



Heavy metal phytoremediation potential of *Vigna radiata* (L.) Wilczek for use in contaminated regions of West Karun River, Iran

Sara Rahdarian^{a,b}, Sakineh Mashjoor^{c,*}, Abidina Abba^{d,**}, Akbar Esmaili^a, Parisa Ziarati^e, Peymaan Hassibi^b

^a Head of Nutrition and Food Sciences Research Center, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

^b Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

^c Marine Pharmaceutical Science Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^d Department of Biology, Faculty of Science, Federal University Lokoja, Lokoja, Nigeria

^e Department of Medicinal Chemistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

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ABSTRACT

Plant-based strategies could provide a key gateway to restoring heavy metal-polluted environments. The present study was aimed to investigate the phytoremediation potential of *Vigna radiata* (L.) Wilczek in the heavy metal contaminated regions by oil industries at West Karun River, Iran. After soil sampling, the plants were grown in pots outdoors and irrigated by distilled water (0 mg/L Cd), Karun River water (0.04 mg/L Cd), and also by 25, 50, 75, and 100 mg/L of cadmium chloride solutions. Plants were harvested at the seedling and ripening stages and their Cadmium (Cd) content was determined. According to the results, the efficiency of *V. radiata* for bioaccumulation of Cd was very high at low concentrations of Cd in Karun River treatments (57% and 21% for shoot and roots, respectively), the highest Transfer Coefficient (TC) was (2.80 ± 0.5), Translocation Factor (TF) (2.78 ± 0.7), and Bioaccumulation Factor (BF) (3.83 ± 0.4). Although our findings shows that *V. radiata* does not possess a high potential of Cd phytoremediation at high concentrations (2.47% and 4.21% in shoot and roots at 50–100 mg/L Cd, respectively), it can provide a safe alternative based at minimum level of Cd concentration. Comparison of heavy metal contents in mung bean plants and soil, shows that there is an antagonistic relationship in Cd uptake and other accessible heavy metals such as Iron (Fe), Zinc (Zn), and Copper (Cu) from the soil at the study area. Thus the *V. radiata* could be considered as a potent candidate for bioremediation and growing food in Cd-polluted environments.

1. Introduction

Heavy metal (HM) is a natural, and poisonous elements in the earth crust (e.g., cadmium (Cd) content is 0.1–0.41 mg/kg) which could be present in different forms as aerosol and also could be present in water and soil as sulfates, chlorides, and oxides [1]. Additionally, it is well-known that crude oil contains heavy metals, and oil spills elicit toxic effects on the environment which negatively affect human health [2]. Heavy metals can stay in the body for long periods [3] and cause serious diseases such as increased blood pressure, iron deficiency, digestion track allergies, cancers, fragile bones, and damage to liver, kidneys, and nervous system [4]. The uptake of HMs by surface soils and water increase health and the food-chain risks [5]. In this regard,

different sources of heavy metals in the environment have set various limits (Table 1).

Chemical and physical procedures for remediation of heavy metal contaminated soils are technique-dependent/site-specific and really expensive (up to \$500/ton soil) [6]. Phytoremediation is an eco-friendly, efficient, and economic approach for removing environmental contaminants [7]. Nevertheless, among the available in-situ and ex-situ remediation techniques, phytoremediation is a suitable solution to heavy metals problems in contaminated soil or water [8–11]. The plant-based remediation approach is regarded as a restoring balance strategy to reduce the associated risks of contaminants from aquatic media or land resource available for agricultural production and/or enhance food security [12,13]. A wide variety of plant species possess

* Corresponding author.

** Corresponding author.

E-mail addresses: s.mashjoor@gmail.com (S. Mashjoor), abidina.abba@fulokoja.edu.ng (A. Abba).

Table 1

Safe limits of Cd concentrations recommended from European Union standards (EU), Food and Agriculture Organization (FAO) and World Health Organization (WHO).

Element	Plant	Fruits vegetable	Soil	water
Cd	0.20 µg/g, 0.02 mg/kg [54, 72]	0.05 mg/kg [73]	3.00 µg/g [52]	0.01 µg/g [74]

detoxification mechanisms, which has been identified as decent accumulators of some anthropogenic toxic metals without incurring any damage to their growth and development [14–17]. In general, there are two main ways for phytoremediation by plants to remove heavy metals from contaminated soils: first, plant roots absorb the contaminants from the soil and water to either accumulate or oxidize the pollutants in biomass (phytostabilization); second, plants excrete the waste product through their root systems into the soil to encourage the growth of rhizospheric organisms (phytoextraction), which would in turn aids in the degradation of pollutants by microbes [16,18]. Research has clearly shown that the achievement of successful phytoremediation depends on plant selection, plant growth rate, contaminants translocation, accumulation potential, and tolerance to pollutants [16,19–22]. However, there is a lot of potential to learn about plant species capacity for phytoremediation [23]. Despite the fact that there is a wealth of research on the remediation of soil contaminants, edible plants also have great potential for phytoremediation [24–26]. The mung bean, *Vigna radiata* (L.) Wilczek from the Fabaceae family can grow in arid and semi-arid regions of the world [27]. It has been very popular in Asian cuisine, and is rich in valuable sources of plant protein, nutrients, minerals, and vitamins [14, 28]. It is adapted to a wide range of well drained soils, but is best suitable for fertile sandy loams. In short growing season of *V. radiata*, the average height of mature plant is 90 cm, the first flowers appear 7–8 weeks after planting and the crop reaches maturity in 12–14 weeks [29]. Although, an induced changes of physiological and biochemical response especially on seed germination of mung bean by some heavy metals were reported [9,26,30,31], there is a need to study the toxicity responses of mung beans in a real contaminated site such as oil industries land in Khuzestan, Iran. However, by using contaminated soil as medium to grow mung bean, the lower and upper limits of Cd levels in irrigation water were examined to simulate the some temporal-seasonal variation of HMs in sediment of West Karun River catchments [32–34]. Therefore following objectives were set in this study: 1-Acertaining the phytoremediation potential of *V. radiata* to clean up heavy metal contaminated soil and water. 2- Health risk assessment of the mung bean product cultivated in contaminated soil and/or water for humans and/or animals. 3- Determining the relationships between cadmium and other heavy metals (Fe, Zn, and Cu) absorption by mung bean.

2. Material and methods

This study was carried out using a complete randomization with 6 treatments and 6 replications. Chemical materials were obtained from Sigma–Aldrich (St. Louis, MO) and/or Merck, Germany. Soil samples (0–20 cm, n = 10 per site) were collected from oil industries site (48° 09'N, 31° 34'E), close to Azadegan oil field at West Karun Region, Iran in May 2019. At the same time, Karun River water samples (0–40 cm, n = 10 per site) were collected from West Karun Region (48° 30'N, 31° 27'E). The samples were placed in plastic bags and transported to the laboratory of Shahid Chamran University of Ahvaz. Heavy metals contents (Cd, Fe, Zn, and Cu) in the soil and water samples were measured by atomic absorption spectrophotometer (model: Analytik Jena, Germany) as described by Xiang et al. [35].

V. radiata (mung bean seeds were purchased from vegetable markets in Ahvaz. The vegetable seeds were steeped for 12 h in warm water, then

transferred into the distilled water (25°C) and allowed to germinate for two to three days. A total of 30 germinated *V. radiata* seeds were placed in a pot (12 cm × 12 cm, n = 36) that filled with contaminated soil in month of June 2019 at the Greenhouse of Botanical Garden, Department of Agronomy and Plant Breeding, Shahid Chamran University of Ahvaz. The plants were grown without pesticides, fertilizers and no addition of any type of manure. Irrigation treatments were undertaken by distilled water (0 mg/L Cd; as experimental control), Karun River water (mean: 0.04 mg/L Cd; as environmental control), and also by four different concentrations of 25, 50, 75, and 100 mg/L of cadmium chloride solutions (CdCl₂ · 2H₂O). Plants were irrigated every 3 days. In view of watering cycle, each pot being irrigated once by Cd (II) treatment solution and followed twice by distilled water. This irrigation cycle continued during the growth period (2 month). The average length of the day and night was 14/10 h, the maximum and minimum daytime temperatures were 45 ± 3 °C and 29 ± 1.5 °C, respectively, and the mean relative humidity during the daytime was 45%. At the seedling stage, cultivated plants were harvested. The plant shoot including leaves and stem were separated and the roots were also removed from the pots separately. At ripening stage, plant samples including previous items plus flower and sheaths containing seeds were taken for analysis of the respective treatments at 45 days after seeding/sowing (DAS). All elements were washed with distilled water then placed in an oven of 70 °C for 24 h and the plant materials were dried, grinded into fine powder. While the acid digestion of plant samples were done by measuring 0.5 g of each sample and was placed in an electric furnace for 2.5 h at 550 °C to form ashes. 5 ml of HCl (2 N) was added to each sample and titrated using Whatman filter paper (Number 41). Distilled water was added into the solution to reach the volume of 50 ml. Final solution obtained was used to determine Cd, Fe, Zn, Cu concentrations using atomic absorption spectrophotometer (model: Analytik Jena, Germany). The calculated parameters are as follows (Equations (1)–(6)): [36–42]

$$\begin{aligned} \text{Microelement (mg/kg)} &= \text{Element concentration (mg/L)} \cdot (V/M) \\ V &= \text{Extract-volume (50-ml)} \cdot M = \text{Dry-Weight-of-plant (g)} \end{aligned} \quad (1)$$

$$\text{Transfer coefficient (TC)} = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration soil}} \quad (2)$$

$$\text{Translocation factor (TF)} = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration in roots}} \quad (3)$$

$$\text{Tolerance index (TI)} = \frac{\text{Dry weight (mg) of shoots in each treatments}}{\text{Dry weight (mg) of shoots in control plant}} \quad (4)$$

$$\text{Bioaccumulation factor (BF)} = \frac{\text{Metal concentration plant or eachorgam}}{\text{Metal concentration in soil}} \quad (5)$$

Where metal concentration in the total plant was considered

$$\text{Uptake index (UI)} = \text{Metal concentration (mg/kg)} \times \text{Total dry Weight (g) of shoot} \quad (6)$$

The significance of the data was analyzed using a statistical package, IBM SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Differences among treatment means were analyzed by Duncan's multiple range tests in ANOVA (analysis of variance). Values of P < 0.01 were assumed significant.

3. Results

The results showed that TC, TF, and BF have a significant difference (P ≤ 0.01), compared to the control (Table 2). According to the comparison of mean values (Table 2; Fig. 1), TC, TF, and BF in the Karun River treatment were higher than the other treatments. The lowest TC, BF, and TF values were found in 50, 75, and 100 mg/L Cd treatments,

Table 2
Effect of different concentrations of Cd on mean values of plant studied parameters.

Parameters	Control (mg/L) Treatments (Cd mg/L)					
	DW	Karun River water (0.04)	25	50	75	100
TC	1.84 ± 0.2 ^b	2.80 ± 0.5 ^a	1.09 ± 0.2 ^c	0.02 ± 0.0 ^c	0.31 ± 0.1 ^c	0.13 ± 0.0 ^c
TF	1.64 ± 0.3 ^b	2.78 ± 0.7 ^a	1.32 ± 0.7 ^b	0.53 ± 0.2 ^c	1.15 ± 0.7 ^{bc}	0.52 ± 0.1 ^c
BF	2.81 ± 0.4 ^b	3.83 ± 0.4 ^a	0.19 ± 0.1 ^c	0.45 ± 0.1 ^c	0.06 ± 0.0 ^c	0.11 ± 0.0 ^c
Soil Cd (mg/Kg)	7.83 ± 0.7 ^c	9.03 ± 0.6 ^c	139.1 ± 18.0 ^c	1075.20 ± 20.0 ^a	787.93 ± 25.0 ^b	706.65 ± 32.0 ^b
Root Cd (mg/Kg)	7.65 ± 0.6 ^c	8.80 ± 0.7 ^c	11.55 ± 0.7 ^c	32.35 ± 2.7 ^b	24.15 ± 1.4 ^{bc}	54.35 ± 12.0 ^a
Stem Cd (mg/Kg)	4.10 ± 0.3 ^c	7.50 ± 0.2 ^a	4.60 ± 0.7 ^c	4.20 ± 0.4 ^c	6.10 ± 0.5 ^b	6.95 ± 0.2 ^{ab}
Leaf Cd (mg/Kg)	5.50 ± 0.2 ^b	11.26 ± 0.7 ^a	6.20 ± 0.7 ^b	7.50 ± 0.3 ^b	14.35 ± 1.8 ^a	11.75 ± 1.7 ^a
Shoot Cd (mg/Kg)	14.43 ± 3.0 ^b	24.26 ± 5.0 ^b	15.20 ± 2.0 ^b	17.15 ± 4.0 ^b	25.05 ± 12.0 ^a	23.33 ± 9.0 ^a
Total plant Cd (mg/Kg)	22.08 ± 4.0 ^d	33.06 ± 7.0 ^{bcd}	26.75 ± 5.0 ^{cd}	42.60 ± 9.0 ^{bc}	49.26 ± 9.0 ^b	77.68 ± 10.0 ^a
Soil Fe (mg/Kg)	406.00 ± 32.0 ^a	128.70 ± 17.0 ^a	396.56 ± 27 ^a	35.98 ± 9.0 ^a	31.43 ± 11.0 ^b	23.10 ± 8.0 ^c
Soil Zn (mg/Kg)	90.35 ± 7.2 ^{bc}	128.70 ± 13.0 ^a	107.60 ± 17.0 ^b	97.85 ± 11.0 ^{bc}	87.30 ± 17.0 ^{bc}	77.40 ± 11.0 ^c
Soil Cu (mg/Kg)	66.30 ± 17.0 ^c	105.00 ± 15.0 ^a	97.00 ± 9.0 ^a	82.85 ± 9.0 ^b	82.20 ± 7.0 ^b	82.00 ± 10.0 ^b
Leaf Cu (mg/Kg)	30.83 ± 3.0 ^b	29.75 ± 6.0 ^b	32.10 ± 8.0 ^b	37.20 ± 9.0 ^a	30.05 ± 6.0 ^b	27.50 ± 6.0 ^b

Note: Data are expressed as mean ± SE of six treatments and six replications. P < 0.05.

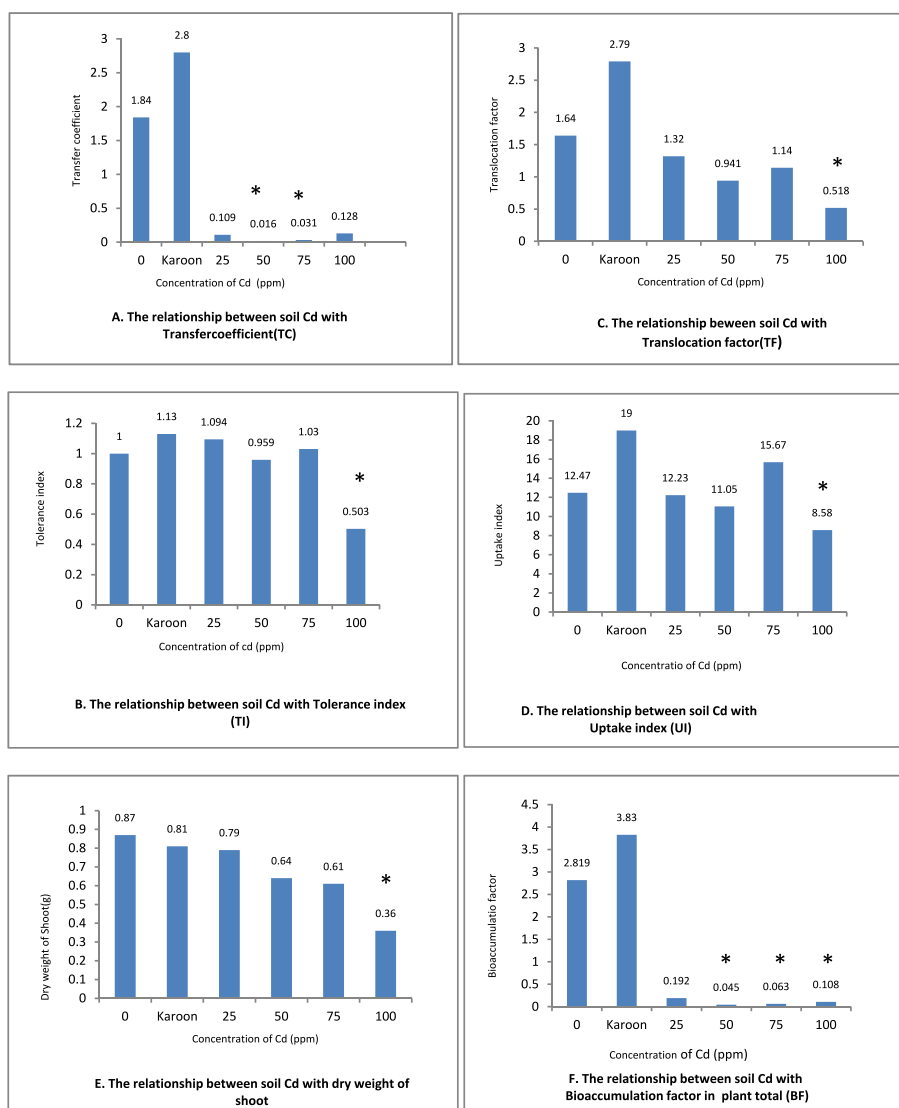


Fig. 1. The relationship between different concentrations of cadmium and plant studied parameters of TC, TI, TF, UI, SDW, and BF. In SDW, control, and other parameters, Karun River has the highest amount. Data are expressed as mean ± SE of six treatments and six replications. *P < 0.05.

respectively. TC, TF, and BF decreased through the addition of soil cadmium. TC has a positive and significant effect on TF. The results showed that in terms of Shoot Dry Weight (SDW), TI, and UI, there was no any significant difference between the treatments compared to the control, but, it has a positive and significant effect on TF, and UI. The result shows the Cd concentrations of 100 mg/L in treatment revealed the highest Cd levels. However, increased soil Cd had a strong potential to become reduced TC, TF, TI, UI, and BF (Table 2; Fig. 1). In other words, there is a reverse relationship between an increase in soil Cd and the plant absorbency levels. These phenomena are due to the existing mechanisms in *V. radiata*, for prevention of cadmium transfer to its shoot. Different treatments showed a significant difference ($p \leq 0.01$) in terms of root, stem, leaf, shoot, and total plant Cd. The highest values were found in 100 mg/L Cd and Karun River treatment, and the lowest levels were observed in the control (Table 2). Also, an increase in the concentration of soil Cd lead to the increase of Cd in root as observed in this study; although a negative and non-significant correlation between soil/root Cd and TC, TF, and BF was observed [43]. This is caused by Cd absorption ratio in the roots being higher than the other organ of the plant. A significant difference was observed between the different treatments on the soils accessible Fe, Zn, and Cu. The lowest amount of soils accessible Fe, Zn, and Cu were observed in 100 mg/L Cd treatments, whereas, the highest plant Fe absorption was observed in the control and the highest Zn and Cu absorption was observed in Karun River treatment (Table 2). Table 3 presents the results of the physico-chemical characteristics and heavy metal concentration in the Karun River water and the soil.

The results of the Pearson's correlation test between the studied parameters are presented in Table 4. As shown in Table 4, there is an antagonistic relationship between soil Cd absorption and uptake of three elements of Fe, Zn, and Cu in soil, which is only significant in soil Cu absorption and between these elements and root. TF has increased by higher concentrations of soil accessible Zn and Cu, but this relationship with Fe and Cu in plant leaves is negative. In other words, higher concentrations of these elements in the soil had increased the Cd transfer from the root to shoot. The higher concentration of leaf Fe causes a decrease in BF while causing an increase in accessible soil Zn. This occurs as Cu has increased BF. A higher concentration of soil Cd has a positive effect on root and total plant Cd but has decreased leaf Zn. Stem Cd also caused increases in leaf and shoot Cd. Soil Fe has shown negative effects on the leaf, shoot, and total plant Cd. Also, plant total Cd increased by higher amounts of soil Cd. Increase in stem Cd, reduced leaf Zn. There is a positive relationship between soil Fe and soil and leaf Cu levels. The highest root Cd was in 100 mg/L Cd treatments and the control showed the lowest. According to these results and in agreement with that of Rezakhani et al. [44], there is a positive relationship between soils accessible Zn and soil Cu (Table 4). Although elevated levels of soil Cd increases the root Cd, and the percentage of root share compared to total stock Cd decreased, in turn. Fig. 2 showed the

Table 3
Physicochemical characteristics and heavy metal concentration in the Karun River water and soil samples were collected from West Karun Region.

Treatment	Characteristics					
	EC	pH	Cd	Fe	Zn	Cu
Karun river	1.77 ± 0.30 (ms/cm ²)	7.48 ± 0.60	0.04 ± 0.01 (mg/L)	0.24 ± 0.02 (mg/L)	0.75 ± 0.04 (mg/L)	0.05 ± 0.01 (mg/L)
Soil (before culture)	2.79 ± 0.50 (ms/cm ²)	7.92 ± 0.40	8.80 ± 0.70 (mg/kg)	358.00 ± 7.00 (mg/kg)	128.70 ± 9.00 (mg/kg)	105.00 ± 12.00 (mg/kg)

Note: Data are expressed as mean ± SE (n = 10).

Table 4
Correlation matrix of parameters (Pearson correlation coefficients (r) per parameter).

	Transfer coefficient (TC)	Translocation factor (TF)	Tolerance index (TI)	Uptake index (UI)	Bioaccumulation factor (BF)	Shoot dry weight	Soil Cd	Root Cd	Stem Cd	Leaf Cd	Sheath Cd	Shoot Cd	Total plant Cd	Soil Fe	Leaf Fe	Soil Zn	Leaf Zn	Soil Cu	Leaf Cu						
Transfer coefficient (TC)	1.000																								
Translocation factor (TF)	0.801**	1.000																							
Tolerance index (TI)	0.219	0.192	1.000																						
Uptake index (UI)	0.385	0.547*	0.672**	1.000																					
Bioaccumulation factor (BF)	0.996**	0.786**	0.211	0.369	1.000																				
Shoot dry weight	0.408	0.503*	0.372	0.697**	0.422	1.000																			
Soil Cd	-0.704**	-0.715**	-0.135	-0.269	-0.718**	-0.572*	1.000																		
Root Cd	-0.525*	-0.732**	-0.320	-0.415	-0.544*	-0.501*	0.614**	1.000																	
Stem Cd	0.285	0.378	-0.063	0.323	0.230	-0.003	-0.096	0.169	1.000																
Leaf Cd	-0.105	0.107	-0.130	0.247	-0.152	-0.185	0.236	0.161	0.746**	1.000															
Sheath Cd	0.359	0.070	-0.387	0.423	0.354	0.176	0.017	-0.50	0.012	-0.098	1.000														
Shoot Cd	0.062	-0.209	-0.063	0.342	-0.010	-0.118	0.205	0.165	0.870**	0.966**	0.059	1.000													
Total plant Cd	-0.425	-0.562*	-0.281	-0.253	-0.454	-0.465	0.487*	0.932**	0.441	0.408	-0.093	0.433	1.000												
Soil Fe	0.334	-0.523*	0.283	0.306	0.354	0.458	-0.443	-0.118	-0.442	-0.492*	0.061	-0.498*	-0.791**	1.000											
Leaf Fe	-0.483*	-0.475*	-0.167	-0.275	-0.479*	-0.235	0.390	0.158	-0.186	0.225	-0.156	0.058	0.098	-0.414	1.000										
Soil Zn	0.563*	0.647*	0.151	0.332	0.545*	0.236	-0.421	-0.626**	0.113	-0.047	0.374	0.049	-0.581*	0.385	-0.256	1.000									
Leaf Zn	0.166	0.128	0.059	0.213	0.213	0.013	-0.330	-0.358	-0.478*	-0.406	-0.111	-0.461	-0.422	0.329	0.062	-0.13	1.000								
Soil Cu	0.491*	0.701**	0.229	0.420	0.481*	0.435	-0.484*	-0.668**	-0.024	-0.152	0.111	-0.099	-0.646**	0.564*	-0.317	0.886**	-0.039	1.000							
Leaf Cu	-0.205	-0.166	0.099	0.001	-0.189	-0.030	0.177	0.006	-0.411	-0.386	0.069	-0.404	-0.087	0.511*	-0.405	-0.005	-0.011	0.078	1.000						

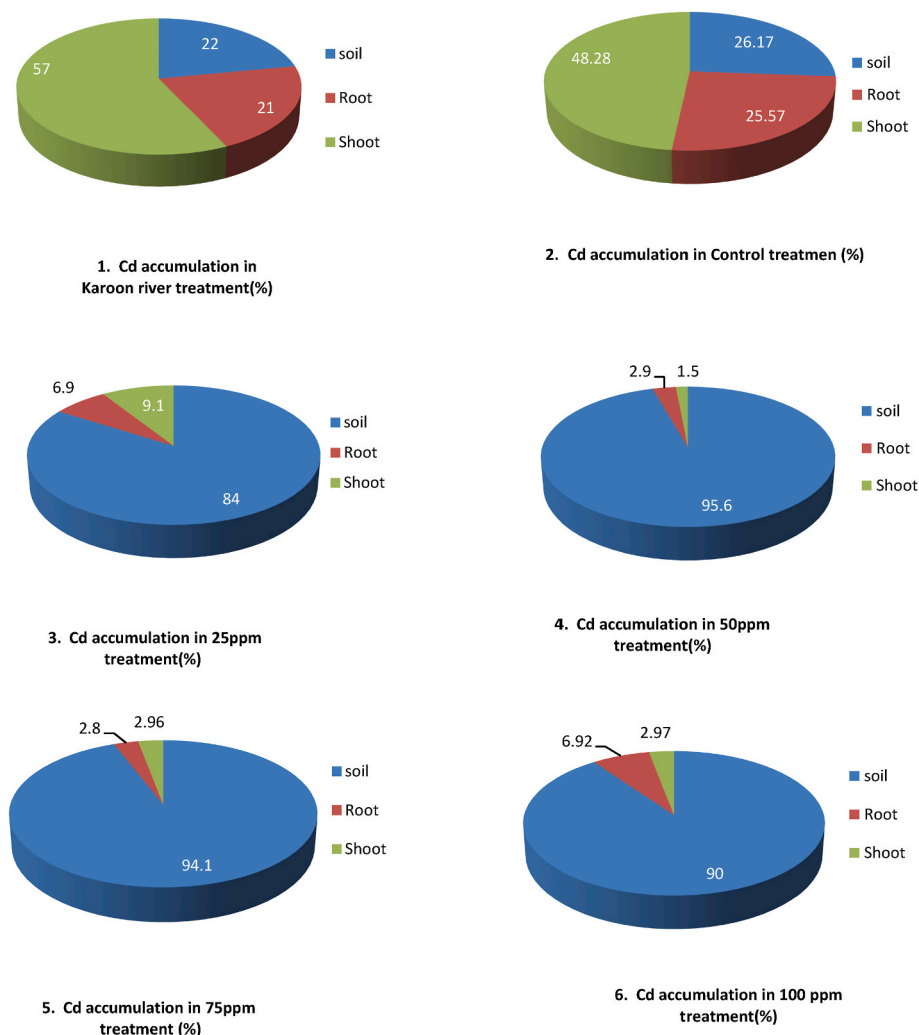


Fig. 2. Comparison of percentage of cadmium accumulation in the soil, roots, and shoots of *V. radiata* in terms of Cd stress treatments (25–100 mg/L Cd), Karun River as environmental control (0.04 mg/L Cd) and experimental control (0 mg/L Cd).

percentage of Cd bioaccumulation in the shoot was more than those of roots in low concentrations (0.04 mg/L Cd).

4. Discussion

The results of the current study revealed that *V. radiata* plants have developed protective strategies to neutralize the side-effects from Cd toxicity or, more controversially, mechanisms that allow them to have a better performance under Cd exposure. Heavy metal accumulation by hyper-accumulator plant species exceeds 0.1–1% of the dry weight [45]. As such, if a plant accumulates a heavy metal e.g., over 1000 mg/kg in its shoot, or its TF ≥ 1 , it is termed a super-absorbent plant [46]. Cadmium ($>5\text{--}10 \mu\text{g}\cdot\text{g}^{-1}$ leaf dry weight (LDW)) is toxic commonly for plants [47], except Cd-hyper-accumulators which can tolerate Cd concentrations of $100 \mu\text{g}\cdot\text{g}^{-1}$ LDW [48]. Conversely, the results of this study showed that in low concentrations of Cd, the remediation efficiency of *V. radiata* is greater and the highest amounts of TC and TF were in Karun River treatments. By increasing Cd concentrations, TC and TF decreased, hence, plant TF < 1 suggests that the plant has adequate stability [49]. As there are no significant differences between treatments in terms of SDW, it can be concluded that *V. radiata* has shown resistance to toxic effects of Cd. However, the potential of heavy metals accumulation in shoot and root and/or plants tolerant to HMs is significant dependent on plant species and growth stage [[50,51]]. These findings were confirmed with the results obtained by Fig. 3 because the symptoms of

Cd toxicity were seen only in some seedlings in different treatments. In agreement, Mao et al. [30] reported that with the rise of heavy metal level, the characteristic symptoms of heavy metal stress such as blackening of roots and chlorosis was observed.

The mung bean subjected to low Cd concentrations demonstrated a greater portion of root Cd accumulation than other treatments, indicating that Cd is a chronically persistent heavy metal in the soil. Moreover, the highest mobility of cadmium from root to shoot was seen in the control and Karun River treatments. Except for the leaves secondary pathway for pollutant uptake, the root system is the uppermost pathway for plant absorption of heavy metals [35]. Therefore, an increase in soil Cd concentration greatly reduced the accumulation of Cd in the shoot, probably due to a limitation in transferring potential of plant vessels for this element [51].

The results of the current study revealed that the phytoremediation power of *V. radiata* was negligible in high Cd concentrations (Fig. 2). In addition, the lowest contamination levels of Cd have been observed in sheaths. Limit values determined for Cd in the soil in this study were comparable to those set by many countries. Recommended soil Cd limit is $> 5 \text{ mg/kg}$ [52], and in many soils worldwide is $\sim 7 \text{ mg/kg}$ [53]. This is fairly similar to our control and Karun River treatment Cd levels. According to the results presented in Table 2, Karun River treatment has shown the highest levels in many traits. Hence, we can conclude that *V. radiata* has the highest phytoremediation potency in the low levels of Cd treatment. Based to the results represented in Table 2, the amount of



Fig. 3. Effect of Cd stress on the morphological changes of leaves, sheaths, and seeds of *V. radiata* (A, D, E, F, and G). No appearance changes in control plants (B, C, and H). Comparison of the root of control plants vs. treated plants (I).

shoot Cd in 25 mg/L treatment is ~ 15.2 mg/kg, which is acceptable for animal feed. Different treatments in terms of sheath Cd compared with the control, have not shown significant difference, whereas the mean of sheath Cd in all treatments is ~ 4.9 mg/kg at maximum. This is a lower cadmium level than that appeared in phytotoxicity standards [54]. These results revealed that beans of *V. radiata* are highly resistant to Cd. As identifying resistant plants in contaminated soil helps us select an appropriate culture, the current results can be a guide to produce a healthier harvest.

The findings further demonstrated that the Cd uptakes of the *V. radiata* plants had an antagonistic effect on the absorption of other heavy metals from the examined soil, including Fe, Zn, and Cu. The results of this study confirmed that Cd absorption and translocation influenced the absorption of Fe, Zn, and Cu. However, Cd and Zn are chemically similar and they can replace each other, but cadmium, unlike zinc, is very toxic. Probably its toxicity is due to the Cd high affinity for -SH functional groups in enzymes and the content of soluble protein. Similar to this, Xiang et al. [35] revealed that heavy metal concentrations in crops were primarily affected by heavy metals in soil and that the interaction of heavy metals is crucial again for pollution prevention and control. Additionally, Mouni et al. [55] discovered that the binding sites at the soil are gradually filled by the solution metals, which has a substantial impact on how the soil's heavy-metal specific behavior is altered by their interactions and/or competition for the accessible surface sites. The absorption and accumulation behavior for many heavy metals in soil varies from species to species, even within the same category of vegetables, regardless of the pH, nature of the soil, organic matter contents, and time on adsorption [35,55,56]. In terms of

V. radiata, the decreasing order of sorption behavior can be identified as $Cd > Cu > Zn > Fe$.

In view of Cd phytoremediation mechanisms, plants have various mechanisms to tackle heavy metal stress. Some of them are 1) creation of a chelate between heavy metal and some organic compounds in cytoplasm root cells, such as amino acids, carboxylic acids, and two groups of peptides of phytochelatin and metallothioneins which are results in Cd capture at the root level [57–59]. This mechanism has been reported in *V. radiata* [60]; 2) dislocation of poisonous metals to vacuoles [57]; 3) utilization of heat-shock proteins [61,62]; 4) accretion of some secretions from root cells (root exudates) [63] exertion influence on some of the enzymes activities [64,65]. Cadmium causes increased transcription of genes that synthesizes glutathione. Glutathione is necessary for phytochelatin synthesis [64]. There are many cysteine amino acids in metallothioneins structure. Metallothioneins play an antioxidant role on the cytoplasm membrane [66]. The bonding of elements with organic compounds probably occurs in plasma membrane. Out-of-root secretions by root cells could bond with heavy metals and induce their absorption. These secretions play an antioxidant role. Another effect of cadmium is induced ATPase enzyme activity in the plasma membrane and increase of synthesis heat-shock proteins [61,62]. These proteins have protection and reparation role. One of the protective proteins is HSP70 which increases in response to Cd stress [61,62]. These proteins are in the nucleus, cytoplasm, and plasma membrane. Likewise, *Paxillus involutus* plant also connects Cd to the cell wall and transfers it to vacuoles. These results were similar to the findings of some previous studies [1,10,67–71].

5. Conclusion

The result of the present study reveals the potential of *V. radiata* for remediation of cadmium content in contaminated soil or in water at West Karun Region, Iran. The Cd content in soil played antagonistic roles in the uptake of Fe, Zn and Cu from soil by *V. radiata* and that their specific behavior is strongly affected by their interactions with other metals. Additionally, there were substantial negative correlation between the Cd levels in soil and in *V. radiata*. This manuscript may help to determine the ecological farming importance of *V. radiata* in Cd-polluted soil and essential remediation strategies to keep the plant growth without accumulation of Cd in their edible parts.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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References

- Palutoglu, B. Akgul, V. Suyarko, M. Yakovenko, N. Kryuchenko, A. Sasmaz, Phytoremediation of cadmium by native plants grown on mining soil, *Bull. Environ. Contam. Toxicol.* 100 (2) (2017) 293–297, <https://doi.org/10.1007/s00128-017-2220-5>.
- Chinedu, C.K. Chukwuemeka, Oil spillage and heavy metals toxicity risk in the Niger Delta, Nigeria, *J. Health. Pollut* 8 (2018), 180905, <https://doi.org/10.5696/2156-9614-8.19.180905>.
- Rafati Rahimzadeh, S. Kazemi, A.A. Moghadamnia, Cadmium toxicity and treatment: an update, *Casp. J. Intern. Med.* 8 (3) (2017) 135–145, <https://doi.org/10.22088/cjim.8.3.135>.
- R.M. Olalekan, O. Adedoyin, T.V. Odubo, Measures of harm from heavy metal content (lead and cadmium) in women Lipstick and Lipgloss in Yenagoa Metropolis, Bayelsa state, Nigeria, *Int. J. Petrochem. Res.* 2 (3) (2018) 236–242, <https://doi.org/10.18689/ijpr-1000141>.
- US EPA, Introduction to Phytoremediation, National Risk Management Research Laboratory, Office of Research and Development, 2000. EPA/600/R-99/107.
- L. Liu, W. Li, W. Song, M. Guo, Remediation techniques for heavy metal-contaminated soils: principles and applicability, *Sci. Total Environ.* 633 (2018) 206–219, <https://doi.org/10.1016/j.scitotenv.2018.03.161>.
- S. Muthusaravanan, N. Sivarajasekar, J.S. Vivek, T. Paramasivan, M. Naushad, J. Prakashmaran, V. Gayathri, O.K. Al-Duaij, Phytoremediation of heavy metals: mechanisms, methods and enhancements, *Environ. Chem. Lett.* 16 (2018) 1339–1359, <https://doi.org/10.1007/s10311-018-0762-3>.
- M. Mirzaei, J. Verrelst, A. Riyahi Bakhtiari, S. Marofi, Potential use of grapevine cv Askari for heavy metal phytoremediation purposes at greenhouse scale, *Environ. Sci. Pollut. Res.* 28 (10) (2021) 12447–12458, <https://doi.org/10.1007/s11356-020-11129-9>.
- S. Behera, S. Bahira, G. Pattnaik, P. Puan, Phytoremediation of chromium by chickpea (*Cicer arietinum* L.) and mung bean (*Vigna radiata* L.): a comparative study, *Int. J. Curr. Sci. Res.* 4 (6) (2018) 1589–1598.
- A.N. Al-Dhaibani, F.S. El-Nakhlawy, S.G. Alsolaimani, F.M. Almeahadi, Phytoremediation of cadmium contaminated soil by sunflower, *Australian J. Basic Appl. Sci.* 7 (7) (2013) 888–894.
- E.R. Aweng, M. Karimah, O. Suhaimi, Heavy metals concentration of irrigation water, soils and fruit vegetables in Kota Bharu, Kelantan, Malaysia, *J. Appl. Sci. Environ. Sanit.* 6 (4) (2011) 463–470.
- V. Shah, A. Daverey, Phytoremediation: a multidisciplinary approach to clean up heavy metal contaminated soil, *Environ. Technol. Innovat.* 18 (2020), 100774, <https://doi.org/10.1016/j.eti.2020.100774>.
- M.A. Ashraf, M.J. Maah, I. Yusoff, Soil contamination, risk assessment and remediation, in: M.C. Hernandez-Soriano (Ed.), *Environmental Risk Assessment of Soil Contamination*, INTECH, Rijeka, 2014, pp. 3–56.
- S. Rahdarian, S. Mashjoor, A. Esmaili, P. Ziarati, P. Hassibi, Effects of cadmium stress on antioxidant responses of *Vigna radiata* L. Wilczek, *Azarian, J. Agri.* 8 (1) (2021) 1–11, <https://doi.org/10.29252/azarinj.047>.
- F.U. Haider, C. Liqun, J.A. Coulter, S.A. Cheema, J. Wu, R. Zhang, M. Wenjun, M. Farooq, Cadmium toxicity in plants: impacts and remediation strategies, *Ecotoxicol. Environ. Saf.* 211 (2021), 111887, <https://doi.org/10.1016/j.ecoenv.2020.111887>.
- A. Yan, Y. Wang, S.N. Tan, M.L.Y. Mohd Yusof, S. Ghosh, Z. Chen, Phytoremediation: a promising approach for revegetation of heavy metal-polluted land, *Front. Plant Sci.* 11 (2020) 359, <https://doi.org/10.3389/fpls.2020.00359>.
- Z.T. Xiong, Pollution-resistant evolution in plants and its gynecological costs, *Chin. J. Ecol.* 16 (1) (1997) 53–57.
- S.M.O.F. Babu, M.B. Hossain, M.S. Rahman, M. Rahman, A.S.S. Ahmed, M. M. Hasan, A. Rakib, T.B. Emran, J. Xiao, J. Simal-Gandara, Phytoremediation of toxic metals: a sustainable green solution for clean environment, *Appl. Sci.* 11 (21) (2021), 10348, <https://doi.org/10.3390/app112110348>.
- A. Kafle, A. Timilsina, A. Gautam, K. Adhikari, A. Bhattarai, N. Aryal, Phytoremediation: mechanisms, plant selection and enhancement by natural and synthetic agents, *Environ. Adv* 8 (2022), 100203, <https://doi.org/10.1016/j.envadv.2022.100203>.
- S. Singh, V. Thorat, C.P. Kaushik, K. Raj, S. Eapen, S.F. Dsouza, Potential of *Chromolaena odorata* for phytoremediation of C137s from solutions and low level nuclear waste, *J. Hazard Mater.* 162 (2009) 743–745, <https://doi.org/10.1016/j.jhazmat.2008.05.097>.
- P. Tanhan, M. Kruatrachue, P. Pokethitiyook, R. Chailayarat, Uptake and accumulation of cadmium, lead and zinc by siam weed [*Chromolaena odorata* (L.) King & Robson], *Chemosphere* 68 (2) (2007) 323–329, <https://doi.org/10.1016/j.chemosphere.2006.12.064>.
- B.J. Alloway, A.P. Jackson, H. Morgan, The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources, *Sci. Total Environ.* 91 (1990) 223–236, [https://doi.org/10.1016/0048-9697\(90\)90300-J](https://doi.org/10.1016/0048-9697(90)90300-J).
- A. Heckenroth, J. Rabier, T. Dutoit, F. Torre, P. Prudent, I. Laffont-Schwo, Selection of native plants with phytoremediation potential for highly contaminated Mediterranean soil restoration: tools for a non-destructive and integrative approach, *J. Environ. Manag.* 83 (3) (2016) 850–863, <https://doi.org/10.1016/j.jenvman.2016.09.029>.
- F.M. Mitton, M. Gonzalez, J.M. Monserrat, K.S.B. Miglioranza, Potential use of edible crops in the phytoremediation of endosulfan residues in soil, *Chemosphere* 148 (2016) 300–306, <https://doi.org/10.1016/j.chemosphere.2016.01.028>.
- A. Salonikioti, S. Petropoulos, V. Antoniadis, E. Levizou, A. Alexopoulos, Wild edible species with phytoremediation properties, *proced. Environ. Sci.* 29 (2015) 98–99, <https://doi.org/10.1016/j.proenv.2015.07.180>.
- T. Yasmeen, Q. Ali, F. Islam, A. Noman, M.S. Akram, M.T. Javed, Biologically treated wastewater fertigation induced growth and yield enhancement effects in *Vigna radiata* L, *Agri. Water. Manag.* 146 (2014) 124–130, <https://doi.org/10.1016/j.agwat.2014.07.025>.
- D. Ratnasekera, A.P.T. Subhashi, Morpho-physiological response of selected mungbean (*Vigna radiata* L) Sri Lankan genotypes to drought stress, *J. AgriSearch* 2 (3) (2015) 162–166.
- P.K. Dahiya, A.R. Linnemann, M.A.J.S. Boekel, N. Khetarpaul, R.B. Grewal, M.J. R. Nout, Mung bean: technological and nutritional potential, *Crit. Rev. Food Sci. Nutr.* 55 (5) (2015) 670–688, <https://doi.org/10.1080/10408398.2012.671202>.
- V. Damera, C. Venkateshwar, S.A. Unnisa, Heavy metals effect in food crop of *Vigna radiata* and its remediation, *J. Chem. Biol. Phys. Sci.* 5 (1) (2015) 917–924.
- F. Mao, G. Nan, M. Cao, Y. Gao, L. Guo, X. Meng, G. Yang, The metal distribution and the change of physiological and biochemical process in soybean and mung bean plants under heavy metal stress, *Int. J. Phytoremediation* 20 (11) (2018) 1113–1120, <https://doi.org/10.1080/15226514.2017.1365346>.
- S.R. Nair, V. Rajani, Effects of heavy metals on seed germination and protein content of *Vigna radiata* (L.) Wilczek, *Int. J. Adv. Res.* 3 (9) (2015) 1306–1317.
- H.R. Pourkhabbaz, F. Hedayatzadeh, M. Cheraghi, Determination heavy metals concentration at water treatment sites in Ahwaz and Mollasani using bioindicator, *Ecopersia* 6 (1) (2018) 55–66.
- M. Razaghi, M.R. Taherizadh, S. Mashjoor, Seasonal variation in heavy metal concentrations in sediment from Khalasi Estuary, Hormozgan, Iran, *Indian J. Fish.* 65 (3) (2018) 130–134, <https://doi.org/10.21077/ijf.2018.65.3.72459-19>.
- V. Diagonanolin, M. Farhang, M. Ghazi-Khansari, J. Jafarzadeh, Heavy metals (Ni, Cr, Cu) in the Karun waterway river, Iran, *Toxicol. Lett.* 151 (1) (2004) 63–67, <https://doi.org/10.1016/j.toxlet.2004.02.018>.
- M. Xiang, Y. Li, J. Yang, K. Lei, Y. Li, F. Li, D. Zheng, X. Fang, Y. Cao, Heavy metal contamination risk assessment and correlation analysis of heavy metal contents in soil and crops, *Environ. Pol.* 278 (2021), 116911, <https://doi.org/10.1016/j.envpol.2021.116911>.
- E. Suchowilska, M. Wiwart, W. Kandler, R. Rrska, A comparison of macro- and microelement concentrations in the whole grain of four Triticum species, *Plant Soil Environ.* 58 (3) (2012) 141–147, <https://doi.org/10.17221/688/2011-PSE>.
- V. Antoniadis, B.J. Alloway, Availability of Cd, Ni and Zn to ryegrass in sewage sludge-treated soil at different temperatures, *Water Air Soil Pollut.* 132 (2001) 201–214, <https://doi.org/10.1023/A:1013202104550>.
- J.K. Adesodun, M.O. Atayese, T.A. Agbaje, B.A. Osadiaye, O.F. Mafe, A.A. Soretire, Phytoremediation potentials of sunflowers (*Thionia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates, *Water, Air, Soil. Pollut.* 207 (1–4) (2010) 195–201, <https://doi.org/10.1007/s11270-009-0128-3>.
- P.K. Padmavathiamma, L.Y. Li, Phytoremediation technology: hyper-accumulation metals in plants, *Water, Air. Soil. Pollut.* 184 (1–4) (2007) 105–126, <https://doi.org/10.1007/s11270-007-9401-5>.
- C. Lamaia, M. Kruatrachue, P. Pokethitiyook, E.S. Upatham, V. Soonthornsarathool, Toxicity and accumulation of lead and cadmium in the filamentous green alga *Cladophora fracta* (OF Muller ex Vahl) Kutzing: a laboratory study, *Sci. Asia* 31 (2) (2005) 121–127, <https://doi.org/10.2306/scienceasia1513-1874.2005.31.121>.
- A. Lux, A. Sotniková, J. Opatrná, M. Greger, Differences in structure of adventitious roots in *Salix* clones with contrasting characteristics of cadmium accumulation and sensitivity, *Physiol. Plantarum* 120 (4) (2004) 537–545, <https://doi.org/10.1111/j.0031-9317.2004.0275.x>.

- [42] Y.B. Sun, G.H. Sun, Q.X. Zhou, Y.M. Xu, L. Wang, X.F. Liang, Y. Sun, X. Qing, Induced-phytoextraction of heavy metals from contaminated soil irrigated by industrial wastewater with *Mirabilis jalapa* L., *Plant Soil Environ.* 57 (8) (2011) 364–371, <https://doi.org/10.17221/148/2011-PSE>.
- [43] Y.M. Zhou, S.S. Long, B.Y. Li, Y.Y. Huang, Y.J. Li, J.Y. Yu, H.H. Du, S. Khan, M. Ming Lei, Enrichment of cadmium in rice (*Oryza sativa* L.) grown under different exogenous pollution sources, *Environ. Sci. Pollut. Res.* 27 (2020) 44249–44256, <https://doi.org/10.1007/s11356-020-10282-5>.
- [44] L. Rezakhani, A. Gholghin, S. Shafiei, Effect of different rates of Cd and Cu on growth and chemical composition of spinach, *Agri. Plant. Breed.* 8 (1) (2012) 87–100.
- [45] V. Kumar, J. Singh, P. Kumar, Heavy metals accumulation in crop plants: Sources, response mechanisms, stress tolerance and their effects, in: V. Kumar, R. Kumar, J. Singh, P. Kumar (Eds.), *Contaminants in Agriculture and Environment: Health Risks and Remediation vol. 1*, Agri. Environ. Sci. Acad, Haridwar, India, 2019, pp. 39–53.
- [46] A.J. Baker, R. Brooks, Terrestrial higher plants which hyperaccumulate metallic elements: review of their distribution, ecology and phytochemistry, *Biorecovery* 1 (1989) 81–126.
- [47] P.J. White, P.H. Brown, Plant nutrition for sustainable development and global health, *Ann. Bot.* 105 (2010) 1073–1080, <https://doi.org/10.1093/aob/mcq085>.
- [48] R.D. Reeves, A.J.M. Baker, Metal-accumulating plants, in: I. Raskin, B.D. Ensley (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment*, John Wiley & Sons Inc, New York, 2000, pp. 193–229.
- [49] M. Zacchini, F. Pietrini, G. Mugnozza, V. Iori, Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics, *Water. Air. Soil. Pollut.* 197 (2008) 23–34, <https://doi.org/10.1007/s11270-008-9788-7>.
- [50] B. Fattahi, K. Arzani, M.K. Souri, M. Barzegar, Morphological and phytochemical responses to cadmium and lead stress in coriander (*Coriandrum sativum* L.), *Ind. Crop. Prod.* 171 (1) (2021), 113979, <https://doi.org/10.1016/j.indcrop.2021.113979>.
- [51] M.K. Souri, M. Hatamian, T. Tesfamariam, Plant growth stage influences heavy metal accumulation in leafy vegetables of garden cress and sweet basil, *Chem. Biol. Technol. Agri.* 6 (25) (2019), <https://doi.org/10.1186/s40538-019-0170-3>.
- [52] European Union (EU), Heavy Metals in Wastes, European Commission on Environment, 2002. http://ec.europa.eu/environment/waste/studies/pdf/heavy_metals_report.pdf.
- [53] J.L. Jia, S. Li, L. Wang, Assessment of soil heavy metals for eco-environment and human health in a rapidly urbanization area of the upper Yangtze Basin, *Sci. Rep.* 8 (2018) 3256, <https://doi.org/10.1038/s41598-018-21569-6>.
- [54] WHO/FAO, Joint FAO/WHO food standard programme codex alimentarius commission 13th session. Report of the Thirty Eight Session of the Codex Committee on Food Hygiene, United States of America, Houston, 2007. ALINORM 07/30/13.
- [55] L. Mouni, L. Belkhir, A. Bouzaza, J.C. Bollinger, Interactions between Cd, Cu, Pb, and Zn and four different mine soils, *Arabian J. Geosci.* 10 (77) (2017), <https://doi.org/10.1007/s12517-017-2864-9>.
- [56] D. Wan, N. Zhang, W. Chen, P. Cai, L. Zheng, Q. Huang, Organic matter facilitates the binding of Pb to iron oxides in a subtropical contaminated soil, *Environ. Sci. Pollut. Res.* 25 (32) (2018) 32130–32139, <https://doi.org/10.1007/s11356-018-3173-x>.
- [57] E. Grill, E.L. Winnacker, M.H. Zenk, Phytochelatin: the principal heavy-metal complexing peptides of higher plants, *Science* 230 (4726) (1985) 674–676, <https://doi.org/10.1126/science.230.4726.674>.
- [58] A.K. Shanker, M. Djanaguiraman, R. Sudhagar, R. Jayaram, G. Pathmanabhan, Expression of metallothionein 3 (MT3) like protein mRNA in *Sorghum cultivars* under chromium (VI) stress, *Curr. Sci.* 86 (2004) 901–902.
- [59] W.E. Rauser, Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin and metallothioneins, *Cell Biochem. Biophys.* 31 (1999) 19–48, <https://doi.org/10.1007/BF02738153>.
- [60] A.K. Shanker, M. Djanaguiraman, R. Sudhagar, C.N. Chandrashekar, G. Pathmanabhan, Differential antioxidative response of ascorbate glutathione pathway enzymes and meta-bolites to chromium speciation stress in green gram (*Vigna radiata* (L.)R.Wilczek, cv CO₄) roots, *Plant Sci.* 166 (2004) 1035–1043, <https://doi.org/10.1016/j.plantsci.2003.12.015>.
- [61] S. Lewis, M.E. Donkin, M.H. Depledge, HSP70 expression in Enteromorphintestinalis (Chlorophyta) exposed to environmental stressors, *Aquat. Toxicol. (Amst.)* 51 (2001) 277–291, [https://doi.org/10.1016/S0166-445X\(00\)00119-3](https://doi.org/10.1016/S0166-445X(00)00119-3).
- [62] D. Neumann, O. Lichtenberger, D. Gunther, K. Tschiersck, L. Nover, Heat-shock proteins induce heavy-metal tolerance in higher plants, *Planta* 194 (3) (1994) 360–367, <https://doi.org/10.1007/BF00197536>.
- [63] Y. Ma, R.S. Oliveira, H. Freitas, C. Zhang, Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation, *Front. Plant Sci.* 7 (2016) 918, <https://doi.org/10.3389/fpls.2016.00918>.
- [64] S.S. Gill, N. Tuteja, Cadmium stress tolerance in crop plants: probing the role of sulfur, *Plant Signal. Behav.* 6 (2) (2011) 215–222, <https://doi.org/10.4161/psb.6.2.14880>.
- [65] S.S. Gill, N.A. Khan, N.A. Anjum Tuteja, Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects, *Plant, Stress* 5 (1) (2011) 1–23.
- [66] B. Ruttkay-Nedecky, L. Nejdil, J. Gumulec, O. Zitka, M. Masarik, T. Eckschlager, M. Stiborova, V. Adam, R. Kizek, The role of metallothionein in oxidative stress, *Int. J. Mol. Sci.* 14 (3) (2013) 6044–6066, <https://doi.org/10.3390/ijms14036044>.
- [67] B. Kaur, B. Singh, N. Kaur, D. Singh, Phytoremediation of cadmium-contaminated soil through multipurpose tree species, *Agrofor. Syst.* 92 (2018) 473–483, <https://doi.org/10.1007/s10457-017-0141-2>.
- [68] W.E. Rauser, Phytochelatin and related peptides. Structure, biosynthesis, and function, *Plant Physiol.* 109 (1995) 1141–1149, <https://doi.org/10.1104/pp.109.4.1141>.
- [69] C.S. Cobbett, Phytochelatin and their roots in heavy metal detoxification, *Plant Physiol.* 123 (2000) 825–832, <https://doi.org/10.1104/pp.123.3.825>.
- [70] R. Howden, P.B. Goldsbrough, C.R. Andersen, C.S. Cobbett, Cadmium-sensitive, cad1, mutants of *Arabidopsis thaliana* are phytochelatin deficient, *Plant Physiol.* 107 (1995) 1059–1066, <https://doi.org/10.1104/pp.107.4.1067>.
- [71] E. Grill, S. Löffler, E.L. Winnacker, M.H. Zenk, Phytochelatin, the heavy-metal-binding peptides of plants, are synthesized from glutathione by a specific glutamylcysteine dipeptidyl transpeptidase (Phytochelatin synthase), *Proc. Natl. Acad. Sci. U.S.A.* 86 (1989) 6838–6842, <https://doi.org/10.1073/pnas.86.18.6838>.
- [72] European Union, Commission Regulation (EC) No. 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs, *Official Journal of European Union* L364/5, 2006.
- [73] FAO/WHO, General Standards for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995), 2014.
- [74] FAO, Water Quality for Agriculture, UNESCO, Publication, Rome, 1985. Paper No. 29 (Rev. 1).