

Research Article

Effects of heavy metals on seed protein fractions in chickpea, *Cicer arietinum* (L.)

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Abstract

Worldwide, different abiotic stresses, such as drought, salinity, and heavy metals, harm crop productivity. Legumes, compared to cereals, are more susceptible to these stresses. The current work aimed to provide more insights into the effects of Cd and Pb on various seed protein characteristics of two cultivars of chickpea (*Cicer arietinum*), HC1 and HC5. At the highest concentrations of Cd, the total seed proteins decreased from 25.2% (control) to 7.1% (30 mg/kg soil), while in the case of the maximum concentration of Pb, 300 mg/kg soil, the protein content decreased to 16.1% from 25.2%. The content of each of the four seed protein fractions viz. albumins, globulins, glutelins and prolamins decreased with an increase in the concentration levels of both heavy metals. The dominating protein fraction, globulins, was reduced by 21.7% in HC1 under Cd stress, while it was reduced by 11.9% in Pb-treated genotype HC5. Electrophoretic analysis of four seed protein fractions on SDS-gels showed only quantitative changes in the polypeptide patterns under varying concentrations of Pb with few qualitative alterations under Cd treatment. The contents of the amino acids tryptophan, cysteine and methionine also decreased with increasing concentrations of heavy metals. Compared to Pb, Cd was found to be more detrimental concerning its influence on seed protein quality. Thus, our analysis revealed how heavy metals impact the quality of chickpea seed proteins by decreasing the content of essential amino acids.

Keywords: Abiotic stresses, *Cicer arietinum*, Heavy metals, Protein fraction

INTRODUCTION

The world's population will be approximately 9.6 billion by 2050 and poses enormous challenges, one of which is achieving food security (Stagnari *et al.*, 2017). Food and Agriculture Organization (FAO, 2020) estimated that 135 million individuals worldwide had been suffering from acute malnutrition before COVID-19, and the current situation is a "crisis within a crisis." To achieve the Sustainable Development Goal (SDG) of ending hunger, access to food for everyone is also a top priority (United Nations, 2019). According to the Worldwide Global Nutrition Report (2020), most countries and India have a high percentage of underweight children due to dietary deficits. Therefore, including food legumes in the diet can improve their health status (Stagnari *et al.*, 2017). With nearly 20,000 species, legumes are the

world's third most crucial crop family after oilseeds and cereals and the second most advantageous crop family (Sita *et al.*, 2017). Therefore, the world's growing population can be secured by ensuring food security through leguminous crops (Foyer *et al.*, 2016; Considine *et al.*, 2017; Liu *et al.*, 2019). With the world's population expected to rise to 11 billion from 7.5 billion by the end of the twenty-first century, 70% more food would be required (United Nations, 2017; Alexandratos, 2009). Chickpeas (*Cicer arietinum* L.) are the principal source of dietary protein for vegetarians among grain legumes (Kudapa *et al.*, 2013). With 20–30% total seed protein, ~41% carbohydrates and 3–6% oil, it is also a good source of minerals such as calcium, phosphorus, iron, zinc, potassium, magnesium and manganese. Food legumes, with high seed protein content, soluble and insoluble fibre, oligosaccharides, phenolics, and essen-

tial nutrients such as vitamins, antioxidants, and biologically active compounds, can provide multiple health benefits to humans and livestock (Meena *et al.*, 2015). However, the seed protein content in food legumes varies considerably, ranging from 16% to 28% in chickpeas (Iqbal *et al.*, 2006). Furthermore, legumes contain a high concentration of globulins and albumins, 70% and 20%, respectively, with prolamins and glutelins serving as minor protein fractions (Duranti, 2006).

Climate change and accompanying biotic and abiotic factors impact crop productivity and yield, as evidenced by the diminishing grain productivity of major crops across the world (Yadav *et al.*, 2015). Drought, heat stress, salinity, and heavy metal (HM) contamination affect legume growth, yield, and quality (Varma and Meena, 2016). The rapid industrial growth, urban development, intensified agricultural practices, and mining have resulted in the significant accumulation of heavy metals in soil and groundwater reserves (Rai *et al.*, 2019). Cd and Pb are released into the environment through mining, improper agricultural practices, and other industrial processes. (Bakirdere *et al.*, 2016). Agricultural soil contaminated with Pb and Cd is a significant concern because it affects consumer health through the food supply (Naveedullah *et al.*, 2013, Hadi *et al.*, 2013). In recent decades, Pb- and Cd-contaminated soils have become major concerns about the potential harm to human health through direct intake and bioaccumulation by the food chain and their effects on the entire ecosystem.

The effects of various heavy metals have been studied on multiple growth parameters in legumes, such as grain quality in groundnut, chickpea and green gram (Farooq *et al.*, 2018), grain oil and vegetative growth in soybean (Farooq *et al.*, 2015; Lebrazi and Fikri-Benbrahim, 2018), chromosomal abnormalities in grass pea (Wei and Cen, 2020), accumulation of seed reserves such as proteins, starch, sugars and minerals, seed quality in chickpea (Valentine *et al.*, 2018), and mineral nutrient balance in peas (Bae *et al.*, 2016). However, studies on the effects of heavy metals on seed protein fractions in chickpea are lacking. Therefore, considering the importance of chickpea in human nutrition and the increasing concentration of heavy metals in soil, the present work was proposed to understand how the rising concentration of heavy metals (Cd and Pb) can impact protein quantity/quality in *Cicer arietinum*.

MATERIALS AND METHODS

The seeds of two cultivars of *C. arietinum* HC1 and HC5 were procured from Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) Hisar, Haryana. A pot experiment was conducted in the experimental plot of the Botany Department, Kurukshetra Uni-

versity, Kurukshetra, Haryana, India, in the last week of October to study the effects of the heavy metals cadmium (Cd) and lead (Pb) on the seed protein fractions of chickpea. For each variety, the pots filled with soil were divided into four sets (i.e., three sets for heavy metal treatments and one set as control) with five replicates in each. Crop thinning was performed to keep five plants per pot. During their vegetative growth, the plants were watered as needed. Seeds were harvested at maturity, dried and ground to seed meal.

Considering the permissible limits of Cd and Pb in the soil and the tolerance range of legumes to these heavy metals, three different concentration levels of each heavy metal, i.e., Cd (as CdCl₂ · H₂O): 10, 20 and 30 mg/kg soil; Pb (as Pb(NO₃)₂): 100, 200 and 300 mg/kg soil, were supplied to plants. The seed meal was defatted using hexane (10 ml/g seed meal) for protein estimation, fractionation of total seed protein into four fractions, estimation of the content of tryptophan, cysteine and methionine in each fraction and electrophoretic separation of fractions on SDS-gels.

Fractionation of seed proteins

For fractionation studies, the Croy *et al.* (1984) method with slight modifications was used. Albumins and globulins were extracted together in 50 mM borate buffer (pH 8) followed by dialysis to separate each one; glutelins and prolamins were extracted in 0.1 N NaOH and 70% ethanol, respectively.

SDS-polyacrylamide gel electrophoresis

The polypeptide patterns of four seed protein fractions were analysed electrophoretically on SDS-gels under reducing conditions following the discontinuous system as given by Davis (1964) and Ornstein (1964) and as per the formulation by Laemmli (1970).

Protein Estimation

For protein estimation, the seed meal's total nitrogen was determined using the semimicro Kjeldahl method as described by Peach and Tracey (1956). Then, the total nitrogen determined was multiplied by 6.25, the standard multiplication factor, to obtain the percentage of total protein in the seed sample.

Proportion of four protein fractions

Bradford's method (1976) was used to determine the proportion of four protein fractions. Using the bovine serum albumin (BSA) standard curve, the final soluble protein concentration in each fraction was calculated.

Tryptophan Estimation

Tryptophan content was determined by following the method given by Spies and Chambers (1948). It is based on the reaction of tryptophan with p-dimethyl amino benzaldehyde and the subsequent development

of blue colour by oxidation with sodium nitrite. For this, 5 ml of dimethyl amino benzaldehyde (0.3% DAB in 19N H₂SO₄) was added to 0.5 ml of a given protein fraction followed by thorough mixing of the contents and incubation at 25 ± 2°C for 18 h in the dark. Then, 0.1 ml of 0.045% sodium nitrite solution was added. The contents were shaken vigorously, and the intensity of colour development was measured by determining the absorbance at 590 nm. Absorbance values were plotted against their respective standard tryptophan concentrations (10-100 mg/ml).

Methionine estimation

The McCarthy and Sullivan (1941) method was used for the estimation of methionine. To 0.5 ml of a given protein fraction, 1.5 ml of Tris-HCl buffer (pH 7.2) containing 0.002 M ethylenediaminetetraacetic acid (EDTA), 0.5% 2-mercaptoethanol and 50 mg of papain enzyme was added. The tubes were incubated for hydrolysis at 50°C for 4 h after vigorous shaking. Subsequently, the tubes were allowed to cool to room temperature, and 50 ml of m-phosphoric acid was added to each tube to stop the reaction. Then, distilled water was added, the volume was raised to 5 ml, the contents were filtered, and aliquots of the filtrate were used for analysis. To 2 ml of the enzyme hydrolysate, 3 ml of distilled water, 1 ml of 5 N NaOH and 2 ml of 3% glycine were added; contents were mixed, and 0.1 ml of 10% freshly prepared sodium nitroprusside solution was added to each. This was followed by incubation of the contents at 40°C for 10 min in a water bath and then cooling to room temperature. For colour development, 2 ml of 85% m-phosphoric acid was added slowly with constant shaking, and the colour intensity was read at 520 nm. The calibration curve prepared using known concentrations (100-1000 mg/ml) of methionine was used to estimate the methionine concentration.

Cysteine estimation

For cysteine estimation, the method given by Goa (1961) was used. It is based on reducing cysteine by

hydrazine hydrate to hydrogen sulfide concentration, which can be determined colorimetrically. To 0.5 ml of protein fraction, 1.0 ml of distilled water and 3 ml of hydrazine hydrate (99-100%) were added. The contents were heated at 120°C for 18 h and then cooled to room temperature. One milliliter of distilled water was added, and the content was shaken. Then, 5 ml of 8N H₂SO₄ was added, and the H₂S produced was collected in 10 ml of bismuth nitrate, which changed to yellow upon reduction with H₂S. The yellow concentration due to bismuth sulfide thus formed was determined spectrophotometrically at 400 nm.

To prepare bismuth nitrate solution, 2.2 g of bismuth nitrate pentahydrate was dissolved in 250 ml of 3.2% mannitol solution in distilled water. To this solution, 80 ml glycerol and 360 ml of 2.5% solution of gum arabic in water were added. This solution was made 1 litre with 0.2 N acetate buffer, pH 4.4 and filtered.

RESULTS

Total seed protein content and four protein fractions

The seed protein content in the analysed chickpea genotypes under varying concentrations of Cd and Pb are shown in Fig. 1. In mature seeds of cultivar HC1, the protein content showed a considerable decrease from 24.5% in the control to 7.1% at the highest concentration (30 mg) of Cd. In the case of Pb treatment, the protein content in cultivar HC5 decreased from 25.2% (control) to 16.1% at the maximum concentration (300 mg/kg).

The effects of different concentrations of Cd and Pb on the four protein fractions are shown in Fig. 2. With the increase in Cd supply from zero in the control to 30 mg/kg, albumins decreased from 30.1 to 17.1 mg/g seed meal; the globulins decreased from 125.2 to 98.1 mg/g seed meal. Glutelins and prolamins also followed a decreasing trend with an increase in concentrations of Cd. An increase in Pb supply also diminished all four protein fractions. Albumins decreased from 31.5 to 19.1 mg/g seed meal, globulins from 130.2 to 114.7

Table 1. Proportion of four seed protein fractions under varying concentrations of Pb and Cd

Genotype	Heavy metals and their concentrations		Proportion of four fractions (%)			
	Cd	Albumins	Globulins	Glutelins	Prolamins	
HC1	Control	14.8	65.6	14.7	4.9	
	10 mg/kg	14.4	67.4	13.8	4.4	
	20 mg/kg	16.2	66.9	12.8	4.1	
	30 mg/kg	17.6	66.0	13.2	3.2	
HC5	Pb					
	Control	14.6	66.7	13.2	5.5	
	100 mg/kg	14.1	66.1	14.8	5.0	
	200 mg/kg	13.6	65.9	15.3	5.2	
	300 mg/kg	12.7	66.4	15.5	5.4	

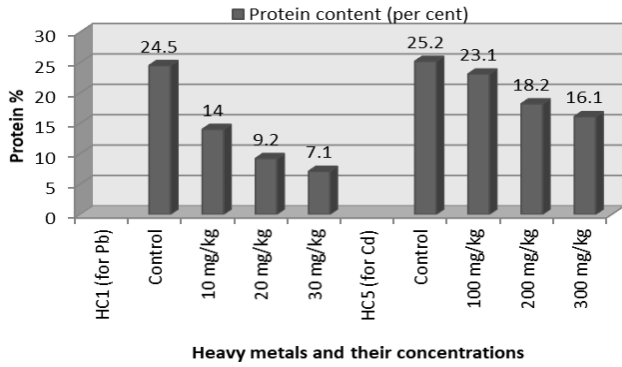


Fig. 1. The seed protein content (%) under different concentrations of Cd and Pb

mg/g seed meal, glutelins from 26.8 to 18.1 mg/g seed meal and prolamins from 5.6 to 4.2 mg/g seed meal. The status of the four protein fractions concerning their relative proportions under varying concentrations of Cd and Pb is given in Table 1. Thus, the relative proportion of albumins and globulins in Cd-treated plants increased from 14.8% to 17.6% and 65.6% to 66.0%, respectively. At the same time, the relative proportion of glutelins and prolamins decreased from 14.7% to 13.2% and 4.9% to 3.2%, respectively. In the case of Pb treatment, the relative proportion of albumins and globulins decreased from 14.6% to 12.7% and 66.7% to 66.4%, respectively. Glutelins increased from 13.2% to 15.5%, while a slight 5.5% to 5.4% reduction was observed in the prolamin fraction.

Amino acids

The four protein fractions analysed for tryptophan, methionine and cysteine content and the relative contribution of each fraction to the total content of each amino acid under increasing levels of both heavy metals are shown in Table 2. Treatment of HC1 with higher levels of Cd resulted in a decrease in total tryptophan content and its contribution due to albumins and glutelins. In contrast, the tryptophan contribution due to globulins and prolamins increased. In HC5, increased Pb concentrations led to a rise in the total tryptophan content as well as its relative contribution due to albumins (15.2% to 18.8%) and glutelins (14.6% to 17.5%). The contribution to tryptophan by globulins and prolamins decreased.

The total methionine and its relative contribution due to albumins, globulins, and prolamins decreased as the Cd concentration increased. After treatment with different Pb levels, total methionine and its relative contribution increased from 21.6% to 23.4% and 6.8% to 11.9% due to glutelins and prolamins, respectively. In contrast, methionine and its relative contribution due to albumins and globulins followed a decreasing trend (25.4% to 22.1% and 46.2% to 42.6%, respectively, as shown in Table 2).

The total cysteine and its relative contribution due to

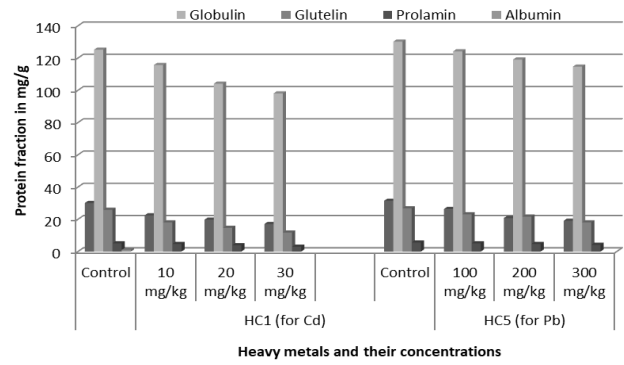


Fig. 2. Effect of heavy metals on four seed protein fractions

glutelins and prolamins followed a decreasing trend as the Cd concentration increased, while albumins and globulins followed an increasing pattern (Table 2). After treatment with different Pb levels, total cysteine and its relative contribution increased from 19.1% to 24.5% and 6.9% to 11.1% for glutelins and prolamins, respectively. In contrast, the albumins and globulins contribution towards the total followed a decreasing trend from 21.5% to 18.5% and 52.5% to 45.9%, respectively.

Polypeptide patterns on SDS-gels

The polypeptide patterns of different seed protein fractions of the genotype HC5 and standard protein molecular weight markers are shown in Fig. 3. Both qualitative and quantitative differences were observed in the polypeptide patterns of protein fractions under the influence of varying levels of Cd (Fig. 4). Globulins are composed of many polypeptides with molecular weights ranging between *Mr* 13 kDa and 75 kDa. The polypeptides of *Mr* 33 kDa and 13 kDa were intense and dark, followed by those of *Mr* 53 kDa and 41 kDa, which were prominent but relatively low intensity. A few polypep-

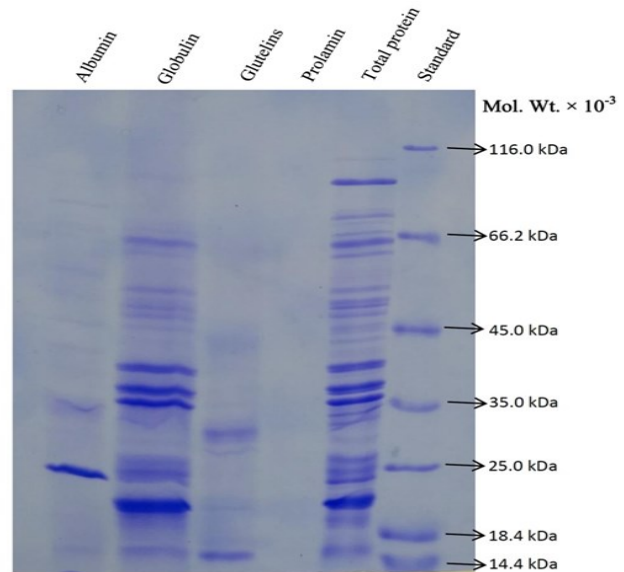


Fig. 3. SDS-PAGE of four seed protein fractions

Table 2. Effect of heavy metals on the proportion of amino acid contributions of four protein fractions

Geno- type	Heavy metals and their con- centrations	Contribution of fractions (g/100gm seed meal)											
		Tryptophan				Methionine				Cysteine			
		al	glo	glu	pro	al	glo	glu	pro	al	glo	glu	pro
Cd													
HC1	Control	23.2	54.4	16.0	6.4	24.8	45.2	19.0	11.0	20.4	46.1	23.2	10.3
	10 mg/kg	21.0	55.8	14.6	8.6	23.2	44.3	22.1	10.4	23.2	46.7	21.5	8.6
	20 mg/kg	18.2	58.2	14.0	9.6	22.5	43.4	24.4	9.7	23.8	48.7	20.3	7.2
	30 mg/kg	16.1	59.2	13.6	11.1	22.0	39.9	28.6	9.5	26.9	49.0	18.5	5.6
Pb													
HC5	Control	15.2	57.7	14.6	12.5	25.4	46.2	21.6	6.8	21.5	52.5	19.1	6.9
	100 mg/kg	16.8	55.6	15.4	12.2	24.9	45.7	22.2	7.2	22.1	47.8	21.8	8.3
	200 mg/kg	17.5	55.2	16.4	10.9	24.1	45.4	23.0	7.5	21.8	46.5	22.7	9.0
	300 mg/kg	18.8	53.7	17.5	10.0	22.1	42.6	23.4	11.9	18.5	45.9	24.5	11.1

(al= albumins, glo= globulins, glu= glutelins, pro= prolamins)

tides of *Mr* 79 kDa, 31 kDa, 19 kDa and 17 kDa are represented by lightly stained bands. Bands with *Mr* 79 kDa, 53 kDa and 19 kDa were absent in the control sample of globulins and appeared as new by different Cd treatments. As shown in Fig. 4, albumins were represented by polypeptides of *Mr* 29 kDa, 19 kDa and 32 kDa. The polypeptides in glutelins were lightly stained, while the polypeptides of prolamins were not visible on the gel.

The globulins in Pb-treated genotype HC5 were represented by polypeptides of *Mr* 13 kDa to 68 kDa (Fig. 5). Major polypeptides of *Mr* 36 kDa, 31 kDa and 29 kDa were intense and dark, but those of *Mr* 68 kDa, 55 kDa, 19 kDa, 18 kDa and 17 kDa were prominent but of relatively low intensity. Polypeptides of *Mr* 65 kDa, 50 kDa, 49 kDa and 13 kDa were represented by very lightly stained bands. No change in polypeptides was observed in globulins with different treatments. Albumins are represented by a polypeptide of *Mr* 20 kDa with varying intensity at different supply levels of Pb. Glute-

lins are comprised of polypeptides of *Mr* 20 kDa, 25 kDa and 38 kDa. Prolamins were not visible on the gel under treatment with lead (Fig. 5).

DISCUSSION

The present investigation suggests that heavy metal (Pb and Cd) inputs in soil affect the seed protein characteristics of both genotypes (HC1 and HC5) of chickpea. The decreasing effects of higher doses of heavy metals on total seed protein content, as in the present study, have also been reported earlier (Farooq *et al.*, 2018; Haider *et al.*, 2021). A decreasing trend of seed protein content under Pb has also been observed in cereals, other pulses and oilseeds (Zulfiqar *et al.*, 2019). The relative proportion of albumins and globulins decreased under Cd supply. In contrast, in the case of Pb, these protein fractions increased, so Cd was more toxic to the major fractions. These results confirm the findings of Chaoui and El Ferjani (2013) in lentils

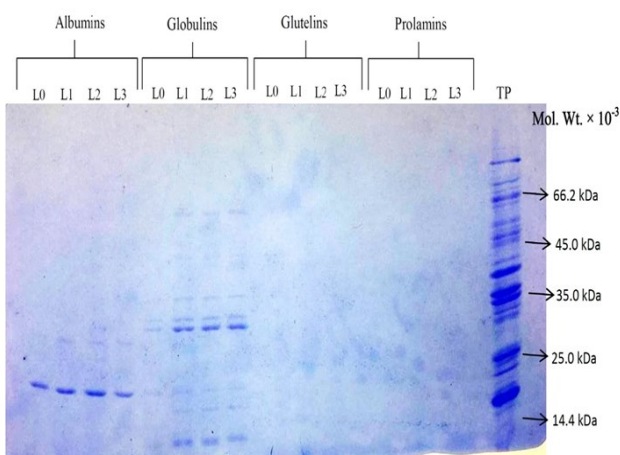


Fig. 4. SDS-PAGE of four fractions from seeds of plants (HC1) grown under different concentrations of Cd (TP= Total proteins, L0= Control, L1= 10 mg/kg, L2= 20 mg/kg, L3= 30 mg/kg)

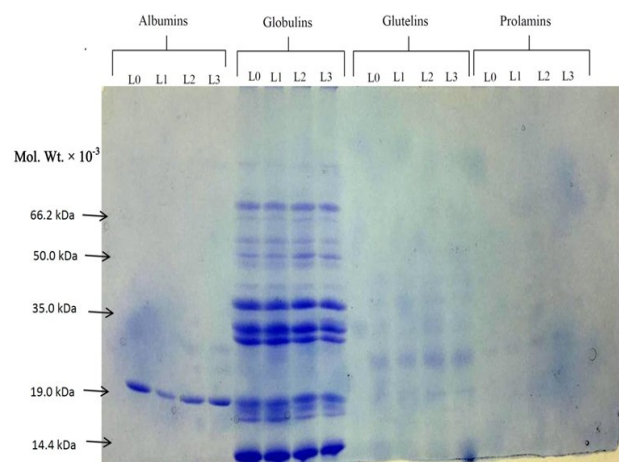


Fig. 5. SDS- PAGE of four fractions from seeds of plants (HC5) grown under different concentrations of Pb (TP= Total proteins, L0= Control, L1= 100 mg/kg, L2= 200 mg/kg, L3= 300 mg/kg)

and Jaouani *et al.*, 2018 in *Pisum sativum*.

Changes in the structure, composition and accumulation patterns of seed proteins under the influence of changing mineral supply levels, salinity, drought, heavy metals, phytohormones, etc., have been studied in different crops, such as wheat, rice, chickpea, and *Phaseolus vulgaris*. (Murumkar and Chavan, 1986; Jacobsen and Shaw, 1989; Soliman *et al.*, 1994; Goldberg, 2003; Bhardwaj *et al.*, 2009; Baxter *et al.*, 2011; Kumar *et al.*, 2017). In contrast to earlier investigations, the present study extended to seed protein fractions and the content of some essential amino acids, such as tryptophan, cysteine and methionine, in chickpea under the influence of Cd and Pb. Protein accumulation during seed formation is the combined outcome of various genetic and environmental factors. The decrease in protein content may be ascribed to a decline in the rate of protein synthesis or an enhanced rate of protein degradation (Balestrasse *et al.*, 2003; Fei *et al.*, 2018). Heavy metals are well recognized to enhance protein degradation (Gadd and Griffith, 1978) and hydrolytic activities of proteases, DNase, and RNase enzymes (Lee *et al.*, 1976a). Heavy metals also induce anatomical changes that affect physiological activities and ultimately seed protein quality (Ali and Malik, 2021). Heavy metal (HM) stress generates hindrances in the folding of nascent proteins; therefore, misfolded/damaged proteins that fail to obtain their native conformation are targeted for degradation by the ubiquitin–proteasome process (UPS) or via autophagy to minimize their accumulation (Liu and Howell' 2016; Hasan *et al.*, 2017). Additionally, soil contamination with HMs greatly reduces the symbiotic nitrogen fixation that leads to reduced accumulation of seed storage proteins.

In the present study, the accumulation of amino acids, tryptophan, cysteine and methionine in chickpea seeds was also substantially affected by heavy metals. Compared to Pb, Cd proved more detrimental in terms of its influence on the content of amino acids, as noted by Gianazza *et al.* (2007) in *Lepidium sativum*. This must be because of the HM-induced reactive oxygen species (ROS) that promote DNA damage, weaken DNA repair mechanisms and disturb the functional membrane integrity, nutrient uptake, and tertiary structure/activity of proteins/enzymes (Tamas *et al.*, 2014). All such changes eventually lead to reduced accumulation/synthesis of amino acids and seed protein fractions (Garcia *et al.*, 2006, Mishra *et al.*, 2006).

Pb and Cd are also known to interact with carboxyl, sulfhydryl, and amine functional groups of proteins, affecting their functions (Tan *et al.*, 2010; Sarkar *et al.*, 2021). As a result of their structural modifications, modified proteins/enzymes cannot function correctly, resulting in cellular malfunctioning. Electrophoretic analysis of seed protein fractions (Fig. 4) in the present study on chickpea under the influence of Cd revealed qualitative

and quantitative changes in polypeptide patterns of protein fractions. However, in the case of Pb (Fig. 5), only quantitative differences were observed. The newly seen polypeptides in protein fractions and polypeptides that exhibited an increase/decrease in their intensity may be considered stress-responsive proteins. The alterations observed reflect the upregulation and down-regulation of genes for different polypeptides and the expression of certain newly induced genes by Cd and Pb. Several proteases have been reported to increase under heavy metal stress (Lee *et al.*, 1976b). Therefore, increasing the activity of these proteases might also account for the decrease in the intensity of certain polypeptides. Accordingly, planning further studies towards understanding the molecular mechanisms that regulate such alterations in protein fractions will be of great interest and significance. The use of forward-looking techniques such as MALDI-TOF-MS should provide novel insight into the precise nature of changes in polypeptides and their role in metabolic changes under Cd and Pb stress.

Conclusion

The present study outlines the impact of Cd and Pb on the accumulation of total proteins, the proportion of four protein fractions and the content of some essential amino acids in chickpea. This study illustrates that the quality of chickpea seed proteins is negatively affected by HMs by decreasing the accumulation of albumin and globulin fractions (rich in essential amino acids). Therefore, to conserve the nutritional quality of chickpea/legume proteins, some novel strategies should be developed to reduce the uptake of HMs and their translocation in the plant system either by chelating HMs in soil or manipulating the plant proteins to restrict the uptake of HMs from the soil.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Alexandratos, N. (2009). World food and agriculture to 2030/50 highlights and views from mid-2009, Expert Meeting on How to feed the World in 2050, Food and Agriculture Organization of the United Nations, Economic and Social Development Department, 24–26 Jun, 2009
2. Ali, Q. & Malik, A. (2021). Genetic evaluation of legume species under heavy metal and biogas wastewater treatment. *Biological and Clinical Sciences Research Journal*, 1–6. <https://doi.org/10.54112/bcsrj.v2021i1.45>
3. Bae, J., Benoit, D.L. & Watson, A.K. (2016). Effect of heavy metals on seed germination and seedling growth of common ragweed and roadside ground cover legumes. *Environmental Pollution*, 213, 112–118. <https://doi.org/10.1016/>

- j.envpol.2015.11.041
4. Bakırdere, S., Bo'lu'cek, C. & Yaman, M. (2016). Determination of contamination levels of Pb, Cd, Cu, Ni, and Mn caused by former lead mining gallery. *Environmental Monitoring and Assessment*, 188, 1-7. 10.1007/s10661-016-5134-5
 5. Balestrasse, K.B., Benavides, M.P., Gallego, S.M. & Tomaro, M.L. (2003). Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants. *Functional Plant Biology*, 30, 57-64 <https://doi.org/10.1071/FP02074>
 6. Baxter, G., Zhao, J. and Blanchard, C. (2011). Salinity alters the protein composition of rice endosperm and the physico-chemical properties of rice flour. *The Journal of the Science of Food and Agriculture*, 91, 2292-2297. <https://doi.org/10.1002/jsfa.4458>
 7. Bhardwaj, P., Chaturvedi, A. K., & Prasad, P. (2009). Effect of enhanced lead and cadmium in soil on physiological and biochemical attributes of *Phaseolus vulgaris* L. *Nature and science*, 7, 63-75.
 8. Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
 9. Chaoui, A., & El Ferjani, E. (2013). β -Estradiol protects embryo growth from heavy-metal toxicity in germinating lentil seeds. *Journal of Plant Growth Regulation*, 32, 636-645. <https://doi.org/10.1007/s00344-013-9332-x>
 10. Considine, M. J., Siddique, K. H. & Foyer, C. H. (2017). Nature's pulse power: Legumes, food security and climate change. *Journal of Experimental Botany*, 68, 1815-1818. <https://doi.org/10.1093/jxb/erx099>
 11. Croy, R. R. D., Houque, M., Gatehouse, J. A. & Boulter, D. (1984). The major albumin proteins from pea (*Pisum sativum* L.) Purification and some properties. *Biochemical Journal*, 218, 795-803. 10.1042/bj2180795
 12. Davis, B.J.B. (1964). Disk electrophoresis. II. Method and application to human serum proteins. *The Annals of the New York Academy of Sciences*, 12, 404-427. 10.1111/j.1749-6632.1964.tb14213.x
 13. Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia*, 77, 67-82. 10.1016/j.fitote.2005.11.008
 14. FAO.(2020).<http://www.fao.org/news/story/en/item/1269721/code/>. Retrieved 15 Sept 2020
 15. Farooq, M., Hussain, M., Usman, M., Farooq, S., Alghamdi, S.S., Siddique & K.H.M. (2018). Impact of abiotic stresses on grain composition and quality in food legumes. *Journal of Agricultural and Food Chemistry*, 66, 8887-8897. <https://doi.org/10.1021/acs.jafc.8b02924>
 16. Farooq, M., Hussain, M., Wakeel, A., Siddique, K.H.M. (2015). Salt stress in maize: Effects, resistance mechanisms and management. A review. *Agronomy for Sustainable Development*. 35, 461-481. <https://doi.org/10.1007/s13593-015-0287-0>
 17. Fei, M., GuanJun, N., Meng, C., Yuqiong, G., Liying, G., Xianxin, M. & Guangde, Y. (2018) The metal distribution and the change of physiological and biochemical process in soybean and mung bean plants under heavy metal stress. *International Journal of Phytoremediation*, 20, 1113-1120. 10.1080/15226514.2017.1365346
 18. Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H., Varshney, R. K. & Colmer, T. D. (2016). Neglecting legumes has compromised human health and sustainable food production. *Nature Plants*, 2, 1-10. <https://doi.org/10.1038/nplants.2016.112>
 19. Gadd, G.M. & Griffith, A.J. (1978). Microorganisms and heavy metal toxicity. *Microbial Ecology*, 4, 303-317
 20. Garcia, J.S., Gratao, P.L., Azevedo, R.A. & Arruda, M.A.Z. (2006) Metal contamination effects on sunflower (*Helianthus annuus* L.) growth and protein expression in leaves during development. *The Journal of Agricultural and Food Chemistry*, 54, 8623-8630. 10.1021/jf061593l
 21. Gianazza, E., Wait, R., Sozzi, A., Regondi, S., Saco, D. & Labra, M. (2007). Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. *Environmental and Experimental Botany*, 59, 179-187. 10.1016/j.envexpbot.2005.12.005
 22. Goa, J. (1961). Micro determination of cysteine plus cystine in proteins. *Acta Chemica Scandinavica*, 15, 853-855
 23. Goldberg, A. L. (2003). Protein degradation and protection against misfolded or damaged proteins. *Nature*, 426, 895-899. 10.1038/nature02263
 24. Hadi, P., Gao, P., Barford, J. P. & McKay, G. (2013). Novel application of the nonmetallic fraction of the recycled printed circuit boards as a toxic heavy metal adsorbent. *Journal of Hazardous Materials*, 252, 166-170. 10.1016/j.jhazmat.2013.02.037
 25. Haider, F.U., Liqun, C., Coulter, J.A., Cheema, S.A., Wu, J., Zhang, R., Wenjun, M. & Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety*, 211, 111887. <https://doi.org/10.1016/j.ecoenv.2020.111887>
 26. Hasan, M., Cheng, Y., Kanwar, M. K., Chu, X. Y., Ahammed, G. J., & Qi, Z. Y. (2017). Responses of plant proteins to heavy metal stress—a review. *Frontiers in plant science*, 8, 1492. <https://doi.org/10.3389/fpls.2017.01492>
 27. Iqbal A, Khalil I. A., Ateeq, N. & Khan, M.S. (2006). Nutritional quality of important food legumes. *Food Chemistry*, 97, 331-335 <https://doi.org/10.1016/j.foodchem.2005.05.011>
 28. Jacobsen, J.V. & Shaw, D.C. (1989). Heat-stable proteins and abscisic acid action in barley aleurone cells. *Plant Physiology*, 91, 1520-1526. <https://doi.org/10.1104/pp.91.4.1520>
 29. Jaouani, K., Karmous, I., Ostrowski, M., El Ferjani, E., Jakubowska, A., & Chaoui, A. (2018). Cadmium effects on embryo growth of pea seeds during germination: investigation of the mechanisms of interference of the heavy metal with protein mobilization-related factors. *Journal of plant physiology*, 226, 64-76. <https://doi.org/10.1016/j.jplph.2018.02.009>
 30. Kudapa, H., Ramalingam, A., Nayakoti, S., Chen, X., Zhuang, W., Liang, X., Kahl, G., Edwards, D. & Varshney, R.K. (2013) Functional genomics to study stress responses in crop legumes: progress and prospects. *Functional Plant Biology*, 40, 1221-1233. 10.1071/FP13191
 31. Kumar, Y., Singh, A., & Matta, N. K. (2017). Proteomics of barley grains under varying salinity levels. *Journal of Proteins and Proteomics*, 8, 49-63.
 32. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. <https://doi.org/10.1038/227680a0>
 33. Lebrazi, S. & Fikri-Benbrahim, K. (2018). Rhizobium-Legume symbioses: Heavy metal effects and principal approaches for bioremediation of contaminated soil. In:

- Legumes Soil Health Sustainable Management* (pp. 205–233). Springer, Singapore
34. Lee, K.C., B.A. Cunningham, K.H. Chung, G.M. Baulsen, G.H. Liang and R.B. Moore. (1976b). Lead effects on several enzymes and nitrogenous compounds in soybean leaf. *Journal of Environmental Quality*, 5, 357-359.
 35. Lee, K.C., Cunningham, B.A., Chung, K.H., Baulsen, G.M., Liang, G.H. & Moore, R.B. (1976a). Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. *Physiologia Plantarum*, 36, 4-6
 36. Liu, J. X., & Howell, S. H. (2016). Managing the protein folding demands in the endoplasmic reticulum of plants. *New Phytologist*, 211, 418-428. <https://doi.org/10.1111/nph.13915>
 37. Liu, W., Min, X., & Wang, Y. (2019). Genome-wide development of microRNA-based SSR markers in *Medicago truncatula* with their transferability analysis and utilization in related legume species. *The Model Legume Medicago truncatula*, 936-945. [10.3390/ijms18112440](https://doi.org/10.3390/ijms18112440)
 38. McCarthy, T. E., & Sullivan, M. X. (1941). A new and highly specific colorimetric test for methionine. *Journal of Biological Chemistry*, 141, 871-876. [10.1016/s0021-9258\(18\)72759-2](https://doi.org/10.1016/s0021-9258(18)72759-2)
 39. Meena, R.S., Meena, V.S., Meena, S.K. & Verma, J.P. (2015) The needs of healthy soils for a healthy world. *Journal of Cleaner Production*, 102, 560-561. [10.1016/j.jclepro.2015.04.045](https://doi.org/10.1016/j.jclepro.2015.04.045)
 40. Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. & Gupta, D.K. (2006). Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere*, 65, 1027-1039. [10.1016/j.chemosphere.2006.03.033](https://doi.org/10.1016/j.chemosphere.2006.03.033)
 41. Murumkar, C.V. & Chavan, P.D. (1986). Influence of salt stress on biochemical processes in chickpea, *Cicer arietinum* L. *Plant Soil*, 96, 439-443.
 42. Naveedullah, N., Hashmi, M. Z., Yu, Ch., Shen, H., Duan, D. & Shen, Ch. (2013). Risk assessment of heavy metals pollution in agricultural soils of siling reservoir watershed in Zhejiang province, China. *Journal of Biomedical and Biotechnology*, 2013, 1-10. [10.1155/2013/590306](https://doi.org/10.1155/2013/590306)
 43. Ornstein, L. (1964). Disc electrophoresis. I. Background and Theory. *Annals of the New York Academy of Sciences*, 121, 321-349. [10.1111/j.1749-6632.1964.tb14207.x](https://doi.org/10.1111/j.1749-6632.1964.tb14207.x)
 44. Peach, K., Tracey, M.V. (1956). *Modern methods of plant analysis*, vol. 1, (pp. 468-502). Springer Verlag, Berlin, Gottingen, Heidelberg
 45. Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F., & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International*, 125, 365-385. <https://doi.org/10.1016/j.envint.2019.01.067>
 46. Sarkar, S., Khatun, M., Era, F.M., Islam, A.K.M., Anwar, M., Danish, S., Datta, R. & Islam, A.K.M. (2021). Abiotic stresses: Alteration of composition and grain quality in food legumes. *Agronomy*, 11, 2238. <https://doi.org/10.3390/agronomy11112238>
 47. Sita, K., Sehgal, A., HanumanthaRao, B., Nair, R. M., Vara-Prasad, P. V., Kumar, S., Gaur, P.M., Farooq, M., Siddique, K.H., Varshney, R.K. & Nayyar, H. (2017). Food legumes and rising temperatures: Effects, adaptive functional mechanisms specific to reproductive growth stage and strategies to improve heat tolerance. *Frontiers in Plant Science*, 8, 1658. <https://doi.org/10.3389/fpls.2017.01658>
 48. Soliman, M.S., Shalabi, H.G. & Campbell, W.F. (1994). Interaction of salinity, nitrogen and phosphorous fertilization on wheat. *Journal of Plant Nutrition*, 17, 1163-1173.
 49. Spies, J.R. & Chambers, D.C. (1948). Chemical determination of tryptophan. Study of color formation reaction of tryptophan, p-dimethylaminobenzaldehyde and sodium nitrite in sulfuric acid solution. *Analytical Chemistry*, 20, 30. <https://doi.org/10.1021/ac60013a006>
 50. Stagnari, F., Maggio, A., Galieni, A., & Pisante, M. (2017). Multiple benefits of legumes for agriculture sustainability: An overview. *Chemical and Biological Technologies in Agriculture*, 4, 2. <https://doi.org/10.1186/s40538-016-0085-1>
 51. Tamas, M. J., Sharma, S. K., Ibstedt, S., Jacobson, T., & Christen, P. (2014). Heavy metals and metalloids as a cause for protein misfolding and aggregation. *Biomolecules*, 4, 252-267. <https://doi.org/10.3390/biom4010252>
 52. Tan, Y.F., O'Toole, N., Taylor, N.L. & Millar, A.H. (2010) Divalent metal ions in plant mitochondria and their role in interactions with proteins and oxidative stress-induced damage to respiratory function. *Plant Physiology*, 152, 747-761. [10.1104/pp.109.147942](https://doi.org/10.1104/pp.109.147942)
 53. United Nations (2017). Department of Economic and Social Affairs, Population Division. World population prospects: the 2017 revision, key findings and advance tables. Working paper No. ESA/P/WP/1248
 54. United Nations (2019). The Sustainable Development Goals Report 2019. <https://unstats.un.org/sdgs/report/2019/The-Sustainable-Development-Goals-Report-2019.pdf>. Retrieved 7 July 2020.
 55. Valentine, A.J., Benedito, V.A. & Kang, Y. (2018). Legume nitrogen fixation and soil abiotic stress: From physiology to genomics and beyond. In: *Nitrogen Metabolism in Plants in the PostGenomic Era* (pp. 207–248). Annual Plant Reviews Book Series; Wiley Online Library: Hoboken, NJ, USA
 56. Varma, D. & Meena, R.S. (2016) Mungbean yield and nutrient uptake performance in response of NPK and lime levels under acid soil in Vindhyan region, India. *Journal of Natural and Applied Sciences*, 8, 860-863
 57. Wei, J. & Cen, K. (2020). Contamination and health risk assessment of heavy metals in cereals, legumes, and their products: A case study based on the dietary structure of the residents of Beijing, China. *The Journal of Cleaner Production*, 260, 121001 <https://doi.org/10.1016/j.jclepro.2020.121001>
 58. Worldwide Global Nutrition Report. (2020). <https://globalnutritionreport.org/reports/global-nutrition-report-2020/>. Retrieved 12 May 2020
 59. Yadav, S. S., Hunter, D., Redden, B., Nang, M., Yadava, D. K., & Habibi, A. B. (2015). Impact of climate change on agriculture production, food, and nutritional security. In: *Crop Wild Relatives and Climate Change* (pp.1-23), Wiley
 60. Zulfiqar, U., Farooq, M., Hussain, S., Maqsood, M., Hussain, M., Ishfaq, M., Ahmad, M. & Anjum, M.Z. (2019). Lead toxicity in plants: Impacts and remediation. *Journal of Environmental Management*, 250, 109557. <https://doi.org/10.1016/j.jenvman.2019.109557>