



## Original article

Lead contamination affects the primary productivity traits, biosynthesis of macromolecules and distribution of metal in durum wheat (*Triticum durum* L.)Mahwish Malik<sup>a</sup>, Seema Mahmood<sup>a,\*</sup>, Sibgha Noreen<sup>a,\*</sup>, Rafia Abid<sup>a</sup>, Shazia Ghaffar<sup>a</sup>, Saman Zahra<sup>a</sup>, Tariq Shah<sup>b</sup>, Ajaz Ahmad<sup>c</sup><sup>a</sup> Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan<sup>b</sup> Agroecology, Université de Bourgogne, Dijon, France<sup>c</sup> Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

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## ABSTRACT

Lead (Pb) pollution emerged as an international issue particularly during second and third industrial revolution and is of serious global concern. Cereal crops have shown different capabilities, innate variability and mechanisms to cope with heavy metals present in their environment. Keeping in view the perspectives of food security and safety with increasing demand for *Triticum durum* L. it becomes imperative to appraise sustainability potential of the crop for Pb contaminated soils. The current study was conducted to test the hypothesis that *T. durum* germplasm holds genetic variability to evolve under Pb contamination through modulations of morpho-biochemical parameters of selective advantage. The performance of nine *T. durum* L. cultivars (CBD25, CBD46, CBD58, CBD59, CBD63, CBD66, CBD68, CBD69 and CBD82) was evaluated following exposure to varying Pb levels (control, 10, 20 and 40 mg kg<sup>-1</sup>) in soil. Growth, biosynthesis of macromolecules and metal distribution in plant parts were assessed using valid procedures and protocols. The cultivars exhibited a differential degree of tolerance to Pb and among the tested germplasm, CBD59 performed better followed by CBD63 and CBD66 for their primary productivity traits, biosynthesis of pigments and other macromolecules (amino acids, proteins and sugar) along with resilience for Pb uptake and its consequent bioaccumulation in grains. The traits used in the study served as strong predictors to provide superior/selective ability to survive under contaminated environment. The study signified that metal tolerance/sensitivity in the cultivars is independent of magnitude of metal stress, growth responses and Pb accumulation in plant parts hence varied in space and time. The existence of genetic variability, which is a pre-requisite for selection can definitely be of great advantage for future breeding projects to develop high yielding varieties/ cultivars of durum wheat with Pb free grains to assure food security and safety.

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## 1. Introduction

Environmental contamination by heavy metals has become a major concern for terrestrial and aquatic ecosystems around the

globe during the past few decades (Kohli et al., 2018; Mallhi et al., 2019; Bali et al., 2018, 2019a,b; Ullah et al., 2020). Heavy metals once introduced into the environment cannot be biodegraded and when entered the food chain pose serious threats to food security and safety hence human health (Azizpour et al., 2020; Souri et al., 2021; Bhat et al., 2021; Siddiqui et al., 2020; Kaya et al., 2020; Ahmad et al., 2020a,b; Alsahli et al., 2020). According to the Agency for Toxic Substances and Disease Registry, Lead (Pb), is a ubiquitous industrial and environmental pollutant that is ranked second based on its occurrence and toxicity to living organisms. In many developing countries, high anthropogenic pressures and industrialization such as metal industry, smelting, mining, lead-acid batteries, petroleum fueled vehicles, radiation

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shields, paint, ceramics, untreated municipal wastewater, industrial effluents, pesticides and application of phosphate fertilizers has contributed substantial contamination of the environment by Pb (Kushwaha et al., 2018).

Being sessile organisms, plants remain exposed to adverse environmental pressures including heavy metals in the growth medium throughout their lifespan. Though, Pb is a non-essential substance but a highly mobile element that can easily penetrate into the roots where its bioaccumulation or further translocation to the aerial tissues takes place (Kohli et al., 2018; Mallhi et al., 2019; Bali et al., 2018, 2019a,b). Subsequently, a significant quantity of the Pb is further translocated to fruits and seeds (Kanwal et al., 2020). Exceeding Pb levels are found to be detrimental for plant growth, development and yield of several crop species (Haider et al., 2021). The hazardous effects of the metal can be identified at different organizational levels in plants ranging from morphological, anatomical, sub-microscopic to molecular level (Ullah et al., 2020). Pb has been widely reported to alter plant responses at different stages of development because it induces changes in metabolic pathways involved in the biosynthesis of all types of fundamental macromolecules (carbohydrates, lipids and proteins), activities of enzymes and stability of biological membranes (Jian et al., 2020; Gao et al., 2021). In some plant species, even minute quantity of Pb in the growth medium can cause cell death (Riaz et al., 2021). Although, the responses of plant species at inter and intra-specific levels vary greatly for heavy metals (Sofy et al., 2020). However, superior survival ability of plants to tolerate metal toxicity has been reported through the alterations of several characteristics/ traits that include morphological expressions, sophisticated and coordinated system of synthesis of biomolecules, enzymatic defense and distinctive pattern of metal accumulation in plant tissues (Abid et al., 2019b). During recent past several species belonging to 45 families of angiosperm including members of the grass family (Poaceae) have been known for their capacity to tolerate elevated levels of heavy metals including Pb (Rasool et al., 2020).

Undoubtedly, the future demand for fiber, food, fuel and land will increase considerably therefore, the quest for balancing between crop production and increasing demand for commodities for rapidly growing global population will continue (Li et al., 2018). Major cereal crops (maize, wheat and rice) fulfill up to 60% of food demand of the world amongst 50,000 edible plants (Thielecke and Nugent, 2018; Haider et al., 2021). World grain utilization was approximately 2646 million tons, during 2018–19 as estimated by The Food and Agriculture Organization (FAO, 2018). However, it is expected to become about 9.8 billion tons by 2050 (UN, 2019) for global food consumption. Consequently, the demand for cereal crops will continue due to their consumption as staple food by the tremendously growing human populations of the world.

Wheat as a staple food is broadly cultivated to feed about one third of the human population all over the world. Moreover, it provides dietary components; carbohydrates, proteins, fats, vitamins, minerals ( $\text{Ca}^{+2}$ ,  $\text{Cu}^{+2}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{Fe}^{+2}$  and  $\text{Zn}^{+2}$ ), antioxidants and fibers (Carcea et al., 2020). *Triticum durum* L. (durum wheat) with a world production of more than 36 million tons occupies 10th position amongst important staple crops of the world (Hamid et al., 2020).

However, the demand of durum wheat has increased considerably because of changing dietary choices and preferences globally. Hence, various food items like burger, couscous, macaroni, pasta and pizza became popular. Consequently, several developed countries started growing *T. durum* as a cash crop to meet the demand of booming food industry. Further, durum wheat has widely been used by several international food chains for making various quality products which clearly suggests its increasing demand for

production and consumption as far as commercial scope and marketing of the crop is concerned.

Furthermore, United Nations has set several targets for sustainability of the developing world. Food security and safety has been recognized as a primary objective to achieve these goals and to address nutritional deficiencies in populations of such countries due to consumption of mono-food diets. Thus, *T. durum* L. is becoming a well-suited diet which can potentially improve health and livelihood. It can serve as an important staple food and industrial crop that can considerably contribute for poverty alleviation and better living for smallholder farmers in addition to food security.

*T. durum* has shown its ability to withstand several stresses (abiotic and biotic) and climate changes (Aprile et al., 2018), thus the crop is mostly grown on marginal lands that are exposed to high temperature, drought and irradiance in an environment which is unsuitable for bread wheat and other crops. Since, *T. durum* is well-adapted to many environmental constraints hence the candidature of the crop can be exploited to address challenges relating to food security and safety in relation to heavy metal contamination of the environment to get maximum yield under metal contaminated soil. Therefore, the identification and selection of the varieties/ cultivars of the crop becomes imperative to improve and preserve natural resources/germplasm by applying strong environmental/ selection pressures (Marzario et al., 2018).

Unlike *T. aestivum* L., studies regarding growth and tolerance potential of *T. durum* for Pb contamination are limited. Therefore, the present study was conducted to examine the performance of *T. durum* at intraspecific level after exposure to varying levels of Pb in the soil. The modulations of several morpho-biochemical traits were appraised in relation to Pb. The uptake and distribution of Pb in different plant parts were assessed to imply possible mechanism of metal tolerance and transport in plants and to ascertain the quality of grains after being exposed to Pb. The main objective of our study was to reveal genetic potential of *T. durum* at intra-specific level to address growing concerns of food security and safety with increasing environmental pollution by Pb.

## 2. Material and methods

### 2.1. Plant growth conditions and Lead (Pb) treatments

A pot experiment was conducted in a wire-netting green house, Botanic Garden, Bahauddin Zakariya University, Multan ( $30^{\circ} 10'N$  and  $71^{\circ} 29'E$ ) altitude 123 m a.s.l. The experiment was carried out under natural conditions (day length 11 h; humidity 62% and temperature  $27 \pm 5^{\circ}C$ ) during 2016–2017. Certified germplasm/ cultivars of *Triticum durum* L. viz., CDB25, CBD46, CBD58, CBD59, CBD63, CBD66, CBD68, CBD69 and CBD82 were obtained from Ayyub Agriculture Research Institute (AARI) Faisalabad, Pakistan.

Garden compost (sand 86.81%; clay 8.51% and silt 4.74%) was used for growth experiment. pH (pH meter, Ino Lab-WTB GmbH, Weilheim, Germany) and electric conductivity (Conductivity Meter, WTW-330i, Germany) were determined and maintained for the soil during the course of experiment. 108 plastic pots (height 40 cm and diameter 25 cm) for nine cultivars, four Pb treatments including control with three replications, were labelled correspondingly were filled with 6 kg of garden compost. As a source of Pb, Lead nitrate;  $\text{Pb}(\text{NO}_3)_2$  was used that was thoroughly mixed with soil @ 10, 20 and 40  $\text{mg kg}^{-1}$  of Pb/kg of soil before sowing. Control (0) pots were filled with soil without Pb. Six pre germinated seeds were transplanted into each pot. Gentle showers for watering were used and experiment continued till maturity of plants without fertilizer, pesticide or herbicide applications

however, weeding was done manually. Various measurements were taken and assays were made from fresh plant material.

## 2.2. Primary productivity traits

The growth was assessed through primary productivity traits (root and shoot) measured in cm after 16 weeks of exposure to Pb. Biomass (fresh and oven dried) of below and above ground plant parts was taken using the digital balance (Chyo Balance Corporation, Japan).

## 2.3. Spectrophotometric estimation of photosynthetic pigments and carotenoid ( $\text{mg g}^{-1}$ )

The chlorophyll (*a*, *b* and total) and carotenoids were estimated following Arnon (1949). Extraction of pigments was carried out with 4 ml acetone (80%) followed by spectrophotometry at corresponding wavelengths (645, 663 and 480 nm) using spectrophotometer (BMS, Biotechnology Medical Service, UV-1900, USA).

## 2.4. Estimation of macro/biomolecules

### 2.4.1. Estimation of total soluble amino acids and proteins ( $\text{mg g}^{-1}$ )

By following Yemm et al. (1955) method soluble amino acids were measured. At 570 nm, the optical densities of the assay were read. For protein content, grains were crushed in phosphate buffer (1 ml) and for 10 min homogenized at 7000g. Then, trichloroacetic acid (1 ml) was added and for 15 min again centrifuged at 8000g. The remains were dissolved in 1 ml of NaOH (0.1 N) and 5 ml Bradford reagent was added (Bradford, 1976).

### 2.4.2. Estimation of total soluble sugars ( $\text{mg g}^{-1}$ ).

Soluble sugar content were estimated by Dubois et al. (1956). 0.5 g of fine plant material was added in test tubes containing 5 ml of ethanol (80%) and placed in water bath for 60 min then heated at 80 °C. The assay (1 ml) was mixed with 1 ml of phenol (18%) and distilled water and for one hour left at room temperature. Finally,  $\text{H}_2\text{SO}_4$  (5 ml) added and vortexed. By using spectrophotometer, the absorbance was read at 490 nm.

## 2.5. Lead uptake and distribution in plant parts (roots, shoots and grains)

For metal content, dried plant (roots, shoots and grains) samples were powdered. Plant material (2 g) was digested in 10 ml of  $\text{HNO}_3$ :  $\text{HClO}_4$  (v/v) in 3:1 ratio (Estefan et al., 2013). The digestion was completed in small glass beakers on a hot plate at 150–185 °C for 4 h. The concentration of Pb in plant parts (grain, shoot and root) was determined through AAS (Atomic Absorption Spectrophotometer, Varian AAS, 1475, California, USA).

## 2.6. Statistical analysis

The growth measurements were taken for all three replicates and biochemical analyses was performed three times. Data presented as mean with standard error (+S.E) for each parameters as bar charts. The data was subjected to 2-way Analysis of Variance using MS Excel 2013 to estimate intra-specific variability among *T. durum* cultivars and also to reveal significant effects of Pb levels and the interaction between two factors for each parameter separately presented in Table 1. Least Significant Differences (LSD) were also calculated by employing Duncan's Multiple Range Test (Duncan, 1955) to elucidate differences among cultivars and Pb levels.

## 3. Results and discussions

### 3.1. Growth attributes

The results depicted that growth attributes in terms of elongations of roots and shoots in *T. durum* cultivars varied under the influence of various concentrations of Pb (Fig. 1a). However, a non-significant effect of Pb on the root length was observed (Table 1). Root length did not show any profound reduction but longer roots of CBD25 and CBD63 were noticed at 40  $\text{mg kg}^{-1}$  Pb while CBD59 and CBD 66 had longer roots at level 20  $\text{mg kg}^{-1}$  Pb when compared with all other cultivars (Fig. 1a). Although, the responses of cultivars also varied with increasing levels of Pb but the metal did not induce any drastic reduction in shoot length. CBD46, CBD68 and CBD 69 had shorter shoots as compared to other cultivars at the highest level of Pb (Fig. 1b).

Table 1 clearly depicted that fresh root biomass differed significantly ( $p < 0.001$ ) in cultivars and a marked influence of different levels of Pb also became evident. The highest level of Pb induced more profound reduction in fresh biomass of roots in CBD66 followed CBD68 and CBD69 (Fig. 2a). The cultivar CBD63 had more root biomass at control but a profound decline was observed in plants grown at 20  $\text{mg kg}^{-1}$  Pb. The maximum reduction in fresh root biomass was observed in CBD66 (about 82%) followed by CBD68 which exhibited a decline of about 65% at the most elevated level of Pb (40  $\text{mg kg}^{-1}$  Pb). The cultivars CBD63 and CBD82 had shown consistently better response for fresh biomass of shoot despite exposure to 20 and 40  $\text{mg kg}^{-1}$  Pb. However, CBD46 and CBD558 showed a gradual decline with increasing Pb levels for fresh biomass of shoots (Fig. 2b). The highest level of Pb induced a profound decline (57%) of shoot fresh biomass in CBD66. On the other hand, CBD59 and CBD82 had no such reduction as both cultivars had more biomass with increasing levels of Pb except for CBD82 at 10  $\text{mg kg}^{-1}$  of Pb. (Fig. 2b). The cultivars and varying levels of Pb exhibited significant variability for fresh biomass of both tissues ( $p < 0.001$ ) and hence a highly significant ( $p < 0.001$ ) interaction between cultivars and Pb levels was observed (Table 1).

Fig. 2c & d depicted responses of cultivars for dry biomass of root and shoot which indicated variable responses of the cultivars under varying levels of Pb. The cultivars uniformly had more biomass of roots for control plants but exposure to Pb caused significant ( $p < 0.001$ ) reduction in root biomass except for CBD25 at 40  $\text{mg kg}^{-1}$  Pb. The extent of decline for root biomass was more drastic in CBD68 which had about 71% decline compared to the lowest decline (33%) in CBD59 at the highest level of Pb. Moreover, CBD59 had more dry biomass of roots at all levels of Pb than all other cultivars (Fig. 2c). The cultivars have distinctive responses for shoot dry biomass but in most of the cultivars increasing levels of Pb did not cause any reduction of dry biomass of shoot. Fig. 2d depicted a steady decline in dry biomass was noticed in CBD59 with increasing level of Pb. Whereas, CBD66 had the lowest shoot dry weight at 40  $\text{mg kg}^{-1}$  of Pb but CBD69 was more influenced by 20  $\text{mg kg}^{-1}$  Pb. Moreover, higher levels of Pb did not adversely affect dry biomass of shoots of durum wheat cultivars.

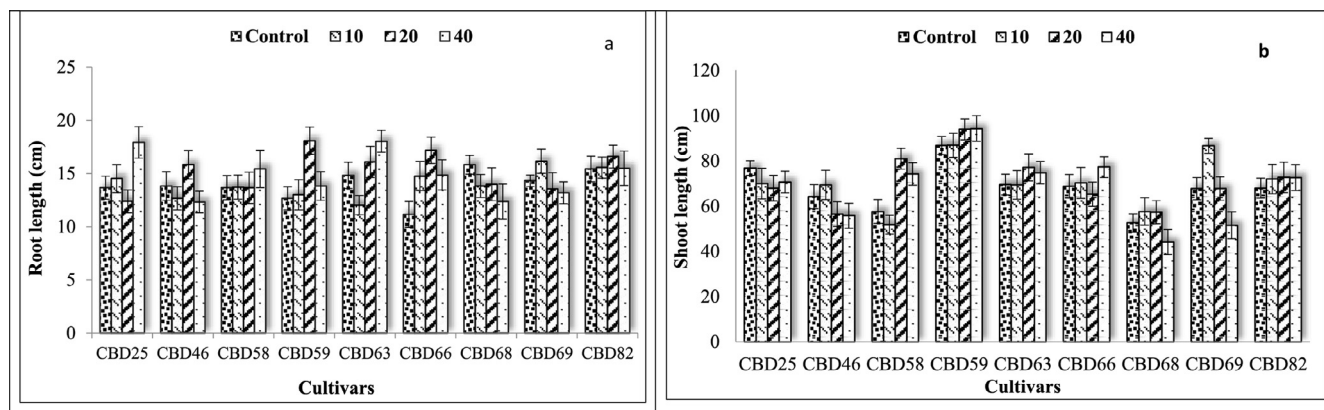
Among primary plant productivity/growth traits, root and shoot development are considered as important determinants to assess their decisively role for plant growth under several environmental constraints (Romdhane et al., 2021) Hence, the results presented clearly depicted that Pb did not cause pronounced inhibitory effects for root and shoot growth but the cultivars exhibited variable responses for the growth traits but more profound changes in cultivars for shoots.

As a principal organ, root anchors the plants and directly encounter with the growth substrate thus all essential and non-essential elements which are present in the soil came in contact

**Table 1**  
Mean square values (two-way ANOVA) of various attributes of nine wheat (*Triticum aestivum* L.) genotypes subjected to varying Pb concentrations.

Attributes	M.S. Cultivars	F	LSD	M.S. Metal concentration	F	LSD	Interaction Cultivar * Metal concentration
Root length (cm)	4.9	N.S	–	11.2	N.S	–	10.5 N.S
Shoot length (cm)	714.2	***	6.1	131.3	N.S	0.00	75.2 N.S
Root fresh biomass (g)	376.4	***	1.2	54.8	***	1.8	287.3***
Shoot fresh biomass (g)	291.7	***	1.7	279.5	***	2.5	240.6***
Dry root biomass (g)	3.9	***	0.3	2.8	***	0.2	3.1***
Dry shoot biomass (g)	7.4	***	0.4	0.8	***	0.2	4.9***
Chlorophyll a (mg g <sup>-1</sup> )	21.6	***	1.5	2.3	N.S	0.00	37.6***
Chlorophyll b (mg g <sup>-1</sup> )	51.2	***	1	39.4	**	1.5	52.7***
Total Chlorophyll (mg g <sup>-1</sup> FW)	77.6	***	3.3	59.7	*	2.2	150.3***
Total Carotenoids (mg g <sup>-1</sup> FW)	0.7	*	0.5	0.5	***	0.098	0.4***
Total soluble amino acids (mg g <sup>-1</sup> FW)	2.2	***	0.3	0.09	N.S	–	1.0***
Total soluble proteins (mg g <sup>-1</sup> FW)	29.2	*	1.9	35.8	*	1.3	35.2***
Total soluble sugars (mg g <sup>-1</sup> FW)	156.1	***	2.2	103.7	***	1.5	60.3***
Pb content (µg g <sup>-1</sup> DW) in roots	351.4	***	1	2070.3	***	0.7	259.1***
Pb content (µg g <sup>-1</sup> DW) in shoots	430.7	***	1.7	1177.4	***	1	313.7***
Pb content (µg g <sup>-1</sup> DW) in grains	0.03	***	0.05	0.33	***	0.03	0.01***

\*\*\*\* = p < 0.05 and 0.001, respectively, N.S = Non-Significant, LSD = Least Significant Difference, M.S. = Mean Square, LSD = Least Significant Difference by Duncan's Multiple Range Test at 5% level of probability.



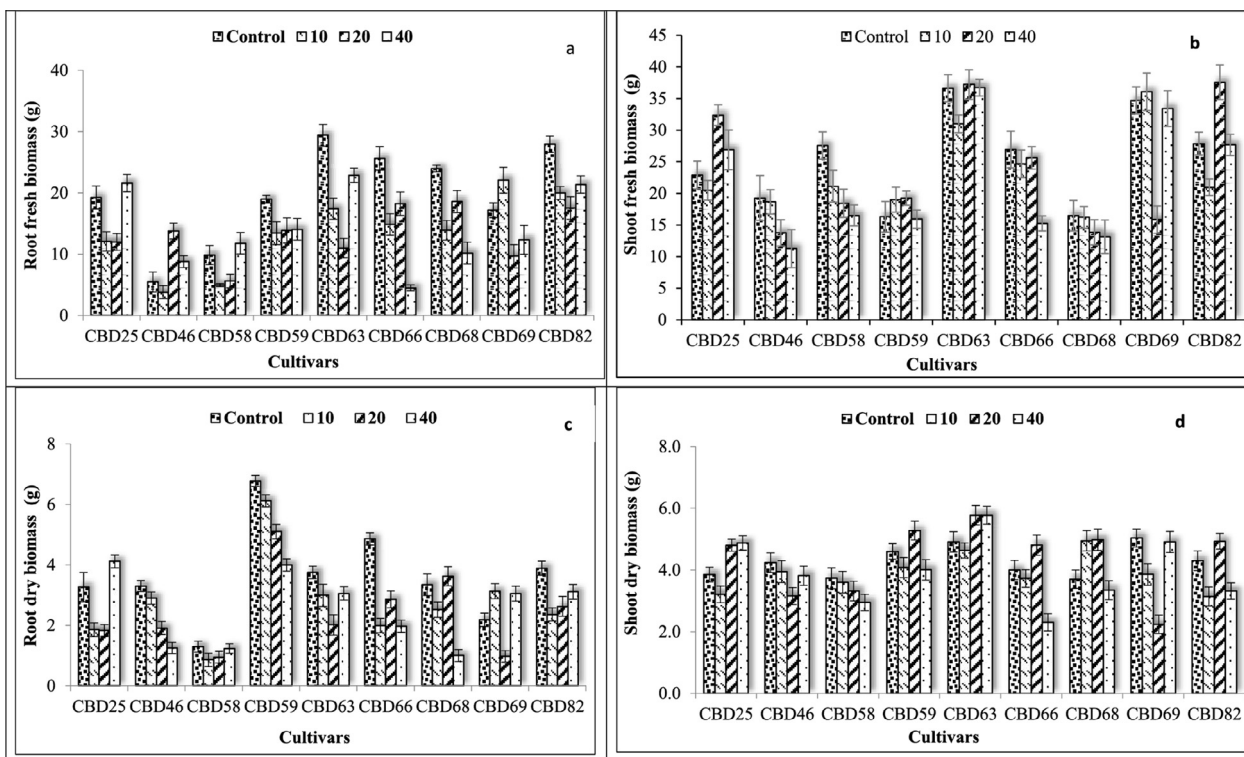
**Fig. 1.** Mean values for root and shoot length (cm) of nine *Triticum durum* L. cultivars following exposure to 0–40 mg kg<sup>-1</sup> of Pb in soil for 16 weeks of each mean is across three replicates. Vertical lines on each bar represents ± S.E.

with it. Therefore, it has been demonstrated that in plants root growth can be a useful marker for stress tolerance including heavy metals (Abid et al., 2019a). Kanwal et al. (2020) attributed obstructed cell division for retardation of root growth in wheat. The alteration of developmental responses has widely been related to metal contents present in the growth medium and their absorption by the roots (Huang et al., 2019; Rasool et al., 2020).

Shoot growth in cultivars of durum wheat seemed to be less influenced by Pb but decline in CBD68 for shoot length became evident at an intermediate metal stress (20 mg kg<sup>-1</sup>). Plant roots act a first line of defense for plants hence, after penetrating the central stellar region of the root, the metals further translocate to aerial plant parts by water flow through vascular system but resilient species had shown restricted transport to above ground tissues. Nevertheless, metal translocation remains dynamic within plants thus the composition of cell saps often change with time (Aslam et al., 2021). Deposition of metals in aerial plant parts may cause several disorders including osmotic effect, nutritional deficiency, ionic imbalance thus declines in many metabolic activities (Sofy et al., 2020). However, long distance, organic ligand-assisted transport of metals has been extensively reported (Figlioli et al., 2019; Gao et al., 2021). Possible ligands include number of small molecules, along with organic acids-carboxylates such as malate, citrate and amino acids as well as peptides and proteins. Thus, in xylem vessels, heavy metal may bind to these substances to form complexes (Zhuang et al., 2021) that

plausibly allow to plants to grow well on metal contaminated soil. The formation of such complexes decrease many free metal ions in plant fluids thus a better threshold to plants hence unaffected growth despite translocation of metals to aerial tissues. Therefore, in the presence of any metal tolerance mechanism, aerial tissues are not adversely affected. Kanwal et al. (2020) and Rizvi et al. (2020) demonstrated formation of metal complexes with components of conducting tissues hence better performance of wheat germplasm in the presence of Pb.

Exposure of plants to metal stress often lead to injurious effects on growth parameters, particularly plants biomass. Although the adverse effects of excessive metal ions vary in plant species, genotypes and even in plant tissues (Riaz et al., 2021). Both fresh and dry biomass are considered as sensitive parameters and potential indicator that reflect cumulative expression to determine the plant's response towards metals toxicity (Alamri et al., 2018). Nevertheless, this collective response is governed by several underlying mechanisms associated with light dependent reaction of photosynthesis and reducing powers that are used in Calvin Cycle for photo-assimilate production (Mohaparta et al., 2018) as well as on the biosynthesis of pigments. Kanwal et al. (2020) described no inhibitory effects on biomass in *T. aestivum* cultivars. *T. durum* also appeared to be a productive as far as biomass in concerned. However, CBD25, CBD59 and CBD63 had shown more carbon assimilation hence greater dry biomass of tissues.

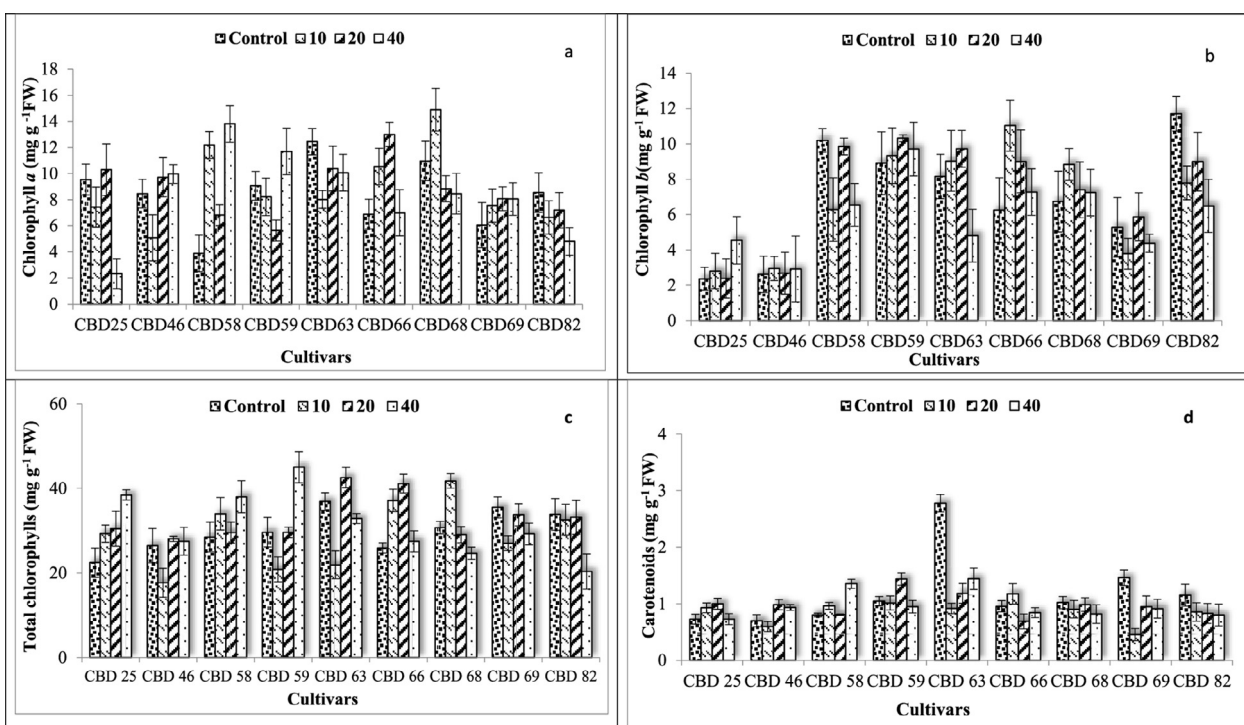


**Fig. 2.** Mean values for root and shoot biomass (g) of nine *Triticum durum* L. cultivars following exposure to 0–40 mg kg<sup>-1</sup> of Pb in soil for 16 weeks. Each mean is across three replicates. Vertical lines on each bar represent ± S.E.

### 3.1.1. Photosynthetic pigments

Pb concentrations had caused not much noticeable changes in chlorophyll *a* and but ANOVA revealed a highly significant ( $p < 0.001$ ) variability for cultivars (Table 1). The most drastic

decline (50%) in chlorophyll was noticed in CBD82 at the most elevated level of Pb (Fig. 3a). On the other hand, the lowest content of chlorophyll *a* were observed for plants of CBD66 grown at control in contrast to CBD68 which had the maximum amount of the pig-



**Fig. 3.** Mean values for chlorophyll *a*, *b* total and carotenoids (mg g<sup>-1</sup> FW) of nine *T. durum* cultivars following exposure to 0–40 mg kg<sup>-1</sup> of Pb in soil for 16 weeks. Each mean is across three replicates. Vertical lines on each bar represent ± S.E.

ment in the absence of Pb. Though, responses of cultivars were inconsistently variable after exposure to different levels of Pb but CBD59 had more chlorophyll *a* than all other cultivars at the most elevated Pb level. The cultivars exhibited significantly ( $p < 0.001$ ) variable responses for chlorophyll *b* with an increase in Pb levels (Fig. 3b) but only CBD82 had a consistent decline with increasing Pb levels and the decline was more pronounced (44%) followed by CBD63 (40%) at the highest level of Pb. The cultivars had shown considerable ( $p < 0.001$ ) variation for total chlorophyll significant difference between Pb levels (Table 1). However, the amount of total chlorophyll was remarkably higher in CBD63 for control plants than all other cultivars but exposure to an initial level ( $10 \text{ mg kg}^{-1}$ ) induced a decline of about 40% (Fig. 3c) but CBD82 had a similar decline at  $40 \text{ mg kg}^{-1}$  Pb. On the other hand, CBD 59 exhibited no decline despite exposure to 20 and  $40 \text{ mg kg}^{-1}$  Pb. Fig. 3(d) depicted that carotenoids content were less effected by Pb applications in all *T. durum* cultivars except CBD63 and CBD69 which had more decline at all levels of Pb. The extent of decline was 67, 57 and 48% with increasing levels of Pb, respectively. Whereas, CBD69 had more profound reduction (69%) of carotenoids at  $10 \text{ mg kg}^{-1}$  Pb. ANOVA (Table 1) depicted a non-significant influence of Pb levels on carotenoids in cultivars of durum wheat.

Metal toxicity is considered as the most serious concern in land plants because of harmful effects of metal not only felt via poor growth, biomass and yield but its influence the photosynthesis which is the most fundamental process of plants (Salih and Aziz, 2019). Durum wheat cultivars seemed to mitigate Pb toxicity without any remarkable reduction in biosynthesis of pigments and enhanced the carotenoids that may serve as non-enzymatic anti oxidative agent. Jian et al., (2020) reported disturbance in content of green pigments due to disorganization of grana, replacement of  $\text{Mg}^{+2}$  with metal ions. Figlioli et al. (2019) described some insights of metal toxicity on photosynthesis and pigment biosynthesis in *Z. mays* and reported robust organization of chloroplast in maize provided the species a better survival ability under supra-optimal levels of metals. The cultivars of durum wheat had shown intraspecific variability for green pigments and carotenoids but lesser influence of Pb levels on chlorophyll *a* and carotenoids thus it appeared that *T. durum* might have the same fundamental mechanism to protect grana from deterioration from the toxic effect of heavy metals as reported for bread wheat. Aslam et al., 2021 recently demonstrated similar results for Pb toxicity in plants.

### 3.2. Total soluble amino acids, proteins and sugars

Table 1 clearly depicted that highly significant ( $p < 0.001$ ) alterations of (amino acids and soluble proteins) with increasing levels of Pb as well as a strong interactive response between Pb levels and cultivars ( $p < 0.001$ ). Fig. 4 showed variable responses of cultivars after exposure to varying Pb levels. The amount of amino acids (Fig. 4a) increased by 29 and 31% in cultivars CBD66 and CBD69, respectively at the most elevated level of Pb ( $40 \text{ mg kg}^{-1}$ ). CBD82 had shown the least amino acids contents and the magnitude of decline ranged between 83, 84 and 91.1% at 10, 20 and  $40 \text{ mg kg}^{-1}$  Pb, correspondingly over respective control.

The data for total soluble proteins (Fig. 4c) exhibited a differential influence of Pb levels and *T. durum* cultivars showed variable responses for this attribute. More increment (33 and 37%) in protein content was observed in CBD58 and CBD68 at elevated levels (20 and  $40 \text{ mg kg}^{-1}$  Pb, respectively, while protein content declined consistently in all cultivars except CBD82 but a rise of about 13% was noticed at an initial level of the metal ( $10 \text{ mg kg}^{-1}$  Pb). Overall, protein content in cultivars increased in relation to Pb

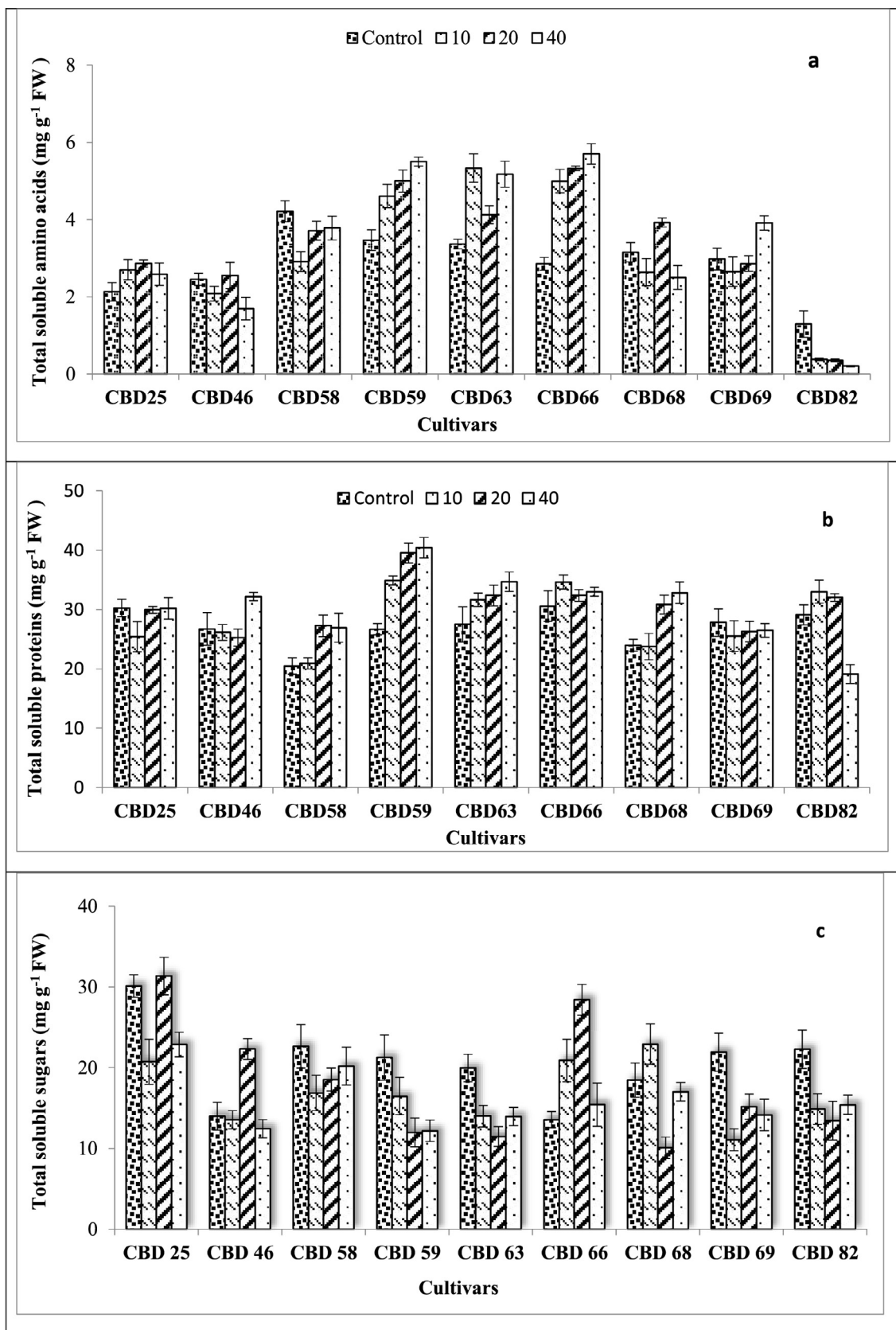
applications. Fig. 4(c) illustrated that the *T. durum* cultivars had distinct responses for total soluble sugars content at various levels of Pb. The most drastic decline (50%) was observed for CBD69 followed by CBD68, which exhibited 45% reduction at 10 and  $20 \text{ mg kg}^{-1}$  Pb, respectively. The degree of decline was least profound in CBD46 and CBD68 where the content reduced only by 3 and 8%, correspondingly, despite exposure to the highest Pb level.

Durum wheat cultivars had variable responses for amino acids and proteins but CBD59, CBD63, CBD68 and CBD82 have more biosynthesis of these biomolecules thus appeared to maintain normal or escalated biosynthesis of amino acids. Nevertheless, the amino acids as constituents of wheat grains are of dietary significance therefore, their biosynthesis under metal stress is of great advantage in addition to their role as osmoprotectants and chelating agents under Pb stress as suggested by Pidatala et al. (2018). Many other workers (Jian et al., 2020) reported an affirmative relationship for biosynthesis of amino acids and stress combating proteins as a result of as a tolerance strategy in various plants. Moreover, contemporarily less amount of amino acids clearly signifies escalated protein synthesis hence existence of genetic basis. However, heavy metal ions can induce enhanced activities of protease, disturbance in N-metabolism thus hydrolysis, degradation or changes in structural configuration of proteins in metal sensitive species and germplasm (Aslam et al., 2021).

Proteins are structurally and functionally the most versatile macromolecules that are important constituents of all of living cells. Proteins play significant roles in signal transduction, regulation, catalysis, inward and outward movement of molecules and nutrients, membrane biosynthesis, structural maintenance and protection at the cellular level. The basic role of a protein is determined by its structural configuration, made up of amino acids chain that are biosynthesized on ribosomes (Pidatala et al., 2018). Plants responses to heavy metals involve alteration of genes expression that encode stress proteins under different environmental constraints. Metal ions can profoundly affect protein homeostasis by interfering with their folding and aggregation of nascent or non-native proteins leading to decreased cell viability. Proteins are crucial for metal chelation in cytoplasm followed by metal ions are compartmentalized in the vacuoles moreover take part in repair, removal and degradation of proteins that fail to achieve their native conformations thus, plants maintain functional and healthy proteomes for survival under erratic environmental settings, thus, recent advances have focused changes in cellular proteins with regards to heavy metal tolerance in plants (Zheng et al., 2021).

The accumulation of sugar contents is governed by several enzymes, rate of photosynthesis and respiration, biosynthesis, transportation and subsequent accumulation. Sugar content are not only acted as metabolic reserves and fundamental components of cells but also help signal regulation of many processes that are essential for plant growth and development (Arshad et al., 2019). Thus, in plants, sugar signaling interact with stress pathways into a complex network to modulate metabolic plant responses. Soluble sugars may either act directly as negative signals or as modulators of plant sensitivity and thus, they can also play important roles in cell responses to stress-induced remote signals. Hence, sugar contents in this context serve as indicative of biochemical and genetic evidences for sugar sensing mechanisms that are well reported in relation to several abiotic stresses (Dutta et al., 2018).

In wheat, an enhancement of sugar contents in plants seemed to be associated with cellular protection under Pb stress owing to the absence of carbohydrates hydrolysis and de-novo synthesis of sugar molecules. Thus the greater sugar content in CBD59 and CBD63 might be an indicative of undisturbed sugar metabolism in the presence of Pb.



**Fig. 4.** Amino acids, proteins and soluble sugar (mg g<sup>-1</sup> FW) in nine *Triticum durum* L. cultivars following exposure to 0–40 mg kg<sup>-1</sup> of Pb in soil for 16 weeks. Each mean is across three replicates. Vertical lines indicate ± S.E.

### 3.3. Uptake and distribution of Pb in below, above and edible part of plant

*T. durum* cultivars showed differential behavior regarding Pb uptake and its distribution in different plant parts, however Pb distribution exhibited a pattern of root > shoot > grain (Fig. 5a–c). CBD68 accumulated more Pb content in root followed by CBD69 but not in a dose-dependent manner (Fig. 5a). The accumulation of the metal varied in shoots in cultivars but an inconsistent manner with increasing levels of Pb. A considerable amount Pb was transported to shoots and among cultivars and the maximum quantity was accumulated in shoots of CBD59 at 20 mg kg<sup>-1</sup> while the least amount of Pb was transported to shoots in CBD25 followed by CBD58 despite their exposure to 40 mg kg<sup>-1</sup> of Pb in soil (Fig. 5b). A subsequent transportation of metal from shoots to grains also became evident in all cultivars. The cultivars did not vary profoundly for metal content in grains at 10, 20 and 40 mg kg<sup>-1</sup> Pb except CBD66 which had strikingly more metal in response to

the highest level of Pb (Fig. 5c). On contrary, CBD59 and CBD82 uniformly had the least amount of Pb in its grains at all levels. The uptake, translocation and accumulation of Pb varied in cultivars irrespective of concentrations of metal in the growth medium hence a differential amount of metal was found in various parts of plant.

Hasanuzzaman et al. (2020) explicitly described mechanism for the uptake of heavy metals by plants from their rhizosphere involves a series of sophisticated mechanisms and through the localized transporters in the plasma-membrane. A complex system of uptake, efflux from cytosol, transport, vascular sequestration and chelation remain functional and govern metal bioaccumulation in plant tissues. The first line of defense is constituted by the extracellular mode of detoxification through immobilization of metals ions by root exudates or mycorrhizal association. Prevention of metal accumulation in a fully reactive form in the cytosol involves phytochelating substances comprised of metallothioneins, organic acids, amino acids, proline and other stress

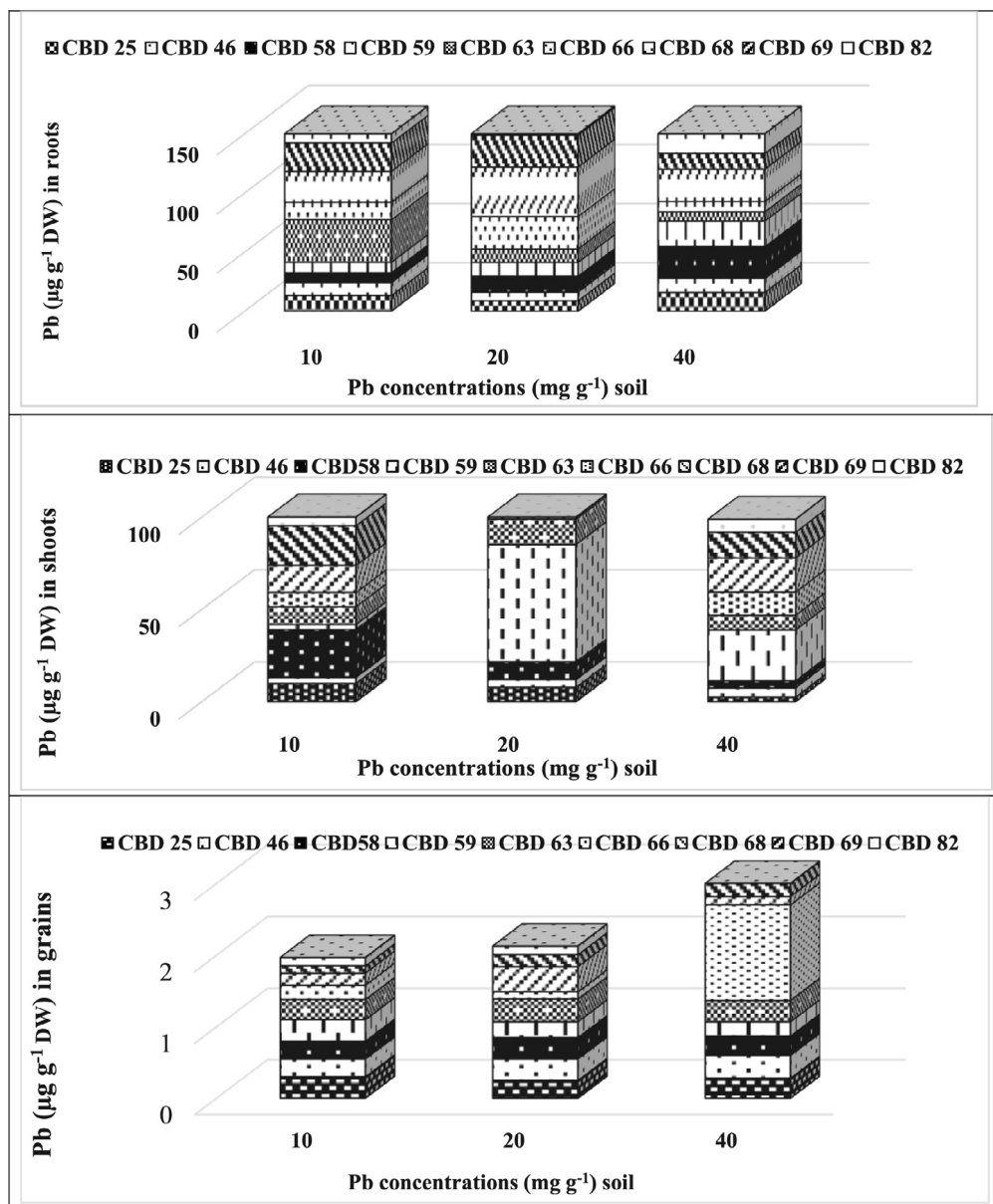


Fig. 5. Lead content (µg g<sup>-1</sup>DW) in root, shoot and grains of nine *Triticum durum* L. cultivars following exposure to 0–40 mg kg<sup>-1</sup> of Pb in soil for 16 weeks. Each value is the mean of three samples analyzed through AAS.



proteins. In addition, signal transduction is considered as the most sophisticated function to activate gene expression for detoxification and homeostasis to cope with the heavy metal stress. The transducing signals involving of second messengers viz.,  $\text{Ca}^{2+}$ , jasmonic acid and  $\text{H}_2\text{O}_2$ , ethylene and salicylic acid are other components of metal uptake and transport mechanisms. The cultivars of durum wheat exhibited distinct mechanism for metal uptake in the roots and its further translocation to above ground tissues. Metal entrance in the root depends upon several variables such as metal concentration, cell wall composition, anatomical features and soil properties/characteristics (Chandra et al., 2018). The robust anatomical feature of roots restrain further translocation of metal (Huang et al., 2019) due to strong root wall/barriers but metal ions can escape through casparian strips and through xylem loading are transported to above ground tissues (Salih and Aziz, 2019). Consequently, a large quantity of metal ions are accumulated in aerial tissues (stem and leaves) and reproductive plant parts (flower and seeds).

However, accumulation of metal in grains (caryopses) takes place through remobilization from leaf blades via phloem and finally deposited into edible part or seed of cereal grasses (Li et al., 2018) under the expression of transporter genes (Rai et al., 2019; Romdhane et al., 2021; Zheng et al., 2021). Guo et al (2018) demonstrated a greater translocation of Cd in wheat but limited uptake of Pb by the roots and a restricted translocation to shoot and grains. This seemed to be of selective/ genetic advantage in wheat and also implied to specific transporter genes for each kind of metal ions (Cd and Pb) in the species. Nevertheless, the presence of heavy metals in edible parts of the plants is a serious issue as far as food safety is concerned (Hamid et al., 2020; Haider et al., 2021). In addition, a distinctive extent of resilience through restricted transport and limited deposition of metal ions in the cultivars can be independent of metal concentration. Hence, our results are in close conformity of other workers (Ashraf et al., 2017; Aprile et al., 2018) who described several potential mechanism of metal uptake, translocation and accumulation, thus different crops have been reported to exhibit variable responses at inter/intra-specific levels. Moreover, the accumulation of metal, in edible parts of some cultivars less than permissible limits reported by FAO (2018) holds significant implications for food safety.

#### 4. Conclusion

The cultivars of *T. durum* were distinctively variable for their performance following exposure to Pb. A differential inhibition of morpho-biochemical responses in cultivars became evident but the most elevated level of Pb induced more profound alterations of parameters studied. The study signified that metal tolerance/sensitivity in the species is independent of magnitude of metal stress, growth responses and Pb accumulation in plant tissues hence varied in space and time. Uniformly, a better response for different growth and biochemical expressions became evident for CBD59 followed by CBD63 and CBD66. The cultivar CBD59 exhibited a greater threshold for primary productivity traits, pigments and biosynthesis of macromolecules (amino acids, proteins and sugar) despite greater Pb uptake in shoots but lesser bioaccumulation in grains. Overall, a better resilience of the cultivars emerged through sustainable biomass production, less degradation of chlorophyll, scavenging of Pb toxicity through enhanced synthesis of biomolecules (amino acids, proteins and sugar) under Pb stress. Hence, these traits are of selective advantage which indicated innate genetic variability and diverse gene pool of the germplasm. Therefore, growth and biosynthesis of major macromolecules served as efficient predictors to understand Pb toxicity in an important cereal crop. Though, capacity of the cultivars to extract

metal from the soil and its subsequent translocation and bioaccumulation in above ground tissues indicated the existence of some molecular mechanism and genetic basis in durum wheat germplasm for potential/ evolution under heavy metal stress hence a positive indicator for food security. In addition, a permissible limit of Pb in grains is particularly of great impact with regard to food safety. The variability at intra-specific level in durum wheat can definitely provide basis for future genetic/ and breeding projects to develop high yielding germplasm with good grain quality to assure food security and safety.

#### Ethics approval

Not Applicable.

#### Consent to participate

All authors consent to participate in this manuscript.

#### Consent for publication

All authors consent to publish this manuscript in Saudi Journal of Biological Science.

#### Availability of data and material

Data will be available on request to corresponding or first author.

#### Code availability

Not Applicable.

#### Author contributions

MM, SM and SN drafted the experimental design and MM performed the experiments. RA, SG, SZ, TS and AA helped in data collection, data analysis and initial draft of manuscript text. All authors read the manuscript before communication.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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