

PHYTOREMEDIATION OF SOIL CADMIUM USING *Chenopodium* SPECIES

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Phytoremediation is effectively used to remove heavy metals from soil. In this research *Chenopodium album* and *Chenopodium murale* were evaluated for their comparative potential to phytoremediate increased (0-750 μM) cadmium (Cd) from soil. Both the *Chenopodium* species showed wide difference for Cd tolerance. Chlorophyll (Chl) *a* was at par with control in at 250 μM Cd *C. album*, but was reduced in *C. murale*. Chl *b* was less reduced by Cd in *C. album* than in *C. murale* resulting in an increased Chl *a:b* ratio. Likewise, carotenoids were reduced minimally in *C. album* and greatly in *C. murale*. Although soluble phenolics were reduced in both the species, a markedly greater accumulation of anthocyanins was noted in *C. album* at all Cd levels. Greater levels of Chl *a* and anthocyanins and minimal Chl *b* contents were important to Cd tolerance in *C. album*. The *C. album* showed a minimal reduction in K^+ , steadier Ca^{2+} , SO_4^{2-} -S, and PO_4^{3-} in shoot and root, NO_3^- in the shoot. *C. album* accumulated more Cd in shoot while *C. murale* in root, and showed greater capacity to adjust metabolites and accumulate macronutrients. From the increased shoot Cd contents, whilst showing better growth, we infer that metabolic changes could help bind and sequester excess of Cd in the vacuole, and thus better Cd tolerance. Greater seed germination, reduced post-germination mortality and enhanced growth of mungbean (used as successive crop) substantiated that *C. album* is a better phytoremediator of Cd than *C. murale*. In crux, better growth and Cd-phytoremediation by *C. album* may have great implications for agricultural practices.

Keywords: Anthocyanins, Cd-toxicity, chlorophyll, nutrients, phytoremediation, successive crop

INTRODUCTION

Heavy metal toxicity is a threat to environment and a looming issue to agriculture especially in the peri-urban areas of the world. This problem arises as a result of discharge of industrial waste in excessive quantities to the environment and the soil, and this problem is more serious in less developed countries (Wahid *et al.*, 2009; Hussain *et al.*, 2010; Sabir *et al.*, 2011). Heavy metals are causing long term risk on environment sustainability by enhancing soil pollution. Soil fertility, crop yields and microbial activities decrease with the passage of time due to pollution caused by heavy metals (Yang *et al.*, 2005). Concentration of heavy metals increases in soils due to atmospheric deposition from sludge, sewage irrigation, utilization of metal-containing farm manures and fertilizers and industrial wastes. Their entry into the food chain has hazardous effects on the biota including human beings (Prasad and Freitas, 2003; Chitmanat and Traichaiyaporn, 2010; Abdollahi *et al.*, 2011). Heavy metals are also very toxic above certain concentrations, affect plant growth, and cause substantial yield reduction (Khan *et al.*, 2007; Jalloh *et al.*, 2009). Heavy metals are found in the form of free or exchangeable ions and complexes (Leyval *et al.*, 1997; Wahid *et al.*,

2009). The metals in the ionic form move to upper parts of plants (Liu *et al.*, 2007). Concentration and duration of exposure and oxidation state are major factors affecting heavy metal toxicity to plants (Cosio *et al.*, 2005).

Plants can easily absorb Cd and move to other parts causing toxicity. Having been absorbed by the plant roots and transported to aerial parts, the Cd may be redistributed to other plants parts via phloem (Unterbrunner *et al.*, 2007; Souza *et al.*, 2008). Leaf chlorosis due to loss of chlorophyll and carotenoids, and plant stunting and root browning are common symptoms of Cd toxicity in plants (Wahid *et al.*, 2008; Perveen *et al.*, 2011; Bahmani *et al.*, 2012). Photosynthetic process especially the CO_2 assimilation is decreased by heavy metals (Iqbal *et al.*, 2010). Applied Cd usually leads to the disruption of membrane functions by increasing the peroxidation of lipids bilayer and increasing the malondialdehyde content (Liu *et al.*, 2011). Cadmium also induces synthesis of secondary metabolites such as phenolics which can bind the heavy metals causes while wall lignification leads to cell death (Wahid *et al.*, 2009).

Some plants species possess potential to extract heavy metals from soil and improve its physico-chemical properties and health for growing successive plants (Chrysafopoulou *et al.*, 2005; Mangkoedihardjo, 2007).

About 400 species of 66 woody plants are hyper accumulator of heavy metals (Kukkola *et al.*, 2000). Such an approach, referred as phytoremediation of heavy metals, has been utilized as an effective means to remove the heavy metals from field soils using the metal hyper accumulator plants (Lone *et al.*, 2008). Furthermore, this is a cost-effective, environmental friendly and highly rewarding technology (Ghosh and Singh, 2005).

Wild plants are important components of ecosystems as their distribution helps in keeping the environment clean. Species of genus *Chenopodium* are common weeds of cultivated fields and widely distributed in many parts of the world (James *et al.*, 2005). *Chenopodium* species are capable of accumulating the excessive quantity of heavy metals in leaf tissues, thereby reducing their quantities in the soil. While exploring the heavy phytoextractability of *Chenopodium* species, Bhargava *et al.* (2008) reported that *C. quinoa* was a better hyper accumulator of nickel, chromium and cadmium, while *C. album* could accumulate copper in large quantities. In their study, Gupta and Sinha (2007) found that *C. album* accumulated greater amounts of heavy metals (chromium, lead and Cd) in leaves followed by stem and root. Several other *Chenopodium* spp. remain yet to be investigated for their heavy metal responses.

Chenopodium album and *C. murale* are common weeds of winter crops in Pakistan, and can grow luxuriantly in stressful conditions. However, the comparative physiological mechanism(s) of Cd tolerance and heavy metals phytoremediation potential of both these species are poorly studied. This study was conducted to elucidate some morphological and physiological mechanisms of Cd tolerance and comparative phytoremediation potential of two common wild species of *Chenopodium* viz. *C. album* and *C. murale* using mungbean (*Vigna radiata*) as successive test crop.

MATERIALS AND METHODS

Experimental plan: Two field plot experiments were performed in the year 2011 for the determination of Cd phytoremediation potential of two *Chenopodium* species. In the first experiment, the comparative responses of *C. album* and *C. murale* was determined, while in second experiment comparative phytoremediation potential of both these species was assessed by growing mungbean as secondary crop in the plots from where the plants of *C. album* and *C. murale* were harvested.

Seeds of *C. album* and *C. murale* were broadcasted in small field plots measuring 1.5 m (long) × 1.0 m (wide) × 0.25 m (deep), lined with polythene sheets containing 600 kg of loam field soil (~30 cm deep). Separate plots were used for each treatment, and sampling was done randomly in triplicate from each plot. The physico-chemical properties of soil were determined with standard methods from dry soil or

soil extracts as the case may be (Tandom, 1993). These properties before the experiment were: sand 37%, silt 32%, clay 31%, organic matter 1.03%, pH 7.02, EC_e, 2.76 dS m⁻¹, CEC 5.76 cmol_c kg⁻¹, and Cd contents not detected. After growing *Chenopodium* species these properties changed to: sand 36%, silt 34%, clay 30%, organic matter 1.24%, pH 7.12, EC_e 3.02 dS m⁻¹ and CEC 6.98 cmol_c kg⁻¹. Cadmium contents were not detectable in fallow plots or in Cd non-contaminated soil where *Chenopodium* species were grown. However, Cd was detected in saturated soil extract of the plots where Cd was added. The Cd values were 15.32, 27.13 and 34.54 µmol kg⁻¹ soil for *C. album* and 17.43, 32.65 and 45.54 µg kg⁻¹ soil for *C. murale* at corresponding levels of applied Cd.

Cd tolerance by *Chenopodium* species: After germination, 20 uniform plants of both species in each plot were grown for one month. Rhizospheric soil of both the species was added with 0 (control), 250, 500 and 750 µM Cd levels prepared from CdCl₂·2.5H₂O, based on the saturation percentage of soil (~30%). One set of plots was kept as blank (no plants grown) without Cd and another blank set was mixed with 750 µM Cd level. Both the plots were kept as fallow for growing successive crop. No fertilizers were added since both the species grow wild, but watering was done when soil surface appeared dry. The plants were grown for two months in Cd contaminated soil and then harvested. The data was recorded for growth characteristics (shoot and root length, number of leaves and roots per plant, fresh and dry weight of shoot and root and leaf area per plant) and physiological and biochemical attributes (chlorophylls, carotenoids, soluble phenolics, anthocyanins in leaves) and ionic analysis (K⁺, Ca²⁺, SO₄²⁻-S and Cd²⁺ in the shoot and root).

Comparative phytoremediation potential of *Chenopodium* species: In this experiment, mungbean (*Vigna radiata* cv. NM-98) was grown in plots from which both the *Chenopodium* species were uprooted (with or without Cd) for a period of one month. For this purpose, 50 seeds were dibbled 2 cm deep in each plot followed by watering. Seed germination was recorded for eight days. After recording germination data the plant density was thinned to 20 uniform plants per plot. The determinations were made for germination percentage, shoot and root length, fresh and dry weight, leaf area per plant and shoot and root Cd contents.

Growth, metabolites and minerals analyses: Among growth parameters, leaf area per plant was determined of intact plants following the method of Carleton and Foote (1965). Shoot and root length of each plant was measured of intact plants whereas root length was measured after uprooting them. Number of leaves and branches was counted before harvesting whereas number of roots was counted after uprooting the plants. Dry weight of shoot and root was taken after drying the fresh harvested plant parts in paper bags at 70°C for seven days.

For the analysis of photosynthetic pigments, 0.1 g fresh leaf material was chopped and extracted with 5 mL of 80% acetone using a pestle and mortar and final volume made to 10 mL using 80% acetone. The absorbance of the extract was taken at 665 and 645 nm using spectrophotometer (U-2001, Hitachi, Tokyo, Japan). The quantities of chlorophyll *a* and *b* were computed as described by Yoshida *et al.* (1976). For carotenoids analysis, the absorbance of above extract was taken at 480 nm while the total quantity was calculated as described by Davies (1976). The 80% acetonetic extract was used for the estimation of soluble phenolics with the method of Julkenen-Titto (1985). For anthocyanins estimation, the fresh plant material was extracted with acidified methanol, centrifuged and absorbance of the extract was taken at 535 nm using spectrophotometer (Stark and Wray, 1989).

To analyze the contents of mineral ions, the dried ground plant material (0.5 g) was digested in concentrated HNO₃ by gradually increasing the temperature of heating block in a fume-hood. A blank, in duplicate, was always run for measurement of various mineral elements. After digestion, and making final volume to 25 mL, the extract was used for the estimation of K⁺ and Ca²⁺ using flame photometer (Jenway PFP7, UK). A graded series of 10–50 mg L⁻¹ was run for making the standard curve for both these ions using KNO₃ and CaCl₂, respectively. Amounts of Cd in the shoot and root were measured using atomic absorption spectrophotometer (AAS 3000, Norwich, CT, USA). For the estimation of sulfur (S), the above extract was taken in 50 mL volumetric flask. The extract was swirled after adding 1 mL of 6 N HCl and 1 mL of 0.5% gum acacia. BaCl₂ crystal (0.5 g) were added and kept for 1 min, swirled until the crystals were dissolved. Transmittance of samples was measured using spectrophotometer (Tandom, 1993).

Statistical analysis: All the determinations were made in triplicate from these completely randomized experiments. The presence or absence of significant differences among different factors was ascertained by performing analysis of variance (ANOVA) while the differences among the treatments were determined by using Duncan's multiple range test (Steel *et al.*, 1997). Computer software MSTAT-C was used for the statistical analysis.

RESULTS

Cd tolerance by *Chenopodium species*: Both the species differed significantly ($P < 0.01$) for shoot length under control and Cd-stress conditions. Under Cd stress in *C. album*, the shoot length increased over control at 250 μ M Cd, declined to the level of control at 500 μ M Cd but further declined at 750 μ M Cd. However, in *C. murale*, the shoot length consistently declined at all Cd levels (Fig. 1a). Root length, although declined consistently in both species at all Cd treatments, it was significantly ($P < 0.01$) greater in *C. album*

than in *C. murale* (Fig. 1b). *C. album*, showing greater number of branches and roots per plant than *C. murale* under control condition, was less affected than *C. murale* under Cd stress for both these attributes (Fig. 1c-d). The number of green leaves per plant was greater than control in *C. album* at 250 μ M but declined at subsequent Cd levels, whilst *C. murale* indicated a consistent decline in this number at all the levels of Cd (Fig. 1e). Leaf area per plant under control condition was lower in *C. album*, but it showed a little decline even at the highest Cd level. However, *C. murale* showed a marked decline in this attribute at all Cd levels (Fig. 1f). For shoot dry weight, *C. album* exhibited no change between control and 250 μ M Cd treatment but displayed a decline at higher Cd levels. However, shoot dry weight was gradually reduced in *C. murale* at all Cd levels (Fig. 1g). Root dry weight was higher in *C. album* than in *C. murale* under control conditions and was not different ($P > 0.05$) from control up to 500 μ M Cd level. However, *C. murale* manifested a substantial decline ($P < 0.01$) in root dry weight at all Cd levels (Fig. 1h).

In Cd treated plants of *C. album*, Chl *a* increased at 250 μ M level compared to control plants, which declined at 500 and 750 μ M Cd. However, Chl *a* consistently declined in *C. murale* at all Cd levels (Fig. 2a). Although Chl *b* declined in both the *Chenopodium* spp., this reduction was greater in *C. murale* (Fig. 2b). In *C. album*, Chl *a:b* ratio indicated a little rise at 250 μ M Cd, which then declined at higher levels. Contrarily, in *C. murale* this ratio decreased at 250 μ M Cd, which then came closer to control values at 500 μ M Cd and increased at 750 μ M Cd level (Fig. 2c). In *C. album*, the carotenoids contents increased at 250 and 500 μ M Cd and then a decrease (at 750 μ M Cd), while *C. murale* displayed a decrease in this character at all Cd levels (Fig. 2d). Leaf soluble phenolics although declined in both the species, the decline was much higher in *C. murale* than in *C. album* with increased Cd stress (Fig. 2e). In *C. album*, the anthocyanin contents increased up to 500 μ M Cd followed by a decline at 750 μ M Cd level. However, in *C. murale*, the anthocyanins declined gradually at all Cd levels (Fig. 2f).

Applied Cd declined shoot and root K⁺ in both the species, although lowly in *C. album* than in *C. murale* (Fig. 3a-b). Cd stress did not affect the shoot Ca²⁺ but nominally affected root Ca²⁺ in *C. album*, which was substantially reduced in both these parts of *C. murale* (Fig. 3c-d). For shoot and root SO₄²⁻-S content under Cd stress, *C. album* indicated its steadier level while *C. murale* showed a reduction in this ion at all Cd levels (Fig. 3e-f). For shoot Cd content under Cd treatment, *C. album* indicated significantly higher Cd content in the shoot than *C. murale* (Fig. 3g). With an increase in Cd level, both the species indicated accumulation of Cd in the roots. However, of the two species *C. album* indicated lesser Cd accumulation in the root than *C. murale* (Fig. 3h).

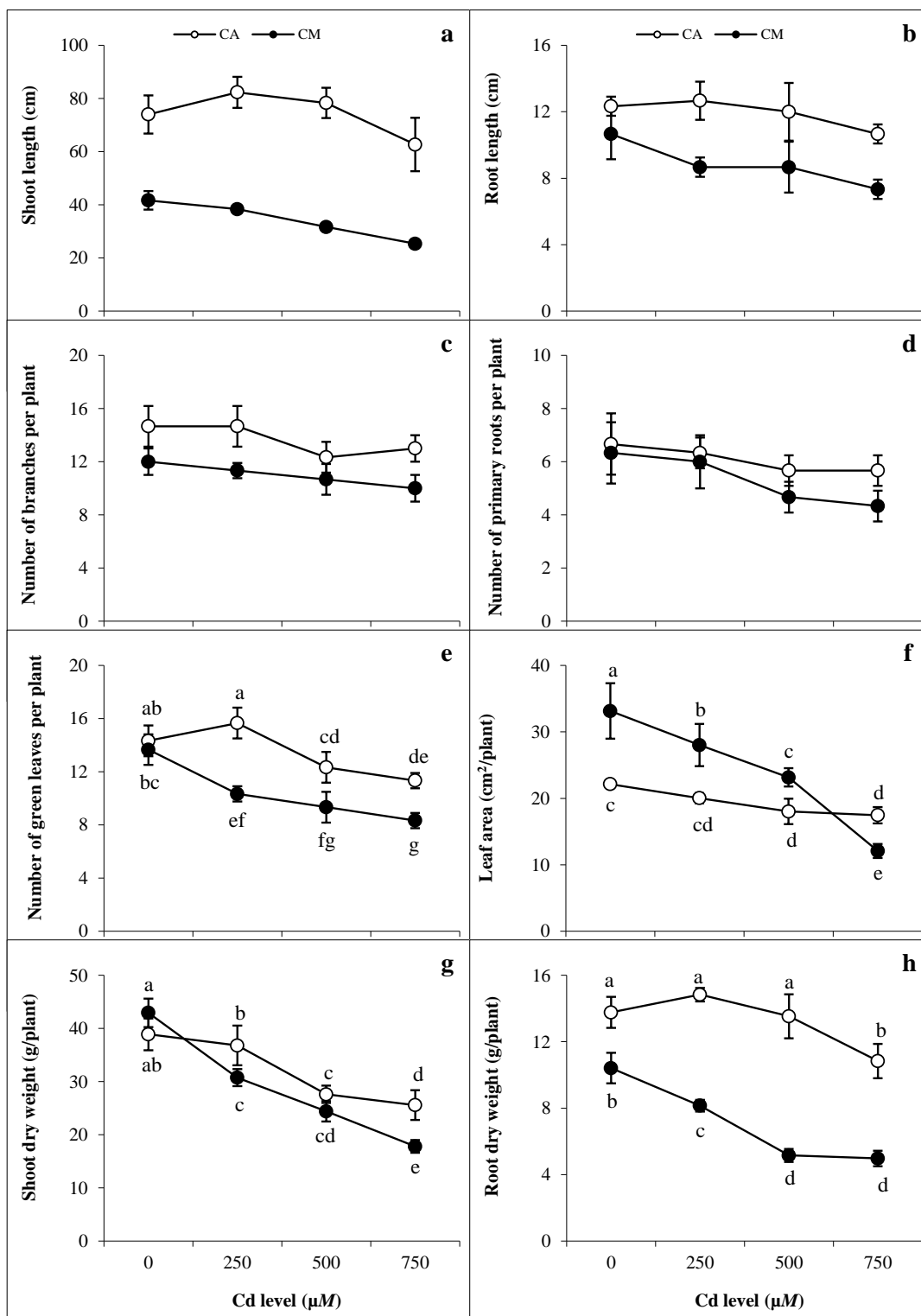


Figure 1. Changes in some growth parameters of *Chenopodium* species grown in field plots contaminated with increased Cd levels. CA and CM stand for *Chenopodium album* and *Chenopodium murale*, respectively

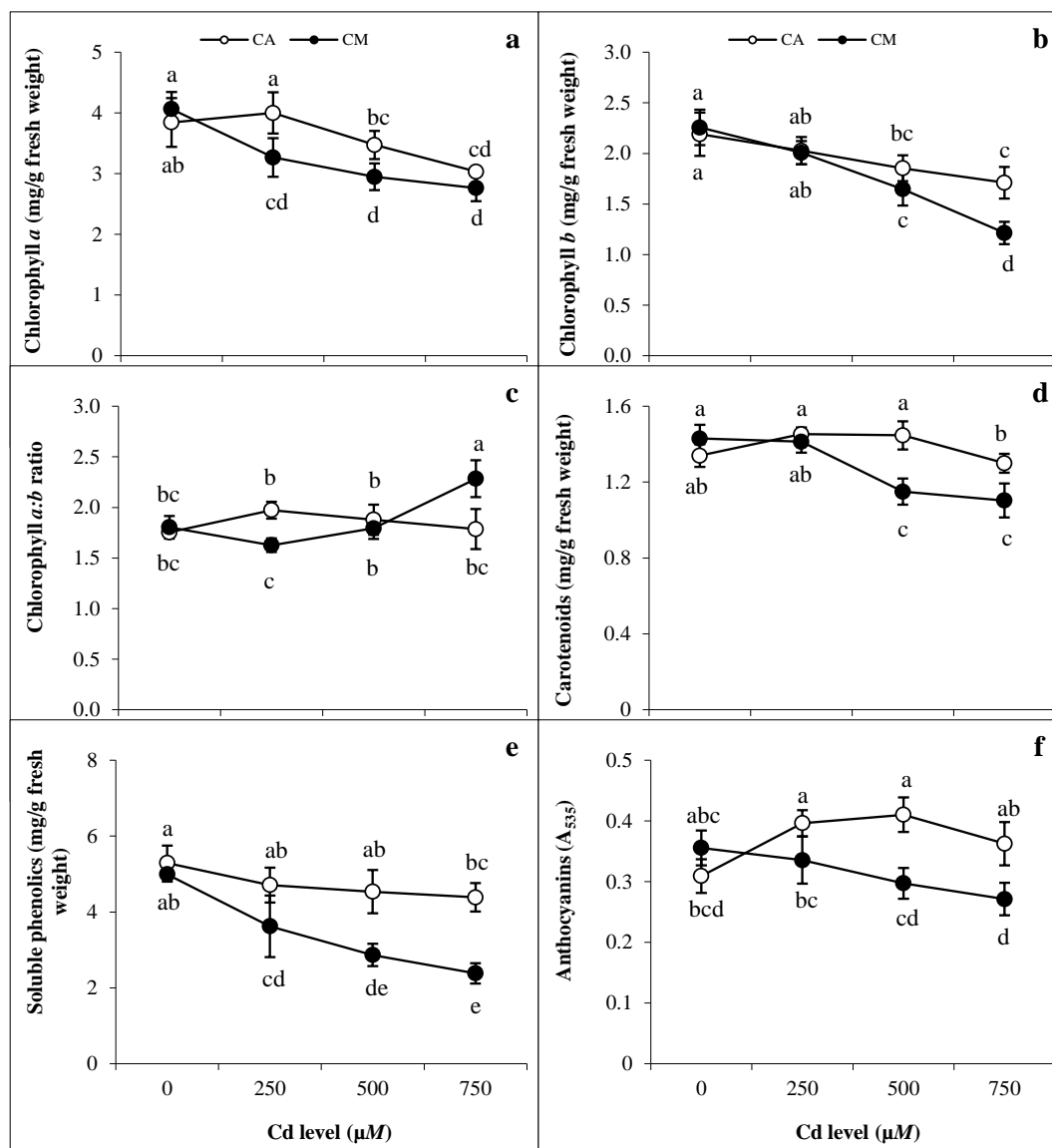


Figure 2. Changes in some metabolite levels of *Chenopodium* species grown in field plots contaminated with increased Cd levels. CA and CM stand for *Chenopodium album* and *Chenopodium murale*, respectively

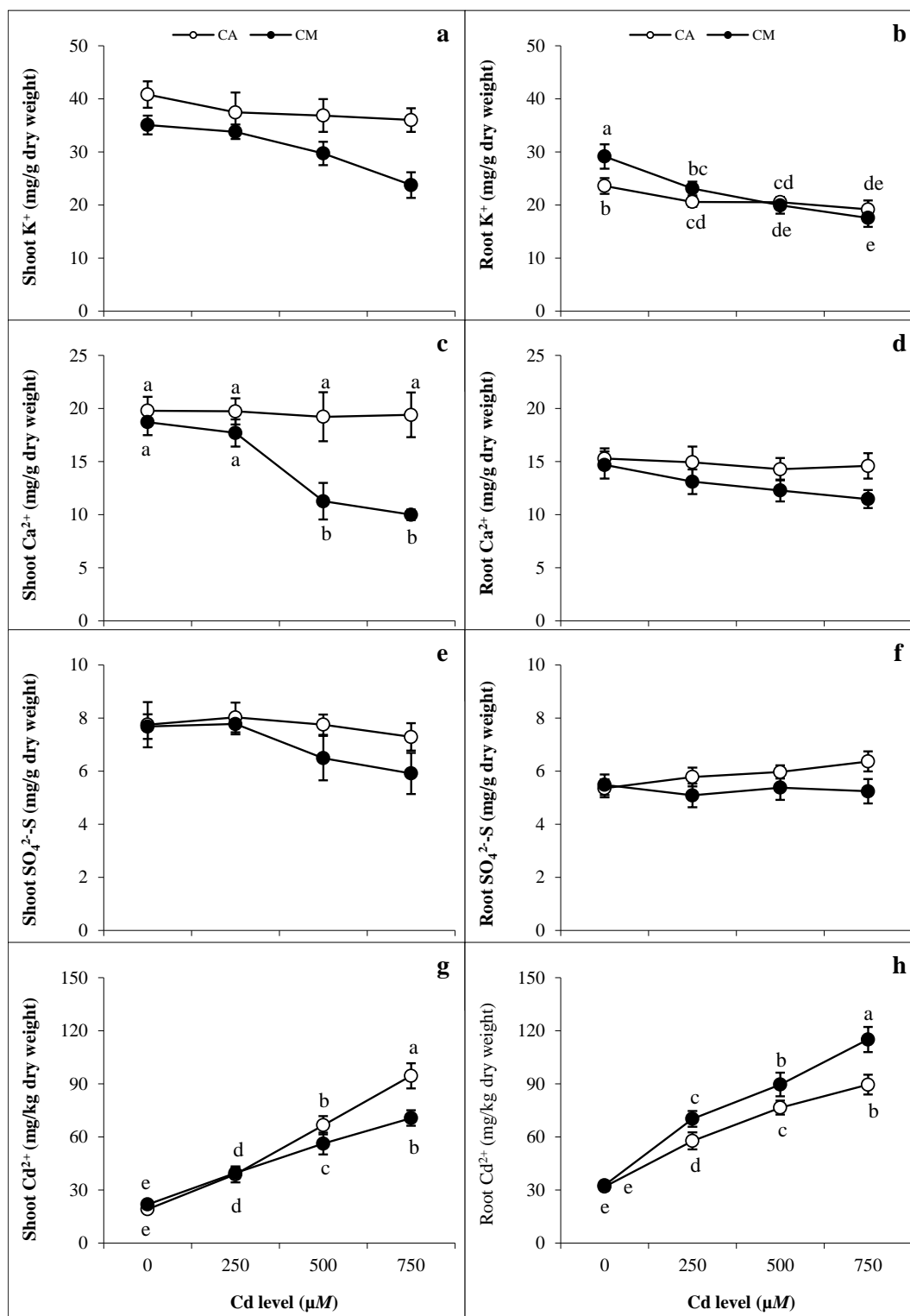


Figure. 3. Changes in some mineral elemental contents of *Chenopodium* species grown in field plots contaminated with increased Cd levels. CA and CM stand for *Chenopodium album* and *Chenopodium murale*, respectively

Cd phytoremediation by *Chenopodium* species: This was determined in terms of changes in germination percentage and post germination seedling mortality in mungbean, and growth attributes of mungbean used as successive crop species. With significant ($P<0.01$) differences among the treatments, the mungbean seeds sown in Cd contaminated plots indicated much lower germination percentage. The order of final germination percentage of mungbean seeds was from the plots: non-contaminated fallow plots > non-contaminated *C. album* plots > non-contaminated *C. murale* plots > 250 μM Cd-*C. album* plots > 250 μM Cd-*C. murale* plots > 500 μM Cd-*C. album* plots > 750 μM Cd-*C. album* plots > 500 μM Cd-*C. murale* plots > 750 μM Cd-*C. murale* plots > 750 μM Cd-fallow plots (Fig. 4a). Likewise, with significant ($P<0.01$) differences amongst treatments, post-germination seedling mortality was the lowest in non-contaminated fallow plots followed by no-Cd *C. album* and *C. murale* plots and *C. album* plots contaminated with 250 μM Cd, while it was the highest in 750 μM Cd contaminated fallow plots followed by 750 and 500 μM Cd contaminated *C. murale* plots (Fig. 4b).

Determinations made for various growth attributes and tissue Cd contents indicated significant ($P<0.01$) differences amongst various treatments in mungbean grown as a successive crop species (Fig. 4a-j). Shoot and root lengths, number of branches per plant and leaf area per plant were the highest in the fallow plots not contaminated with Cd followed by the *C. album* and *C. murale* plots not applied with Cd and those applied with 250 μM Cd. These parameters were the lowest in plots applied with 750 μM Cd followed by *C. murale* plots contaminated with 750 and 500 μM Cd. A highest number of roots and leaves per plant and shoot dry weight was noted in the mungbean grown in *C. album* plots contaminated with 250 μM Cd followed by the *C. album* and *C. murale* plots not contaminated with Cd, while it was the lowest in fallow plots contaminated with 750 μM Cd followed by from the *C. album* and *C. murale* plots applied with 750 and 500 μM Cd, respectively (Fig. 4d).

Higher shoot and root Cd was analyzed in mungbean grown in fallow plots applied with 750 μM Cd followed by those grown in *C. murale* plots treated with 750 and 500 μM Cd. Contrarily, lowest shoot Cd was noted in mungbean grown in fallow plots not contaminated with Cd followed by those grown in from the *C. murale* and *C. album* plots not contaminated with Cd.

DISCUSSION

This study on two native wild species of *Chenopodium*, i.e. *C. album* and *C. murale*, revealed that both these species showed great differences in their response to increased levels of Cd. Although both species indicated a reduction in their growth with increased substrate Cd concentrations, the

performance of *C. album* was better than *C. murale* at all Cd levels. This was evident from the fact that for most of these parameters *C. album* indicated an increased value at 250 μM Cd and incurred a significantly less reduction at 750 μM Cd (Fig. 1a–h). This suggested that *C. album* entails some specific mechanism(s) to withstand the Cd toxicity. In this context, *Chenopodium* species have been reported to manifest great differences for accumulating various heavy metals in the aerial parts (Porębska and Ostrowska, 1999; Bhargava *et al.*, 2008).

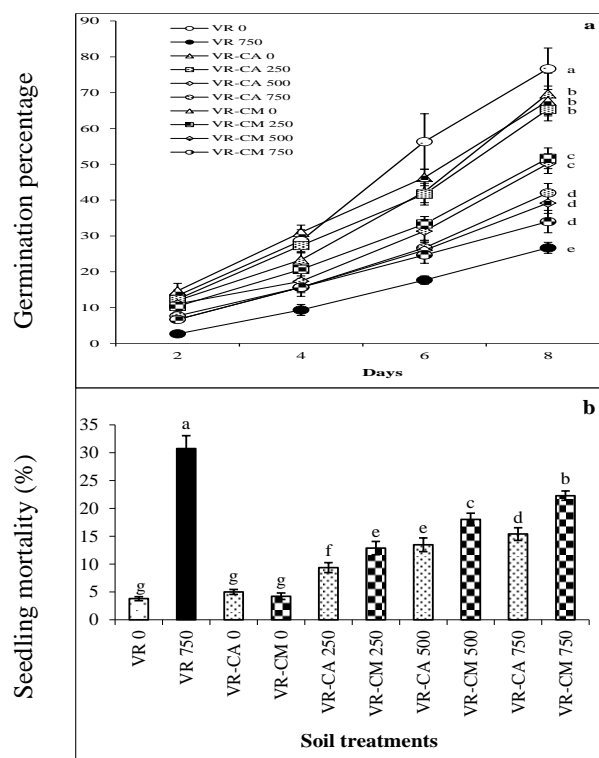


Figure 4. Germination percentage of mungbean seed sown in the plots after harvesting the *Chenopodium* species (*C. album* and *C. murale*). Post-germination seedling mortality was recorded based on death of seedling within three days of emergence. In legends: VR, *Vigna radiata*, CA and CM are *C. album* and *C. murale*, respectively and 0, 250, 500 and 750 are levels of Cd (μM).

To compare metabolic and nutritional responses of *C. album* and *C. murale*, the determinations were made for changes in chlorophylls, carotenoids, soluble phenolics and anthocyanins (Fig. 2a–f). Loss of chlorophyll as a result of damage to chlorophyll protein complexes in thylakoids are often taken as important criteria of sensitivity to metal stress (Fatoba and Udoh, 2008; Wahid *et al.*, 2008). Carotenoids

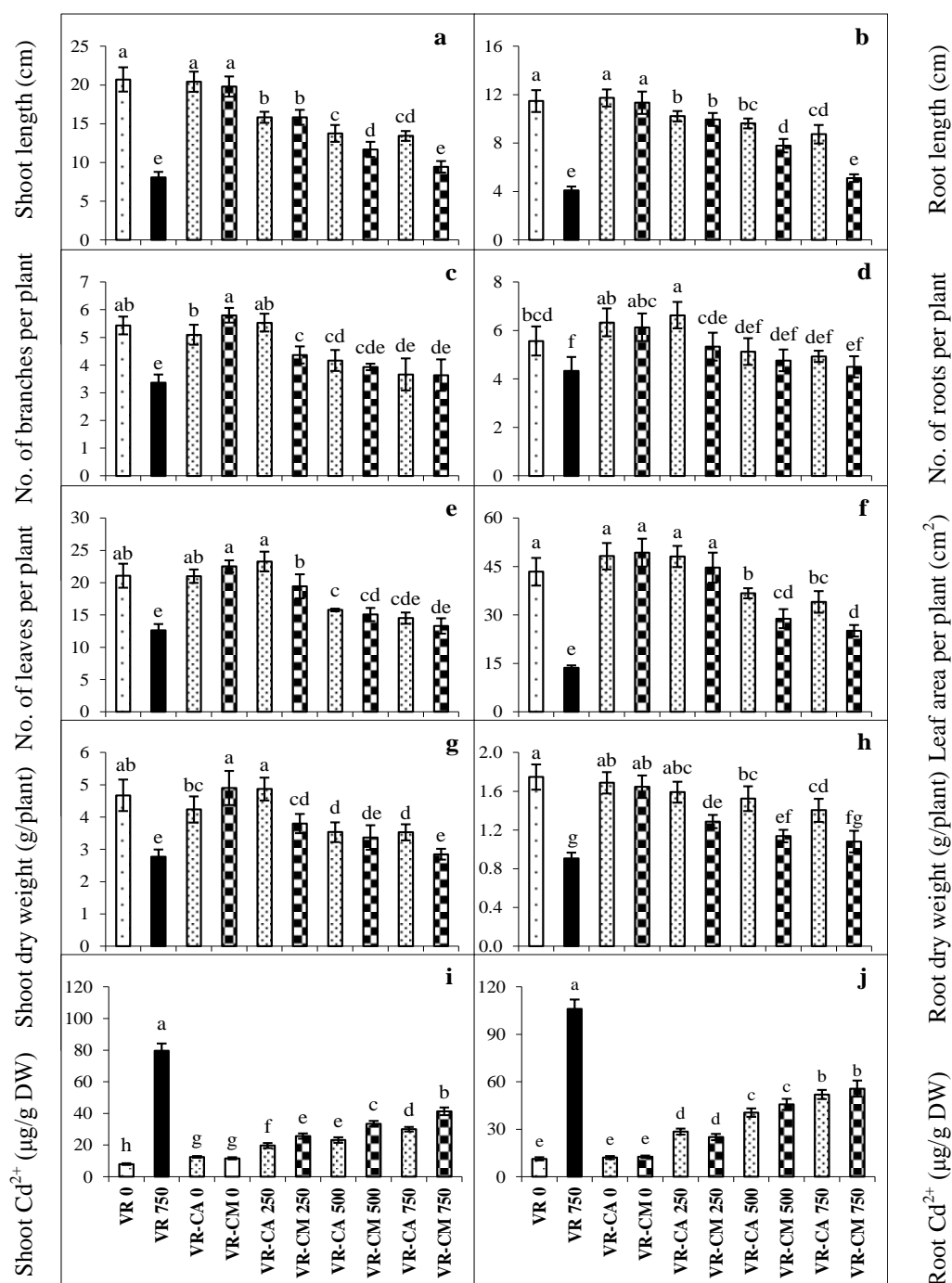


Figure 5. Changes in growth and Cd accumulation properties of mungbean grown as succeeding crop in field plots from where *Chenopodium* was harvested. Thin dotted bars are control; dark shaded bars are Cd contaminated plots where no *Chenopodium* was grown; thick dotted bars pertain to *C. album* and checkered bars represent *C. murale*. In legends: VR, *Vigna radiata*, CA and CM are *C. album* and *C. murale*, respectively and 0, 250, 500 and 750 are levels of Cd (μM).

have multiple roles in plant stress tolerance as their contents may change depending on the type and severity of stress (Wahid *et al.*, 2008). Accumulation of soluble phenolics and anthocyanins has been regarded as an indicator of stress tolerance in plants (Chalker-Scott, 1999; Hale *et al.*, 2001). Data on photosynthetic pigments for both the species in this study revealed that both chlorophyll *a* and *b* species were minimally reduced in *C. album* than *C. murale* and that chlorophyll *a* was increased at 250 μM Cd level in the former species, resulting in increased chlorophyll *a:b* ratio in the former species. Likewise, carotenoids were reduced lowly in *C. album* and substantially in *C. murale*. With wide differences, soluble phenolics although were reduced in both the species, there was markedly greater accumulation of anthocyanins in *C. album* than *C. murale* at all levels of applied Cd. These data suggested that *C. album* could reduce the loss of chlorophyll *a* and anthocyanins even at higher levels of Cd. Here, the role of anthocyanins in Cd tolerance appears to be exclusive since they are reported to complex with heavy metals and sequester them in the vacuole (Chalker-Scott, 1999; Hale *et al.*, 2001). In addition, maintenance of greater carotenoid content is also advantageous in nullifying oxidative damage caused by heavy metals (Ünyayar *et al.*, 2005). In morpho-anatomical terms, *C. album* has more elaborate epidermal cells and succulent leaves than *C. murale* (Ahmad *et al.*, 2009), which may be an adaptive advantage in terms of keeping toxic ions away from physiologically active sites.

It is reported that Cd interferes for the transport of ions such as Ca^{2+} and K^{+} at plasmalemma, thus reducing their concentration in the cytosol and causing toxicity after being competitively taken up (Adhikari *et al.*, 2006; Wahid *et al.*, 2009). However, a cooperative uptake of mineral ions has been reported with Cd (Liu *et al.*, 2007). Heavy metals reduced the assimilation of certain macronutrients when present in soluble form (Gill and Tuteja, 2011). Our results revealed that *C. album*, as compared to *C. murale*, tended to show minimal reduction in shoot and root K^{+} (Fig. 3a,b) no significant change in shoot Ca^{2+} but a reduction in root Ca^{2+} (Fig. 3c,d) as well as reduction in shoot and root $\text{SO}_4^{2-}\text{-S}$ (Fig. 3e, f). Analysis of Cd contents in shoot and root indicated that *C. album* accumulated greater Cd in the shoot than *C. murale*. On the other hand, Cd contents of root were greater in *C. murale* than *C. album* (Fig. 3g, h). Potassium is metabolically important in cytosol and chloroplast, while Ca^{2+} is found both in the cytosol and as structural part of cell wall and certain macromolecules (Epstein and Bloom, 2005). Sulfur is more important nutrient in heavy metal stress tolerance because it is part of phytochlatins and metallothioneins, which are crucially important in cytosolic binding of heavy metals and thus reducing their toxicities and improving plant growth (Liu *et al.*, 2011, 2012). These findings implied that *C. album* had greater capacity to absorb and assimilate the available nutrients than *C. murale*,

which appeared to enable it show better growth under Cd stress. From the increased shoot Cd contents in *C. album* it can be inferred that above-mentioned metabolic changes, especially anthocyanins and plausibly greater synthesis of Cd binding and inactivating proteins could help this species to bind and sequester the excess of Cd in the vacuole (Chalker-Scott, 1999; Hale *et al.*, 2001).

From the changes in the shoot Cd contents of both the species, it was noticed that *C. album* could accumulate excess of Cd in the shoots, which indicates its potential to phytoremediate the soil. To test this possibility, mungbean was grown as successive crop due to its sensitivity to Cd toxicity (Wahid and Ghani, 2008), in those plots in which *C. album* and *C. murale* were grown without or with increased Cd levels as well as in the fallow plots not contaminated with and contaminated with 750 μM Cd for comparison purpose. The results regarding germination (Fig. 4a) and post-emergence mortality of seedlings (Fig. 4b) revealed that both these important parameters were far better in the *C. album* grown plots. Growth data further substantiated that mungbean displayed appreciably better growth in the plots where the *C. album* and *C. murale* were grown as compared to 750 μM Cd contaminated plots fallow plots (Fig. 5a–j). A comparison of both these species revealed that *C. album* was more effective phytoextractor of Cd from soil than *C. murale*, and this effect was related to exclusion of most of the root absorbed Cd to the shoot followed by either its binding by the phytochelations or anthocyanins and sequestration into the vacuole.

We conclude from the above that *C. album* compared to *C. murale* is more efficient phytoremediator of marginally Cd contaminated soils especially in the peri-urban areas. The possible mechanisms involved are fast growth, distinct morpho-anatomical features, metabolic adjustments, Cd sequestration with anthocyanins and maintenance of greater nutrients contents in *C. album*, which otherwise were minimally observed in *C. murale*.

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REFERENCES

- Abdollahi, H., M. Fekri and M. Mahmodabadi. 2011. Effect of heavy metals pollution on pistachio trees. *Int. J. Agric. Biol.* 13:599–602.
- Adhikari, T., E. Tel-Or, Y. Libal and M. Shenker. 2006. Effect of cadmium and iron on rice (*Oryza sativa* L.) plant in chelator-buffered nutrient solution. *J. Plant Nutr.* 29:1919–1940.
- Ahmad, K., M.A. Khan, M. Ahmad, M. Zafar and Zulqarnain. 2009. Morpho-palynological and leaf

- epidermal anatomy of weeds of district Tank, N.W.F.P., Pakistan. Pak. J. Weed Sci. Res. 15:309–320.
- Bahmani, R., M.R. Bihamta, D. Habibi, , P. Forozeshand S.I. Ahmad. 2012. Effect of cadmium chloride on growth parameters of different bean genotypes (*Phaseolus vulgaris* L.) ARPJ J. Agric. Biol. Sci. 7:35–40.
- Bhargava, A., S. Shukla, J. Srivastava, N. Singh, and D. Ohri. 2008. *Chenopodium*: a prospective plant for phytoextraction. Acta Physiol. Plant. 30:111–120.
- Tandon H.L.S. (ed). Fertilizer Development and Consultation Organization, New Delhi, India, pp: 49–82.
- Chitmanat, C. and S. Traichaiyaporn. 2010. Spatial and temporal variations of physical-chemical water quality and some heavy metals in water, sediments and fishes of the Mae Kuang River, Northern Thailand. Int. J. Agric. Biol. 12:816–820.
- Carleton, A.F. and H.W. Foote. 1965. A comparison of methods for estimating total leaf area of barley plant. Crop Sci. 5:602–603.
- Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress responses. Photochem. Photobiol. 70:1–9.
- Chrysafopoulou, E., J. Kadukova and N. Kalogerakis. 2005. A whole plant mathematical model for the phytoextraction of lead (Pb) by maize. Environ. Int. 31:255–262.
- Cosio, C., L. DeSantis, B. Frey, S. Diallo and C. Keller. 2005. Distribution of cadmium in leaves of *Thlaspi caerulescens*. J. Exp. Bot. 56:765–775.
- Davies, B.H. 1976. Carotenoids. In: T.W. Goodwin (ed.). Chemistry and Biochemistry of Plant Pigments. Academic Press London, pp. 138–165.
- Epstein, E. and A.J. Bloom. 2005. Mineral Nutrition of Plants: Principles and Perspectives, 2nd edition. Sinauer Associates, Massachusetts, USA.
- Fatoba, P.O. and E.G. Udoh. 2008. Effect of some heavy metals on chlorophyll accumulation. Ethnobot. Leaflets 12:776–783.
- Ghosh, M. and S.P. Singh. 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. Appl. Ecol. Environ. Res. 3:1–18.
- Gill, S.S. and N. Tuteja. 2011. Cadmium stress tolerance in crop plants. Probing the role of sulfur. Plant Signal Behav. 6:215–222.
- Gupta, A.K. and S. Sinha. 2007. Phytoextraction capacity of the *Chenopodium album* L. grown on soil amended with tannery sludge. Bioresour. Technol. 98:442–446.
- Hale, K.L., S.P. McGrath, E. Lombi, S.M. Stack, N. Terry, I.J. Pickering, G.N. George and E.A.H. Pilon-Smits. 2001. Molybdenum sequestration in *Brassica* species. A role for anthocyanins? Plant Physiol. 126:1391–1402.
- Hussain, A., G. Murtaza, A. Ghafoor, S.M.A. Basra, M. Qadir and M. Sabir. 2010. Cadmium contamination of soils and crops by long term use of raw effluent, ground and canal waters in agricultural lands. Int. J. Agric. Biol. 12:851–856.
- Iqbal, N., A. Masood, R. Nazar, S. Syeed and N.A. Khan. 2010. Photosynthesis, growth and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in cadmium tolerance. Agric. Sci. China 9:519–527.
- James T.K., A. Rahman and J.M. Mellsop. 2005. Fathen (*Chenopodium album*): A biotype resistant to dicamba. New Zealand Plant Prot. 58:152–156.
- Jalloh, M.A., J. Chen, F. Zhen and G. Zhang. 2009. Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress. J. Haz. Mat. 162:1081–1085.
- Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Agric. Food Chem. 33:213–217.
- Khan, N.A., S.S. Samiullah and R. Nazar. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J. Agron. Crop Sci. 193:435–444.
- Kukkola, E., P. Rautio and S. Huttunen. 2000. Stress indicators in copper and nickel exposed scots pine seedlings. Environ. Exp. Bot. 43:197–210.
- Leyval, C., K. Turnau and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: Physiological, ecological and applied aspects. Mycorrhiza 7:139–153.
- Liu, Y.J., Y.G. Zhu and H. Ding. 2007. Lead and cadmium in leaves of deciduous trees in Beijing, China: Development of a metal accumulation index (MAI). Environ. Pollut. 145:387–390.
- Liu, G.-Y., Y.-X. Zhang and T.-Y. Chai. 2011. Phytochelatin synthase of *Thlaspi caerulescens* enhanced tolerance and accumulation of heavy metals when expressed in yeast and tobacco. Plant Cell Rep. 30:1067–1076.
- Liu, Z., C. Gu, F. Chen, D. Yang, K. Wu, S. Chen, J. Jiang and Z. Zhang. 2012. Heterologous expression of a nelumbo nucifera phytochelatin synthase gene enhances cadmium tolerance in *Arabidopsis thaliana*. Appl. Biochem. Biotechnol. 166:722–734.
- Lone, M.I., Z.-L. He, P.J. Stoffella and X.-E. Yang. 2008. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J. Zhejiang Univ. Sci. B 9:210–220.
- Mangkoedihardjo, S. 2007. Phytotechnology integrity in environmental sanitation for sustainable development. J. Appl. Sci. 3:1037–1044.

- Perveen, A., A. Wahid and F. Javed. 2011. Varietal differences in spring and autumn sown maize (*Zea mays*) for tolerance against cadmium toxicity. *Int. J. Agric. Biol.* 13:909–915.
- Porębska, G. and A. Ostrowska. 1999. Heavy metal accumulation in wild plants: Implications for phytoremediation. *Polish J. Environ. Stud.* 8:433–442.
- Prasad, M.N.V. and H.M.O. Freitas. 2003. Metal hyperaccumulation in plants biodiversity prospecting for phytoremediation technology. *Electr. J. Biotechnol.* 6:285–321.
- Sabir, M., A. Ghafoor, Saifullah, M.Z.U. Rehman, H.R. Ahmad and T. Aziz, 2011. Growth and metal ionic composition of *Zea mays* as affected by nickel supplementation in the nutrient solution. *Int. J. Agric. Biol.*, 13:186–190
- Souza, V.L., A.F. Almeida, B.T. Hora, A.S. Gesteira and J.C.M. Cascado. 2008. Preliminary analysis of expressed sequences of genes in *Genipa americana* L. Plant root exposed to cadmium in nutrient solution. *Genet. Mol. Res.* 7:1282–1288.
- Stark, D. and V. Wray. 1989. Anthocyanins: In: *Methods in Plant Biology; Plant Phenolics*. Academic Press, London. Vo. 1, pp. 325–356.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition McGraw Hill Co., New York, USA.
- Unterbrunner, R., M. Puschenreiter, P. Sommer, G. Wieshammer, P. Tlustos, M. Zupan and W.W. Wenzel. 2007. Heavy metal accumulation in trees growing on contaminated sites in Central Europe. *Environ. Pollut* 148:107–114.
- Ünyayar, S., Y. Keleş and F.Ö. Çekiç. 2005. The antioxidative response of two tomato species with different drought tolerances as a result of drought and cadmium stress combinations. *Plant Soil Environ.* 51:57–64.
- Wahid, A. and A. Ghani. 2008. Varietal differences in mungbean (*Vigna radiata*) for growth, yield, toxicity symptoms and cadmium accumulation. *Ann. Appl. Biol.* 152:59–69.
- Wahid, A., A. Ghani and F. Javed. 2008. Effect of cadmium on photosynthesis, nutrition and growth of mungbean. *Agron. Sustain. Dev.* 28:273–280.
- Wahid, A., M. Arshad and M. Farooq. 2009. Cadmium phytotoxicity: responses, mechanisms and mitigation strategies. In: *Advances in Sustainable Agriculture – Book Series*. Ed. E. Lichtfouse, Vol 1, pp. 371–403.
- Yang, X., X.F. Jin, Y. Feng and E. Islam. 2005. Molecular mechanisms and genetic bases of heavy metal tolerance/hyperaccumulation in plants. *J. Integr. Plant Biol.* 47:1025–1035.
- Yoshida, S., D.A. Forno, I. Cock and K.A. Gomez. 1976. *Laboratory Manual for physiological studies of Rice*, IRRI. Los Banos, Philippines.