Pak. J. Bot., 55(6): 2243-2251, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-6(37)

EVALUATION OF MORPHO-ANATOMICAL CHANGES IN CULINARY HERBS IN RESPONSE TO CADMIUM AND LEAD TO EXPLORE THEIR ROLE IN PHYTOREMEDIATION

RIMSHA ANWAR, FARHAN AHMAD KHAN SURI, SABA BUTT AND AISHA SALEEM KHAN*

Kauser Abdulla-School of Life Sciences, Forman Christian College, Lahore, Pakistan
**Corresponding author's email: aishasaleemkhan@fccollege.edu.pk*

Abstract

Main purpose of this research work was to check whether coriander and mint leaves available in various vegetable markets and nurseries of Lahore are safe to consume and free of heavy metals traces. A field experiment was also conducted to evaluate the potential of the selected plants in accumulating Cd and Pb in their different parts in order to further explore their role in phytoremediation through various morpho-anatomical parameters and spectroscopic techniques. Treated plants were analyzed for fresh and dry weight, chlorophyll estimation, stomatal count, changes in cortical and vascular tissues caused in response to Cd and Pb. Metals uptake in plant tissues was confirmed by atomic absorption spectroscopy (AAS). In coriander, less uptake of both metals was reported in aerial parts. However, mint accumulated relatively higher amount of both metals in aerial parts which was evident through reduction in length of plants, leaf area and through inhibition of vascular tissues. AAS further revealed that metals were also translocated to leaves of mint, suggesting that plant can be used for phytoremediation of Cd and Pb.

Key words: Cadmium, Herbs, Lead, Phytoremediation.

Introduction

Increase in industrialization and mining activities including excessive applications of herbicides and pesticides, and traffic exhaust are responsible for release of heavy metals in the environment. Heavy metals like arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) are harmful for plants and animals as reported by many workers (Garcia *et al*., 2006; Shahid *et al*., 2012; Su & Liang, 2015; Harguinteguy *et al*., 2016 and Jiang *et al*., 2018). They are known to affect overall growth of plants (Khan *et al*., 2016; Haider *et al*., 2021) as their uptake is related with inhibition in seed germination (Soudek *et al*., 2010) and reduction in vascular tissues (Khan & Rehan, 2014; Wafee *et al*., 2018).

Uptake of heavy metals like Cd and Pb is known to interfere with micronutrients levels, results in an increase in oxidative damage and decrease in chlorophyll content (Gallego *et al*., 2012) by distrupting major metabolic pathways (Rizwan *et al*., 2016; Candido *et al*., 2020); thereby affecting the overall growth and development of plants (Haider *et al*., 2021). Similarly Pb is also known to cause damage to photosynthetic pigments (He *et al*., 2016; Jiang *et al*., 2018 ; Taylor, 2019; Aslam *et al*., 2021) and delay in floral initiation (Chaudhry & Khan, 2006).

Many plants have developed detoxifying mechanism to overcome the effects of heavy metals therefore, ecofriendly techniques like phytoremediation are gaining more attention in the past few decades which aim to remove pollutants from the environment through the use of hyperaccumulators which can accumulate heavy metals (Yan *et al*., 2020). Main approaches being used in phytoremediation include rhizofiltration, phytoextraction, phytodegradation and phytovolatilization (Rascio & Navari-Izzo, 2011; Suman *et al*., 2018; Shojaei *et al*., 2021). Hyperaccumulators are plant species with potential to accumulate heavy metals almost 50-100 times more as compared with non-hyperaccumulators without showing any toxicity symptoms (McGrath & Zhao, 2003; Amirmoradi, 2012; van der Ent *et al*., 2013; Wang *et al*., 2017; Emamverdiani & Ding, 2018). These plant species either accumulate metals in different tissues, prevent their uptake to aerial parts, detoxify them or move them into smaller number of cells so they do not interfere their metabolic activities. Some hyperaccumulators like *Brassica pekinensis* and *Pelargonium* are capable of transporting higher amount of Pb to aerial parts without showing any damage to metabolic functions (Humberto *et al*., 2020). Plants like water hyacinth and rice can also produce proteins which bind with Cd to reduce its toxic effects (Prasad, 1995).

Material and Methods

Sample collection and experiment: Almost 6 to 7 young plants (*C. sativum*) and (*M. piperita*) of same height were placed in beakers of 500 ml capacity, containing metal solutions and were labelled. Different concentrations of $CdCl_2$ and $PbCl_2$ applied individually for a month were 50 ppm, 100 ppm, 150 ppm, and 300 ppm. Control plants were treated with tap water. Hoagland nutrient solution was also provided to these plants twice a week. All plants were placed under same conditions.

Internal morphology: Length and width of cells were measured using ocular micrometer in transverse and longitudinal sections and slides were photographed under light microscope. Cortical, vascular and pith region were measured in stems tissus.

Chlorophyll estimation: For the chlorophyll analysis of coriander and mint leaves, acetone was used as solvent because chlorophyll requires a polar solvent for being solvated. Approximately 4 cm leaf was cut, weighed and crushed using pestle and mortar. Then 2 ml of acetone was used to grind the leaf material and another 2 ml of

acetone was used for washing. The resulting solution was added into centrifuge tube and the final volume was made up to 7ml by adding more acetone. This was done for each sample. The obtained solution was then centrifuged at 10,000 rpm for 5 minutes. The supernatant had the amount of chlorophyll present in the samples. Later, 2-3 ml of this supernatant was transferred into a cuvette and the absorption was measured and recorded at two different wavelengths for estimating the amount of chlorophyll a and chlorophyll b. Acetone was used as blank in spectrophotometry. The absorbance of samples for estimating chlorophyll 'a' and 'b' was measured at 663nm and 645nm respectively (Arnon, 1949).

Stomatal count: In control and treated plants, lower epidermis of coriander and mint leaves was used to count stomata through an indirect method. Transparent nail polish was applied on the lower side of leaf and was allowed to dry for half a minute. Then the scotch tape cuttings were placed over that polished area and was pressed carefully to get impregnated image of stomata on the tape. These tape cuttings were placed over slides and were viewed under light microscope at 40X magnification. For each plant, an average number of open stomatal pores were recorded.

Atomic absorption spectroscopy (AAS): Samples were taken from roots, stems and leaves and were digested for atomic absorption spectroscopy. For digestion, 0.1 gram of plant material was weighed and digested in a mixture containing 25 ml of HNO_3 and 10 ml of H_2SO_4. Mixture was carefully added in approximately 50 ml of distilled water, samples were filtered and tested through flame atomic absorption spectrometry (Varian AA240FS, US). The same procedure was followed for the samples placed at different metal concentrations and all the reading were carefully recorded.

Fresh and dry weight: For fresh weight, treated plants were carefully pat dried using blotting paper and were weighed using weighing balance. The weights were recorded, and these same samples were left to dry in oven, at 70°C, overnight. After 24 hours, these samples were taken out and weighed again. This weight was recorded as dry weight. Same was done with all the plant samples of coriander and mint.

Growth measurements: Growth was expressed relative to control plants and data given were the average of at least three independent experiments ± standard deviation, calculated according to the following expression.

$$\text{Relative growth inhibition (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Results

Coriander and mint treated with $CdCl_2$ and $PbCl_2$ showed considerable changes in their root and shoot length. Plants treated with these metals showed wilting as compared to the control (Figs. 1, 2). Noticeable decrease in stomatal count of treated plants was observed in comparison with control. Cd-treated coriander plants evidently showed differences in fresh and dry weights (Fig. 3) while some Pb-treated plants showed gradual decrease in fresh and dry weights in comparison with control group (Fig. 4). Similar results were recorded for fresh and dry weight in case of mint treated with Cd and Pb (Figs. 5, 6). Moreover, there was no noticeable change in leaf area of both coriander and mint plants.

Fig. 1. Plants treated with $CdCl_2$.

Fig. 2. Plants treated with $PbCl_2$.

In coriander, maximum reduction in average diameter of root cell cortex was 13% as compared to control, at 300 ppm $CdCl_2$ while maximum decrease in cortical width at same concentration of $PbCl_2$ was 31% (Table 1, Figs.7-11). In case of Pb, an immediate gradual decrease was observed in vascular bundles of roots which caused an inhibition in overall growth of root (Figs. 12-16). Cortical region of stem cells showed relatively less changes in treated plants as compared to the cortex of root. Significant decrease of 23% and 27% was observed in cortical region of stem cells treated with 150 ppm and 300 ppm of $CdCl_2$ respectively. (Table 2). However, magnitude of inhibition was wider in stems of Pb treated coriander plants as compared to Cd treated coriander plants (Figs. 17-26).

In transverse and longitudinal sections of both plants, similar pattern of changes in cell sizes were observed. In case of stem cells, average diameter of cortex and vascular bundles showed a gradual decrease in width (Tables 3-7) (Figs. 27-31) with the exception that average diameter of pith region in mint stem cells increased gradually with increase in metal concentrations (Figs. 32-41).

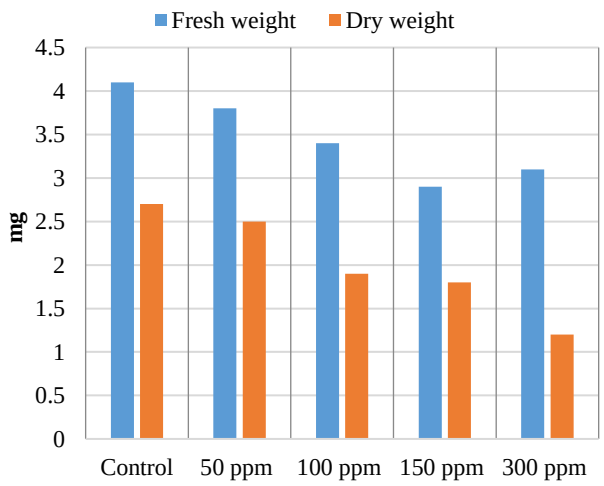

Fig. 3. Fresh weigh and dry weight of coriander (whole plant) treated with $CdCl_2$..

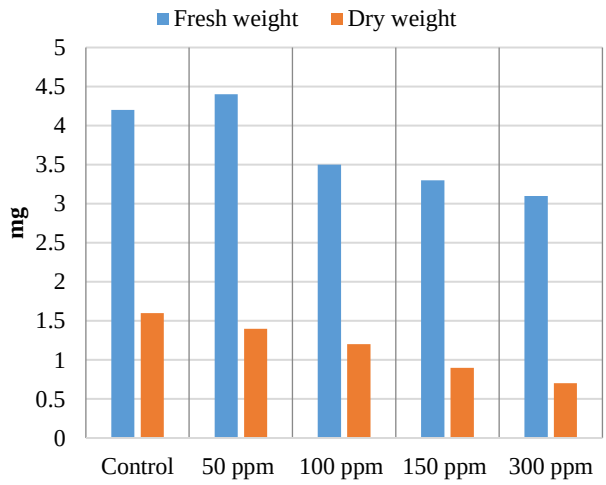

Fig. 4. Fresh weigh and dry weight of coriander (whole plant) treated with $PbCl_2$.

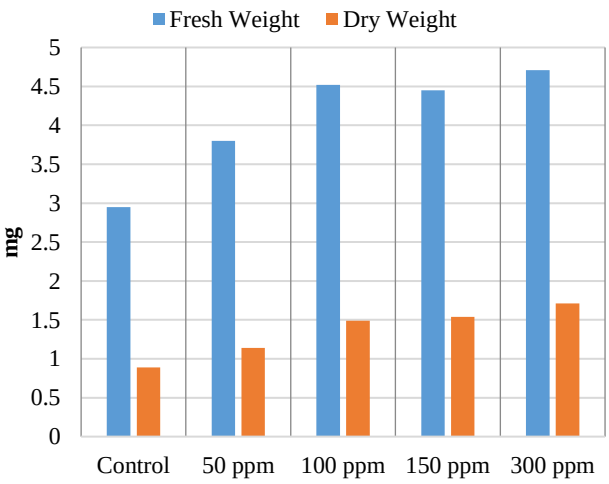

Fig. 5. Fresh weight and dry weight of *M. piperita* (whole plant) treated with $CdCl_2$.

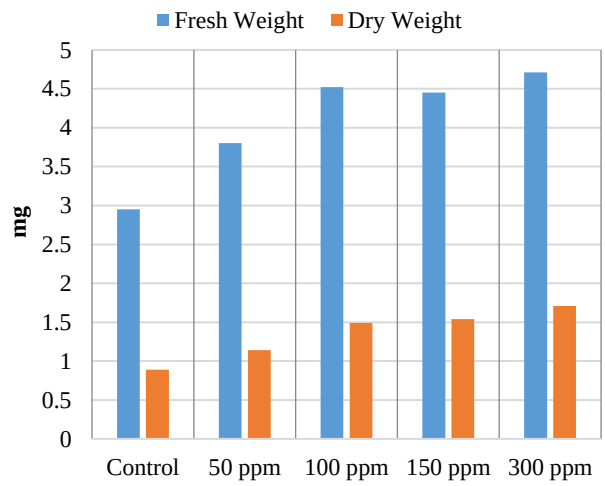

Fig. 6. Fresh weigh and dry weight of *M. piperita* (whole plant) treated with $PbCl_2$.

Table 1. Effects of doses of $CdCl_2$ on cell sizes in transverse sections of *C. sativum* roots.

Treatments (ppm)	Average diameter of xylem (µm)	Average diameter of cortex (µm)
Control	792	2013
50 ppm $CdCl_2$	693	1881
100 ppm $CdCl_2$	561	1875
150 ppm $CdCl_2$	528	1782
300 ppm $CdCl_2$	496	1756

Table 2. Effects of doses of $PbCl_2$ on cell sizes in transverse sections of *C. sativum* roots.

Treatments (ppm)	Average diameter of xylem (µm)	Average diameter of cortex (µm)
Control	1815	2805
50 ppm $PbCl_2$	1650	2640
100 ppm $PbCl_2$	1584	2508
150 ppm $PbCl_2$	1186	2244
300 ppm $PbCl_2$	1050	1948

Table 3. Effects of doses of $CdCl_2$ on cell sizes in transverse section of *C. Sativum* stems.

Treatments (ppm)	Average diameter of xylem (µm)	Average diameter of cortex (µm)
Control	396	1551
50 ppm $CdCl_2$	363	1485
100 ppm $CdCl_2$	366	1188
150 ppm $CdCl_2$	260	1185
300 ppm $CdCl_2$	231	1122

Table 4. Effects of doses of $PbCl_2$ on cell sizes in longitudinal section of *C. sativum* stems.

Treatments (ppm)	Average diameter of xylem (µm)	Average diameter of cortex (µm)
Control	660	1716
50 ppm $PbCl_2$	495	1584
100 ppm $PbCl_2$	429	1353
150 ppm $PbCl_2$	330	1320
300 ppm $PbCl_2$	297	1250

Table 5. Effects of doses of $CdCl_2$ on cell sizes in transverse section of *M. piperita* roots.

Treatments (ppm)	Average diameter of vascular bundle	Average diameter of cortex	Average diameter of epidermis
Control	891	1056	1485
50 ppm $CdCl_2$	726	726	1320
100 ppm $CdCl_2$	495	693	1155
150 ppm $CdCl_2$	759	825	1419
300 ppm $CdCl_2$	627	759	1352

Table 6. Effects of doses of $CdCl_2$ on cell sizes in transverse section of *M. piperita* stems.

Treatments (ppm)	Average diameter of vascular bundle	Average diameter of pith	Average diameter of cortex
Control	792	1188	1848
50 ppm $CdCl_2$	825	1188	1881
100 ppm $CdCl_2$	660	1320	1749
150 ppm $CdCl_2$	528	1485	1650
300 ppm $CdCl_2$	330	1551	1320

Table 7. Effects of doses of $PbCl_2$ on cell sizes in transverse section of *M. piperita* stems.

Treatments (ppm)	Average diameter of vascular bundle	Average diameter of pith	Average diameter of cortex
Control	792	858	1914
50 ppm $PbCl_2$	825	924	2145
100 ppm $PbCl_2$	660	990	1980
150 ppm $PbCl_2$	462	1221	2046
300 ppm $PbCl_2$	396	1386	1914

For chlorophyll estimation, chlorophyll 'a' and chlorophyll 'b' both were measured in treated plants as well as in control groups. Coriander plants treated with $CdCl_2$ and $PbCl_2$ showed a some decrease in chlorophyll content, however, in some cases, an increase in chlorophyll content was recorded when compared with control (Figs. 42, 43). For mint leaves, chlorophyll 'a' and 'b' revealed a change in comparison with control (Figs. 44, 45).

AAS results showed that the Cd uptake in coriander was least in roots of control group i.e, 0.004 mg/L (Fig. 46). The amount of Cd uptake in roots was increased with increase in metal concentrations. At 100 ppm $CdCl_2$, the amount of Cd was 0.008 mg/L which increased to 0.01 mg/L under 150 ppm $CdCl_2$. However, maximum Cd uptake of 0.027 mg/L was reported at 300 ppm $CdCl_2$. Coriander plant when treated with Pb, reported relatively more Pb content in roots and leaves as compared to plants treated with Cd. In case of root tissues, it was revealed through AAS that the amount of Pb in control was 0.38 mg/. With application of 50 ppm $PbCl_2$, the amount of Pb increased by 0.51 mg/L as compared to control. At 100 ppm and 150 ppm $PbCl_2$ the amount of Pb was 0.69 mg/L and 0.6 mg/L respectively. However, at 300 ppm $PbCl_2$, the amount of Pb increased to 0.85 mg/L (Figs. 47, 48).

In case of leaves treated with Cd, there was no considerable uptake of Cd at any concentration amount of Pb in control group was 0.32 mg/L that increased by 0.11 mg/L with application of 150 ppm $PbCl_2$. Cd uptake reported at 300 ppm $PbCl_2$ was 0.31 mg/L (Fig. 49).

In case of mint plants, results revealed that amount of Cd in leaves of control plants was 0.05 mg/L. At 50 ppm and 100 ppm $CdCl_2$ the amount of Cd increased by 0.06 mg/L and 0.1 mg/L as compared to control. However, amount of Cd decreased to 0.25 mg/L as compared to 100 ppm $CdCl_2$. At 300 ppm amount of Cd was reported to be 0.18 mg/L (Fig. 50). Likewise, for root tissues, the amount of Cd was 0.003 mg/L. On application of 50 ppm and 100 ppm $CdCl_2$, amount of Cd increased to 0.2 mg/L and 0.357 mg/L respectively. Accumulation of 0.32 mg/L was reported at 150 ppm $CdCl_2$. However, 0.21 mg/L uptake was recorded at 300 ppm $CdCl_2$. Amount of Pb in control root was 0.2 mg/L (Fig. 51). After application of 50 ppm $PbCl_2$, the amount of Pb increased by 0.1 mg/L. Similarly, 100 ppm and 150 ppm $PbCl_2$ concentrations also showed an increase in amount Pb by 0.2 mg/L and 0.5 mg/L respectively. Furthermore, amount of Pb accumulated at 300 ppm $PbCl_2$ was 0.4 mg/L. In case of leaves of control group, amount of Pb was 0.22 mg/L and increased by 0.09 mg/L upon treating plants with 50 ppm $CdCl_2$ and 100 ppm $CdCl_2$. At 150 ppm and 300 ppm $CdCl_2$ amount of Pb recorded was 0.33 mg/L. 0.39 mg/L respectively. Hence, leaves reported less metal accumulation as compared to roots. Mint samples collected from vegetable markets of 5 different areas ; Gulberg, Thokar niaz baig, Shadrah, Jallo park and Allama Iqbal Town reported Pb in them. Results through AAS revealed maximum of 0.48 mg/L in samples collected from Shadrah (Fig. 52). However almost all the samples showed negligible or no Cd uptake at all.

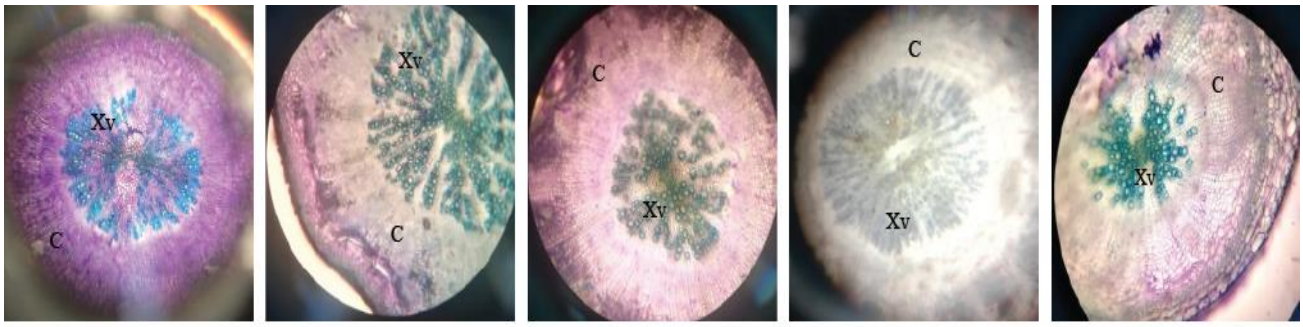

Fig. 7 Fig. 8 Fig. 9 Fig. 10 Fig. 11

Figs. 7-11: T.S of roots of *C. sativum* under 10X magnification (Fig. 7) Control root (Fig. 8) Root treated with 50 ppm $CdCl_2$ (Fig. 9) Root treated with 100 ppm $CdCl_2$ (Fig. 10) Root treated with 150 ppm $CdCl_2$. (Fig. 11) Root treated with 300 ppm $CdCl_2$.

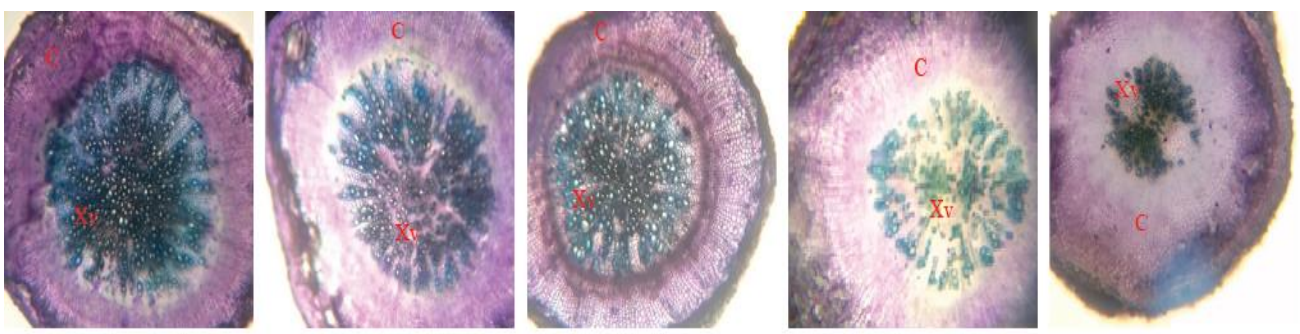

Fig. 12 Fig. 13 Fig. 14 Fig. 15 Fig. 16

Figs. 12-16. T.S of *C. sativum* root under 4X magnification. (Fig. 12) Control root (Fig. 13) Root treated with 50 ppm $PbCl_2$. (Fig. 14) Root treated with 100 ppm $PbCl_2$ (Fig. 15) Root treated with 150 ppm $PbCl_2$ (Fig. 16) Root treated with 300 ppm $CdCl_2$.

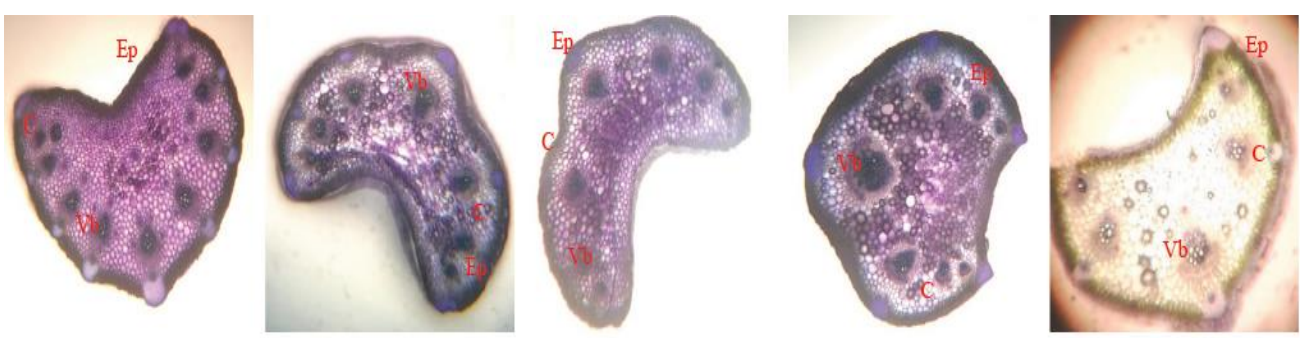

Fig. 17 Fig. 18 Fig. 19 Fig. 20 Fig. 21

Fig. 17-21. T.S of stems of *C. sativum* treated with $CdCl_2$, under 4X magnification. (Fig. 17) Control stem (Fig. 18) Stem treated with 50 ppm $CdCl_2$. (Fig. 19) Stem treated with 100 ppm $CdCl_2$ (Fig. 20) Stem treated with 150 ppm $CdCl_2$ (Fig. 21) Stem treated with 300 ppm $CdCl_2$.

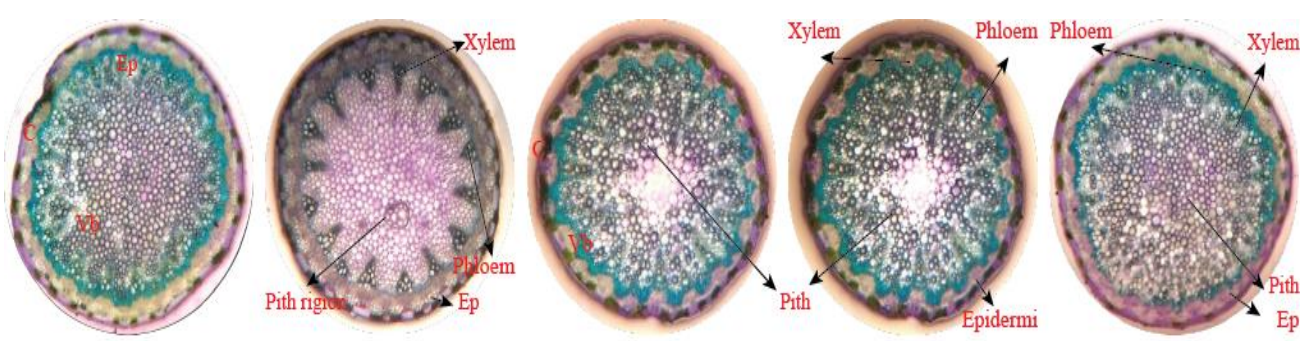

Fig. 22 Fig. 23 Fig. 24 Fig. 25 Fig. 26

Figs. 22-26. T.S of stems of *C. sativum* treated with PbCl2, under 4X magnification. (Fig. 22) Control stem (Fig. 23) Stem treated with 50 ppm $PbCl_2$.(Fig. 24) Stem treated with 100 ppm $PbCl_2$ (Fig. 25) Stem treated with 150 ppm $PbCl_2$ (Fig. 26) Stem treated with 300 ppm $PbCl_2$.

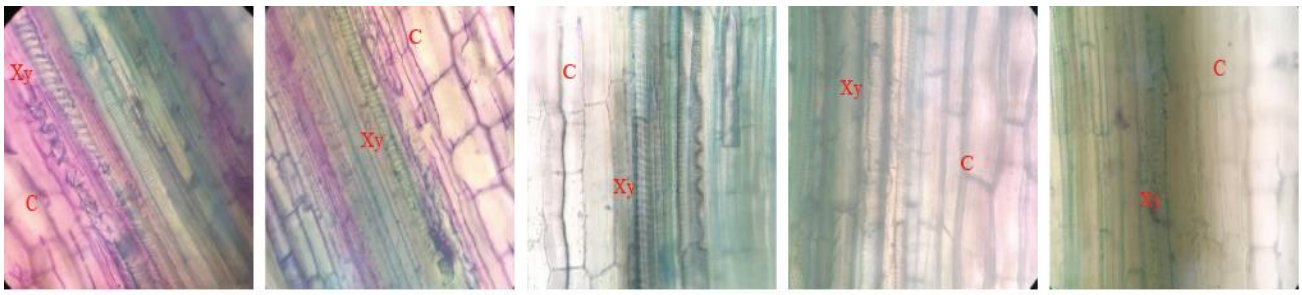

Fig. 27 Fig. 28 Fig. 29 Fig. 30 Fig. 31

Figs. 27-31. L.S sections of control stems of *M. piperita.* Control stem (Fig. 27) Stem of *M. piperita* treated with $CdCl_2$ (Fig. 28) Stem treated with 100 ppm $CdCl_2$. (Fig. 29) Stem treated with 150 ppm $CdCl_2$ (Fig. 30) Stem treated with 300 ppm $CdCl_2$ (Fig. 31).

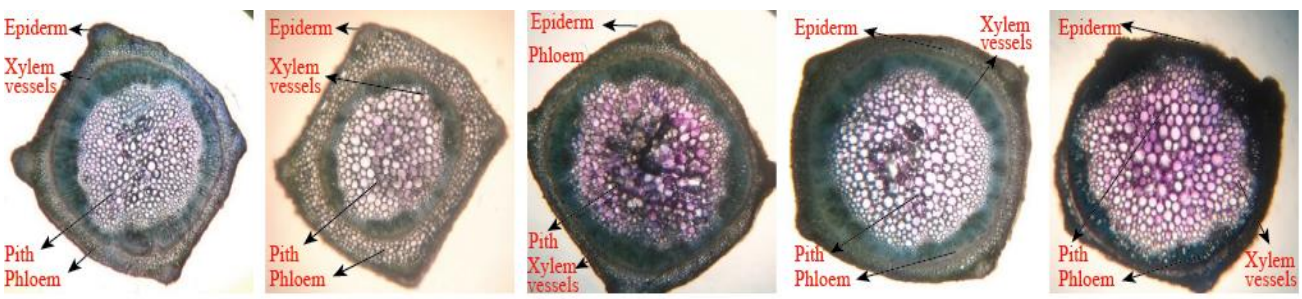

Fig. 32 Fig. 33 Fig. 34 Fig. 35 Fig. 36

Figs. 32-36. T.S sections of stems of *M. piperita* treated with $CdCl_2$. Control stem (Fig. 32) Stem treated with $CdCl_2$ (Fig. 33) Stems treated with 100 ppm $CdCl_2$. (Fig. 34) Stem treated with 150 ppm $CdCl_2$ (Fig. 35) Stem treated with 300 ppm $CdCl_2$. (Fig. 36).

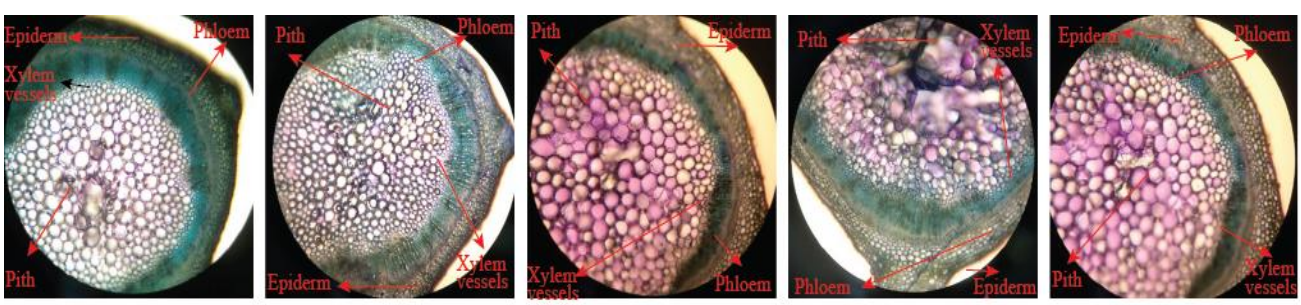

Fig. 37 Fig. 38 Fig. 39 Fig. 40 Fig. 41

Figs. 37-41. T.S sections of stems of *M. piperita* treated with $PbCl_2$. Control stem (Fig. 37) stem treated with 50ppm $PbCl_2$. (Fig. 38) Stem treated with 100 ppm $PbCl_2$. (Fig. 39) Stem treated with 150 ppm $PbCl_2$. (Fig. 40) Stems treated with 300 ppm $PbCl_2$ (Fig. 41).

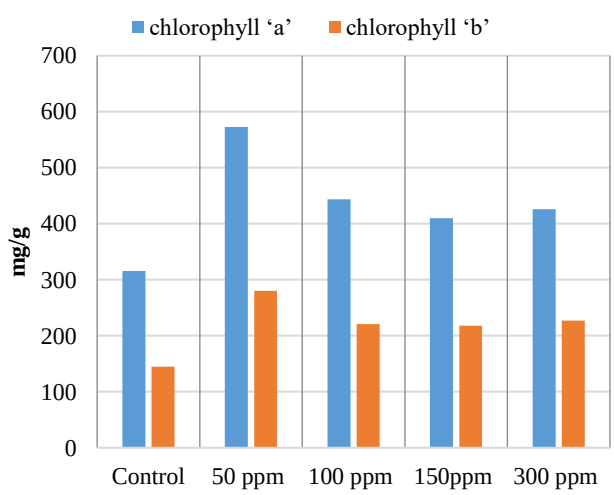

Fig. 42. Comparison of chlorophyll 'a' and 'b' in coriander leaves treated with $CdCl_2$.

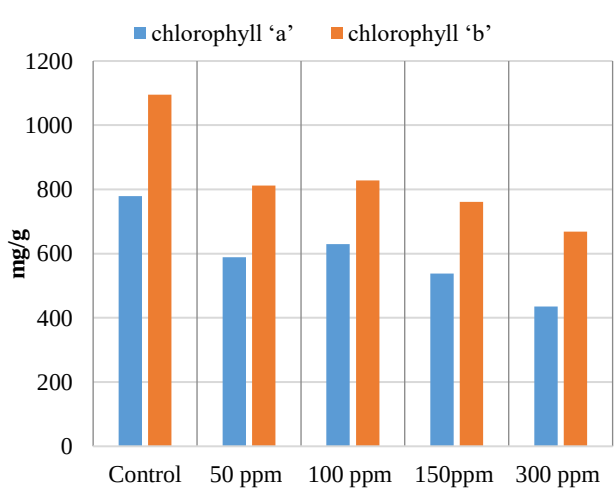

Fig. 43. Comparison of chlorophyll 'a' and 'b' in coriander leaves treated with $PbCl_2$.

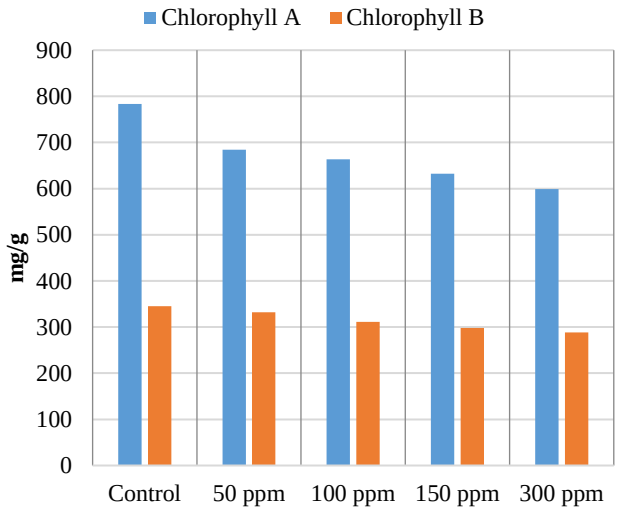

Fig. 44. Comparison of chlorophyll 'a' and 'b' in *M. piperita* leaves treated with $CdCl_2$.

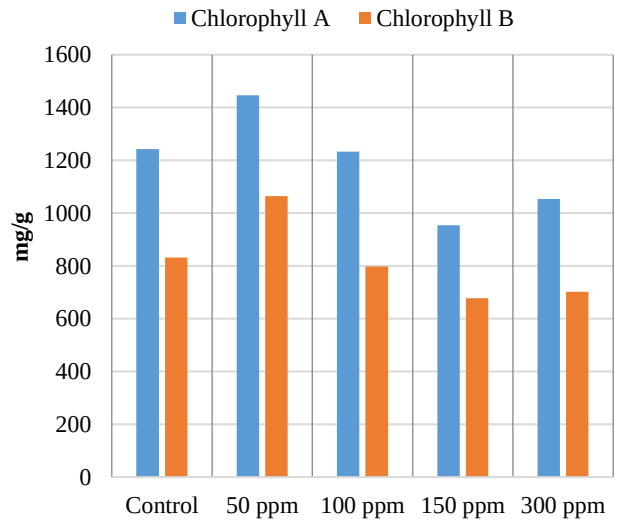

Fig. 45. Comparison of chlorophyll 'a' and 'b' in *M. piperita* leaves treated with $PbCl_2$

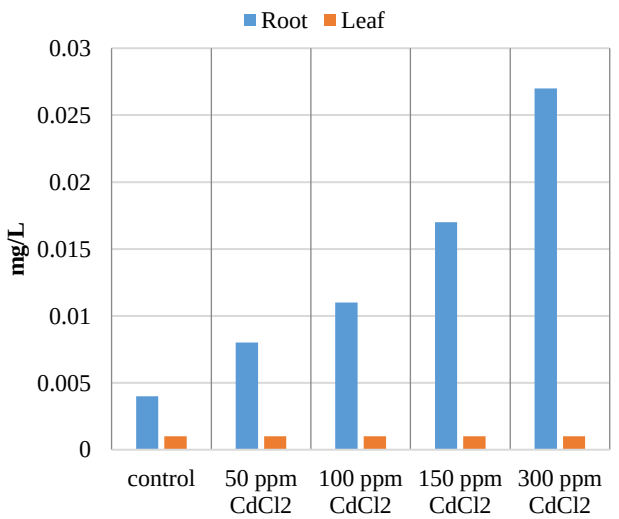

Fig. 46. Accumulation of $CdCl_2$ (mg/L) in roots and leaves *of C. sativum* through atomic absorption spectroscopy.

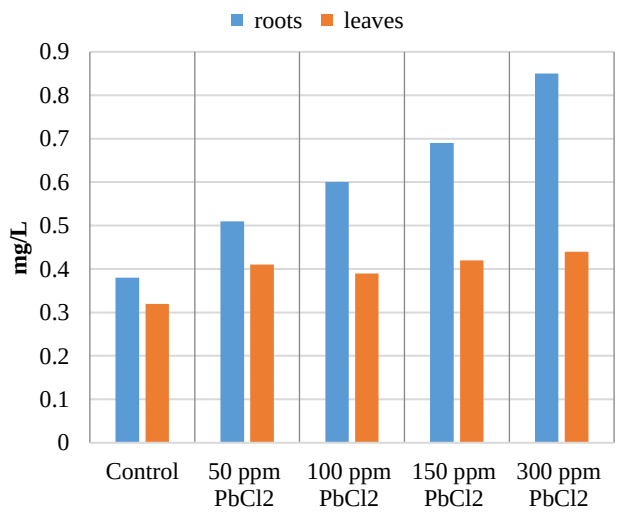

Fig. 47. Accumulation of $PbCl_2$ (mg/L) in roots and leaves *of C. sativum* through atomic absorption spectroscopy.

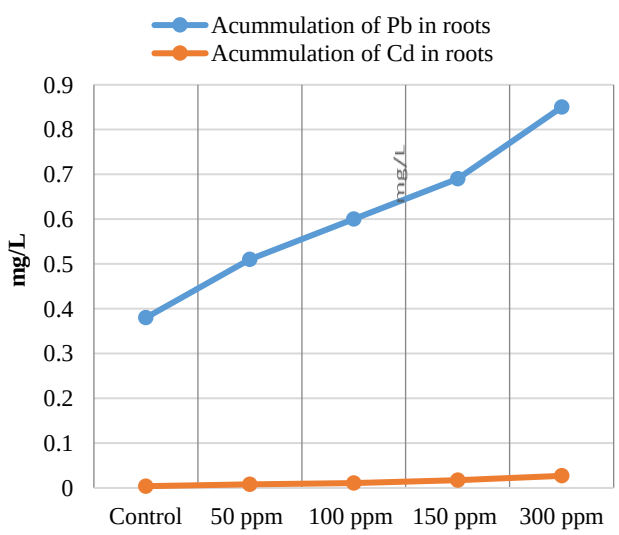

Fig. 48. Comparison of Cd and Pb uptake (mg/L) in roots of *C. sativum*.

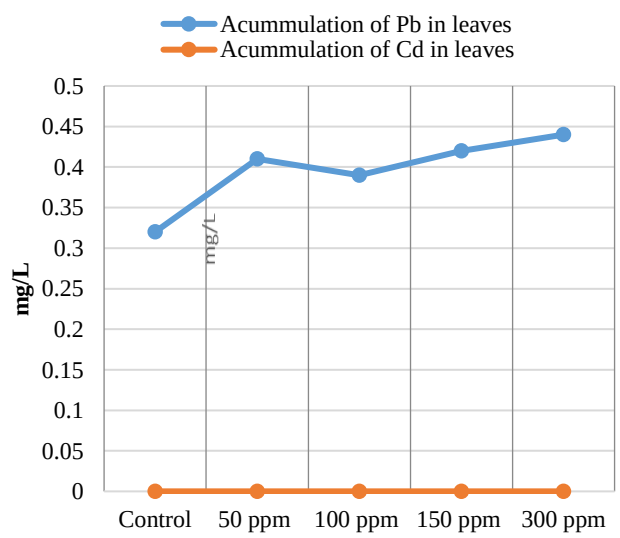

Fig. 49. Comparison of Cd and Pb uptake (mg/L) in leaves of *C. sativum*.

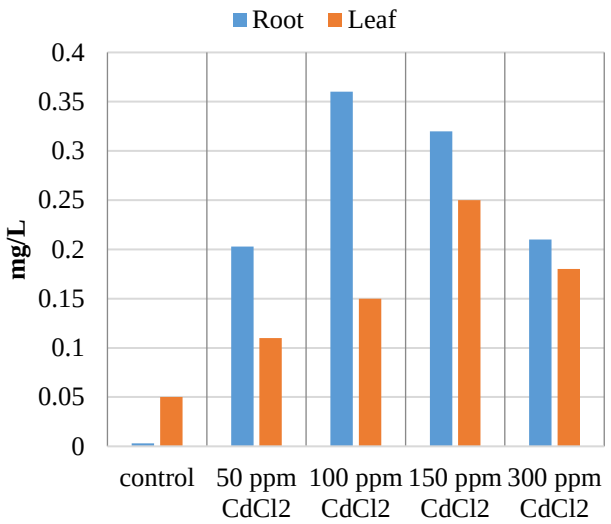

Fig. 50. Accumulation of $CdCl_2$ (mg/L) in roots and leaves of *M. piperita* through atomic absorption spectroscopy.

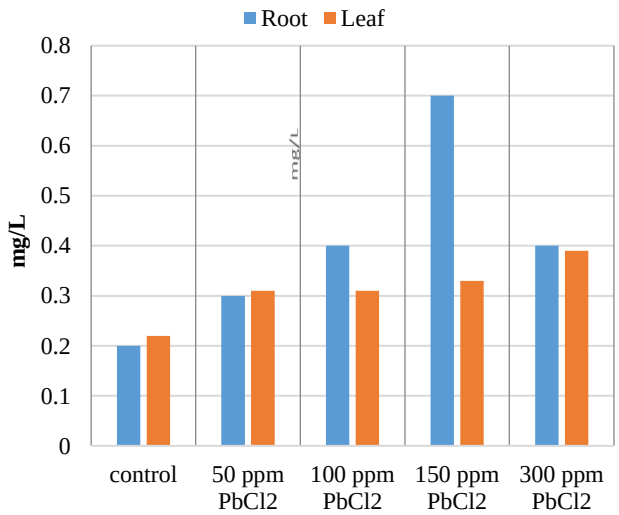

Fig. 51. Accumulation of $PbCl_2$ (mg/L) in roots and leaves of *M. piperita* through atomic absorption spectroscopy.

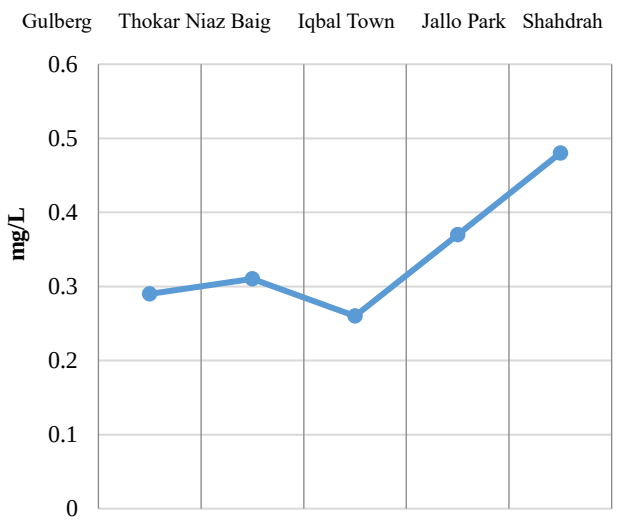

Fig. 52. Comparison of Pb toxicity in samples collected from different areas of Lahore.

Discussion

The present work revealed that under Cd and Pb stress, *C. sativum* showed reduction in chlorophyll content and caused inhibition of vascular region in both roots and stem. Coriander has also been previously reported to accumulate Pb and As in significant amounts (Gaur *et al*., 2017). However, in current work, reduction in vasuclar region of stem indicates that plant is capable of uptake and translocation of heavy metals from roots to stem. This is in accordance to work reported by Asdeo & Rathore (2012) and Wafee & Khan (2018) that Cd and Pb are translocated to aerial parts of plants through root. However, at 100 ppm $CdCl_2$, coriander showed negligible effects but at doses of 150 and 300 ppm, reduction in vascular tisssues was observed which was further confirmed by AAS that these changes are due to upake of Cd as plant accumulated Cd in aerial parts. However, when treated with $PbCl_2$, plant showed relatively less reduction in growth, suggesting that coriander is more sensitive to Cd as compared with Pb which might be due to the different mechanisms of metal uptake (Sharma & Dubey, 2005; Dragunski, 2014; Candido *et al*., 2020; Aslam *et al*., 2021).

In case of *M. piperata,* reduction in cotrical and vascular region of root, stem and leaves was reported. When treated with Cd and Pb doses, width of cortical region and xylem vessels of root and stem showed inhibited however, phloem remain unaffected. Inhibition in leaf area and chlorophyll content was also recorded in mint leaves which might be due to well-known effect of Pb which can cause reduction in chlorophyll formation in many plants by reducing the uptake of essential elements like magnesium and iron and replaces magnesium in chlorophyll 'b' (Nas & Ali, 2018). Pb also causes disorganization of chloroplast ultrastructure and inhibition of electron transport process including diversion of electrons from PSI. In the current work, inhibition in leaf area and chlorophyll biosynthesis might be due to interference of Pb with PSI and thereby reducing the rate of photosynthesis.

In current work, reduction in chlorophyll content of both treated plants indicates that Cd and Pb are translocated to their leaves, though inhibition was not observed at lower doses. Amount of Pb reported in mint leaves at 300 ppm was 0.85 mg/L, which can cause toxicity in cells (Kadir *et al*., 2008). AAS further revealed that mint roots can accumulate significant amount of Pb at 150 ppm, which is quite toxic to human health if leaves are consumed. According to a study, Pb levels i.e 10-80 µg/dl (which is equal to 0.1-0.8 mg/L) result in neurotoxicity, hypertension, renal impairment and altered cognitive functions (Kadir *et al*., 2008). However, level of Cd in mint was upto 0.36 mg/L which is also above safety levels in Pakistan. Therefore, due to accumulation of high amounts of Cd and Pb by mint, it can be used for phytoremediation of soil but it is not recommended for use as culinary herb as consuming contaminated leaves of mint can lead to many health hazards.

References

Amirmoradi, S. 2012. Effect of cadmium and lead on quantitative and essential oil traits of peppermint (*Mentha piperita* L.). *Not. Sci. Biol.*, 4(4): 101-109.

Arnon, D. 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in betavulgrais. *Plant. Physiol.*, 24(1): 1-15.

Asdeo, A. and S. Rathore. 2012. Bio-accumulation of trace metals in leafy vegetables due to effluent water irrigation in Peri urban Area of Western Rajasthan. *Adv. Appl. Physiol. Chem. Sci*., 87-93.

Aslam, M., A. Aslam, M. Shearz, B. Ali, Z. Ulhassan, U. Najeeb, W. Zhou and R.A. Gill. 2021. Lead toxicity in cereals: mechanistic insight into toxicity, mode of action, and management. *Front. Plant Sci*., 4(11): 587785.https://doi.org/10.3389/fpls.2020.587785.

Baker, A.J.M. 1981. Accumulators and excluders-strategies in the response of plants to heavy metals. *J. Plant. Nutr.*, 3(1-4): 643-654.

Candido, G.S., F.R. Lima, I.C. Vasques, K.R. Souza, G.C. Martins, P. Pereira, M. Engelherdt, R. Reis and J.Marques. 2020. Lead effects on sorghum and soybean physiology in oxisols, in *Arch. Agron. Soil Sci*, 1–15. doi: 10.1080/03650340.2020.1723004.

Chaudhry, N. and A. Khan. 2006. Improvement of pistillate flowers yields with GA_3 in heavy metals treated plants. *Plant. Growth. Regul.*, 50: 211-217.

Dragunski, C. 2014. Phytoaccumulation and effect of lead on yield and chemical composition of *Mentha crispa* essential oil. *Desalin. Water. Treat.*, 53(11): 3007-3017.

Emamverdiani, A. and Y. Ding. 2018. Effects of inhibition threshold in heavy metals on the growth and development of *Arundinaria fortune. J. Bot.*, 50(3): 887-892.

Gallego, S.M., L.B. Pena, R.A. Barcia, C.E. Azpilicueta, M.F. Iannone, E.P. Rosales and M.P. Benavides. 2012. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. *Environ. Exp. Bot*., 83: 33-46.

Garcia, J., P. Gratao, R. Azevedo and M. Arruda. 2006. Metal contamination effects on sunflower (*Helianthus annuus* L.) growth and protein expression in leaves during development. *J. Agric. Food Chem*., 54(22): 8623-8630.

Gaur, N., A. Kukreja, M. Yadav and A. Tiwari. 2017. Assessment of phytoremediation ability of *Coriander sativum* for soil and water co-contaminated with lead and arsenic: a small-scale study. 3. *Biotech*., 7(3): 196.

Haider. F.U., C. Liqun, J.A. Coulter, S.A. Cheema, J. Wu, R. Zhang, M. Wenjun and M. Farooq. 2021. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicol Environ Saf*., 2021 Mar 15;211:111887. doi: 10.1016/j.ecoenv.2020.111887. Epub 2021 Jan 12. PMID: 33450535.

Harguinteguy, C.A., M.N. Fernández-Cirelli and M.L. Pignata. 2016. The macrophytes *Potamogeton pusillus* L. and *Myriophyllum aquaticum* (Vell.) Verdc. as potential bioindicators of a river contaminated by heavy metals. *Microchem. J*., 124: 228-234.

He, J., Z. Xji, Q. Wang, C. Liu and Y. Zhou. 2016. Effect of Cu and Pb pollution on the growth and antioxidant enzyme activity of *Suaeda heteroptera*. *Ecol. Eng.*, 87(5): 102-109.

Humberto, A., M. Paula, B. Butler, P. Jorge, F. Matus, C. Merino and K. Yakov. 2020. Meta-analysis of heavy metal effects on soil enzyme activities. *Sci. Total Environ*., 139744 https://doi.org/10.1016/j.scitotenv.2020.139744.

Jiang, L., L. Zhang and C. Tian. 2018. Copper-induced similar changes in growth and physiological responses of plants grown from dimorphic seeds of *Suaeda salsa*. *Pak. J. Bot.*, 50(3): 871-877.

Kadir, M., Z. Janjua, S. Kristensen, Z. Fatmi and N. Sathiakumar. 2008. Status of children's blood lead levels in Pakistan: Implications for research and policy. *Public. Health*., 122(7): 708-715.

Khan, A. and S. Rehan. 2014. Environmental factors affect calcium oxalate crystals formation in *Tradescantia pallida* (Commelinaceae). *Pak. J. Bot.*, 46(2): 477-482.

Khan, A., M. Hussain and K. Malik. 2016. A possibility of using waterlily (*Nymphaea alba* L.) for reducing the toxic effects of chromium (Cr) in industrial wastewater. *Pak. J. Bot.*, 47(47): 1447-1452.

McGrath, S. and F. Zhao. 2003. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol*., 14(3): 277-282.

Nas, S.F. and M. Ali. 2018. The Effect of Lead on plants in terms of Growing and Biochemical parameters: MOJ, *Eco. Environ. Sci*., 3(4): 265-268.

Prasad, A.S. 1995. Zinc: An Overview. *Nutr*., 11: 93-99.

Rascio, N. and F. Navari-Izzo. 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci*., 180: 169-181.

Rizwan, M., J.D. Meunier, H. Miche and C. Keller. 2012. Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination, *J. Hazard. Mater*., 209-210-326-334.

Shahid, M., M. Arshad, M. Kaemmerer and E. Pinelli. 2012. Long-term field metal extraction by pelargonium: phytoextraction efficiency in relation to plant maturity. *Int. J. Phytorem*., 14: 493-505.

Sharma, P. and R. Dubey. 2005. Lead Toxicity in Plants. *Braz. J. Plant Physiol*., 17(1): 35-52.

Shojaei, S., A. Jarapour, S. Shojaei, Y. Gyasi-Agyei and J. Rodrigo-Camino. 2021. Heavy metal uptake by plants from wastewater of different pulp concentrations and contaminated soils. *J. Clean. Prod*., http://doi.org/10.1016/j.jclepro.2021.126345.

Soudek, P., A. Katrusakova, L. Sedlacek, S. Petrova, V. Koci, P. Marsik, M. Griga and T. Vanek. 2010. Effect of heavy metals on inhibition of root elongation in 23 cultivars of flax (*Linum usitatissimum* L). *Arch. Environ. Contam. Toxicol*., 59(2): 194-20.

Su, Y. and Y. Liang. 2015. Foliar uptake and translocation of formaldehyde with Bracket plants (*Chlorophytum comosum*), *J. Hazard. Mater*., 291: 120-128.

Suman, J., O. Uhlik, J. Victorova and T. Macek. 2018. Phytoextraction of heavy metals: A promising tool for clean-up of polluted environment?, *Front. Plant Sci*.,9:1-15.https://doi.org/10.3389/fpls.2018.01476

Taylor, F. 2019. Effect of chromium and lead on yield, chemical composition of essential oil, and accumulation of heavy metals of mint species. *Comm. Soil. Sci. Plant. Anal*., 41(16-19): 2170-2186.

van der Ent, A., A. Baker, A.J.M. Reeves, R.D., Pollard, A.J. and H. Schat. 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. *Plant Soil,* 362: 319-334. doi: 10.1007/s11104-012-1287-3.

Wafee, C., A. Khan and M.R. Siddiqui. 2018. Phytoremediation Potential of *Catharanthus roseus* L.and effects of lead toxicity on its morpho-anatomical features: *Pak. J. Bot.*, 323-1336.

Wang, L., B. Ji, Y. Hu, R. Liu and W. Sun. 2017. A review on in situ phytoremediation of mine tailings. *Chemosphere,* 184: 594-600. doi: 10.1016/j.chemosphere.2017.06.025

Yan, A., W. Yamin, S.N. Tan, M. Yusof, M. Lokman, G. Subhadip and C. Zhong. 2020. Phytoremediation: A promising approach for revegetation of heavy metal-polluted land, *Front. Plant Sci*., 11.359 DOI=10.3389/fpls.2020.00359.

(Received for publication 25 May 2022)