The Effects of Lead and Zinc Stress on Some Anatomic Properties of Barley and Radish Leaves*

Aslıhan CESUR TURGUT

Burdur Food Agriculture and Livestock Vocational School
Burdur Mehmet Akif Ersoy University
Burdur, Türkiye
acesur@mehmetakif.edu.tr

Kudret KABAR

Department of Biology,
Faculty of Arts and Science
Süleyman Demirel University
Isparta, Türkiye
kudretkabar@sdu.edu.tr

ABSTRACT

In this work, the effects of various concentrations of zinc and lead on the various anatomical particularities of barley (*Hordeum vulgare* L. cv. Tarm) and white radish (*Raphanus sativus* L.) seedlings leaves are examined. Seedlings were grown for 21 days in a growth chamber under conditions of 25±1ºC constant temperature, continuous fluorescent light (8.000 lux), and 60%±5 moisture.

In some anatomical investigations of leaf anatomy, the number of stomata, stomatal index, and width and length of stomata parameters of barley seedlings often gave similar responses to heavy metals. The numbers of stomata on almost all the leaf surfaces in zinc and lead medium, except the lower surface of the leaf in zinc medium, were usually fluctuating compared to the control. Both heavy metals caused a decrease in the stomatal index on both upper and lower surfaces. The width and length of the stoma, except leaf stomata length in zinc media, excluding the lower surface and remaining leaf surfaces under the effect of zinc and lead, led to fluctuations compared to the control. Mentioned parameters of radish seedlings had different reactions than barley in zinc and lead medium: while the number of stomata, in the presence of zinc and lead, decreased in general in the lower surfaces, fluctuated in the upper surfaces in zinc media, and increased upwards from 4 mM in lead media. While both surfaces stomatal index decreased in zinc medium, it fluctuated in lead medium. The width of the stoma, which generally decreased in zinc medium, fluctuated in the presence of lead on both surfaces. While the length of the stoma in zinc medium fluctuated on the upper surface, it usually increased on the lower surface. The length of the stoma in the presence of lead, a reduction in the upper surface, and a fluctuation in the lower surface, were observed. The only common effect of zinc was that it reduced the stomatal index of both species on the lower and upper surfaces. The numbers', widths, and lengths of the epidermis cells, leaf thickness, and distance between vascular bundles gave similar responses to zinc and lead mediums in both species: While heavy metals led to an increase in the epidermis cells on the upper surfaces of both species, they caused an increase in barley and a decrease in radish's lower surface. The lengths of epidermis cells were decreased on both surfaces of both species by the effects of the heavy metals. While decreases in epidermis cell length were observed in both species in zinc medium, fluctuations were observed in lead medium, except for the length of epidermis cells on barley's upper surface. While both heavy metals caused a decrease in leaf thickness in barley, they generally led to fluctuating results in radish leaves. Both species' distances between vascular bundles gave similar responses to zinc and lead, and they decreased.

Keywords: Zinc, lead, leaf anatomy, stomatal movement.

I. INTRODUCTION

A. What is the Heavy Metal Stress in Plants?

The phenomenon of heavy metal stress in plants pertains to the detrimental consequences resulting from increased levels of heavy metals, including both essential and non-essential elements, on the physiological processes and overall progress of plant organisms. The excessive presence of heavy metals, including copper (Cu), zinc (Zn), lead (Pb), cadmium (Cd), and mercury (Hg), has been found to impede plant growth and elicit signs of toxicity. Plants have developed diverse cellular strategies to mitigate the harmful effects of heavy metals and exhibit resilience in the face of metal-induced stress. These mechanisms encompass:

1. It has been seen that the process of attaching heavy metals to plant cell walls and then letting them out into the extracellular exudates makes them less bioavailable.

* This chapter is part of Aslıhan CESUR TURGUT's PhD thesis.

2. Diminished uptake or efflux pumping: Plants possess the ability to regulate the absorption of heavy metals by diminishing their uptake from the soil or aggressively expelling them from the cells.

In the cytosol, plants employ a mechanism known as chelation to bind metals. This process involves the production of peptides called phytochelatins, which effectively sequester heavy metals. By chelating these metals, plants are able to mitigate their harmful effects and facilitate their containment within the cytosol. The repair of stress-induced protein damage is a crucial process in plant cells, particularly in response to heavy metal stress. According to Hall (2002), plants possess mechanisms that enable them to repair or degrade proteins that have been damaged, thereby ensuring the maintenance of cellular function. The process of compartmentalization of metals within the vacuole is observed in plants, whereby heavy metals are effectively sequestered within vacuoles. This mechanism serves to limit the accumulation of heavy metals in the cytosol, therefore mitigating their harmful effects [1].

Understanding the cellular processes involved in heavy metal detoxification and tolerance is the most important step in coming up with solutions to help plants deal with the negative effects of heavy metal stress. One example is the use of silicon (Si) supplements, which have been shown to help reduce the negative effects of heavy metal stress on plants. According to Bhat et al. (2019), silicon has the potential to decrease the bioavailability of heavy metals, activate antioxidant defense mechanisms, and improve the regulation of genes associated with metal transport [2].

In addition, it has been observed that exposure to heavy metal stress in plants can lead to alterations in gene expression and modifications in DNA methylation patterns. These changes have the potential to be passed on to subsequent generations [3].

B. The Importance of Heavy Metal Stress in Sustainable Agriculture

The harmful impact that heavy metal stress has on soil, water, plants, and eventually human health plays a crucial part in sustainable agriculture. An efficient method for resolving the issue of heavy metal stress in agricultural systems is phytoremediation, which is a technology that makes use of metal-accumulating plants that have been particularly selected and designed. This strategy makes use of phytoextraction, rhizofiltration, and phytostabilization, all of which aim to either remove hazardous metals from polluted soil and water or limit the bioavailability of these metals in soil [4].

There has been a significant amount of research conducted on the regulatory networks and mechanisms that are involved in the heavy metal stress response in plants. According to DalCorso et al. (2010), the focus of these investigations is on the signal pathways that are responsible for sensing and transducing the "metal signal" inside plant cells. This, in turn, leads to the activation of transcription factors and the production of genes that provide plants with the ability to combat heavy metal stress [5].

According to Gupta et al. (2013), plants have developed a variety of mechanisms that allow them to withstand and detoxify heavy metals, particularly lead (Pb). The induction of cellular processes such as adsorption to the cell wall, compartmentation in vacuoles, augmentation of active efflux, and the creation of metal chelates such as phytochelatins are all included in these techniques. Phytochelatins are an essential part of the method that plants use to detoxify themselves and play a key role in the process of sequestering lead ions [6].

There is a need for effective measures to manage the amount of heavy metals that are present in animal feeds, manure, soil, and products that originate from animals. This influence of heavy metals on livestock-related activities has also been noted. According to Hejna et al. (2018), in order to develop sustainable ways for the management of heavy metals in agriculture, it is essential to take into account the intricate interrelationships that exist between rural activities, farming practices, soil conditions, and climate variables [7].

The accumulation of heavy metals in dietary vegetables and cultivated soil in an organic farming system has shown that atmospheric deposition of heavy metals can have a destabilizing effect on sustainable agricultural practices and increase the dietary intake of toxic metals [8]. This was found in a study that looked at the accumulation of heavy metals in dietary vegetables and cultivated soil in an organic farming system.

C. The Effects of Lead (Pb) and Zinc (Zn) in Plants

The presence of lead (Pb) in plants has been found to have numerous adverse consequences for their morphology, physiology, and biochemistry. Lead (Pb) has been found to hinder the process of photosynthesis, disturb the uptake of essential minerals and water balance, modify hormone levels, and impact the structure and permeability of cell membranes. The aforementioned consequences can result in inhibited growth, chlorosis (a condition characterized by the yellowing of leaves), and the darkening of the root system [9-11]. Plants have evolved many methods to effectively detoxify and tolerate lead (Pb) contamination. Putting lead (Pb) in the vacuole, making organic molecules like phytochelatins and glutathione that bind to Pb, and turning on antioxidant defense systems are all ways to reduce the toxicity of lead (Pb). Plants can handle Pb because they can keep it in their cell walls, make osmolytes, and make antioxidant enzymes work [9, 12]. The absorption of lead (Pb) in plants is subject to various parameters, including soil pH, particle size, cation exchange capacity, and root exudation. The

presence of an excessive amount of lead (Pb) in plants has been found to have detrimental effects on their metabolic processes, impede their growth and development, and perhaps result in plant mortality. The presence of lead in soils and sediments is a prevalent concern that arises from many human, agricultural, and industrial practices. The potential of phytoremediation and rhizofiltration methods in the treatment of soils contaminated with lead has been demonstrated. These methodologies employ the use of plants with the purpose of mitigating or eliminating lead (Pb) from the surrounding environment. Also, lead-induced stress has the potential to change the make-up and behavior of bacterial communities that are closely linked to plants, such as those that live in biofilms that stick to plants that are underwater. Elevated levels of lead (Pb) have the potential to impede the diversity of bacterial communities and result in a decline in enzyme function [9, 13].

The effects of zinc (Zn) in plants can vary depending on the concentration and duration of exposure. While zinc is an essential micronutrient for plants, excessive levels of zinc can be toxic and have detrimental effects on plant growth and development. High levels of zinc in the soil can inhibit many plant-metabolic functions, resulting in retarded growth and senescence. Zinc toxicity can limit the growth of both roots and shoots and cause chlorosis in younger leaves, which can extend to older leaves with prolonged exposure to high soil zinc levels. The chlorosis may arise partly from induced iron (Fe) deficiency, as hydrated zinc and iron ions have similar radii. Zinc toxicity can also affect photosynthesis in plants. With increasing zinc concentration, photosystem II (PSII) efficiency parameters can decline, leading to impaired photosynthetic electron transport and a decrease in bio-membrane permeability. However, it is worth noting that some plants have the ability to resist and even accumulate zinc in their tissues. These plants, known as zinc hyperaccumulators, display morphological, physiological, and biochemical adaptations resulting from the activation of molecular zinc hyperaccumulation mechanisms [14, 15].

In this chapter, it is aimed to examine various anatomical parameters (Stomatal number (SN), epidermis cell number (ECN), stomatal width (SW), stomatal length (SL), and stomatal index (SI) in superficial sections; In cross-sections, epidermis cell width (ECW), epidermis cell length (ECL), leaf thickness (LT), and distance between vascular bundles (DBVB)) of barley and radish leaves grown under lead and zinc stress.

II. MATERIALS AND METHODS

A. Seed Germination

The plant materials for our research were the seeds of barley (*Hordeum vulgare* L. cv. "Tarm") and radish (*Raphanus sativus* L.) from the Ankara Agricultural Research Institute. As heavy metals, zinc ($Zn(NO_3)_2.4H_2O$) and lead ($Pb(NO_2)_2$) salts were used.

The seeds have undergone surface sterilization before harvesting. To do this, the seeds are kept in 1% sodium hypochlorite for 10 minutes, then washed with pure water five times and dried at room temperature on filter paper [16]. Filter paper, petri dishes, and other glass materials are sterilized and dried in the Pasteur oven.

25 seeds of approximately the same size, in full appearance, 8 ml of pure water (control group) or two layers of filter paper, containing Zn and Pb solutions at different concentrations specified above, are placed on a 12 cm diameter petri dish, covered with filter paper, and allowed to germinate in continuous darkness for 7 days in a container adjusted to 20℃.

B. Anatomical Examination and Measurements

The following concentrations were determined in barley seedlings for seedling growth after germination: Together with the Control (Hoagland) group, 4-6-8-10 mM Zn and 2- 3- 4- 6-8 mM Pb were prepared with the Hoagland solution. In radish seedlings after germination, 0.50-0.75- 1.0-1.25-1.50 mM Zn and 1- 2- 4- 6-7-8 mM Pb were prepared with Hoagland solution together with the Control (Hoagland) group.

From the seedlings of barley and radish seeds germinated in distilled water and Zn and Pb levels mentioned above for 7 days; in distilled water Hoagland (control), Zn and Pb concentrations prepared with Hoagland solution in pots containing 50% perlite and 50% washed, sieved, sterilized stream sand containing Zn and Pb solutions at the above-mentioned concentrations in sufficient numbers (4 for each concentration). pots and 5 seedlings in each pot) were transferred and grown in a plant growth room at 25±1 ºC constant temperature, continuous light (8.000 lux), and 60±5% relative humidity for 21 days (Figures 1-3).

Figure 1. Radish seedlings treated with Zn in the plant growth chamber

Figure 2. Radish seedlings treated with Pb in the plant growth chamber

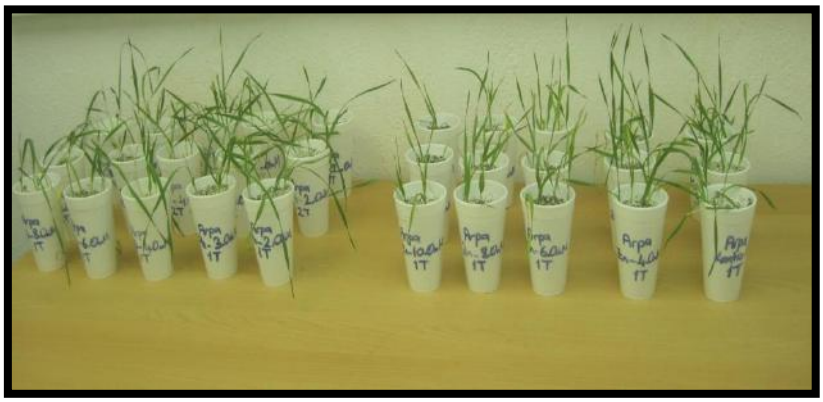

Figure 3. Barley seedlings treated with Zn and Pb in the plant growth chamber

- **Anatomical examinations**

After transfer to pots, the seedlings were grown in the climate chamber for 21 days. Some of the secondary leaves of the seedlings were used to obtain upper and lower superficial sections, and the sections were prepared using Canadian balsam on the slide. Other secondary leaves were preserved in 70% alcohol. These samples underwent the sequential dehydration process as shown in Table 1.

Table 1. Dehydration series and duration

Alcohol and Alcohol + Xylol Series		Waiting Times
1	80% alcohol	1/2 hour
2	96% alcohol	1/2 hour
3	100% alcohol	1/2 hour
4	100% alcohol (again)	1/2 hour
5	200 ml alcohol + 100 ml xylol	1/2 hour
6	100 ml alcohol + 100 ml xylol	1/2 hour
7	100 ml alcohol + 200 ml xylol	1/2 hour
8	Pure xylol	1/2 hour
9	50% Paraffin + 50% Xylol (60 ºC)	1/2 hour
10	Pure paraffin (60 ºC)	6 Hours

After the serialization process was completed, the samples were carefully paraffin-blocked in the cross-section directions [17]. Excess paraffin around the paraffin blocks was trimmed with a razor blade, and 5-6 µm thick cross-sections were taken with the help of a Leica RM2255 rotary microtome (German). Sections adhered to the slide with Albumin-Glycerin were stained with Periodic Acid Schiff (P.A.S.) stain (Hotchkiss Mc Manus 04-130802, Milan/Italy). The sections, whose staining process was completed, were closed with entellan and made continuous, and their photographs were taken with the help of the microscope's internal camera.

Stomatal number (SN), epidermis cell number (ECN), stomatal width (SW), stomatal length (SL), and stomatal index (SI) in superficial sections; In cross-sections, epidermis cell width (ECW), epidermis cell length (ECL), leaf thickness (LT), and distance between vascular bundles (DBVB) parameters were measured in 3 repetitions, 10 times in each leaf, and the averages were taken. The sizes of the relevant parameters in anatomical examinations were determined as µm using an ocular micrometer.

In addition, stoma index (SI) was obtained by counting the stomatal and epidermis cells in a unit area of 1 mm^2 with the help of an ocular micrometer, and these counts were performed 10 times on both the upper and lower surfaces of each leaf, and their averages were calculated. After the determination of the number of stomata and epidermis cells in the leaf unit area, the stomatal index was calculated according to the method of Meidner and Mansfield [18].

$$\text{Stoma index} = \frac{\text{The number of stomata per unit area}}{\text{“The number of stomata + the number of epidermis cells” per unit area}} \times 100$$

Experiments were arranged in four repetitions, and statistical evaluation of all parameters was made according to Duncan's multiple range test using SPSS 14.0.

III. RESULTS

Some anatomical parameters were measured on the leaves 21 days after the transfer of barley and radish seedlings to the plant growth chamber. These are the stomatal number (SN), epidermis cell number (ECN), stoma width (SW), stoma length (SL), and stomatal index (SI), epidermis cell width (ECW), epidermis cell length (ECL), leaf thickness (LT), and distance between vascular bundles (DBVB) parameters.

A. Anatomical Results in Barley Seedlings

The findings related to the anatomical parameters examined in the leaves taken from the barley seedlings grown in the plant growth cabinet are presented in Tables 2 and 3.

Table 2. The effect of zinc on some anatomical parameters of the seedlings 21 days after the transfer of barley seedlings to the plant growth chamber

Zn (mM)	Stomatal Number (SN)		Stomatal Width (SW) (μm)		Stomatal Length (SL) (μm)		Stomatal Index (SI)	
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**
0.0	*5.6 ± 0.6^{ab}	**4.5 ± 1.0^{a}**	17.3 ± 1.2^{a}	**16.7 ± 2.0^{b}**	33.6 ± 3.2 ^{a}	**36.3 ± 2.3^{c}**	23.5	**24.5**
4	5.1 ± 0.9^{a}	**4.6 ± 0.9^{a}**	17.2 ± 2.3^{a}	**17.3 ± 1.7^{b}**	35.6 ± 2.5 ^{b}	**35.5 ± 2.6^{bc}**	19.5	**21.0**
6	5.6 ± 0.7^{a}	**5.8 ± 1.0^{b}**	18.6 ± 2.3^{b}	**15.5 ± 1.5^{a}**	39.1 ± 3.2 ^{c}	**33.8 ± 2.7^{a}**	20.8	**21.2**
8	6.1 ± 1.6^{b}	**4.8 ± 1.0^{a}**	17.3 ± 2.1^{a}	**16.4 ± 1.7^{b}**	36.6 ± 2.9 ^{b}	**34.3 ± 3.3^{ab}**	20.6	**18.9**
10	5.1 ± 0.7^{a}	**6.3 ± 1.3^{b}**	16.6 ± 2.2^{a}	**16.6 ± 1.9^{b}**	32.9 ± 3.2 ^{a}	**34.1 ± 3.0^{ab}**	18.8	**22.2**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

Table 2. The effect of zinc on some anatomical parameters of the seedlings 21 days after the transfer of barley seedlings to the plant growth chamber (continued)

Zn (mM)	Epidermis Cell Number (ECN)		Epidermis Cell Width (ECW) (μm)		Epidermis Cell Length (ECL) (μm)		Leaf Thickness (LT) (μm)	Distance Between Vascular Bundles (DBVB) (μm)
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**		
0.0	*18.4 ± 2.0^{a}	**13.9 ± 1.4^{a}**	26.3 ± 6.0^{b}	**23.1 ± 5.5^{b}**	22.0 ± 6.4^{d}	**15.2 ± 4.4^{c}**	141.7 ± 24.4^{d}	**329.8 ± 56.3^{d}**
4	21.0 ± 3.1^{b}	**17.4 ± 1.8^{b}**	18.2 ± 7.6^{a}	**18.5 ± 5.0^{a}**	9.7 ± 3.6^{a}	**10.8 ± 3.6^{a}**	77.1 ± 9.3^{a}	**186.1 ± 41.0^{a}**
6	21.3 ± 2.1^{b}	**21.6 ± 1.9^{cd}**	25.9 ± 8.9^{b}	**23.6 ± 5.3^{b}**	11.5 ± 2.7^{ab}	**12.3 ± 5.9^{ab}**	111.9 ± 11.9^{c}	**315.6 ± 89.4^{d}**
8	23.7 ± 3.4^{c}	**20.6 ± 2.2^{c}**	17.3 ± 4.9^{a}	**16.4 ± 5.0^{a}**	15.7 ± 3.6^{c}	**14.2 ± 4.1^{bc}**	103.6 ± 13.8^{b}	**216.9 ± 33.3^{b}**
10	22.0 ± 2.7^{b}	**22.0 ± 2.5^{d}**	16.1 ± 5.0^{a}	**15.9 ± 4.4^{a}**	13.1 ± 3.7^{b}	**11.5 ± 3.8^{a}**	78.6 ± 15.9^{a}	**194.5 ± 30.7^{ab}**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

In barley leaves, Zn had different effects on the number of stomata on the upper (adaxial) and lower (abaxial) surfaces. For example, while the SN values on the upper surface were irregular, it was determined that the SN on the lower surface showed an increase in all Zn levels compared to the control. When the SW is examined, a statistical difference is observed only at 6 mM on the upper and lower surfaces compared to the control group. At this Zn level, while the SW on the upper surface was the highest with 18.6 µm, it had the lowest value with 15.5 µm on the lower surface. While SL shows irregular increases on the upper surface, there is a decrease in all Zn levels on the lower surface compared to the control. A situation similar to SW draws attention here as well; at 6 mM, SL reached the highest value at 39.1 µm on the upper surface and the lowest value at 33.8 µm on the lower surface. SI showed decreases in both lower and upper surfaces compared to the control at all concentrations (Figures 4-5).

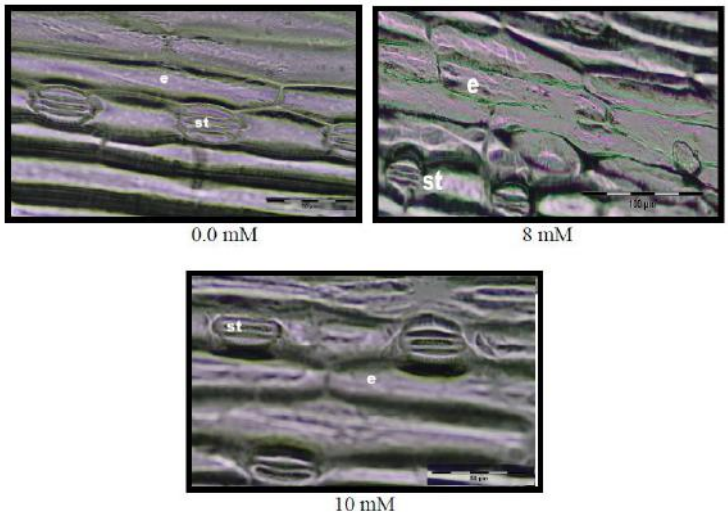

Figure 4. Leaf superficial (upper surface) sections of barley seedlings germinated and grown at various Zn concentrations; e: epidermis, st: stomata

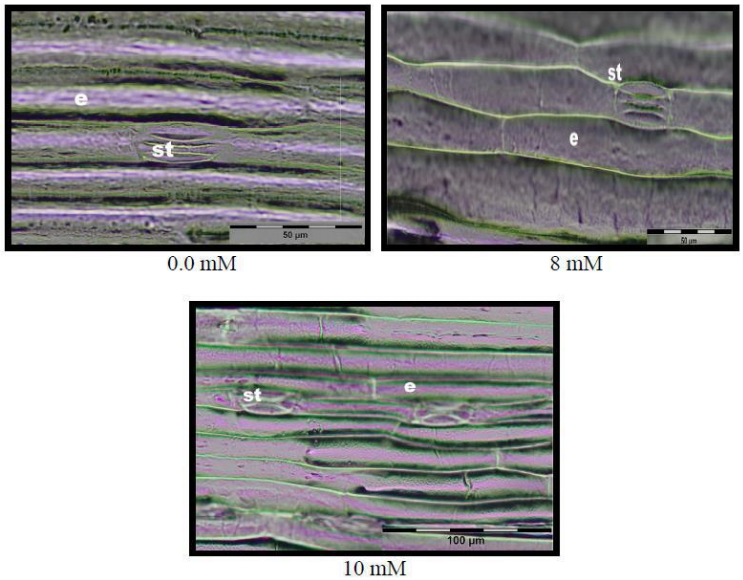

Figure 5. Leaf superficial (lower surface) sections of barley seedlings germinated and grown at various Zn concentrations; e: epidermis, st: stomata

ECN, on the other hand, increased at all Zn levels on both the upper and lower surfaces of the leaf compared to the control. ECN, which was 18.4 in the upper surface control, reached the highest value with 23.7 in 8 mM Zn. The value, which was 13.9 in the control on the lower surface, had a maximum value of 22.0 at the highest concentration, 10 mM. ECW and ECL showed similar increase and decrease on both the upper and lower surfaces, and decreases were noted at all concentrations compared to the control (Figure 6).

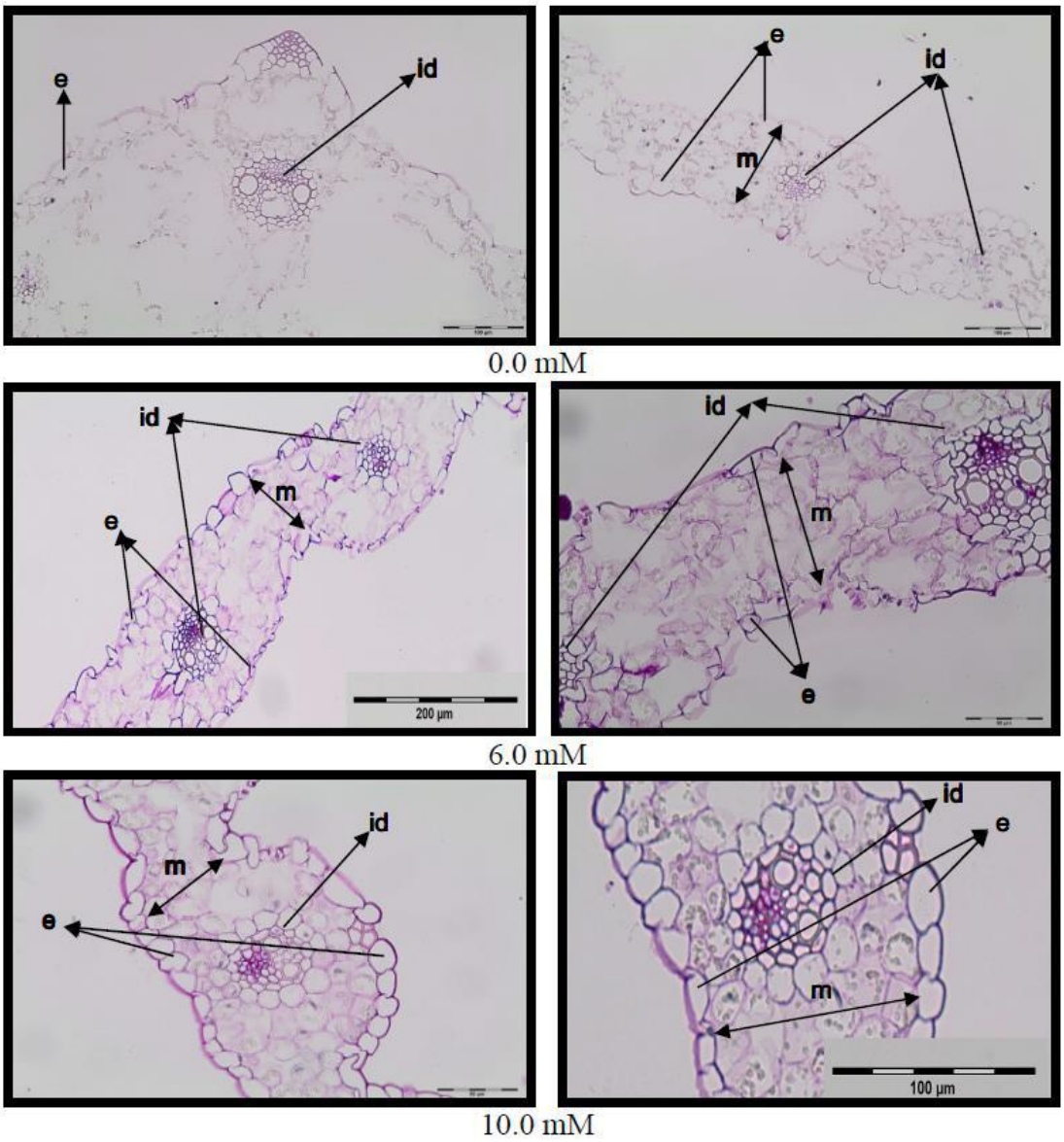

Figure 6. Leaf cross-sections of barley seedlings germinated and grown at various zinc concentrations; e: upper and lower epidermis, id: vascular bundle, m: mesophyll

LT and DBVB measurement results are also similar to ECW and ECL. When compared to the control, both parameters went down in all concentrations. The sharp drop in LT and DBVB in 4 mM zinc was especially interesting. For example, while LT was 141.7 µm in the leaves of the control group, the lowest value was measured as 77.1 µm at 4 mM, with a decrease of approximately 50%. DBVB was determined to be 329.8 µm in the control group. Parallel to the LT, it had its lowest value at 4 mM and was measured as 186.1 µm.

As seen in Table 3, Pb had different effects on the leaves of Barley seedlings 21 days after the transfer to the plant growth chamber.

Table 3. The effect of lead on some anatomical parameters of the seedlings 21 days after the transfer of barley seedlings to the plant growth chamber

Pb (mM)	Stomatal Number (SN)		Stomatal Width (SW) (μm)		Stomatal Length (SL) (μm)		Stomatal Index (SI)	
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**
0.0	*5.6 ± 0.6^{c}	**4.5 ± 1.0^{c}**	17.3 ± 1.2^{bc}	**16.7 ± 2.0^{b}**	33.6 ± 3.2^{b}	**36.3 ± 2.3^{a}**	23.5	**24.5**
2	6.0 ± 0.8^{c}	**5.4 ± 1.0^{d}**	17.6 ± 1.5^{c}	**17.4 ± 3.0^{b}**	34.9 ± 2.1^{bc}	**38.3 ± 2.9^{b}**	20.4	**23.8**
3	5.1 ± 0.8^{b}	**3.6 ± 0.8^{b}**	19.5 ± 2.6^{d}	**19.3 ± 3.9^{c}**	36.3 ± 3.1^{d}	**37.3 ± 2.9^{ab}**	19.1	**19.1**
4	4.7 ± 0.8^{a}	**2.9 ± 0.4^{a}**	16.6 ± 1.5^{ab}	**14.2 ± 1.5^{a}**	30.3 ± 2.2^{a}	**36.2 ± 2.2^{a}**	20.1	**14.9**
6	4.3 ± 0.8^{a}	**3.7 ± 0.7^{b}**	16.2 ± 1.7^{a}	**16.2 ± 1.8^{b}**	37.9 ± 2.5^{e}	**40.2 ± 2.5^{c}**	19.9	**18.6**
8	5.7 ± 0.8^{c}	**4.6 ± 0.9^{c}**	16.2 ± 2.2^{a}	**14.9 ± 1.2^{a}**	36.1 ± 2.3^{cd}	**36.3 ± 2.8^{a}**	20.3	**19.8**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

Table 3. The effect of lead on some anatomical parameters of the seedlings 21 days after the transfer of barley seedlings to the plant growth chamber (continued)

Pb (mM)	Epidermis Cell Number (ECN)		Epidermis Cell Width (ECW) (μm)		Epidermis Cell Length (ECL) (μm)		Leaf Thickness (LT) (μm)	Distance Between Vascular Bundles (DBVB) (μm)
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**		
0.0	*18.4 ± 2.0^{ab}	**13.9 ± 1.4^{a}**	26.3 ± 6.0^{b}	**23.1 ± 5.5^{c}**	22.0 ± 6.4^{c}	**15.2 ± 4.4^{abc}**	141.7 ± 24.4^{bc}	**329.8 ± 56.3^{b}**
2	23.2 ± 2.1^{d}	**17.2 ± 1.4^{d}**	19.6 ± 6.0^{a}	**18.0 ± 4.6^{a}**	21.5 ± 6.5^{c}	**17.9 ± 5.4^{c}**	116.3 ± 19.1^{a}	**258.8 ± 65.0^{a}**
3	21.8 ± 2.1^{c}	**15.1 ± 1.6^{b}**	23.8 ± 6.4^{b}	**22.8 ± 6.9^{c}**	14.6 ± 4.6^{b}	**16.0 ± 5.4^{bc}**	135.3 ± 18.6^{b}	**262.4 ± 44.4^{a}**
4	18.5 ± 2.1^{b}	**16.8 ± 1.5^{cd}**	23.7 ± 6.3^{b}	**19.6 ± 5.4^{ab}**	16.4 ± 4.8^{b}	**14.4 ± 4.8^{ab}**	149.0 ± 22.2^{c}	**251.1 ± 29.0^{a}**
6	17.4 ± 1.8^{a}	**16.1 ± 1.3^{c}**	26.0 ± 8.1^{b}	**21.5 ± 6.0^{bc}**	10.9 ± 4.0^{a}	**12.7 ± 5.3^{a}**	111.4 ± 22.8^{a}	**263.2 ±20.7^{a}**
8	22.2 ± 1.9^{cd}	**18.5 ± 1.6^{e}**	23.0 ± 6.9^{a}	**18.6 ± 5.1^{ab}**	21.3 ± 5.7^{c}	**14.2 ± 4.5^{ab}**	108.8 ± 25.7^{a}	**251.0 ±29.8^{a}**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

SN was wavy on both the upper and lower surfaces of the leaf, and while an increase was observed at 2 mM on both surfaces compared to the control, an unexpected decrease was observed at higher levels (3-4-6 mM). SW and SL were also observed to fluctuate like SN (Figures 7–8). An increase was observed for SW compared to control at low concentrations (2–3 mM) on both the upper and lower surfaces of the leaf, while a decrease was observed in all remaining Pb levels compared to control. Non-regular increases were observed on both surfaces compared to the control at other concentrations except 4 mM in SL. SI, on the other hand, showed a decrease in all Pb levels on both the lower and upper surfaces compared to the control. While the value of 23.5 at the upper surface control was 19.1 at 3 mM, it was observed that the SI, which was 24.5 at the lower surface control, had the lowest value with 14.9 at 4 mM.

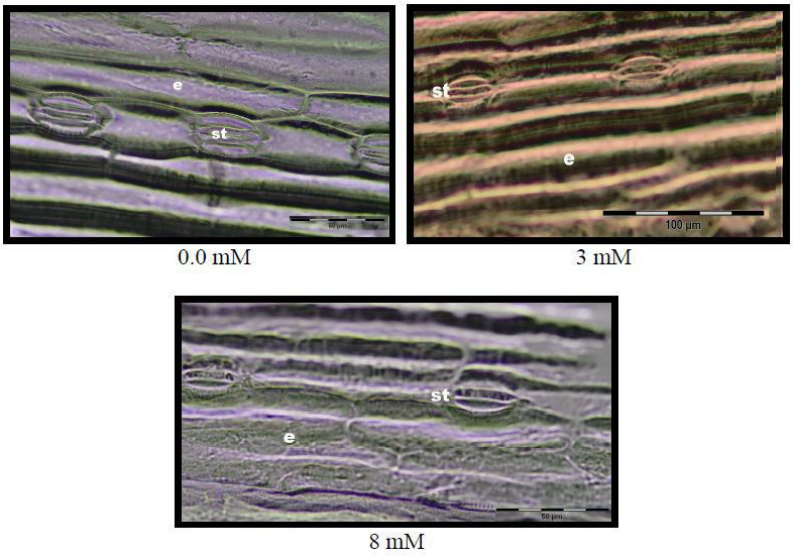

Figure 7. Leaf superficial (upper surface) sections of barley seedlings germinated and grown at various Pb concentrations; e: epidermis, st: stomata

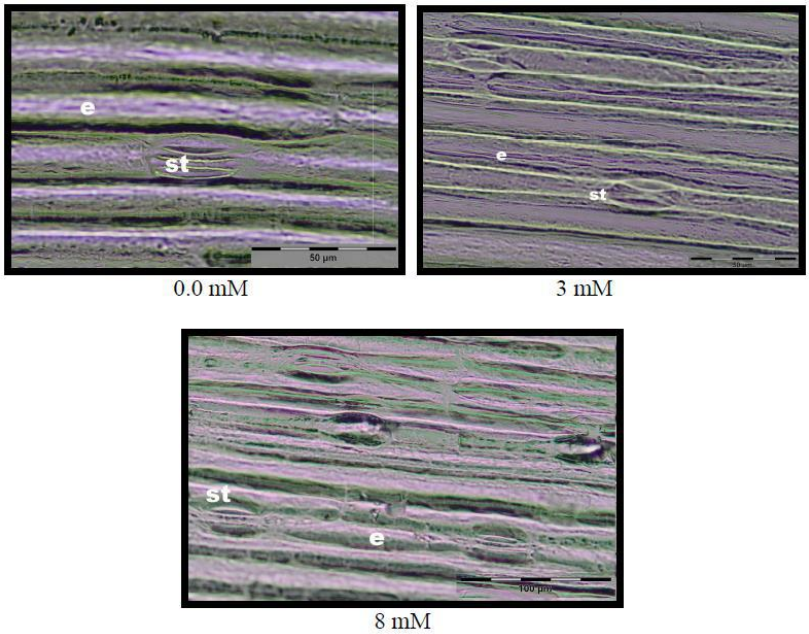

Figure 8. Leaf superficial (lower surface) sections of barley seedlings germinated and grown at various Pb concentrations; e: epidermis, st: stomata

Pb caused an increase in ECN of barley leaves compared to control at almost all concentrations on both the upper and lower surface. The control value was determined as 18.4 on the upper surface, and an increase was observed at all levels except 6 mM. A decrease was observed in all Pb levels on both the upper and lower surfaces of EHW compared to the control values, with the lowest values detected at 2 mM (Figure 9). While the control values on the upper and lower surfaces were 26.3 µm and 23.1 µm, respectively, these values decreased to 19.6 µm and 18.0 µm at 2 mM, respectively. The lowest values of ECL on the upper and lower surfaces compared to the control were determined at 6 mM. While a decrease is observed in all Pb levels on the upper surface compared to the control, a wavy course is observed on the lower surface. For example, while the ECL on the lower surface was 15.2 µm in the control, it followed a wavy course, 17.9 µm at 2 mM and 12.7 µm at 6 mM. Compared to control, Pb caused a decrease in LT at almost all levels. It was determined that LT, which was 141.7 µm in the control, increased only at 4 mM and decreased at all remaining levels. It had its lowest value at 8 mM. DBVB decreases at all Pb concentrations. DBVB, which was found to be 329.8 µm in the control, showed statistically insignificant decreases in other Pb levels.

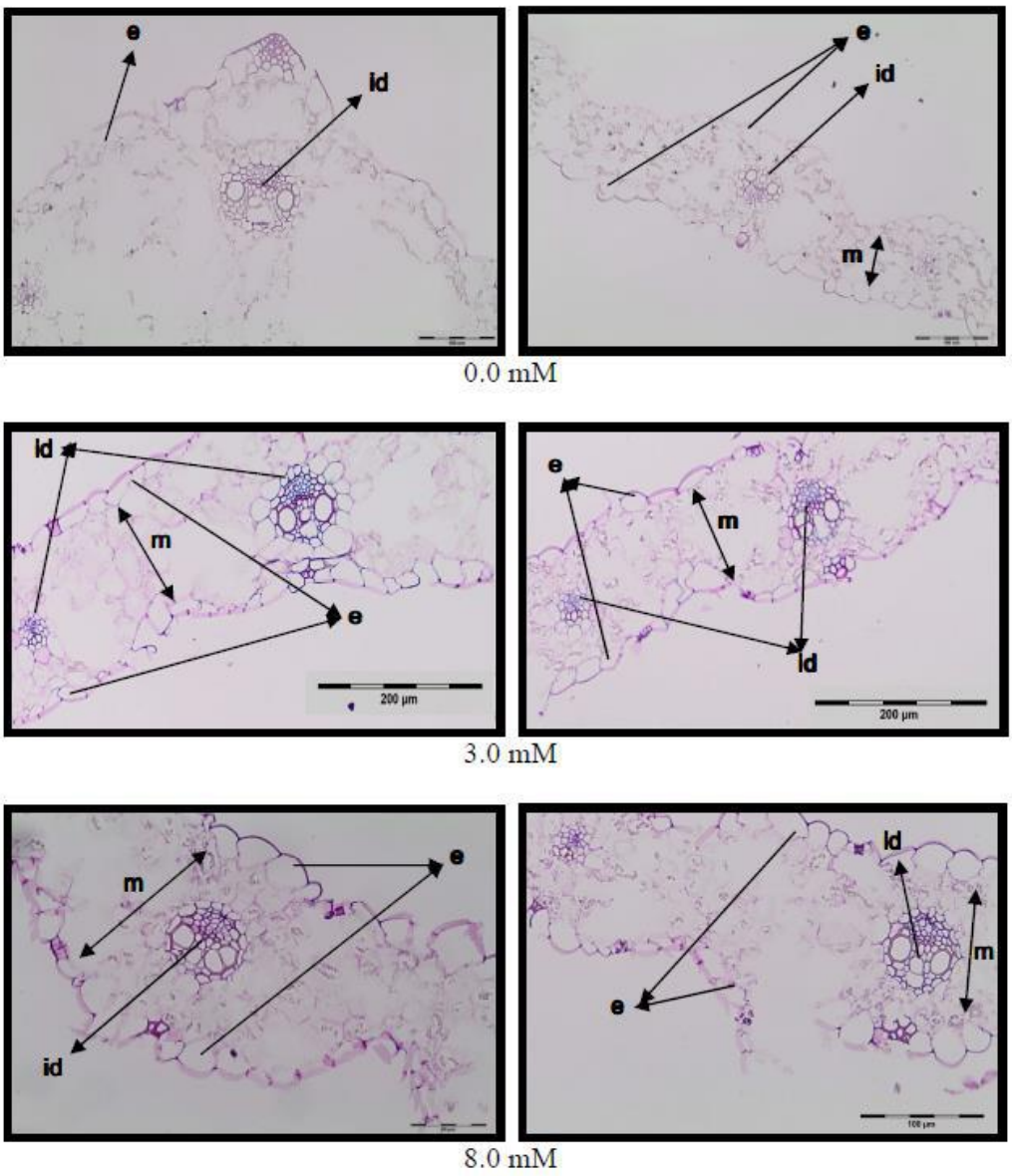

Figure 9. Leaf cross-sections of barley seedlings germinated and grown at various lead concentrations; e: upper and lower epidermis, id: vascular bundle, m: mesophyll

B. Anatomical Results in Radish Seedlings

The findings related to the anatomical parameters examined in the leaves taken from the radish seedlings grown in the plant growth cabinet are presented in Tables 4 and 5.

Table 4. The effect of zinc on some anatomical parameters of the seedlings 21 days after the transfer of radish seedlings to the plant growth chamber.

Zn (mM)	Stomatal Number (SN)		Stomatal Width (SW) (µm)		Stomatal Length (SL) (µm)		Stomatal Index (SI)	
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**
0.0	*1.4 ± 0.6^{b}	**3.6 ± 0.8^{c}**	10.3 ± 1.3^{bc}	**10.7 ± 1.6^{c}**	18.3 ± 1.9^{d}	**16.1 ± 2.3^{ab}**	18.8	**18.3**
0.50	1.0 ± 0.2^{a}	**1.3 ± 0.5^{ab}**	10.2 ± 0.6^{bc}	**10.2 ± 0.6^{abc}**	16.8 ± 1.8^{c}	**16.9 ± 1.4^{b}**	12.2	**10.5**
0.75	1.1 ± 0.3^{a}	**1.4 ± 0.6^{b}**	10.5 ± 1.0^{c}	**10.3 ± 1.0^{abc}**	17.5 ± 1.6^{cd}	**16.3 ± 1.8^{ab}**	12.5	**11.2**
1.0	1.5 ± 0.7^{bc}	**1.5 ± 0.5^{b}**	10.1 ± 0.8^{bc}	**9.9 ± 0.5^{a}**	17.3 ± 1.9^{c}	**15.8 ± 1.8^{a}**	13.3	**12.9**
1.25	1.8 ± 0.7^{cd}	**1.0 ± 0.2^{a}**	9.8 ± 0.6^{ab}	**10.6 ± 1.1^{bc}**	15.3 ± 1.6^{b}	**18.7 ± 1.8^{c}**	13.3	**10.3**
1.50	1.9 ± 0.8^{d}	**1.5 ± 0.6^{b}**	9.6 ± 0.9^{a}	**10.1 ± 0.5^{ab}**	14.1 ± 1.9^{a}	**16.0 ± 1.8^{ab}**	11.9	**11.3**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

Table 4. The effect of zinc on some anatomical parameters of the seedlings 21 days after the transfer of radish seedlings to the plant growth chamber (continued).

Zn (mM)	Epidermis Cell Number (ECN)		Epidermis Cell Width (ECW) (µm)		Epidermis Cell Length (ECL) (µm)		Leaf Thickness (LT) (µm)	Distance Between Vascular Bundles (DBVB) (µm)
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**		
0.0	*6.1 ± 1.1^{a}	**16.2 ± 2.9^{c}**	46.4 ± 15.0^{bc}	**42.8 ± 19.2^{c}**	15.5 ± 3.7^{b}	**16.6 ± 3.6^{c}**	194.5 ± 19.8^{c}	**4922.5 ± 143.3^{c}**
0.50	7.4 ± 1.2^{b}	**11.1 ± 1.5^{b}**	39.0 ± 17.6^{abc}	**30.1 ± 10.5^{b}**	14.8 ± 4.4^{ab}	**18.3 ± 4.0^{d}**	174.5 ± 16.8^{b}	**2270.0 ± 854.8^{b}**
0.75	7.5 ± 1.8^{b}	**11.1 ± 1.6^{b}**	36.7 ± 13.3^{a}	**26.1 ± 10.4^{ab}**	15.3 ± 4.3^{ab}	**11.1 ± 2.0^{b}**	161.8 ± 13.5^{a}	**1548.1 ± 558.0^{a}**
1.0	10.0 ± 1.6^{c}	**9.9 ± 1.6^{a}**	47.5 ± 21.2^{c}	**38.6 ± 13.4^{c}**	19.0 ± 3.6^{c}	**16.1 ± 4.0^{c}**	222.7 ± 15.5^{e}	**2492.2 ± 77.5^{b}**
1.25	11.9 ± 1.9^{d}	**9.0 ± 1.4^{a}**	39.3 ± 10.1^{abc}	**19.8 ± 6.1^{a}**	13.3 ± 3.4^{a}	**12.0 ± 0.7^{a}**	203.8 ± 21.1^{d}	**2012.2 ± 867.4^{ab}**
1.50	14.3 ± 2.5^{e}	**11.8 ± 1.6^{b}**	38.5 ± 13.2^{ab}	**21.0 ± 7.5^{a}**	13.4 ± 3.5^{ab}	**10.5 ± 3.1^{b}**	176.4 ± 19.9^{b}	**2563.5 ± 223.3^{b}**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

While SN on the upper (adaxial) surface of radish leaves decreased at low concentrations compared to control, it increased from 1 mM with the increase in Zn level. On the lower (abaxial) surface, the SN decreased compared to the control value (3.6) at all concentrations. The lowest SN was found to be 1.0 at 1.25 mM. SW decreased at almost all concentrations on both the upper and lower surfaces of radish leaves. The value on the upper surface, which was 10.3 µm in the control, increased only at 0.75 mM. All remaining Zn levels were low compared to control, with the lowest SG being 9.6 µm at 1.50 mM. On the lower surface of the leaf, a decrease was observed at all concentrations compared to the control. Irregular reductions in SL were detected on the upper surface of the leaf compared to the control. For example, SL, which was 18.3 µm in the control, was 16.8 µm at 0.50 mM, 17.5 µm at 0.75 mM, and 14.1 µm at the highest concentration of 1.50 mM. On the lower surface, there is usually an increase, but there are decreases at 1 mM and 1.50 mM. It is surprising that while minimum SL (15.8 µm) is observed at 1 mM, maximum SL (18.7 µm) is observed at an upper Zn level of 1.25 mM, and this value decreases again (16.0 µm) at the highest Zn level (1.50 mM). Zn caused both upper and lower surface reductions in SI compared to the control group at all levels.

Zn had an opposite effect on ECN in radish leaves on the upper and lower surfaces. With this surprising effect, an increase was observed at all concentrations on the upper surface compared to the control. For example, EHS peaked at 1.50 mM, the highest concentration, which was 6.1 in the control, and became 14.3 with an increase exceeding 100%. On the lower surface, however, the situation was reversed; the value of 16.2 in the control decreased at all Zn levels and took its lowest value of 9.0 at 1.25 mM. ECW was decreased at almost all Zn levels compared to the control on both surfaces of the leaf (Figure 10). ECL decreased at almost all concentrations on both surfaces. The results at 1 mM on the upper surface and 0.50 mM on the lower surface were surprising. While irregular values were observed in LT, a decrease was observed in all Zn levels in DBVB compared to the control group.

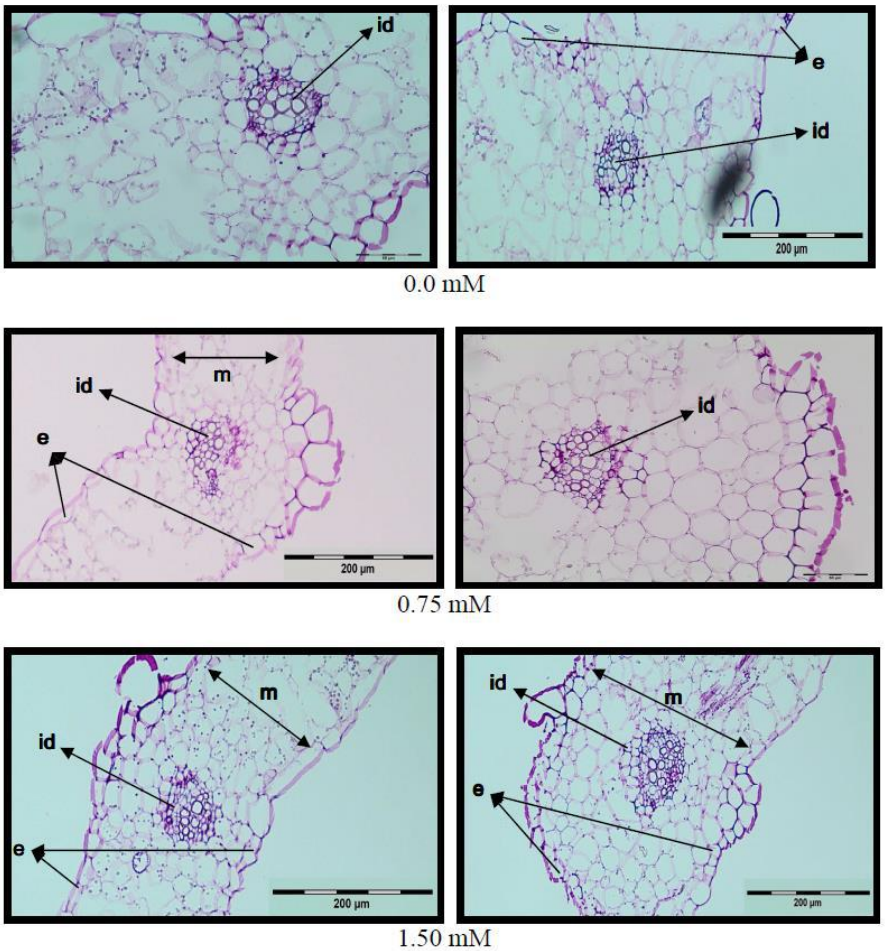

Figure 10. Leaf cross-sections of radish seedlings germinated and grown at various zinc concentrations; e: upper and lower epidermis, id: vascular bundle, m: mesophyll

As seen in Table 5, Pb produced different effects on the leaves of Radish seedlings 21 days after the transfer to the plant growth chamber.

Table 5. The effect of lead on some anatomical parameters of the seedlings 21 days after the transfer of radish seedlings to the plant growth chamber

Pb (mM)	Stomatal Number (SN)		Stomatal Width (SW) (μm)		Stomatal Length (SL) (μm)		Stomatal Index (SI)	
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**
0.0	*1.4 ± 0.6^{ab}	**3.6 ± 0.8^{d}**	10.3 ± 1.3^{ab}	**10.7 ± 1.6^{ab}**	18.3 ± 1.9^{d}	**16.1 ± 2.3^{ab}**	18.8	**18.3**
1	1.2 ± 0.4^{a}	**2.5 ± 0.8^{c}**	10.1 ± 1.0^{ab}	**10.1 ± 1.2^{a}**	17.3 ± 1.2^{bc}	**15.1 ± 1.8^{a}**	12.7	**17.3**
2	1.4 ± 0.5^{ab}	**2.1 ± 0.5^{bc}**	9.8 ± 0.8^{a}	**10.6 ± 1.1^{ab}**	15.5 ± 1.5^{a}	**16.8 ± 1.5^{bc}**	16.0	**15.6**
4	2.1 ± 0.5^{c}	**3.9 ± 0.8^{d}**	11.0 ± 1.2^{cd}	**10.2 ± 0.6^{ab}**	17.8 ± 2.1^{cd}	**16.8 ± 1.2^{bc}**	21.3	**21.2**
6	2.1 ± 0.6^{c}	**2.1 ± 0.9^{bc}**	10.5 ± 1.2^{bc}	**10.7 ± 1.3^{ab}**	16.8 ± 1.7^{bc}	**15.3 ± 1.8^{a}**	23.7	**16.3**
7	2.3 ± 0.6^{c}	**1.8 ± 0.6^{ab}**	11.4 ± 1.4^{d}	**10.8 ± 1.5^{b}**	17.1 ± 2.2^{bc}	**18.3 ± 2.3^{d}**	23.7	**13.1**
8	1.5 ± 0.6^{b}	**1.6 ± 0.8^{a}**	10.3 ± 0.8^{ab}	**10.7 ± 1.1^{ab}**	16.3 ± 1.8^{ab}	**17.3 ± 2.0^{c}**	13.7	**12.5**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

Table 5. The effect of lead on some anatomical parameters of the seedlings 21 days after the transfer of radish seedlings to the plant growth chamber (continued)

Pb (mM)	Epidermis Cell Number (ECN)		Epidermis Cell Width (ECW) (μm)		Epidermis Cell Length (ECL) (μm)		Leaf Thickness (LT) (μm)	Distance Between Vascular Bundles (DBVB) (μm)
	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**	Adaxial Surfaces	**Abaxial Surfaces**		
0.0	*6.1 ± 1.1^{a}	**16.2 ± 2.9^{d}**	46.4 ± 15.0^{b}	**42.8 ± 19.2^{d}**	15.5 ± 3.7^{b}	**16.6 ± 3.6^{bc}**	194.5 ± 19.8^{b}	**4922.5 ± 143.3^{d}**
1	8.0 ± 1.6^{c}	**11.8 ± 2.1^{b}**	38.7 ± 12.3^{ab}	**34.1 ± 15.8^{c}**	16.3 ± 6.2^{b}	**15.8 ± 4.1^{bc}**	221.1 ± 19.1^{c}	**3001.9 ± 237.1^{c}**
2	7.5 ± 1.4^{c}	**11.5 ± 1.4^{ab}**	44.8 ± 20.1^{b}	**34.7 ± 13.0^{c}**	17.0 ± 5.2^{bc}	**15.0 ± 5.4^{bc}**	196.6 ± 17.1^{b}	**2854.1 ± 885.3^{bc}**
4	7.9 ± 1.5^{c}	**14.5 ± 1.6^{c}**	34.1 ± 13.6^{a}	**26.2 ± 11.2^{ab}**	12.3 ± 4.8^{a}	**26.6 ± 15.7^{d}**	177.7 ± 10.3^{a}	**2767.1 ± 160.7^{bc}**
6	6.7 ± 1.1^{ab}	**10.6 ± 1.5^{a}**	34.7 ± 13.2^{a}	**31.6 ± 16.8^{bc}**	18.2 ± 5.6^{bc}	**16.9 ± 5.8^{c}**	180.8 ± 12.0^{a}	**2370.3 ± 408.7^{b}**
7	7.3 ± 1.7^{bc}	**11.8 ± 1.3^{b}**	43.8 ± 18.9^{b}	**21.0 ± 6.9^{a}**	19.3 ± 4.7^{c}	**12.7 ± 2.9^{ab}**	250.5 ± 33.6^{d}	**1841.8 ± 378.9^{a}**
8	9.3 ± 1.6^{d}	**11.4 ± 1.8^{ab}**	44.2 ± 15.4^{b}	**28.5 ± 10.6^{abc}**	11.4 ± 2.6^{a}	**10.9 ± 2.2^{a}**	215.9 ± 19.8^{c}	**1501.3 ± 621.2^{a}**

*The difference between the values indicated with the same letter in each parameter column is insignificant at the ($p<0.05$) level.

While SN increased from a 4 mM Pb level on the upper surface of the leaf compared to the control, it decreased in all Pb levels except 4 mM on the lower surface. SW showed an irregular increase and decrease compared to the control on both surfaces. While SL showed a decrease in all Pb levels on the upper surface of radish leaves compared to the control group, it showed an irregular decrease or increase on the lower surface. Similarly, irregular increases and decreases in SI were observed on both the upper and lower surfaces of the leaf compared to the control. While ECN increased on the upper surface of the leaf compared to the control, the situation was reversed on the lower surface and decreased compared to the control. ECW was decreased on both the lower and upper surfaces compared to the control. In the ECL, on the other hand, wavy results were observed on both surfaces (Figure 11).

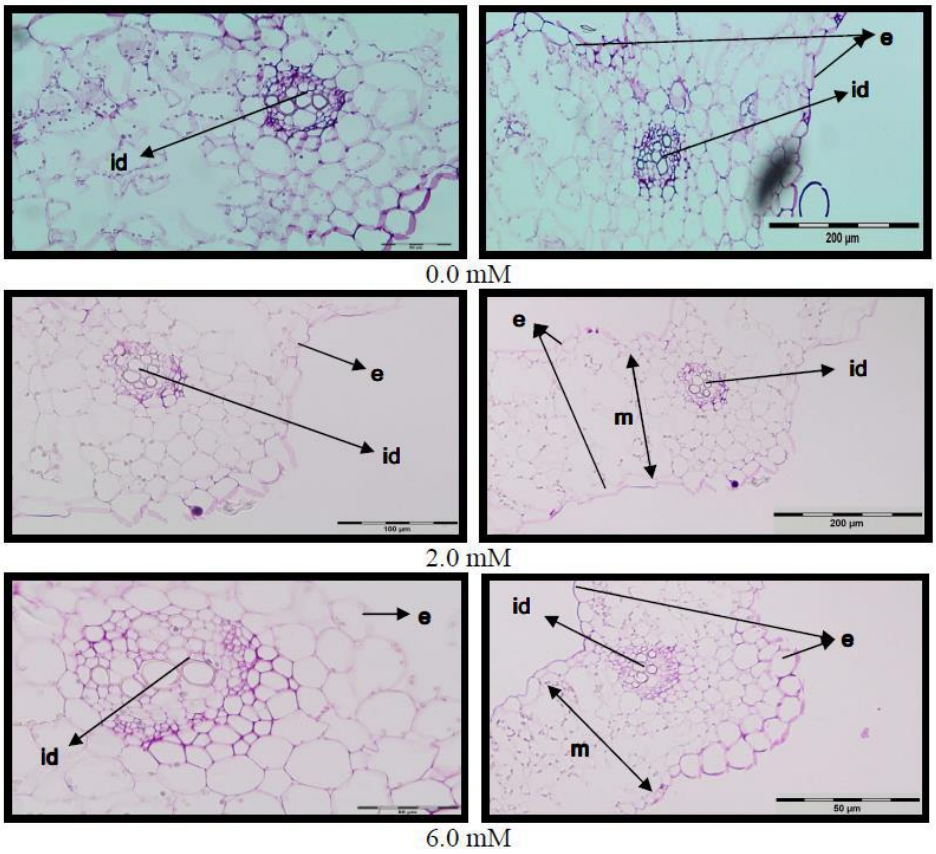

Figure 11. Leaf cross-sections of radish seedlings germinated and grown at various lead concentrations; e: upper and lower epidermis, id: vascular bundle, m: mesophyll

An increase is observed in LT compared to the control at other concentrations except 4 and 6 mM. While the thickness of the control group leaves was 194.5 µm, the lowest value was recorded as 177.7 µm at 4 mM, and the highest value was recorded as 250.5 µm at 7 mM. DBVB decreased at all Pb levels compared to control, and the lowest value was found at 8 mM. For example, DBVB, which was 4922.5 µm in the control, showed a large decrease at the highest concentration of 8 mM and decreased to 1501.3 µm.

IV. DISCUSSION AND CONCLUSION

The anatomical parameters of both plants are summarized below:

• Stoma number, stoma index, stoma width, and stoma length parameters generally gave similar responses to barley seedlings in zinc and lead applications:

Generally, irregular decreases and increases were observed on almost all leaf surfaces in SN, Zn, and Pb concentrations compared to control, except for the leaf underside in Zn. Both heavy metals caused a decrease in SI on both the upper and lower surfaces. SW and SL showed wavy results compared to control on all remaining leaf surfaces under the influence of Zn and Pb, except for SL on the lower leaf surface at Zn levels.

Radish seedlings gave different responses in Zn and Pb compared to barley in the listed parameters:

While SN generally decreases in the presence of Zn and Pb on the lower surfaces, it increases from 4 mM in the wavy Pb medium to 5 mM in the Zn medium on the upper surfaces. While SI decreased on both surfaces at the Zn levels, in the presence of Pb, it followed a wavy course on both surfaces. While SW showed a

general decrease on both the upper and lower surfaces at the Zn levels, it followed a wavy course on both surfaces in the presence of Pb. While SL followed a wavy course on the upper surface at the Zn levels, it generally increased on the lower surface. In the presence of Pb, while a decrease was observed on the upper surface, a wavy course was observed on the lower surface. The common effect of zinc in both species is that it reduces SI only on the lower and upper surfaces.

- ECN, ECW, ECL, LT and DBVB parameters gave similar responses at Zn and Pb levels in both plant species:

While Zn and Pb caused an increase in the upper surfaces of both species in ECN, they caused an increase in the lower surfaces in barley and a decrease in the lower surfaces in radish. Similarly, ECW values decreased on both surfaces in the presence of Zn and Pb in both species. While decreases were observed in ECL in both species at the Zn levels, fluctuations were observed at Pb levels, except for barley upper surface ECL. While both heavy metals caused a decrease in LT values in barley, they caused fluctuating results in radish in general. In both species, DBVB responded similarly and decreased to zinc and lead.

When the leaf structures of seedlings that grew in heavy metal-stressed environments were looked at (Table 2–5), the results were different depending on the species studied, but the effects of different heavy metals on the same species were the same. In barley, almost all leaf surfaces in SN, Zn, and Pb media, except for the lower leaf surface of Zn, were generally observed to be bumpy compared to control. Both heavy metals caused a decrease in SI on both the upper and lower surfaces. The basis of the decrease in SI can be evaluated as the increase in ECN in both heavy metals on both the lower and upper surfaces of the leaf. Shortenings in the ECL may also be a reason for this. With the effects of Zn and Pb, SW and SL were found to be going up and down compared to the control on all the other leaf surfaces, except for SL on the lower leaf surface of Zn. In radish, SN generally decreases on the lower surfaces due to the heavy metal effect, while it is generally in the form of fluctuations in Zn on the upper surfaces and increases in Pb. While SI showed a decrease in Zn on both surfaces, in the presence of Pb, it followed a wavy course on both surfaces. While SW showed a general decrease in Zn on both the upper and lower surfaces, it followed a wavy course on both surfaces in the presence of Pb. While SL followed a wavy course on the upper surface of Zn, it generally increased on the lower surface. In the presence of Pb, while a decrease was observed on the upper surface, a wavy course was observed on the lower surface. The only common effect of zinc observed in both species is that it reduces SI on both the lower and upper surfaces.

Both species' ECN, ECW, ECL, LT, and DBVB parameters had the same response to Zn and Pb: Zn and Pb made the upper surfaces of both species' ECN go up, the lower surfaces go up in barley, and the lower surfaces go down in radish. Similarly, ECW values decreased on both surfaces in the presence of Zn and Pb in both species. In Zn, decreases in ECL were observed in both species, while fluctuations were observed in Pb, except for barley top surface ECL. It is thought that both heavy metals generally cause decreases in ECL and ECW in both radish and barley and may have led to decreases in SIs by increasing their ECN.

Both heavy metals caused reductions in LT in barley. This may be due to reductions in ECW and ECL. In radish, fluctuating results were observed in LT. The DBVB values of both species decreased, with similar results in zinc and lead. The decrease in DBVB may also be the result of the decrease in leaf area due to the increase in concentration.

In the literature, generalizations are made about the response of various growth parameters, such as root growth, stem growth, and leaf growth, to heavy metal pollution, such as pressure and inhibition of heavy metal stress [19, 20]. However, the literature on the effects of different heavy metals on different species is both very limited and mostly species-specific. On the other hand, it is not possible for all plants to give the same response(s) to a stress factor, which means neglecting the fact that species may have different equipment. In coping with stress, there should be different ways and mechanisms besides similar reactions.

As seen in this chapter, it should not be ignored that different species may react differently to the same heavy metal. At least, by increasing similar and detailed studies with the same species, common (similar) and different responses should be tried to be learned, including all plants at the species level or in general terms, and then a generalization should be made. Essentially, different plant species may have similar morphological, physiological, anatomical, and biochemical responses to different environmental stress factors, as well as species-specific responses at the species level. Both situations are seen in this study.

As in all living things, there is always environment-structure-function coordination in plants. In this coordination, there may be similarities in many plant species, and there may also be mechanisms different from other species that will serve the life of the plant and contribute to coping with stress at the species and even variety level.

REFERENCES

[1] J. Hall, "Cellular mechanisms for heavy metal detoxification and tolerance", Journal of Experimental Botany, vol. 53, no. 366, p. 1-11, 2002. https://doi.org/10.1093/jxb/53.366.1

[2] J. Bhat, S. Shivaraj, P. Singh, D. Navadagi, D. Tripathi, P. Dashet al., "Role of silicon in mitigation of heavy metal stresses in crop plants", Plants, vol. 8, no. 3, p. 71, 2019. https://doi.org/10.3390/plants8030071

[3] W. Cong, Y. Miao, L. Xu, Y. Zhang, C. Yuan, J. Wanget al., "Transgenerational memory of gene expression changes induced by heavy metal stress in rice (oryza sativa l.)", BMC Plant Biology, vol. 19, no. 1, 2019. https://doi.org/10.1186/s12870-019-1887-7

[4] D. Salt, M. Blaylock, N. Kumar, V. Dushenkov, B. Ensley, I. Chetet al., "Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants", Nature Biotechnology, vol. 13, no. 5, p. 468-474, 1995. https://doi.org/10.1038/nbt0595-468

[5] G. DalCorso, S. Farinati, & A. Furini, "Regulatory networks of cadmium stress in plants", Plant Signaling &Amp; Behavior, vol. 5, no. 6, p. 663-667, 2010. https://doi.org/10.4161/psb.5.6.11425

[6] D. Gupta, H. Huang, & F. Corpas, "Lead tolerance in plants: strategies for phytoremediation", Environmental Science and Pollution Research, vol. 20, no. 4, p. 2150-2161, 2013. https://doi.org/10.1007/s11356-013-1485-4

[7] M. Hejna, D. Gottardo, A. Baldi, V. Dell'Orto, F. Cheli, M. Zaninelliet al., "Review: nutritional ecology of heavy metals", Animal, vol. 12, no. 10, p. 2156-2170, 2018. https://doi.org/10.1017/s175173111700355x

[8] J. Pandey, "Accumulation of heavy metals in dietary vegetables and cultivated soil horizon in organic farming system in relation to atmospheric deposition in a seasonally dry tropical region of india", Environmental Monitoring and Assessment, vol. 148, no. 1-4, p. 61-74, 2008. https://doi.org/10.1007/s10661-007-0139-8

[9] P. Sharma and R. Dubey, "Lead toxicity in plants", Brazilian Journal of Plant Physiology, vol. 17, no. 1, p. 35-52, 2005. https://doi.org/10.1590/s1677-04202005000100004

[10] A. Cesur Turgut and K. Kabar, "The effects of lead and zinc metals on some morphological and physiological parameters of barley and white radish seedlings", International Chemistry & Biology Conference'18 (ChemBioCon'18), Sharm El Sheikh, 11th-14th July 2018.

[11] F. Nas and M. Ali, "The effect of lead on plants in terms of growing and biochemical parameters: a review", MOJ Ecology &Amp; Environmental Sciences, vol. 3, no. 4, 2018. https://doi.org/10.15406/mojes.2018.03.00098

[12] N. El-Shanhorey and O. Emam, "Response of lantana camara plants to foliar applied citric acid for decreasing the harmful effect of heavy metals pollution in the irrigation water (c) effect of lead", Scientific Journal of Flowers and Ornamental Plants, vol. 7, no. 2, p. 93-107, 2020. https://doi.org/10.21608/sjfop.2020.96209

[13] W. Chang, X. Zhu, J. Sun, Y. Pang, & S. Zhang, "Effects of lead pollution on bacterial communities in biofilm attached to submerged plants", Water Science and Technology, vol. 86, no. 6, p. 1358-1372, 2022. https://doi.org/10.2166/wst.2022.279

[14] P. Nagajyoti, K. Lee, & T. Sreekanth, "Heavy metals, occurrence and toxicity for plants: a review", Environmental Chemistry Letters, vol. 8, no. 3, p. 199-216, 2010. https://doi.org/10.1007/s10311-010-0297-8

[15] H. Balafrej, D. Bogusz, Z. Triqui, A. Guedira, N. Bendaou, A. Smouniet al., "Zinc hyperaccumulation in plants: a review", Plants, vol. 9, no. 5, p. 562, 2020. https://doi.org/10.3390/plants9050562

[16] Ş. Baltepe, H. H. Mert, "Bazı Cucurbitaceae Türlerinin Hipokotil Büyümesi Üzerinde Gibberellik Asit ve İndol Asetik Asitin Etkileri", Tubitak IV. Bilim Kongresi Tebliği, 1973.

[17] G. Algan, "Bitkisel Dokular için Mikroteknik", Fırat Üniversitesi Fen Fakültesi Yayınları, Botanik. No:1, 94s, İstanbul, 1981.

[18] H. Meidner, T. A. Mansfield, "Physiology of Stomata", Graw-Hill, 179, New York, 1968.

[19] A. D. Bradshaw, T. McNeilly, "Evolution and Pollution", Institute of Biology Studies in Biology No. 130, London: Arnold, 1981.

[20] A. J. M. Baker, P. L. Walker, "Chemical Speciation and Bioavailibility", 1(3), 7-17, 1989.