Cadmium and Lead Differentially Affect Growth and Metal Accumulation in Guar (*Cyamopsis Tetragonoloba* L.) Varieties

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Abstract

Phytoremediation is a strategy to employ plants to recover high quantities of metals in the soil into the harvestable parts, such as shoots and roots. High levels of Cd and lead (Pb) in the soil cause several stress symptoms in plants, including a decrease in growth, reduced root growth, and carbohydrate metabolism. In this study, Saravan and HGS-867 as local landrace and Indian guar variety were selected to investigate the effect of the application of Pb (0, 40, 150, and 200 mg/l) and the cadmium (0, 25, 50, and 100 mg/l) on phonological, yield parameters and phytoremediation assessment. The results showed that Pb translocation factor (TF) was significant in Pb×Cd and Pb×Cd×G (genotype) treatments at 1% and in Pb×G at 5%. Pb bioconcentration factor (BCF) was significant in all treatments except Cd and Cd×G at 1%. Mean comparison of the data showed that the number of flowers, leaves, and clusters in plant decreased significantly with increasing Pb content. With increasing Cd concentration, the number of branches, height, the number of seeds, clusters, and leaves for each plant decreased significantly at the level of 1%. The maximum TF was observed in Pb at 40 mg/l in the HG-867 variety. Also, the Saravan landrace in Cd (100 mg/l) showed the highest amount of BCF (Cd). With increasing the concentrations of Pb and Cd, the gum percentage decreased significantly. The results of Pearson's correlation analysis indicated that plant height, number of pods/plant, root length, biomass, and pod length had a positive correlation with seed yield and a negative correlation with TF (Pb) and BCF (Pb). The results suggest that according to TF, BCF, and BAC, *C. tetragonoloba* L. can be effectively used as a good accumulator.

1. Introduction

Heavy metals, like Cadmium (Cd) and Lead (Pb) are particularly toxic to the majority of plants and animals even at low concentrations (Shukla et al., 2007). Heavy metals at their toxic concentration inhibit various stages of plant development, especially seed formation and physiological and biochemical processes. Pb is the second most dangerous substance, after arsenic, considering the occurrence frequency, toxic effects, and the potential for human exposure. Its transfer from polluted soils to plants has been extremely evaluated, particularly regarding food quality and use in phytoremediation (Uzu et al. 2009). Contamination of the soil with toxic trace metals as well as their accumulation in soil affect agricultural production because of the adverse impacts on crop growth; for example, metal phytotoxicity and soil microorganisms (Nagajyoti et al., 2010). Pb may exist in soil as a free metal ion and as complexes with inorganic constituents (like CO_3^{2-} , HCO_3^- , SO_4^{2-} , and Cl^-), can exist as organic ligands (such as amino acids and humic acids), or can be adsorbed on particle surfaces (like biological material, peroxides, clay particles, and organic matter) (Uzu et al. 2009). Phytoremediation is a strategy to employ plants to recover high quantities of metals in the soil into the harvestable segments, such as roots and shoots (Mahmood, 2010). Harvesting of the biomass can be safely done using composting, drying, ashing, and strating at a landfill, pure plant oil manufacturing, anaerobic digestion, microbial, physical, or other chemical means (Ginneken et al., 2007).

High levels of Cd in the soil can elicit different stress symptoms in plants, like a decrease in growth, particularly root growth, impairments in mineral nutrition, and carbohydrate metabolism (Moya et al. 2004), leading to a reduction in biomass production.

In some earlier reports by Stoyanova and Tchakalova (1999) Cd at toxic levels disturbed the chloroplast envelope as well as the integrity of the membrane system resulting in an increase in plastoglobule number, alteration in the lipid composition, and the levels of the major structural constituents of thylakoid membranes.

Various plant species belonging to the Fabaceae family show phytoremediation potential (Ginneken & Gawronski, 2007; Anjum et al., 2014). Legumes are rich in phytoremediation and provide additional N-compounds to the soil leading to an improvement in soil fertility and capacity to support biological development (Hao et al., 2014). *Cyamopsis tetragonoloba* L. Guar (*Cyamopsis tetragonoloba* L.) is a low-input and annual fast-growing legume species that is almost cultivated in arid and semi-arid areas (Mefthahzade et al., 2019). Guar has been recently widely considered because of the general tendency toward the application of polysaccharides from plants in different industrial applications. Guar is a multipurpose crop with economic values cultured for forage, vegetable (green pods), and seed gum that is widely used in various industries, such as food processing (as a novel natural additive), paper manufacturing (as a fiber deflocculants and beater additive), textile and carpet printing, mining explosive, drilling, pharmaceutical, cosmetics, agrochemistry, and oil and gas exploration because of the high galactomannans content of its endosperm (Abidi et al., 2015; Gresta et al., 2017; Mudgil et al., 2014). Guar seed has seed coat (14–17%), endosperm or gum (35–42%), and germ (43–47%) (Sabah El Khir et al., 2008). The demand for guar seed and its derivatives has been recently increased globally.

Guar is fast-growing with a high development rate and shoot biomass. Also, it can be employed for phytoremediation. We investigated tolerance of the landrace (Saravan) and HG-867 variety of guar to Pb and Cd treatments in the soil during growth periods.

2. Materials And Methods

2.1. A summary of the site, plant materials, and experimental design

This experiment was performed in a research greenhouse located in Jahanabad Meybod industrial town. The seeds of Guar (*Cyamopsis tetragonoloba* L.) were prepared and authenticated from Industrial and Medicinal Plant Research Institute, Ardakan, Iran. Saravan as Iranian landrace and HGS-867 as Indian variety were selected for the current experiment to evaluate the effect of Cd and Pb on tested parameters. The study was carried out with three replications of 3n factorial based on Randomized Complete Design (RCD). The scale of the plot was 9 m² (3×3 m). Five seeds were sown per hole, and the plants were thinned to three per hole after two weeks of seeding. The combinations of Cd nitrate and Pb nitrate were used. The Pb element was used at four levels (0, 40, 150, and 200 mg/l) and the Cd element was used at four levels (0, 25, 50, and 100 mg/l) with three replications.

2.2. The composition of the soil

The soil texture was sandy loam (0.87 ds m^{-1} , pH 6.9) with organic matter content of 8.0 g kg^{-1} soil, field capacity of 26.7%, total nitrogen ($\text{N}_{\text{kjeldhal}}$) of 0.03%, available phosphorus (P) of $14 \text{ mg}^{-1} \text{ kg}^{-1}$ soil, and available potassium (K) of $227 \text{ mg}^{-1} \text{ kg}^{-1}$. Weeding was performed manually 25 and 50 days after planting (DAP). When plants have four leaves, heavy metal treatments of Pb and Cd (each at four levels with three replications) were applied and till harvesting continued.

2.3. Morphological, phonological, yield parameters and phytoremediation assessment

Harvest was done at the maturity phase of different landrace and variety. Guar plants were regarded sufficiently mature if the majority of the pods (80%) were brown, and subsequently, data related to morphological parameters, like plant height (at maturity stage), number of leaves per plant (at pod initiation phase), and leaf area of the plant were recorded from 5 randomly selected plants of each plot.

3.1. Estimation of photosynthetic pigments

The chlorophyll was extracted based on the method reported by Arnon (1949). In brief, fresh leaves (1g) were ground with 80% acetone (20–40 mL) and the mixture was subjected to centrifugation (10000 rpm / 5 min). The solution absorbance was read at 645 nm and 663 nm against the blank (acetone). Total chlorophyll level was determined by the below equation:

$$\text{Total chlorophyll (mg/g FW)} = 20.2 (A_{645}) + 8.02 (A_{663}) \times V/1000W$$

Where A_{645} and A_{663} were the absorbance values determined at 649 and 665 nm, V was the volume of the extract (mL), and W was the weight of the sample (g).

For determining gum%, the guar flour dry weight achieved following the milling process was determined at 90°C.

2.3.2. Phonological parameters

Phonological parameters, like days to 50% flowering, days to 50% podding, and time to start pod harvesting were estimated for two landraces and variety (Patil, 2014).

2.3.3. Yield and yield components

Pod length, the number of pods for each plant and each cluster, the number of seeds for each pod and each plant, seed yield, and weight of 100 seeds were determined at harvesting time.

2.3.4. Evaluation of phytoremediation efficiency (Cd, Pb, BCF, BAC, and TF)

That plants were oven-dried at 80°C for obtaining a constant weight and their dry weights were noted. To determine Cd and Pb accumulation in different plant tissues (roots and shoots), harvested plant parts were rinsed completely using tap water, and deionized (DI) water for cleaning the adhered components of soil followed by oven-drying at 80°C until reaching a steady weight. Pb concentration after extraction of samples with 2N hydrochloric acid was read using atomic absorption spectrometry. To measure the concentration of Cd in the shoots and roots of the plant, a sample of the dried plant weighing 2 g was poured into a porcelain crucible and placed in an oven at 30°C. The oven temperature was gradually raised to 550°C within two hours. After holding for 4 to 12 hours at 550°C, the oven was turned off and the crucibles were removed from the oven. After cooling, the ash was soaked in a little water and covered with a watch glass, and at the same time, 10 ml of 2 M hydrochloric acid was slowly added. The crucibles were then placed in a water bath to release the first white vapors at 80°C. The contents of the crucible were filtered through Whatman No.1 filter paper into a 100 ml jogger balloon, and after rinsing the crucible, the contents of the jogger balloon were measured. The extract was used to read the concentration of Cd by atomic absorption spectrometer. For quantifying the Pb content in the soil, a specimen of 1 g soil was digested using a wet acid digestion approach through HNO_3 and HCl (3:1) followed by heating on a hot plate for 2 h until observing a clear solution. Following cooling, the volume was completed to 50 mL through the addition of distilled water. The solution was filtered through Whatman's filter paper and Pb contents were examined with Atomic Absorption Spectrophotometer.

To evaluate the uptake of Cd and Pb in cultivated plants, indices, such as TF and bioconcentration factor (BCF) were estimated (Monni et al., 2000). The TF was determined as a ratio of Cd and Pb concentration in plant shoot to that in plant root and the BCF was obtained from the ratio of Cd and Pb concentration in the root to its level in the soil. The bioaccumulation coefficient (BAC) was defined as the Pb and Cd ratio in the shoot to that in soil.

2.4. Data analysis

The normality of data was assessed by Skewness and Kurtosis test prior to analysis of variance (ANOVA). Differences between treatments were determined using a two-way analysis of variance (ANOVA) by SAS software. The mean analysis was done by Duncan's Multiple Range Test (DMRT). The significant differences were calculated at $p < 0.01$. The principal component analysis (PCA) was applied for assessing possible relationships between the treatments by SPSS 10 software. Cluster analysis (UPGMA) using Euclidean distance and scatter plot diagram was done by the PAST software.

3. Results And Discussion

According to the ANOVA, plant height was affected by all treatments except Cd and Pb interaction at a significance level of 1%. The number of branches per plant was not significant by genotype. But there was a significant difference using other treatments at the level of 1%. The number of flowers and leaves was significant by all treatments, except G and the interaction of G (genotype) in Cd at the level of 1%. The number of clusters per plant was significant in all treatments, but no significant difference was found between the two genotypes at the level of 1%. Chlorophyll content was not significantly affected by Cd and Pb interaction. Pod length and the number of seeds per plant were also significant by Pb and the interaction of Pb and Cd and Pb in Cd at the level of 1%. The

number of pods/plant and seed yield were also signed by the Pb, G, Pb in G, Pb in Cd, and Pb in Cd in G at the level of 1%. Also, 50% of flowering was not affected by any of the treatments. However, 50% of pods showed a significant difference by all treatments except genotype at the level of 1%. The weight of 100 seeds using Pb, Cd, and Pb ×Cd was significantly different at the level of 1% (Table 1). The gum percentage was significantly different only by the Pb ×Cd treatment at the level of 1% and the root depth showed a significant difference using all treatments at the level of 1%.

Pb TF was also significant using Pb×Cd and Pb×Cd×G at 1% and Pb×G at 5%. Pb BCF was significantly different by all treatments except Cd and Cd×G at the level of 1%. Cd TF was significantly different by Pb×Cd and Pb×Cd×G at the level of 1%. Cd BCF showed a significant difference by Cd, Pb×Cd, Cd×G, and Pb×Cd×G treatments, whereas, BAC (Cd) was not significantly different by any tested treatments, while BAC (Pb) was significantly different by Pb, G, Pb×G, and Pb×Cd×G significant at the level of 1% (Table 1).

3.1. Simple impact of Pb, Cd, and genotypes

Mean comparison of the data showed that the height and number of branches in the control and Pb (50 mg/l) treatments showed the highest rate. The number of flowers, leaves, and clusters in the plant decreased significantly by increase in Pb content. The chlorophyll content was elevated significantly by increase in the Pb concentration. The pod length and number of seeds per plant in the Pb (100 mg/l) treatment had the lowest rate. Regarding the number of pods per plant and seed yield per plant, Pb (25 mg/l) treatments had the lowest rate. The weight of 100 seeds in the Pb (25 and 100 mg/l) had the lowest amount (2.01 and 1.77, respectively). Finally, regarding the plant biomass, Pb (100 mg/l) treatments showed the lowest level. A reduction in plant biomass can be related to disturbed metabolic activities because of decreased uptake of essential nutrients while growing under Pb stress (Gopal & Rizvi, 2008; Kopittke et al., 2007). Similar results have been reported by other researchers, Islam et al. 2008 reported that Pb toxicity in *Elsholtzia argyi* inhibited growth, significantly reduced plant height and root length, reduced fresh and dry weight of shoots, discolored leaves, and folded them. Compared with control treatments, Pb stress at 1000 mg kg⁻¹ reduced root fresh weight (8.15 g plant⁻¹) and shoot fresh weight (21.13 g plant⁻¹) in *C. tetragonoloba* (Amin et al., 2018).

The gum percentage was also affected by the concentration of Pb. In Pb (100 mg/l) treatment, the gum was 32.2% (Table 2). A reduction in gum in the treatment of high concentration of Pb may be due to the release of polysaccharides in the pericellular space that largely determines the course of allelopathic processes of sorption, desorption, ion exchange, and cell protection from extreme influences (Lombardi and Vieira, 1999).

The total level of metals accumulated in the shoots is regarded as the most important factor for evaluating the potential of phytoextraction in plants (Zaier et al., 2010). The highest level of BAC (Pb) was observed in Pb (25 mg/l). BCF (Pb) increased with increasing the concentration of Pb. In Pb (100 mg/l) treatment, the BAC (Pb) was 4.11. The mobility, as well as availability of heavy metals in the soil, are commonly low, particularly when the soil is high in pH, clay and organic matter (Rosselli et al., 2003). Thus, it seems that at the low concentration of these heavy metals, their concentration in soil is so high. The highest Pb TF from root to shoot was 2.9, 3.7, and 3.54, respectively. This result emphasis that guar can accumulate Pb in root and can be a good source of Pb phytoremediation. It seems that Pb accumulation will occur in the apoplast cell of the root. Kopittke et al. (2007) reported that Pb reduces the fresh weight of buds in *Vigna unguiculata*. Also, their examinations using TEM microscopy showed that most of the root Pb accumulates in the apoplast of the outer cell layers of the root. Smaller amounts of Pb were also observed in the central cylinder apoplast. It was also observed that the amount of Pb accumulated in the roots is 10-50 times higher than in the buds (Kopittke et al., 2007).

Mean comparison of different levels of Cd in the studied traits showed that with increasing Cd concentration, height, the number of branches, clusters, seeds, and leaves per plant decreased significantly at the level of 1%. Also, the increase in Cd levels has caused the number of days to 50% of pods to be increased. However, the maximum days to 50% pods maturity was observed in the Cd (40 mg/l) (51.21 days) (Table 3). Root depth and gum percentage also showed a significant decrease with increasing Cd concentration at the level of 1%. Although in Cd (40 and 150 mg/l) treatment, the gum percentage compared with the control showed a significant increase, at 250 mg/l, this rate decreased (31.19%). Bioaccumulation coefficient (Cd) increased significantly with increasing Cd levels, at 200 mg/l Cd and the BAC (Cd) was 3.97 (Table 3). Mihalescu et al. (2010) reported that increasing the concentration of Cd decreased the length of the maize plant, especially at concentrations of 100 and 200 ppm.

Shanker et al. (2005) stated that Pb and Cd that are transferred to the aerial part of the plant, reduce the plant height due to disturbances in the cellular metabolism of the aerial part. The reason for this is the loss of cell dilation and the reduction of mitotic activity or inhibition of cell elongation. Cd in cells inhibits cell proliferation by affecting the cell walls and middle wall and increasing cross-linking between cell wall compounds.

Rai et al. (2005) studied the effects of Cd on *Phyllanthus amarus* and achieved the following results: Severe fresh and dry weight loss, a decrease in root depth, and a decrease in sugar, carotenoids, protein, and chlorophyll. These findings are consistent with our results. Investigation of different effects of Cd (9, 6, 3, 3, and 12 mg/kg) on cultivars of *Vigna radigna* showed that with increasing the amount of Cd, the fresh and dry weight of the plant decreased in all cultivars. But the response of cultivars to weight loss was different (Ghani, 2010), which is in agreement with our finding that varieties have different responses to BCF (Cd and Pb). It seems that the reduction of shoot growth due to the effect of Cd can be due to the reduction of chlorophyll content and photosynthetic activity (Shah et al., 2008).

The mean comparison on the studied variety and landrace showed that HG-867 showed superiority to Saravan landrace in terms of plant height, the number of flowers, seed/ plant, root depth, and BCF (Cd and Pb). However, gum percentage and biomass in the Saravan landrace were higher than the other variety (36.6% gum vs. 33.6%) (Table 4). The current results showed that HG-867 has a stronger accumulator rather than Saravan in the case of Pb and Cd accumulation in roots.

3.2. Interaction of Pb, Cd, and genotypes

The maximum TF (Pb) was observed in Pb (40 mg/l) in the HG-867 variety (Fig 4A). Also, the Saravan landrace in Cd (100 mg/l) treatment showed the highest amount of BCF (Cd) (Fig 4B). The HG-867 variety had the highest amount of BAC (Pb) compared with the Saravan landrace in Pb (40 mg/l) treatment (Fig 4C).

With increasing the levels of Cd, its BAC (Pb) did not increase (Fig 4C). Regarding BCF (Pb), the Saravan landrace in Pb treatment (200 mg/l) showed the highest BCF (Pb) (Fig 4D). Ouzounidou et al. (1995) declared that the uptake of heavy metals can cause chromosomal aberration and division of cells to become unusual that significantly reduced plant growth.

The interaction of Pb in Cd in terms of TF (Pb) showed that Cd (100 mg/l) and Pb (200 mg /l) had the highest TF (Fig 5A). Regarding the BCF (Pb), the results indicated that Cd (0) and Pb (200 mg /l), as well as Cd (25 mg/l) and Pb (200 mg/l), had the highest BCF (Pb) (Fig 5B). Also, as can be seen in Fig 5C, the highest amount of TF (Cd) was observed in Cd (50 mg/l) and Pb (40 mg/l) treatments (Fig 5C). in addition, with increasing Cd concentration, the BCF (Cd) increased. The interaction of Cd (100 mg/l) and Pb (0, 40 mg/l) showed the highest amount of BCF (Cd) (Fig 5D).

Regarding the effect of interaction treatments on root length, the results showed that interaction of Cd (25 mg/l) and Pb (control), Cd (50 mg/l), Pb (control), and Pb (150 mg/l) caused the highest root depth (27, 26, and 25 cm, respectively) (Fig 6A). The interactions of Pb and Cd regarding gum percentage showed that treatment with Cd (25 mg/l) and Pb (control) (40 and 150 mg/l) showed the highest gum percentage (38, 39, and 40%). However, with increasing the concentrations of Pb and Cd, the gum percentage decreased significantly (Fig 6B). Regarding biomass trait, with increasing Cd and Pb, the amount of biomass decreased significantly (Fig 6C). Regarding the interaction effect of Cd and Pb on seed yield per plant, in control treatments, Cd (25 mg/l) and Pb (150 mg/l) and Cd (100 mg/l) and Pb (150 mg/l) were found with the highest amount of seed per plant. By further increasing the concentration of Pb and Cd, the amount of seed yield in the plant decreased significantly (Fig 6D). Fouda and Arafa (2002) assessed soybeans and reported that treatment with high concentrations of Cd reduced plant height, number of leaves, and leaf area, while Pb also caused water stress, thereby reducing leaf area, photosynthesis, plant dry weight, and plant height and it was also effective in reducing the number of nodes.

As can be seen in Fig 3, the maximum weight of 100 seeds was observed in Pb (50 mg/l) and Cd (150 mg/l) treatments, while the number of branches was more in Pb (0) ×Cd (150 mg/l), Pb (0) ×Cd (25mg/l), and control treatments.

Chlorophyll content decreased by the interaction of Cd × Pb, especially at high concentrations. It seems that a reduction in protochlorophyllide reductase enzyme activity because of heavy metals toxicity is the main cause for less production of total chlorophyll. Cd can inhibit the photoactivation of photosystem-II, which is the main reason for less pigment generation in plants while growing in Cd contaminated soils (De Filippis and Pallaghy, 1994).

A TF greater than 1 indicates that the accumulation in shoots is more than roots and soil, respectively (Marrugo-Negrete et al., 2016). In the current research, TF in Cd×Pb treatment was more than 1, which means that guar is a good accumulator of Cd and Pb in the shoot. This phenomenon occurs at a high concentration of the used heavy metals.

3.3. Correlation matrix

Table 5 lists the results of Pearson's correlation analysis. As shown in this table, plant height, number of pods/plant, root length, biomass, and pod length showed a positive correlation with seed yield, while TF (Pb) and BCF (Pb) were negative. A gum percentage was another main factor in guar. In the current research, plant height, weight of 100 seeds and pod number showed a positive correlation with gum percentage, while chlorophyll content was found with a negative correlation. there was a negative relationship between the effect of Cd and the specific surface area of the leaf. Cd reduces water absorption and transpiration, too (Vassilev et al. 1997). Decreased water uptake in Cd-treated plants can be well justified by reducing root growth.

3.4. Multivariate analyses

We conducted principal component analysis (PCA) for finding trends in the collected dataset and defining the multivariate relationships between the studied parameters (Table 6). In Tables 6 and 7, the individual values, the percentage of variance, the cumulative percentage, as well as the loading of the components are also given. Only the first four major components (PC1, PC2, PC3, and PC4) were found with values greater than one (6.59, 2.2, 1.95, and 1.38, respectively); thus, data can be divided into four variables comprising 73.21% of the overall variance.

These high values influence the distinction between landraces, and the high integrity of the relationships observed. In Table 7, the equation of the first four components is shown. The coefficients in this table are ordinary, and thus their numerical values reflect their weight when the corresponding component is formed. The highest coefficients were for the first part, plant height, branch number per plant, number of flower per plant, and number of clusters per plant (Fig. 1).

Cluster analysis (UPGMA) was also conducted through the Euclidean distance coefficient and the average method of linkage based on all the tested traits (Fig. 2) for approximating the relationship between the used treatments. The clusters were divided into two primary clusters (I and II) and two sub-clusters (IA, IB, and IIA, IIB) for Cd ×Pb treatment (Fig. 2). For example, Pb₃Cd₁ and Pb₃Cd₂ were most closely clustered (Fig. 2).

4. Conclusions

The BCF, BAC, and TF values are used for identifying the effectiveness of plants for phytoremediation (phytoextraction or phytostabilization) through explaining the accumulation features and translocation behaviors of metals in plants. Plants with BCF, BAC, and TF values > 1 can be promising phytoextractors, appropriate for phytoextraction, while those with BCF and TF < 1 are regarded potential. It can be concluded that guar, especially HG-867 variety at high doses of Pb and Cd showed values of more than 1, which indicates that it is effective for translocation and phytostabilization at high concentrations of Pb and Cd.

Declarations

Ethical Approval

This material is the authors' own original work, which has not been previously published elsewhere. Also, the manuscript is not currently being considered for publication elsewhere. This manuscript reflects the authors' own research and analysis in a truthful and complete manner.

Consent to Participate

All authors have been personally and actively involved in substantial work leading to the manuscript, and will take public responsibility for its content.

Consent to Publish

All authors have agreed to submit the manuscript in its current form for consideration and possible publication in "*Environmental Science and Pollution Research*".

Authors Contributions

S. Sanaei performed the experiment; M. Sadeghinia and H. Meftahizade supervised the research, wrote and revised the manuscript; A. Fatahi Ardakani and M. Ghorbanpour advised the research and contributed to statistical analyses. All authors read and approved the final manuscript version.

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Competing Interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable

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Tables

Table 1. Analysis of variance of studied traits of Guar varieties.												
S.O.V	df	H	Br (NO)	Fl (NO)	Clu (NO)	L (NO)	CH	L. A	P. L	S (NO)	P (NO)	S.Y
Rep	2	67.167 ^{ns}	2.260 ^{ns}	9317.469 ^{ns}	30.406 ^{ns}	861.792 ^{ns}	54.214 ^{ns}	44.667 ^{ns}	0.512 ^{ns}	1.377 ^{ns}	1.766 ^{ns}	83.305
Pb	3	11411.705 ^{**}	62.010 ^{**}	90674.306 ^{**}	968.083 ^{**}	21682.667 ^{**}	415.904 ^{**}	670.944 ^{**}	4.739 ^{**}	7.217 ^{**}	40.062 ^{**}	2104.5
Cd	3	1971.455 ^{**}	36.788 ^{**}	16026.250 [*]	214.972 ^{**}	6517.056 [*]	162.819 ^{ns}	313.028 ^{ns}	1.552 ^{ns}	3.723 [*]	6.635 ^{ns}	425.23
G	1	1211.260 ^{**}	11.344 ^{ns}	315.375 ^{ns}	24.000 ^{ns}	13.500 ^{ns}	200.971 ^{ns}	280.167 ^{ns}	0.66 ^{ns}	0.388 ^{ns}	29.040 ^{**}	954.42
Pb*Cd	9	3001.227 ^{**}	24.721 ^{**}	22827.916 ^{**}	245.002 ^{**}	6583.181 ^{**}	228.985 ^{**}	279.995 [*]	2.055 ^{**}	3.621 ^{**}	13.104 ^{**}	713.46
Pb* G	3	5080.879 ^{**}	28.820 ^{**}	43331.500 ^{**}	444.857 ^{**}	9973.786 ^{**}	228.448 [*]	342.190 [*]	2.523 [*]	4.246 ^{**}	21.916 ^{**}	1074.6
Cd* G	3	1153.569 ^{ns}	17.986 [*]	8733.5 ^{ns}	132.619 [*]	3493.071 ^{ns}	157.058 ^{ns}	196.595 ^{ns}	1.608 ^{ns}	2.644 ^{ns}	7.483 ^{ns}	361.18
Pb*Cd* G	9	1606.265 ^{**}	13.537 ^{**}	13456.940 ^{**}	141.526 ^{**}	3723.564 [*]	145.912 [*]	200.473 ^{ns}	1.814 ^{**}	2.963 ^{**}	8.356 ^{**}	438.91
Error	62	117.349	3.078	4819.877	32.223	1753.609	77.357	160.892	0.72	1.018	2.793	142.34
S.O.V.: Source of variation, H: height, Br(NO): Number of branches, Fl (NO): Number of flowers, Clu (NO): Number of clusters, L (NO): Number of leave, CH: chl Leaf Area,												
P. L: Pod length, S (NO): Number of seeds, P (NO): Number of pods, S.Y: Seed yield, 50% p: 50% pods												

Continued Table 1. Analysis of variance of studied traits of Guar varieties.													
S.O.V	df	50% f	W 100 S	B	TSPH	Gum%	RL	Pb TF	Cd TF	BCF Cd	BAC Cd	BCF Pb	BA
Rep	2	4.667 ^{ns}	0.929 ^{ns}	3.690 ^{ns}	11.760 ^{ns}	29.729 ^{ns}	2.260 ^{ns}	0.0830 ^{ns}	0.0163 ^{ns}	11.22 [*]	148.11 ^{ns}	5.3034 ^{ns}	4.8
Pb	3	60.038 ^{ns}	2.332 ^{**}	119.967 ^{**}	603.8 ^{**}	124.987 ^{ns}	694.4 ^{**}	0.5109 ^{ns}	4.2685 ^{ns}	3.778 ^{ns}	134.62 ^{ns}	51.895 ^{**}	11.
Cd	3	27.955 ^{ns}	2.557 ^{**}	8.545 ^{ns}	445.9 ^{**}	220.775 ^{ns}	134.42 ^{**}	0.7380 ^{ns}	0.1376 ^{ns}	32.65 ^{**}	148.14 ^{ns}	7.3208 ^{ns}	1.8
G	1	102.094 ^{ns}	0.492 ^{ns}	28.276 [*]	187.04 [*]	218.297 ^{ns}	225.09 ^{**}	0.6439 ^{ns}	1.7222 ^{ns}	4.061 ^{ns}	103.02 ^{ns}	13.041 ^{**}	30.
Pb*Cd	9	33.727 ^{ns}	1.807 ^{**}	29.816 ^{**}	276.3 ^{**}	191.022 [*]	196.56 ^{**}	0.4975 ^{**}	1.6234 ^{**}	11.52 ^{**}	129.545 ^{ns}	15.878 ^{**}	3.3
Pb* G	3	43.546 ^{ns}	1.200 ^{ns}	57.126 ^{**}	307.8 ^{**}	107.683 ^{ns}	356.70 ^{**}	0.5568 [*]	2.2207 ^{ns}	4.065 ^{ns}	127.39 ^{ns}	24.657 ^{**}	11.
Cd* G	3	41.212 ^{ns}	1.39 ^{ns}	11.679 ^{ns}	253.4 ^{**}	136.977 ^{ns}	120.486 [*]	0.4159 ^{ns}	0.3638 ^{ns}	14.87 ^{**}	132.30 ^{ns}	6.073 ^{ns}	6.6
Pb*Cd* G	9	34.287 ^{ns}	1.077 [*]	17.522 ^{**}	159.3 ^{**}	154.747 ^{ns}	124.28 ^{**}	0.4296 ^{**}	1.2243 ^{**}	7.219 ^{**}	125.73 ^{ns}	9.0274 ^{**}	3.6
Error	62	28.290	0.536	4.988	33.051	88.609	17.798	0.2056	0.6275	2.7669	127.571	1.9658	1.9
S.O.V.: Source of variation, 50% f: 50% flowering, W 100 S: Weight of 100 seeds, B: Biomass, TSPH: Time to start pod harvesting, Gum%: gum Percent, RL: f length,													
Pb TF: Translocation factor (Pb), Cd TF: Translocation factor (Cd), BCF Cd: Bioconcentration factor (Cd), BAC Cd: Bioaccumulation coefficient (Cd),													
BCF Pb: Bioconcentration factor (Pb), BAC Pb: Bioaccumulation coefficient (Pb)													

Table 2. Mean Comparison of traits measured at different levels of Pb.															
Pb (mg/l)	H	Br (NO)	Fl (NO)	Clu (NO)	L (NO)	CH	L. A	BCF pb	RL	P. L	B	P (NO)	S. Y	50% p	W 100 S
0	87.1 ^a	12.5 ^a	154.4 ^a	20.4 ^a	117.96 ^a	62.5 ^{ab}	23.04 ^{ab}	0.9247 ^c	25.17 ^a	5.900 ^a	2.90 ^a	4.7 ^{ab}	34.3 ^a	50.5 ^a	6.4296 ^a
25	47.7 ^b	9.5 ^b	28.5 ^c	7.5 ^{bc}	57.63 ^{bc}	60.4 ^b	15.13 ^{ab}	1.122 ^{bc}	14.42 ^b	5.092 ^b	2.27 ^{ab}	2.8 ^c	18.3 ^b	50.0 ^{ab}	2.0158 ^c
50	85.8 ^a	11.4 ^a	88.4 ^b	11.5 ^b	88.63 ^{ab}	67.8 ^a	22.21 ^{ab}	2.117 ^b	21.88 ^a	5.521 ^{ab}	1.85 ^b	5.8 ^a	38.2 ^a	50.7 ^a	4.6942 ^b
100	49.7 ^b	9.1 ^b	23.0 ^c	6.4 ^c	53.63 ^c	69.1 ^a	12.29 ^b	4.11 ^a	14.58 ^b	4.916 ^b	1.11 ^c	3.7 ^{bc}	22.8 ^b	49.3 ^b	1.7704 ^c
H: Height, Br (NO): Number of Branches, Fl (NO): Number of Flowers, Clu (NO): Number of Clusters, L (NO): Number of Leave, CH: Chlorophyll, L. A: Leaf Area, Bioconcentration Factor (Pb), RL: Root Length, P. L: Pod Length, B: Biomass, P (NO): Number of pods, S. Y: Seed yield, 50% p: 50% pods, W 100 S: Weight of 1 Bioaccumulation Coefficient (Pb),															
Gum%: Gum Percentage.															

Table 3. Mean Comparison of traits measured at different levels of Cd.											
Cd (mg/l)	H	Br (NO)	S.P (NO)	Clu (NO)	L (NO)	S. Y	50% pd	TSPH	RL	Gum%	BAC Cd
0	76.17 ^a	11.21 ^a	6.68 ^{ab}	14.54 ^a	90.42 ^a	33.56 ^a	49.54 ^b	41.58 ^b	20.88 ^a	34.9 ^a	1.662 ^b
40	72.79 ^{ab}	11.50 ^a	6.75 ^a	12.83 ^a	86.25 ^{ab}	29.96 ^{ab}	49.42 ^b	50.17 ^a	19.88 ^a	38.4 ^a	1.5628 ^b
150	65.96 ^b	11.17 ^a	6.42 ^{ab}	11.08 ^{ab}	86.25 ^{ab}	23.90 ^b	49.50 ^b	41.79 ^b	19.75 ^a	36.02 ^a	1.6981 ^b
200	55.63 ^c	8.83 ^b	5.88 ^b	7.54 ^b	54.92 ^b	26.43 ^{ab}	51.21 ^a	41.29 ^b	15.54 ^b	31.19 ^b	3.973 ^a
H: Height, Br (NO): Number of Branches, S. P (NO): Seed/ Pod, Clu (NO): Number of Clusters, L (NO): Number of Leave, S. Y: Seed yield, 50% P: Days to 50% podding, TSPH: Time to start pod harvesting, RL: Root Length, Gum%: Gum Percentage, BAC Cd: Bioaccumulation Coefficient (Cd)											

Table 4. Mean Comparison of traits measured in two landrace and variety.

Genotype	H	Fl (NO)	S. P	B	Gum%	RL	BCF (Pb)	BCF (Cd)
Saravan	64.1 ^b	71.8 ^b	25.316 ^b	3.185 ^a	36.68 ^a	17.49 ^b	1.969 ^b	7.641 ^b
HG-867	71.1 ^a	75.4 ^a	31.622 ^a	4.270 ^b	33.67 ^b	20.54 ^a	4.396 ^a	12.030 ^a

H: Height, Fl (NO): Number of flower/ plant, S. P: Seed/plant, B: Biomass, Gum%: Gum Percent, RL: Root Length, BCF (Pb): Bioconcentration factor (Pb), BCF (Cd): Bioconcentration factor (Cd).

Table 5. Correlation analysis among studied traits in different guar varieties under various Pb and Cd.

Traits	H	Br (NO)	Fl (NO)	Clu (NO)	L (NO)	CH	P. L	S (NO)	P (NO)	S. Y	50%P	W 100 S	B	RL	Gum%	Cd TF	Pb TF
H	1																
Br (NO)	.680**	1															
Fl (NO)	.594**	.460**	1														
Clu (NO)	.639**	.634**	.769**	1													
L (NO)	.564**	.576**	.466**	.592**	1												
CH	.103	.316**	-.149	-.093	.081	1											
P. L	.450**	.500**	.299**	.399**	.291**	.190	1										
S (NO)	.433**	.481**	.345**	.462**	.282**	.163	.898**	1									
P (NO)	.672**	.385**	.434**	.261*	.219*	.236*	.298**	.245**	1								
S. Y	.719**	.436**	.500**	.359**	.253*	.203*	.448**	.438**	.969**	1							
50 %P	.124	-.056	.184	.052	.044	-.151	.033	-.009	.279**	.288**	1						
W 100 S	.313**	.323**	.079	.150	.251*	.288**	.151	.186	.297**	.294**	-.088	1					
B	.656**	.551**	.596**	.586**	.601**	.004	.260*	.270**	.369**	.410**	.086	.006	1				
RL	.734**	.700**	.641**	.591**	.611**	.251*	.374**	.351**	.524**	.551**	.007	.277**	.698**	1			
G%	.317**	.301**	.234**	.222*	.241*	-.027	.176	.187	.266**	.282**	.141	.373**	.110	.252*	1		
Cd TF	.088	-.013	.037	.008	.049	-.037	-.048	-.049	.001	.008	-.061	.014	.035	-.112	.024	1	
BCF Cd	-.007	-.002	-.008	.013	.010	-.108	.094	.119	.011	.047	-.089	.017	-.027	.040	.074	.137	1
Pb TF	.013	-.075	-.056	-.161	-.110	.099	.008	-.051	.302**	.263**	.315**	.069	-.009	-.004	.085	-.035	1
BCF Pb	-.058	.053	-.057	-.004	.017	-.075	.065	.032	-.090	-.077	-.084	-.011	-.046	.019	.053	-.023	1

H: Height, Br (NO): Number of Branches, Fl (NO): Number of flowers, Clu (NO): Number of Clusters, L (NO): Number of Leave, CH: Chlorophyll, P. L: Pod Length Seeds,

P (NO): Number of pods, S. Y: Seed yield, 50% p: 50% podding, W 100 S: Weight of 100 seeds, B: Biomass, Gum%: Gum Percent, RL: Root length, Cd TF: Translocation factor (Cd), Pb TF: Translocation factor (Pb), BCF Pb: Bioconcentration factor (Pb).

Table 6. PCA table of the Roselle landraces and genotypes under Pb and Cd application.

Explained Variance (Eigenvalue)			
Value	Eigenvalue	% of Variance	Cumulative%
PC1	6.593	31.394	31.394
PC2	2.200	10.475	41.869
PC3	1.950	9.285	51.154
PC4	1.386	6.598	57.752
PC5	1.350	6.430	64.182
PC6	1.139	5.425	69.607

Table 7. PCA table of the guar genotypes under Pb and Cd application.									
Components loadings	PC1	PC2	PC3	PC4	Components loadings	PC1	PC2	PC3	PC4
Height	.888	-.138	.017	.034	50% p	.144	-.474	.337	-.400
Br (NO)	.785	.275	-.093	.046	50% f	.279	.570	.004	.041
Fl (NO)	.752	-.294	-.286	-.154	W 100 S	.372	.139	.293	.624
Clu (NO)	.762	-.054	-.427	-.108	B	.710	-.129	-.276	-.268
L (NO)	.669	.056	-.327	.025	RL	.836	.049	-.071	-.060
CH	.172	.511	.459	.135	Gum%	.392	-.189	.143	.410
L.A	.442	-.587	-.171	.334	Cd TF	.023	-.212	-.151	.488
P. L	.602	.540	.116	-.162	BCF Cd	.037	.060	-.093	.319
S (NO)	.603	.515	.043	-.117	Pb TF	.027	-.207	.662	-.203
P (NO)	.682	-.276	.546	.008	BCF Cd	-.027	.136	-.085	.006
S. Y	.762	-.198	.489	-.023					

H: Height, Br (NO): Number of Branches, Fl (NO): Number of flowers, Clu (NO): Number of Clusters, L. A: Leaf Area, CH: Chlorophyll, P. L: Pod Length, S (NO): Number of Seeds, P (NO): Number of pods, S. Y: Seed yield,

50% p: 50% podding, 50% f: 50% flowering, W 100 S: Weight of 100 seeds, B: Biomass, RL: Root length,

Gum%: Gum Percent, Cd TF: Translocation factor (Cd), BCF Cd: Bioconcentration factor (Cd),

Pb TF: Translocation factor (Pb), BCF Pb: Bioconcentration factor (Pb).

Figures

Figure 1
Biplot of the first two principal components (PC1 and PC2) based on the examined parameters which were significant following interaction of Pb and Cd.

Figure 2

Dendrogram of cluster analysis for the interaction of Pb and Cd on investigated traits.

Figure 3

The comparison of the impact of interaction Pb in Cd on weight of 100 seeds and number of branches.

Figure 4

Interaction of genotypes and Pb/Cd on the TF (Pb) (A), BCF (Cd) (B), BAC (Pb) (C) and BCF (Cd).

Figure 5

Interaction of Cd and Pb on Pbtf (A), bcf Pb in root (B), Cd tf (C) and bcf Cd in root (D).

Figure 6

Interaction of Cd and Pb on seed yield, biomass, gum% and root length.