015
Cd accumulation (mg/DBM)	Pearson correlation Sig. (1-tailed)	0.350	0.276	0.254	0.246	0.743**	1	0.964**	0.721*	0.369	0.320	0.562*
	Pearson correlation Sig. (1-tailed)	0.161	0.220	0.240	0.026	0.007	0.964**	0.000	0.011	0.147	0.184	0.045
Free proline (ppm)	Pearson correlation Sig. (1-tailed)	0.421	0.324	0.311	0.578*	0.763**	0.964**	1	0.349	0.438	0.403	0.642*
	Pearson correlation Sig. (1-tailed)	0.113	0.181	0.191	0.040	0.005	0.000	0.349	0.161	0.103	0.124	0.023
Total phenolics (ppm)	Pearson correlation Sig. (1-tailed)	0.783**	0.892**	0.386	-0.430	0.680*	0.621*	0.349	1	0.932**	0.981**	0.787**
	Pearson correlation Sig. (1-tailed)	0.004	0.000	0.135	0.107	0.015	0.011	0.161	0.981**	0.000	0.000	0.003
Chlorophyll a (ppm)	Pearson correlation Sig. (1-tailed)	0.722**	0.900**	0.304	-0.358	0.738**	0.369	0.638	0.932**	1	0.985**	0.890**
	Pearson correlation Sig. (1-tailed)	0.009	0.000	0.197	0.155	0.007	0.147	0.010	0.000	0.985**	0.000	0.000
Chlorophyll b (ppm)	Pearson correlation Sig. (1-tailed)	0.764**	0.912**	0.348	-0.398	0.723**	0.320	0.563	0.981**	0.985**	1	0.856**
	Pearson correlation Sig. (1-tailed)	0.005	0.000	0.162	0.127	0.009	0.184	0.012	0.000	0.000	0.856**	0.001
Carotenoids (ppm)	Pearson correlation Sig. (1-tailed)	0.603*	0.683*	0.300	-0.132	0.684*	0.562*	0.642*	0.787**	0.890**	0.856**	1
	Pearson correlation Sig. (1-tailed)	0.032	0.015	0.200	0.358	0.015	0.045	0.023	0.003	0.000	0.001	0.001

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Table 19 Correlations among different parameters in leaves of *R. communis* plant grown in 100 ppm Cd-contaminated soil

	Length (cm)	Fresh biomass (g)	Dry biomass (g)	Total water content (g)	Cd concentration (ppm)	Cd accumulation (mg/DBM)	Free proline (ppm)	Total phenolics (ppm)	Chlorophyll a (ppm)	Chlorophyll b (ppm)	Carotenoids (ppm)
Length (cm)	1	0.721**	0.863**	-0.468	0.702*	0.068	0.179	0.607*	0.593*	0.064	0.456
Fresh biomass (g)		1	0.001	0.086	0.012	0.426	0.310	0.031	0.035	0.430	0.093
Dry biomass (g)			1	-0.717**	0.905**	0.147	0.241	0.791**	0.775**	-0.035	0.575*
Total water content (g)				1	0.000	0.343	0.251	0.003	0.004	0.461	0.041
Cd concentration (ppm)					1	-0.012	0.073	0.266	0.258	0.115	0.214
Cd accumulation (mg/DBM)						1	0.420	0.228	0.236	0.376	0.277
Free proline (ppm)							1	-0.265	-0.336	-0.311	-0.165
Total phenolics (ppm)								1	0.171	0.191	0.325
Chlorophyll a (ppm)									1	0.869**	0.714*
Chlorophyll b (ppm)										1	0.721**
Carotenoids (ppm)											1

*Correlation is significant at the 0.05 level (1-tailed)

**Correlation is significant at the 0.01 level (1-tailed)

Correlation among different parameters

Strong correlations between total phenolics and Cd accumulation in plant roots and leaves were found, suggesting a significant role of phenolic compounds in protection of plant cells against the toxic effects of Cd ions (Ahmad et al. 2015; Khatamipour et al. 2011; Sun et al. 2007). Similarly, free proline also demonstrated positive correlations with Cd accumulation and dry biomass of the plant.

Conclusions and recommendations

R. communis is a good candidate for cadmium phytoextraction because of its fast growth, massive biomass, heavy metal tolerance and capacity for hyperaccumulation. Mo demonstrated significantly positive effect on Cd phytoextraction and on plant growth even under cadmium stress. 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. The correlation between total phenolics, dry biomass and Cd accumulation in different parts of the plant, under different treatments of Mo, was found to be statistically significant. It is recommended to further study various concentrations of Mo foliar spray to find the optimum concentration for plant growth and Cd phytoremediation. 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.

Acknowledgments The Pakistan Science Foundation (PSF) is highly acknowledged for the full financial support. PSF has funded this project under Pak-US Natural Sciences Linkage Programme (NSLP) vide project No. PSF/NSLP/KP-UM (432).

References

Ahmad A, Hadi F, Ali N (2015) Effective phytoextraction of cadmium (Cd) with increasing concentration of total phenolics and free proline in *Cannabis sativa* (L) plant under various treatments of fertilizers, plant growth regulators and sodium salt. *Int J Phytoremed* 17: 56–65

Ali N, Hadi F (2015) Phytoremediation of cadmium improved with the high production of endogenous phenolics and free proline contents in *Parthenium hysterophorus* plant treated exogenously with plant growth regulator and chelating agent. *Environ Sci Pollut Res* 22: 13305–13318

Allen SE (1974) *Chemical Analysis of Ecological Materials*. Blackwell Scientific, Oxford (London)

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *J Plant Soil* 39:205–207

Bavi K, Kholdebarin B, Moradshah A (2011) Effect of cadmium on growth, protein content and peroxidase activity in pea plants. *Pak J Bot* 43:1467–1470

Bhattacharjee S, Mukherjee AK (1994) Influence of cadmium and lead on physiological and biochemical responses of *Vigna unguiculata* (L). Walp. Seedling germination behaviour, total protein, proline content and protease activity. *Pollut Res* 13:269–277

Citterio S, Santagostino A, Fumagalli P, Prato N, Ranalli P, Sgorbati S (2003) Heavy metal tolerance and accumulation of Cd, Cr and Ni by *Cannabis sativa* L. *Plant Soil* 256:243–252

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie* 88:1707–1719

De B, Mukherjee AK (1998) Mercury induced metabolic changes in seedlings and cultured cells of tomato. *Geobios* 23:83–88

Falkowska M, Pietryczuk A, Piotrowska A, Bajguz A, Grygoruk A, Czerpak R (2011) The effect of gibberellic acid (GA₃) on growth, metal biosorption and metabolism of the green algae *Chlorella vulgaris* (chlorophyceae) Beijerinck exposed to cadmium and lead stress. *Pol J Environ Stud* 20:53–59

Genrich I, Burd D, George D, Glick BR (2000) Plant growth promoting bacteria that decrease heavymetal toxicity in plants. *Can J Microbiol* 46:237–245

Gouia H, Ghorbala HH, Meyer C (2000) Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiol Biochem* 38:629–638

Gupta UC (1997) Symptoms of molybdenum deficiency and toxicity in crops. In: Gupta UC (ed) *Molybdenum in agriculture*. Cambridge University Press, Cambridge

Hadi F, Bano A (2009) Utilization of *Parthenium hysterophorus* for the remediation of lead-contaminated soil. *Weed Biol Manag* 9(4):307–314

Hadi F, Asghari B, Fuller MP (2010) The improved phytoextraction of lead (Pb) and the growth of maize (*Zea mays* L.): the role of plant growth regulators (GA₃ and IAA) and EDTA alone and in combinations. *Chemosphere* 80:457–462

Hadi F, Ali N, Ahmad A (2014) Enhanced phytoremediation of cadmium-contaminated soil by *Parthenium hysterophorus* plant: effect of gibberellic acid (GA₃) and synthetic chelator, alone and in combinations. *Bioremed J* 18(1):46–55

Handique GK, Handique AK (2009) Proline accumulation in lemongrass (*Cymbopogon flexuosus* Stapf.) due to heavy metal stress. *J Environ Biol* 30:299–302

Haouari CC, Nasraoui AH, Bouthour D, Houda MD, Daieb CB, Mnai J, Gouia H (2012) Response of tomato (*Solanum lycopersicon*) to cadmium toxicity: growth, element uptake, chlorophyll content and photosynthesis rate. *African J Plant Sci* 6:1–7

Hesberg C, Haensch R, Mendel RR, Bittner F (2004) Tandem orientation of duplicated xanthine dehydrogenase genes from *Arabidopsis thaliana*. *J Biol Chem* 279:13547–13554

Hristozkova M, Geneva M, Stancheva I (2006) Response of pea plants (*Pisum sativum* L.) to reduced supply with molybdenum and copper. *Int J Agric Biol* 8(2):218–220

Huang D, Zhang Y, Qi Y, Chen C, Weihong J (2008) Global DNA hypomethylation, rather than reactive oxygen species (ROS), a potential facilitator of cadmium-stimulated K562 cell proliferation. *Toxicol Lett* 179:43–47

John R, Ahmad P, Gadgil K, Sharma S (2009) Heavy metal toxicity: effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *Intl J Agron Plant Prod* 3:65–76

Joseph P (2009) Mechanisms of cadmium carcinogenesis. *Toxicol Appl Pharm* 238:272–279

Kaiser BN, Gridley KL, Brady JN, Phillips T, Tyerman SD (2005) The role of molybdenum in agricultural plant production. *Ann Bot* 96: 745–754

Kaznina NM, Laiidinen GF, Titov AF (2006) The effect of cadmium on shoot apical meristems of barley. *Ontogenez* 37:444–448

- Khatamipour M, Piri E, Esmacilian Y, Tavassoli A (2011) Toxic effect of cadmium on germination, seedling growth and proline content of milk thistle (*Silybum marianum*). *Ann Biol Res* 2(5):527–532
- Krocova Z, Macela A, Kroca M, Hernychova L (2000) The immunomodulatory effect(s) of lead and cadmium on the cells of immune system in vitro. *Toxicol in Vitro* 14:33–40
- Lalk I, Dorfling K (1985) Hardening, ABA, proline and freezing resistance in the winter wheat varieties. *Physiol Plant* 63:287–229
- Linger P, Ostwald A, Haensler J (2005) *Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis. *Biol Plant* 49(4):567–576
- Mendel RR, Haensch R (2002) Molybdoenzymes and molybdenum cofactor in plants. *J Exp Bot* 53:1689–1698
- Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 15:523–530
- Rana M, Dhamija H, Prashar B, Sharma S (2012) *Ricinus communis* L.—a review. *Int J Pharm Tech Res* 4(4):1706–1711
- Rix RM (1999) *Annuals and biennials*. Macmillan, London, p. 106
- Rogers NJ, Franklin NM, Apte SC, Batley GE (2007) The importance of physical and chemical characterization in nanoparticle toxicity studies. *Integr Environ Assess Manag* 3:303–304
- Sagi M, Scazzocchio C, Fluhr R (2002) The absence of molybdenum cofactor sulfuration is the primary cause of the flacca phenotype in tomato plants. *Plant J* 31:305–317
- Sakishima Y, Yamasaki H (2002) Lipid peroxidation induces by phenolics in conjunction with aluminium ions. *Biol Plant* 45:249–254
- Sheirdil RA, Bashir K, Hayat R, Akhtar MS (2012) Effect of cadmium on soybean (*Glycine max* L) growth and nitrogen fixation. *Afr J Biotechnol* 11:1886–1891
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16:144–158
- Sumanta N, Haque CI, Nishika J, Suprakash R (2014) Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. *Res J Chem Sci* 4(9):63–69
- Sun RL, Zhou QX, Sun FH, Jin CX (2007) Antioxidative defense and proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator, *Solanum nigrum*. *Environ Exp Bot* 60:468–476
- Takiguchi M, Achanzar W, Qu W, Li G, Waalkes M (2003) Effects of cadmium on DNA-(cytosine-5) methyltransferase activity and DNA methylation status during cadmium induced cellular transformation. *Exp Cell Res* 286:355–365
- Tassi E, Pouget J, Petruzzelli G, Barbafieri M (2008) The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. *Chemosphere* 71:66–73
- Uraguchi S, Watanabe I, Yoshitomi A, Kiyono M, Kuno K (2006) Characteristics of cadmium accumulation and tolerance in novel Cd-accumulating crops, *Avena strigosa* and *Crotalaria juncea*. *J Exp Bot* 57:2955–2965
- Varalakshmi LR, Ganeshamurthy LN (2009) Effect of cadmium on plant biomass and cadmium accumulation in amaranthus (*Amaranthus tricolor*) cultivars. *Indian J Agri Sci* 79:226–250
- Williams RJ, Frausto da Silva JJR (2002) The involvement of molybdenum in life. *Biochem Biophys Res Commun* 292:293–299
- Zadeh BM, Savaghebi-Firozabadi GR, Alikhani HA, Hosseini HM (2008) Effect of sunflower and Amaranthus culture and application of inoculants on phytoremediation of the soils contaminated with cadmium. *Amer-Eurasian J Agric Environ Sci* 4:93–103
- Zengin FK, Munzuroglu O (2006) Toxic effects of cadmium (Cd⁺⁺) on metabolism of sunflower (*Helianthus annuus* L.) seedlings. *Acta Agric Scand Sect B Plant Soil Sci* 56:224–229