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Effects of lead heavy metal exposure on anatomical structure of bread wheat (*Triticum aestivum* L. cv. Krasunia Odeska)

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ABSTRACT: This study aimed to investigate the effects of various concentrations of lead (Pb) heavy metal on the anatomical structure of bread wheat (*Triticum aestivum* L. cv. Krasunia Odeska). Wheat seedlings were cultivated under controlled conditions and treated with different concentrations of Pb (0, 10, 15 mM). After 28 days, root, stem, and leaf samples were collected for anatomical analysis. Pb stress caused thickening of the epidermis, exodermis, and endodermis in the roots, increased the diameter of cortex cells, and decreased the diameter of the stele and vascular bundles. In the stem, the epidermis and collenchyma cells thickened, while the diameter of the tracheids in the vascular bundles decreased. In the leaves, the length of the epidermis cells decreased, the length and width of bulliform cells increased, the sclerenchyma tissue thickened, and the thickness of the phloem in the vascular bundles decreased. In conclusion, lead stress induced significant anatomical changes in the root, stem, and leaf tissues of bread wheat. These alterations can be interpreted as the plant's attempts to develop tolerance to Pb stress.

KEYWORDS: Bread wheat, lead, heavy metal stress, anatomical structure, adaptation

INTRODUCTION

Soil pollution with toxic heavy metals and their accumulation in soil is of great concern in agricultural production owing to the adverse effects on crop growth [1, 2]. The excess heavy metals have been recognized to have a significant toxicity for animals, plants, microorganisma and human beings as well as environment [2, 3]. Lead (Pb) is a prevalent heavy metal pollutant, primarily derived from the combustion of liquid and solid fuels, the application of chemical fertilizers to soil, and its use in various economic sectors due to its low melting point [4]. Lead (Pb) exhibits limited solubility and demonstrates a high affinity for binding to soil colloidal particles. This characteristic results in its prolonged persistence in soil environments, subsequently inducing various direct and indirect effects on plant metabolic processes and development [5]. As a heavy metal contaminant, lead significantly disrupts soil microbial activity, leading to a degradation of soil fertility. Furthermore, lead contamination adversely affects various physiological mechanisms in plants, with chlorosis of leaves being the most prominent manifestation of lead toxicity [6]. When bound on the cell surface and also within the cell, Pb ions interact with the functional groups of proteins, nucleid acids and polysaccharides and substitute other metal ions already bound to these functional groups than can lead to various matabolic disorders and reduction in growth [7]

Toxic heavy metal contamination adversely impacts soil properties and diminishes the availability of essential nutrients to plants, consequently affecting their morphological and anatomical structures [8]. In response to heavy metal stress, roots exhibit several anatomical changes, including changes in root diameter, conductive tissues, pericycle, central vessel, epidermal and parenchymatic cells [9, 10]. Additionally, heavy metal exposure can lead to thickening of the exodermis and endodermis, as well as suberization, lignification, and increased cell wall thickness in root tissues [11, 12]. Furthermore, Hajihashemi et al., [13] observed significant reductions in wheat root diameter and xylem vessel size when irrigated with complex heavy metal wastewater. Numerous studies have documented the adverse effects of heavy metals on various plant stem structures. For instance, Chaudhari et al., [14] reported abnormalities of ruptured cortex and epidermis cells due to irregular division of cells in response to Pb stress. Similarly, as heavy metals are transported to aboveground organs through vascular tissues, anatomical alterations are frequently observed in vascular tissues and their surrounding cells [9]. Zarinkamar et

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al., [15] noted significant alterations in the leaf surface, cuticle, and collenchymatous tissues of *Hypericum perforatum L.* plants treated with Pb. Furthermore, excessive Pb application led to an increase in the number of epidermal cells, although it caused a decrease in the diameter of these cells [16].

Wheat, a globally important product, is known for its rich nutritional value in terms of carbohydrates, proteins, lipids, fiber, and vitamins. It provides a significant portion of the world's food energy and is preferred more than other products [17]. However, environmental pollution has led to the accumulation of heavy metals in wheat flour, raising concerns about their potential transfer to humans through the food chain [18]. Due to its high economic value, detailed studies have been carried out on the anatomical and morphological characteristics of wheat [19, 20]. Nevertheless, further research is needed to elucidate the anatomical effects of Pb exposure on wheat. This study aims to investigate the anatomical changes in bread wheat (*Triticum aestivum* L. cv. Krasunia Odeska) subjected to different levels of Pb stress (0, 10, 15 µmol).

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of the bread wheat cultivar Krasunia Odeska were sourced from agricultural fields in Suluova, Amasya, Turkey. Seedlings were subsequently transplanted into plastic pots filled with a sand-soil mixture. A modified half-strength Hoagland solution served as the nutrient medium. Plants were subjected to different concentrations of Pb (0, 10, 15 µM) at alternate days for a duration of 28 days. Pots containing clean, uncontaminated soil were maintained as controls. The experimental design was a completely randomized design with three replicates per treatment.

Anatomical parameter

For anatomical analysis, root, stem, and leaf segments (10-15 cm) were preserved in 70% ethyl alcohol. Hand sections were prepared, and the thinnest sections exhibiting the desired anatomical features were selected. These sections were immersed in a 0.2% sodium rhodizonate solution for 30 minutes, followed by the addition of one drop of acetic acid buffer solution (pH 2.8). After rinsing with distilled water, the sections were mounted on glass slides and examined under a Nikon Coolpix 5100 light microscope for detailed observation and photography [21]. Images were captured using a digital camera attached to the microscope.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) followed by the least significant difference (LSD) test at a significance level of $p < 0.05$ to compare treatment means. Statistical analyses were performed using SPSS version 20.

RESULTS AND DISCUSSION

The data presented in Table 1 provide a comparative analysis of root, anatomical characteristics in plants grown under control and Pb-stressed conditions. Transverse sections of *T. aestivum* cv. Krasunia Odeska roots revealed notable changes in tissue structure induced by Pb exposure (Figure 1A-B). Notably, the epidermis, exodermis, and endodermis tissues exhibited thickening at higher Pb concentrations (Table 1). The thickening of exodermis and endodermis has been reported in various plant species as a strategy to minimize Pb translocation [22, 23]. Similar observations were reported by Lux et al., [24], who noted that exodermis and endodermis thickness is a characteristic of plants with high heavy metal tolerance. The thickening of cell walls in roots creates a barrier for heavy metal retention, reducing their translocation to leaves [21, 25]. As the root is the primary organ for Pb uptake, the metal initially moves through the apoplastic pathway, following the water flow until it reaches the endodermis. The thickening of endodermal cells is an adaptive response to block Pb translocation into the stele [26].

Our findings revealed an increase in the diameter of cortex cells, likely due to the accumulation of Pb (Table 1). The enlargement of parenchyma cells may be attributed to impaired root elongation, as suggested by Maksimović et al., (27). Additionally, disintegration or collapse of root cortical tissues has been reported as a response to heavy metal stress [28]. Sridhar et al., [29] observed distortion of cortical cells in Pb-treated wheat (*Triticum aestivum*) roots. Similar effects of heavy metals on cortical cells have been reported in other plant species [30, 31]. These results align with those of Singh et al., [32], who reported a loss of shape in root cortical cells following heavy metal treatment. Compared to the control, Pb-treated roots exhibited a reduced stele and vessel diameter. This reduction in vessel diameter became more pronounced with increasing metal concentration (Table 1). A similar decrease in vascular tissue proportion was observed in *Oryza sativa* L. following Pb stress [33]. Other studies have reported that a reduction in vessel diameter is associated with the accumulation and translocation of heavy metals within the cell wall system [34, 35].

Table 1. Root anatomical characters of *T. aestivum* treated with different lead concentrations.

		Concentration of lead (mM)		
	Characters	**0**	**10**	**15**
Root	The length of epidermis cells	31.78±0.738^{a}	32.11±0.945^{a}	36.46±0.786^{b}
	The width of epidermis cells	23.01±0.597^{a}	26.47±0.555^{b}	33.52±0.787^{c}
	Thickness of exodermis	53.70±1.855^{a}	56.50±1.596^{a}	77.03±3.420^{b}
	The diameter of exodermis cells	18.23±0.576^{a}	18.95±0.694^{a}	22.815±0.863^{b}
	The diameter of cortex cells	30.72±1.141^{a}	32.80±1.027^{a}	38.05±1.123^{b}
	Thickness of cortex issue	115.16±4.036^{a}	122.87±3.278^{ab}	129.20±2.337^{b}
	The length of endodermis cells	19.85±0.622^{a}	22.50±0.915^{ab}	23.83±0.806^{b}
	The width of endodermis cells	17.06±0.566^{b}	14.17±0.277^{a}	14.07±0.411^{a}
	The diameter of stele	366.4±4.667^{b}	301.71±7.419^{a}	289.80±5.614^{a}
	The diameter of vessel elements	52.63±1.509^{b}	50.19±1.452^{b}	43.82±1.201^{a}

Data are mean values ±SE of 30 measurements. Values in each column with the similar letters are not statistically nonsignificant ($P<0.05$).

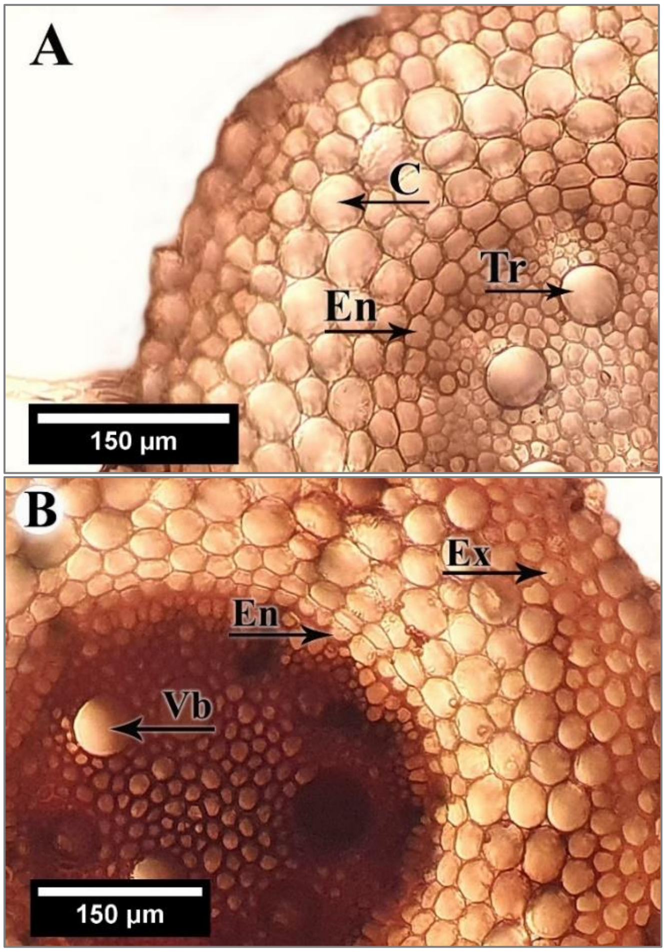

Figure 1. Light micrographs showing a transverse section of the root. A) control B) root tissue treated with 15 mM Pb and stained with sodium rhodizonate. C: cortex, E: epiderma, En: endoderma, Ex: exoderma, Tr: trachea, Vb: vascular bundle.

Lead accumulation was observed in the root tissues, including epidermal, exodermal, and endodermal cells, cortical layers, and vascular conducting systems, as indicated by dark coloration (Figure 1B). The intensity of the coloration correlated with the concentration of accumulated lead.

Pb stress significantly impacted the dimensions of stem epidermal cells in wheat plants (Table 2). The length of epidermal cells increased from 11.02 ± 0.297 µm in control plants to 14.04 ± 0.420 µm in plants treated with 0.15 mM Pb (Table 1). Concurrently, an increase in epidermal cell width was observed compared to control plants (Table 2, Figure 2A-B). This thickening of epidermal cells is a common response to Pb absorption by plant cells. Previous studies have also reported that Pb absorption can affect plant epidermal cells [36]. The thickness of collenchyma in Pb-treated stems increased compared to control stems (Table 2). Conversely, the diameter of vessel elements significantly decreased in the stems of Pb-treated *T. aestivum* cv. Krasunia Odeska (Table 2, Figure 2B). The development of narrower vessels in plants exposed to stress has also been reported in *Avicennia marina* and *Cicer arietinum* [37, 38]. Prolonged exposure to heavy metals can alter xylem structure and vessel characteristics, and these anatomical modifications are considered physiological adaptations to heavy metal stress [28, 39].

In the stem, lead deposits were observed primarily on the epidermis, collenchyma cells and vascular system and appeared as dark spots (Figure 2B). These deposits on cell walls suggest the binding of lead to the cell wall matrix, leading to cell wall thickening. Metal deposition on the vascular bundle resulted in a reduction in vessel element diameter [35, 38, 40]. Wheat plants exposed to Pb exhibited modified leaf anatomical characteristics (Figure 3A-B). The length of epidermal cells decreased with increasing Pb concentration compared to control leaves (Table 3). However, no significant changes were observed in the width of leaf epidermal cells in Pb-treated plants. The dimensions (length and width) of bulliform cells were greater in Pb-treated leaves compared to controls (Table 3, Figure 3B).

Table 2. Stem anatomical characters of T. aestivum treated with different lead concentrations.

	Characters	Concentration of lead (mM)		
		0	**10**	**15**
Stem	The length of epidermis cells	11.2±0.297^{a}	11.66±0.300^{a}	14.04±0.420^{b}
	The width of epidermis cells	8.59±0.376^{a}	8.98±0.255^{ab}	9.69±0.232^{b}
	Thickness of collenchyma tissue	25.41±0.925^{a}	32.89±1.526^{b}	36.37±1.038^{b}
	The diameter of collenchyma cells	9.83±0.394^{a}	10.31±0.445^{a}	10.82±0.275^{a}
	Thickness of phloem	29.08±0.891^{a}	34.55±0.314^{b}	35.06±1.208^{b}
	Thickness of xylem	49.61±1.704^{a}	51.3267±1.942^{a}	69.26±2.450^{b}
	The diameter of vessel elements	25.33±0.593^{a}	20.29±0.507^{b}	17.12±0.628^{c}

Data are mean values ±SE of 30 measurements. Values in each column with the similar letters are not statistically nonsignificant ($P<0.05$)

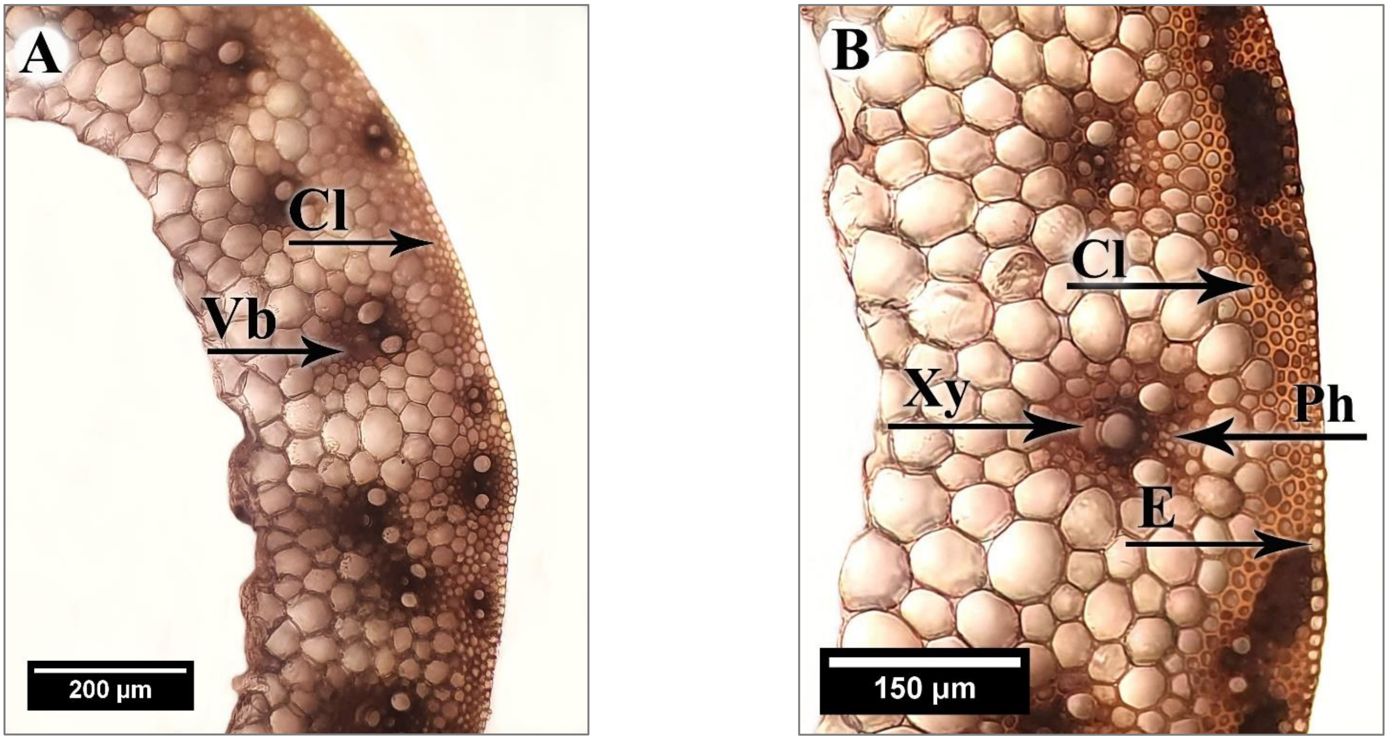

Figure 2. Light micrographs showing a transverse section of the stem. A) control B) stem tissue treated with 15 mM Pb and stained with sodium rhodizonate. E: epiderma, Cl: collenchyma, Ph: phloem, Vb: vascular bundle, Xy: xylem.

Table 3. Leaf anatomical characters of *T. aestivum* treated with different lead concentrations.

	Characters	Concentration of lead (mM)		
		0	**10**	**15**
Leaf	The length of epidermis cells	19.63±0.640^{a}	19.35±0.530^{b}	16.20±0.440^{b}
	The width of epidermis cells	16.24±0.402^{a}	15.19±0.467^{a}	14.92±0.364a
	The length of bulliform cells	24.33±1.254^{a}	31.49±1.608^{b}	33,04±1.049^{b}
	The width of bulliform cells	20.89±0.909^{a}	25.30±1.279^{b}	27.29±0.900^{b}
	Thickness of sclerenchyma tissue	40.54±1.770^{a}	54.31±1.375^{b}	55.83±2.908^{b}
	Thickness of phloem	44.25±0.906^{b}	29.70±1.093^{a}	28.89±1.319^{a}
	Thickness of xylem	45.05±1.048^{a}	45.84±0.949^{a}	46.55±0.800^{a}
	The diameter of vessel elements	27.86±0.678^{b}	24.20±0.464^{a}	23.28±0.406^{a}

Data are mean values ±SE of 30 measurements. Values in each column with the similar letters are not statistically nonsignificant ($P<0.05$)

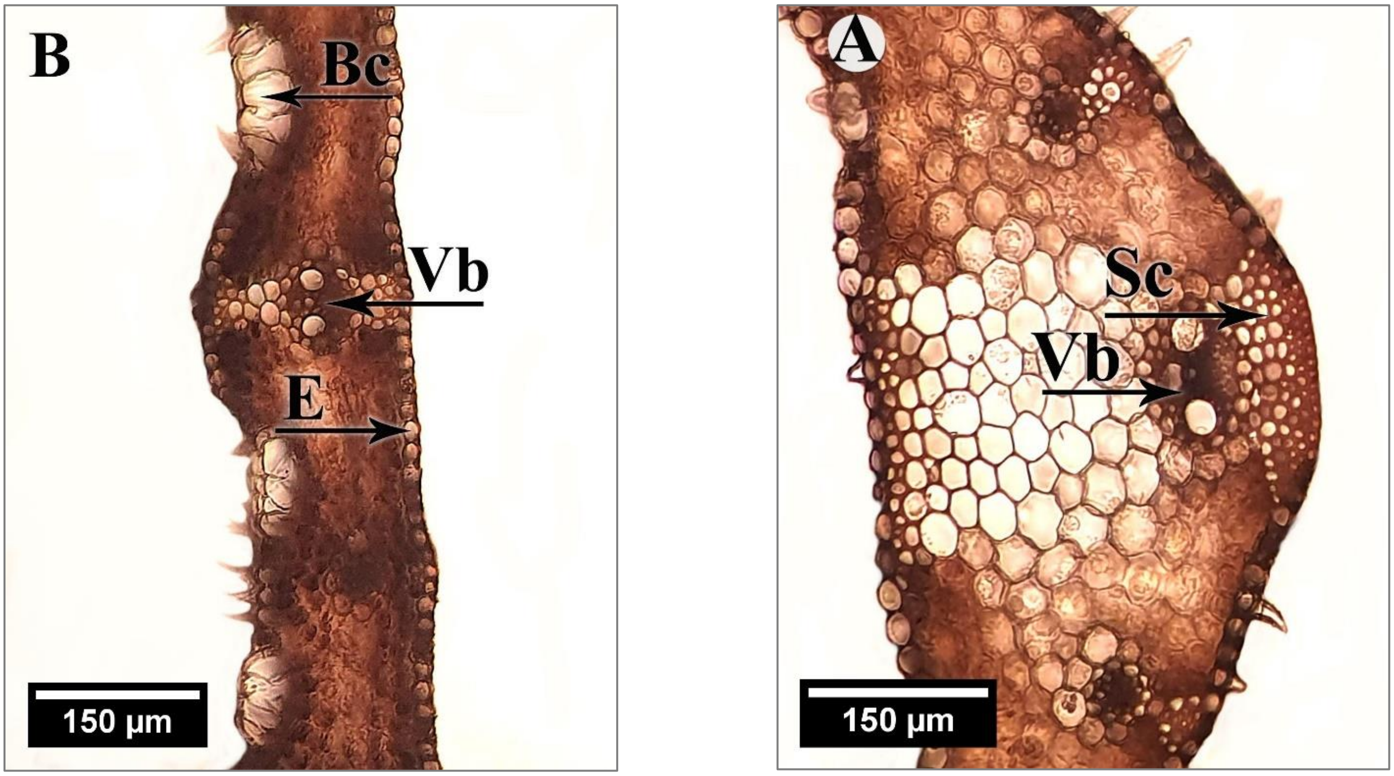

Figure 3. Light micrographs showing a transverse section of the leaf. A) control B) leaf tissue treated with 15 mM Pb and stained with sodium rhodizonate. Bc: bulliform cells, E: epiderma, Sc: sclerenchyma, Vb: vascular bundle.

While heavy metals accumulate primarily in roots, some heavy metals, such as lead (Pb), have been shown to accumulate in leaf tissue even at low concentrations, leading to different anatomical adaptations. In *Triticum aestivum* cv. Krasunia Odeska, one of the important adaptations observed under lead stress was an increase in the size of bulliform cells. Similarly, an increase in the size of bulliform cells has been reported in leaves of rice plants treated with aluminum [41]. Gomez et al., [25] suggested that these enlarged bulliform cells could be a strategy used to reduce water loss through transpiration. The reduction in transpiration and evaporation may limit the transport of heavy metals from roots to leaves. Furthermore, leaf curling mediated by bulliform cells may contribute to a more humid microenvironment around the stomata by reducing the evaporating surface area of the leaf [42]. This phenomenon is thought to be an adaptation mechanism that inhibits the transport of heavy metals into leaves [43].

This study also revealed a significant increase in the thickness of leaf sclerenchyma tissues under Pb stress (Table 3; Figure 3B). The increased thickness of sclerenchyma tissues in *T. aestivum* cv. Krasunia Odeska leaves, induced by heavy metals, may be related to the adsorption of heavy metals onto cell walls, providing an alternative pathway for metal allocation and preventing their translocation to photosynthetic tissues. Directing the deposition of heavy metals to non-photosynthetic tissues could be a plant strategy to tolerate toxic metal levels [25].

Numerous studies have documented the adverse effects of heavy metals on conductive tissues. Heavy metals are transported from roots and stems to leaves through vascular tissues, which can cause anatomical changes in some tissues of the leaves. Our findings indicate a significant decrease in phloem thickness in wheat leaves treated with 0.10 mM Pb compared to control leaves. However, no significant changes were observed in xylem thickness in Pb-treated plants (Table 3). Similarly, De Jesus et al., [44] reported disruptions in plant water status due to alterations in vascular bundles. Liza et al., [45] observed a reduction in vascular tissues in Cu-stressed sorghum leaves. Additionally, Gwayed and Almagharabi, [46] reported a decrease in the size of conductive tissues in maize exposed to copper, and Kouhi et al., [47] found a reduction in vascular bundle size in *Brassica napus* treated with zinc.

The present investigation revealed a decrease in leaf vessel diameter in Pb-treated plants compared to control plants (Table 3). A reduction in the number of vessel elements has been documented in the literature as a potential adaptive mechanism to maintain water flow [48]. Weryszko-Chmielewska and Chwil, [49] reported that Pb exposure can significantly impact the transport chain, leading to a substantial reduction in xylem and phloem tissue and a decrease in xylem vessel diameter.

Reddish deposits were particularly noticeable on the epidermal cell walls and vascular bundles of the leaves (Figure 3B). This deposition on the epidermal cell wall may serve as a strategy to prevent lead from entering chloroplast cells, where it could disrupt the CO_2 fixation process of photosynthesis [50]. Additionally, metal deposition within cells has been reported to reduce the size of vascular bundles [21].

The present study revealed distinct anatomical alterations in the root, stem, and leaf cross-sections of *Triticum aestivum* cv. Krasunia Odeska under Pb stress. Notably, the severity of these anatomical changes correlated with increasing Pb concentrations. Based on these findings, further investigation of the relationship between anatomical structure, growth and biochemical changes will provide a better understanding of plant responses to Pb stress in plant species.

CONCLUSION

This study investigated the anatomical responses of read wheat (*Triticum aestivum* L. cv. Krasunia Odeska) to lead (Pb) stress, revealing significant alterations in root, stem, and leaf tissues. Under Pb exposure, the roots exhibited thickening of the epidermis, exodermis, and endodermis, along with an increase in cortex cell diameter and a reduction in stele and vascular bundle diameter. These changes are likely adaptive mechanisms to minimize Pb translocation and protect the plant's vascular system. In the stems, Pb stress led to thickening of epidermal and collenchyma cells, while the diameter of tracheids in vascular bundles decreased, indicating a potential response to maintain structural integrity under stress. In the leaves, Pb exposure resulted in decreased epidermal cell length, increased bulliform cell dimensions, and thickened sclerenchyma tissues, which may help reduce water loss and prevent Pb from reaching photosynthetic tissues. Additionally, a reduction in phloem thickness was observed, suggesting impaired nutrient transport.

In conclusion, Pb stress significantly altered the anatomical structures of bread wheat roots, stems, and leaves. These alterations indicate that the plants developed tolerance mechanisms against heavy metal toxicity. The findings highlight the importance of understanding the mechanism of adaptation to environmental stress such as heavy metals. Future physiological and genetic studies will provide comprehensive insights into the mechanisms of Pb stress tolerance. This will enable the development of cultivars capable of tolerating stress factors such as heavy metals, facilitating healthier crop production under challenging environmental conditions.

DECLARATION

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Authorship Contributions

Concept and Writing: Assoc. Prof. Dr. Adnan Akcin, Prof. Dr. Tulay Aytas Akcin; Data Collection and Interpretation: Assoc. Prof. Dr. Adnan Akcin, Prof. Dr. Tulay Aytas Akcin, Prof. Dr. Erkan Yalcin

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Competing interests

The authors declared that there is no conflict of interest.

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