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EFFECTS OF MERCURIC CHLORIDE ($HGCL_2$) ON ROOT OF MINT (*MENTHA SPICATA* L.) IN SAND CULTURE

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Abstract

In this study, the effects of mercury II chloride ($HgCl_2$) at concentrations of 0.5, 1, 1.5, 2, 2.5 and 3 mg/L on seed germination speed, seed germination rate, mercury accumulation in the roots and various root parameters in mint (*Mentha spicata* L.) plant were investigated. The sand culture experiment was conducted using a randomized block design with five replicates, lasted for 60 days.

It was observed that the growth of the plants was retarded, especially at 2.5 and 3 mg/L mercury concentrations. The amount of mercury accumulated in the root increased with the increasing mercury concentration. It was determined that negative effect was increased with the increase concentration of mercury in all studied parameters, which comprised of seed germination speed, seed germination rate, mercury accumulation in the roots, root fresh weight, root dry weight, root area, root length, root xylem area, root phloem area, total area of root tracheal lumens, root mean tracheal lumen area, total area of root tracheid lumens, total wall area of root xylem, root trachea number and root tracheid number.

The objective of this study was to investigate the effects of different concentrations of $HgCl_2$ on seed germination, root growth, mercury accumulation, and root anatomical characteristics of *Mentha spicata* L. in sand culture. This investigation aimed to provide new insights into how mercury toxicity affects mint, an economically and medicinally important aromatic plant, particularly during its early developmental stages.

Key words: Heavy metal; Mercury; Sand culture; *Mentha spicata* L.; Root anatomy

Introduction

There are many studies on the negative effects of heavy metals on plants. These negative effects include impacts on seed germination, seedling and root growth, mineral nutrition, plant water status and membrane permeability, senescence, photosynthesis, enzyme activity, respiratioffn and mitochondria, chlorophyll biosynthesis and carotenoid levels, sex change, nitrogen fixation, disruption of hormone balance, flower number, pollen and tube growth, as well as genotoxic effects (Palit *et al.*, 1994; Patra & Sharma, 2000; Munzuroğlu & Gür, 2000; Shekar *et al.*, 2011; Azevedo & Rodriguez, 2012).

Mercury stands out among heavy metals due to its high toxicity. In fact, mercury alone can cause the negative effects listed above for heavy metals in plants. It has been reported that Hg and Pb have no known physiological function or beneficial effects in plants and animals (Allan, 1997; Chibuike & Obiora, 2014).

As a result of human activities such as burning fossil fuels, breaking rocks containing mercury in the mining sector, leakage from solid waste storage areas, random disposal of used batteries, and the use of agricultural herbicides containing mercury, twenty thousand tons of mercury are released into the environment annually. The most important sources of mercury pollution in agricultural areas are the unconscious use of disinfectants, herbicides and fertilizers containing organic mercury (Cavallini *et al.*, 1999; Şener, 2010; Azevedo & Rodriguez, 2012).

Plants have developed various tolerance mechanisms to resist heavy metal accumulation in the soil. One of these is limiting the uptake of heavy metals by the roots. Plant roots secrete organic acids such as citric acid, malic acid, succinic acid, and oxalic acid into the rhizosphere to reduce the uptake of heavy metals. These organic compounds bind to heavy metals and prevent their mobility. Another mechanism by which plants limit heavy metal uptake is increasing the pH of the rhizosphere. This, like organic acids, causes a decrease in metal mobility (Mishra & Dubey, 2006; Ghori *et al.*, 2019; Küçük & Almaca, 2020).

After heavy metals are taken up by the plant roots, different tolerance mechanisms come into play. Heavy metals enter the root via apoplastic and symplastic pathways. In the symplastic pathway, the activity of carrier proteins located in the cell membrane is involved. The transport of nutrients also occurs via this same pathway, which explains how heavy metals are transported against the concentration gradient and how they cause nutritional deficiencies. Apoplastic transport is limited by the cation exchange capacity of the cell wall and must pass through the endodermis to enter the symplastic pathway (Raskin *et al.*, 1994; Ghosh & Singh, 2005; Pasricha *et al.*, 2021).

As soon as heavy metal stress is detected, a complex signal transduction network is activated, characterized by the synthesis and activation of stress-related proteins and various signaling molecules. The signal transduction pathway typically leads to the activation of transcription factors that induce the expression of various genes sensitive to metal stress. Different signaling pathways are activated in

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response to different types of metal stress. These signaling pathways include mitogen-activated protein kinase cascades, hormonal signaling, reactive oxygen species signaling pathways, and the calcium-calmodulin pathway. The genes stimulated by these signals initiate the synthesis of necessary ligands in response to stress. Ligands are molecules that can donate pairs of electrons to a central metal ion or atom. Metals that bind with two or more ligands form structures known as chelates. Compounds acting as ligands include phytochelatins, metallothioneins, amino acids (such as proline and histidine), and organic acids. In these mechanisms, metals are bound by ligands, converted into less toxic forms, and then transported to metabolically inactive parts of the cell, such as the vacuole and cell wall (Ghori *et al.*, 2019).

Against the free radicals promoted by the increase in heavy metals in plant cells, another tolerance mechanism involves the activity of antioxidant enzymes (such as catalase, superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, peroxiredoxins, and thioredoxins) and hormones (such as auxins, jasmonic acid, ethylene, abscisic acid, and salicylic acid). Some plants convert the heavy metals they absorb into less toxic and volatile forms, which are then released into the atmosphere through transpiration. This process is known as phytovolatilization (Emamverdian *et al.*, 2015; Bücker-Neto *et al.*, 2017; Ghori *et al.*, 2019; Yan *et al.*, 2020).

When these tolerance mechanisms fail to control heavy metal accumulation sufficiently, both the quantity and quality of the crop are adversely affected. In addition, the accumulation of heavy metals in plants can render the crops unusable. This situation negatively affects both the production of plants in sufficient quantities to meet the demand and their quality, which determines market value. This study investigated the effects of mercuric chloride ($HgCl_2$) on the root anatomy, morphology, and seed germination of mint (*Mentha spicata* L.) grown in a sand culture. The study aimed to determine the effects of different $HgCl_2$ concentrations on critical parameters such as seed germination rate and speed, root growth, mercury accumulation, and anatomical changes in the root structure. Additionally, the tissue-level effects of $HgCl_2$ were examined. By focusing on these parameters, this study aims to contribute to a deeper understanding of the toxicological effects of mercury on plants and to evaluate the potential impacts of mercury contamination in agricultural soils on plant growth and productivity.

Material and Methods

Experimental design and material used: In the study, the seeds of the hairless form of mint plant (*Mentha spicata* cv. Spearmint, no: 581) were planted in sand culture and exposed to different concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg/L) of $HgCl_2$ (The Merck Index, 215465) together with 50% concentration of Hoagland-I solution for 60 days. For the control group, only 50% Hoagland-I solution was used. The experiment was designed as a randomized complete block design with five replications (John *et al.*, 2009; Manikandan *et al.*, 2015; Mehdizadeh *et al.*, 2024).

The Hewitt sand purification method was used for the purification of quartz sand of 0.4-0.6 mm size (Hewitt, 1966). After washing with deionized water, the dried sand was filled into nested polyethylene pots, having a capacity of 600 mL, using 600 grams per pot to ensure equal water-holding capacities (Gusmao, 2010).

The pH of the application solutions was adjusted to 5.5 - 6.5 at 25 °C using a potassium hydroxide (KOH - The Merck Index, 484016) solution (Hoagland & Arnon, 1950).

Thirty-five pots filled with 600 g of quartz sand were labeled. Application solutions were added to each pot at a volume of 178 mL. The seeds were divided into groups of fifty. Subsequently, these seeds were sown superficially in the pots, the tops of which were covered with stretch film, and then left to germinate. The temperature in the experimental room was set to 25±2 ºC, relative humidity to 53±3% and the illumination lux to 5750 lux per 0.825 m^2.

Every five days, 4 mL of application solution was added to each pot, and then the tops were covered with stretch film again. This process was repeated three times in a 15-day period. Germination percentage and germination speed were determined based on this 15-day period. From the 16th day onwards, an amount of solution that would not drain from the bottom of the pots was given equally to all groups.

Sampling and analysis procedures: To analyze germination parameters, the fifty seeds sown in each pot were monitored daily for 15 days after sowing, and the number of germinated seeds was recorded. Germination rate was calculated according to the formula: Germination rate (%) = (Number of germinated seeds / Total number of seeds) x 100. Germination speed was calculated according to the formula: Germination speed = n1/t1 + n2/t2 + ……. + nn/tn (Batabyal *et al.*, 2014).

To determine the amount of mercury accumulated in the roots, root samples from three different pots in each experimental group were heated in an oven at 70°C until they reached a constant weight. Then, the samples weighed as 0.2 g were subjected to inductively coupled plasma mass spectrometry (ICP-MS) analysis. In the laboratory, 6 mL of nitric acid (HNO_3 - The Merck Index, 1004562500) and 2 mL of hydrogen peroxide (H_2O_2 - The Merck Index, 107298) were added to the samples placed in digestion tubes. Subsequently, the tubes were subjected to a digestion process in the microwave device (Milestone, Ethos Easy) at 200ºC, 40 minutes and 90 bar pressure. Following digestion, the samples were filtered through filter paper and then completed to a final volume of 25 mL with deionized water. Then, the measurements were made with the ICP-MS device (THERMO, X SERIES 2) in three replicates (Wu ShaoLe *et al.*, 1997).

To determine root lengths, the root lengths of five plants in each of three pots per experimental group were measured. The measurements were conducted using photographs of the plants placed on millimeter paper and analyzed with Photoshop CS5 (Adobe Systems, USA).

For fresh weight determination, root samples from three plants in each of three pots per experimental group were weighed using a precision balance. Samples with recorded fresh weights were placed in an oven at 60-70°C for four days until they reached a constant weight. Then, the weights of the samples were measured again using a precision balance to determine their dry weights (Huang *et al.*, 2017).

For each experimental group, root samples from three plants in each of three pots were fixed in 70% ethanol. To analyze root anatomical parameters, cross-sections were taken from the roots at the junction with the stem. The sections were stained using the safranin-fast green double staining method (Johansen, 1940). Photographs of the stained sections were taken with a microscope equipped with a LEICA ICC 50 HD imaging system, using 4x, 10x, and 40x objectives. Measurements were made from these photographs using Photoshop CS5 and ImageJ, Version 1.54m (National Institutes of Health, Bethesda, MD, USA). For the measurement of root phloem area, root xylem area, total area of root tracheal lumens, root average trachea area, total area of root tracheid lumens, root xylem total wall area, root trachea number, root tracheid number parameters, at least 1/8 of the central cylinder was taken as the sample area. Measurements were made on this area and proportioned to the entire central cylinder area.

Mean values of all parameters were subjected to one-way ANOVA to study the treatment effect and Duncan's multiple range test was used to determine the significant differences between any two means. Comparisons were made at the 5% probability level. The data were analyzed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Germination rate and germination speed: In a preliminary trial to determine the range of mercury ($HgCl_2$) doses to be used in the experiment, 0.5, 1, 2, 3, 5, 10 and 15 mg/L concentrations of mercury were applied to plants along with 50% Hoagland solution for 45 days. The maximum mercury concentration in which plants could survive during the 60-day trial period was determined as 3 mg/L. 10 and 15 mg/L mercury concentrations completely inhibited seed germination.

The highest concentration of mercury (3 mg/L $HgCl_2$) reduced the germination rate and germination speed by 54.64% and 65.04%, respectively, compared to the control. The results revealed that as the amount of applied mercury increased, seed germination rate and seed germination speed decreased significantly (Fig. 1). Statistically, it was determined that increasing mercury concentration caused a significant difference between the group mean vectors in both germination rate and germination speed parameters (Table 1).

Root fresh and dry weight: The highest concentration of mercury (3 mg/L $HgCl_2$) reduced the fresh weight by 87.63% and the dry weight by 90.02% compared to the control. The results showed that as the amount of applied mercury increased, the fresh and dry weights of the plant roots were decreased significantly (Fig. 2, Table 2).

Table 1. Effect of different $HgCl_2$ concentrations on germination rate and germination speed.

Concentration (mg/L)	GR		GS	
	%	RC (%)	Value (day^{-1})	RC (%)
0	77.60 ± 0.37^{a}	-	5.60 ± 0.11^{a}	-
0.5	74.00 ± 0.45^{ab}	-4.64	5.06 ± 0.11^{b}	-9.67
1	72.00 ± 0.71^{ab}	-7.22	4.91 ± 0.06^{bc}	-12.45
1.5	68.00 ± 1.38^{b}	-12.37	4.59 ± 0.13^{c}	-18.16
2	61.60 ± 0.86^{c}	-20.62	3.91 ± 0.09^{d}	-30.27
2.5	52.40 ± 1.71^{d}	-32.47	2.93 ± 1.17^{e}	-47.70
3	35.20 ± 0.75^{e}	-54.64	1.96 ± 0.18^{f}	-65.04

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), GR= Germination rate, GS= Germination speed (day^{-1})

Table 2. Effect of different $HgCl_2$ concentrations on fresh weight and dry weight.

Concentration (mg/L)	FW		DW	
	g	RC (%)	g	RC (%)
0	0.36 ± 0.03^{a}	-	0.06 ± 0.01^{a}	-
0.5	0.33 ± 0.03^{ab}	-9.48	0.05 ± 0.01^{a}	-2.67
1	0.29 ± 0.02^{ab}	-20.42	0.04 ± 0.00^{b}	-31.19
1.5	0.22 ± 0.04^{bc}	-39.74	0.03 ± 0.01^{c}	-54.37
2	0.14 ± 0.02^{cd}	-60.43	0.02 ± 0.00^{c}	-63.81
2.5	0.10 ± 0.02^{de}	-71.70	0.02 ± 0.00^{cd}	-71.48
3	0.05 ± 0.01^{e}	-87.63	0.01 ± 0.00^{d}	-90.02

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), FW= Fresh weight, DW= Dry weight

Table 3. Effect of different $HgCl_2$ concentrations on root mercury accumulation, root length and root area.

Concentration (mg/L)	RMA		RL		RA	
	ppm	RC (%)	cm	RC (%)	mm^2	RC (%)
0	0.002	-	10.52 ± 0.48^{a}	-	0.87 ± 0.09^{a}	-
0.5	0.017	+714	08.58 ± 0.31^{b}	-18.44	0.87 ± 0.01^{a}	+0.23
1	0.033	+1434	07.70 ± 0.31^{b}	-26.80	0.81 ± 0.06^{a}	-7.34
1.5	0.088	+3994	07.84 ± 0.54^{b}	-25.47	0.72 ± 0.09^{a}	-17.77
2	0.290	+13326	06.30 ± 0.37^{c}	-40.11	0.66 ± 0.06^{ab}	-24.54
2.5	0.522	+24056	04.60 ± 0.37^{d}	-56.27	0.45 ± 0.07^{bc}	-48.05
3	0.920	+42466	02.73 ± 0.18^{e}	-74.04	0.26 ± 0.04^{c}	-70.30

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), RMA= Root mercury accumulation, RL= Root length, RA= Root area

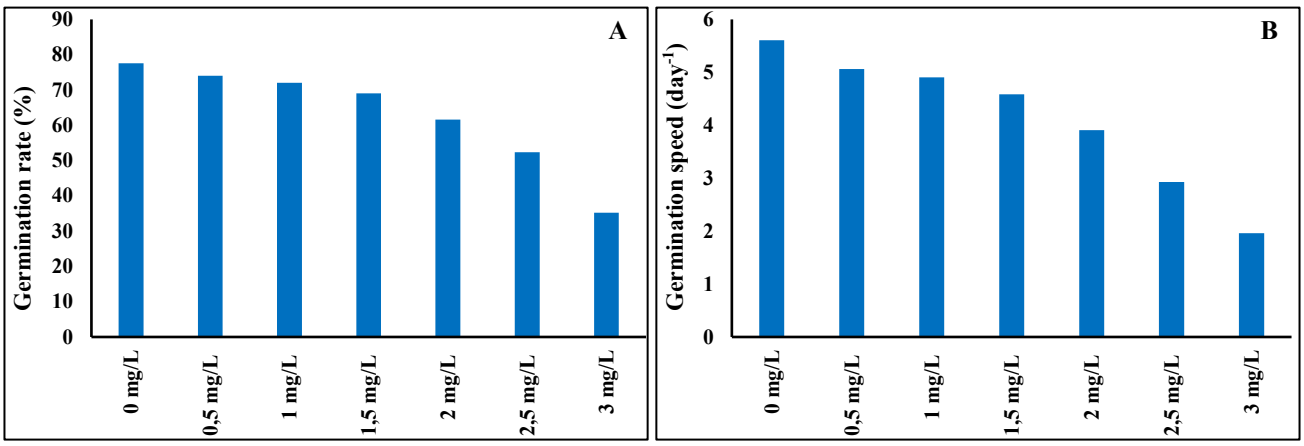

Fig. 1. Effect of different $HgCl_2$ concentrations on germination rate and germination speed: A, Germination rate (%); B, Germination speed (day^{-1}).

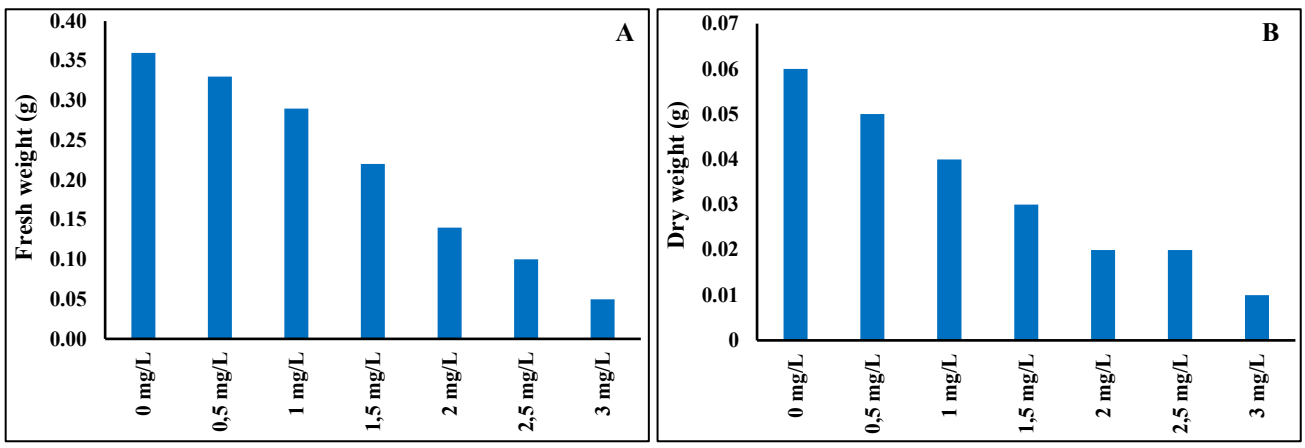

Fig. 2. Effect of different $HgCl_2$ concentrations on fresh weight and dry weight: A, Fresh weight (g); B, Dry weight (g).

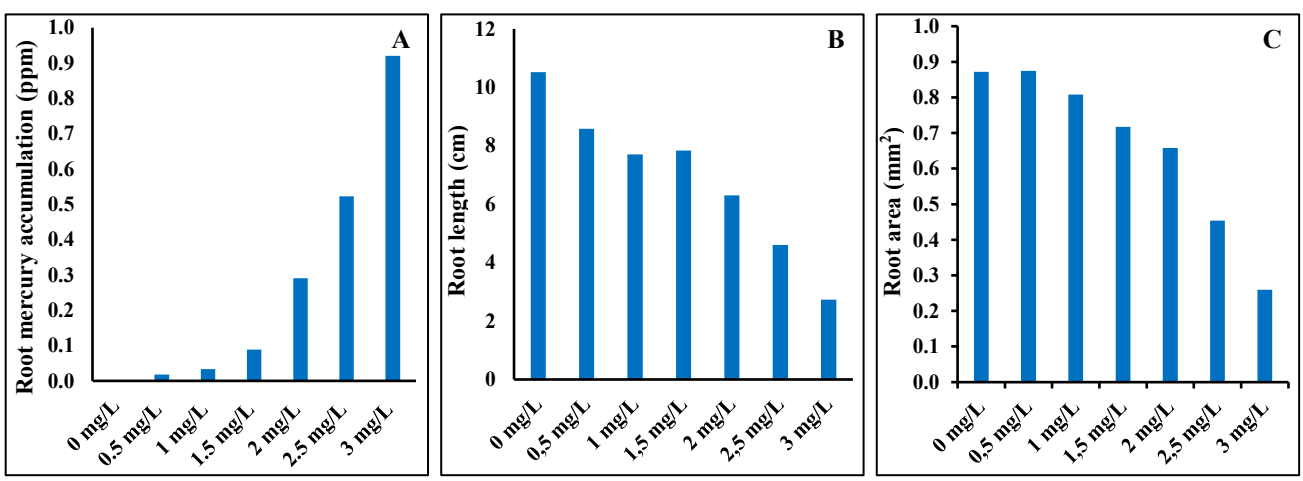

Fig. 3. Effect of different $HgCl_2$ concentrations on root mercury accumulation, root length and root area: A, Root mercury accumulation (ppm); B, Root length (cm); C, Root area (mm^2).

Root mercury accumulation, root length and root area: The highest concentration of mercury (3 mg/L $HgCl_2$) reduced the root length by 74.04% and the root area by 70.30% compared to the control, while mercury accumulation in the roots at this concentration was 42466% higher than the control. The results demonstrated that as the amount of applied mercury increased, accumulation of root mercury was also increased, while both root length and root area were decreased significantly (Fig. 3, Table 3).

Anatomical examination of the root: In order to distinguish between tracheae and tracheids in cross-sections, the widest tracheids were identified in radial sections of the roots. The diameters of the widest tracheids ranged from 13.85 to 14.35 µm (Fig. 4).

After identifying the widest tracheids in radial sections, tracheids with diameters ranging from 13.85 to 14.35 µm were detected in root cross-sections and their areas were measured. The average of 25 area measurements was determined as 104.33 $µm^2$. Based on this result, xylem conducting elements with an area greater than 104.33 $µm^2$ in cross-sections were classified as tracheae, while those with an area of 104.33 $µm^2$ or smaller were classified as tracheids (Fig. 5).

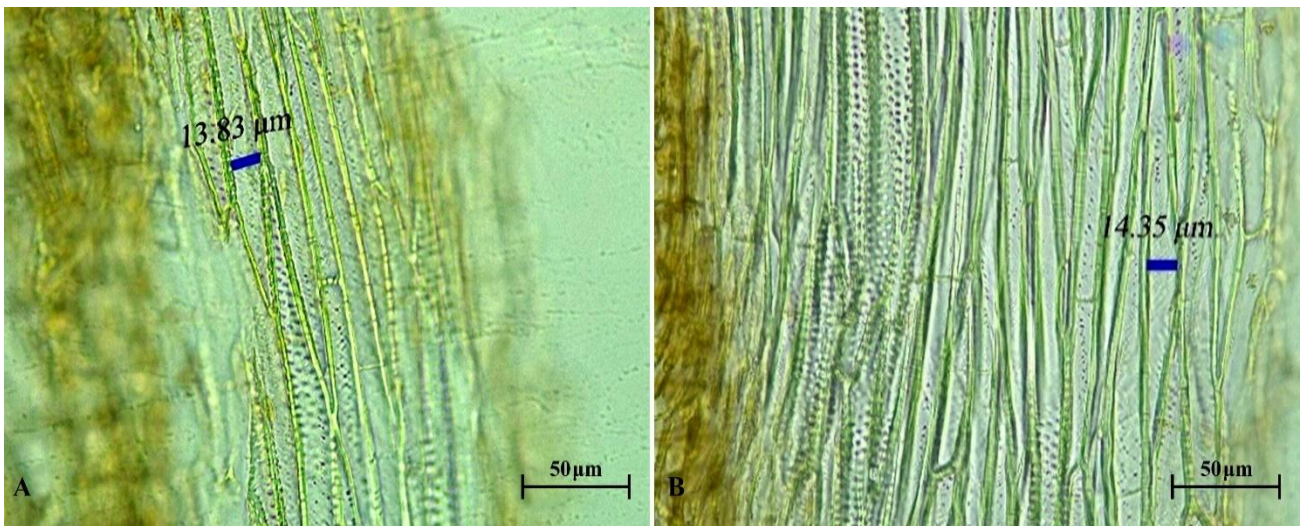

Fig. 4. The widest tracheids determined in root radial sections: A, 2.5 mg/L $HgCl_2$; B, 0 mg/L $HgCl_2$.

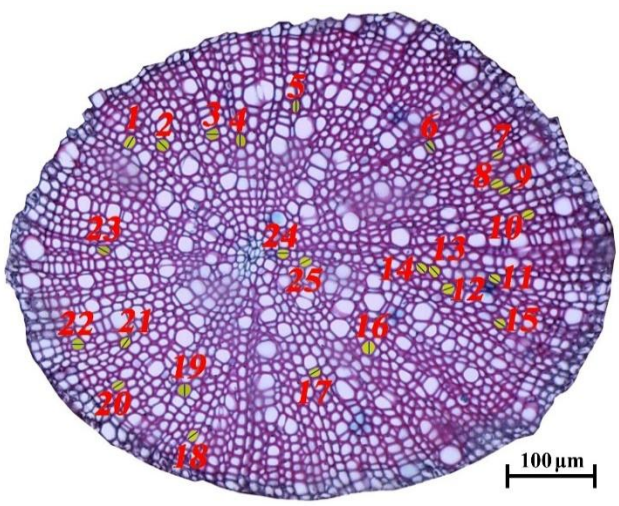

Fig. 5. Tracheids whose diameters were determined to be 13.85 - 14.35 µm in cross sections and whose areas were measured (0 mg/L $HgCl_2$).

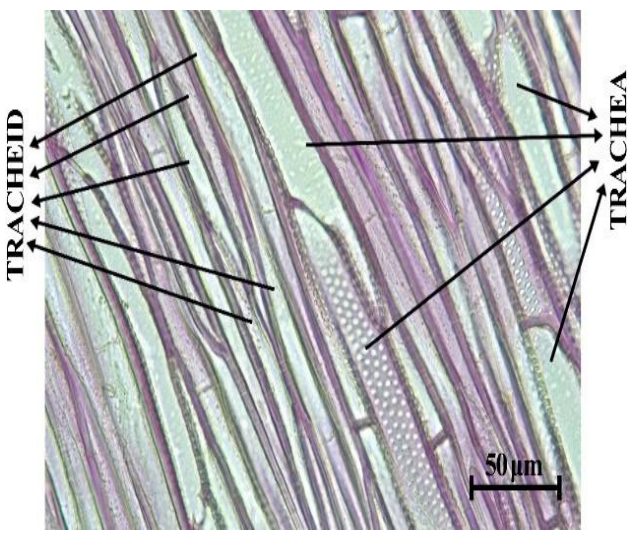

Fig. 6. View of tracheas and tracheids in root radial sections.

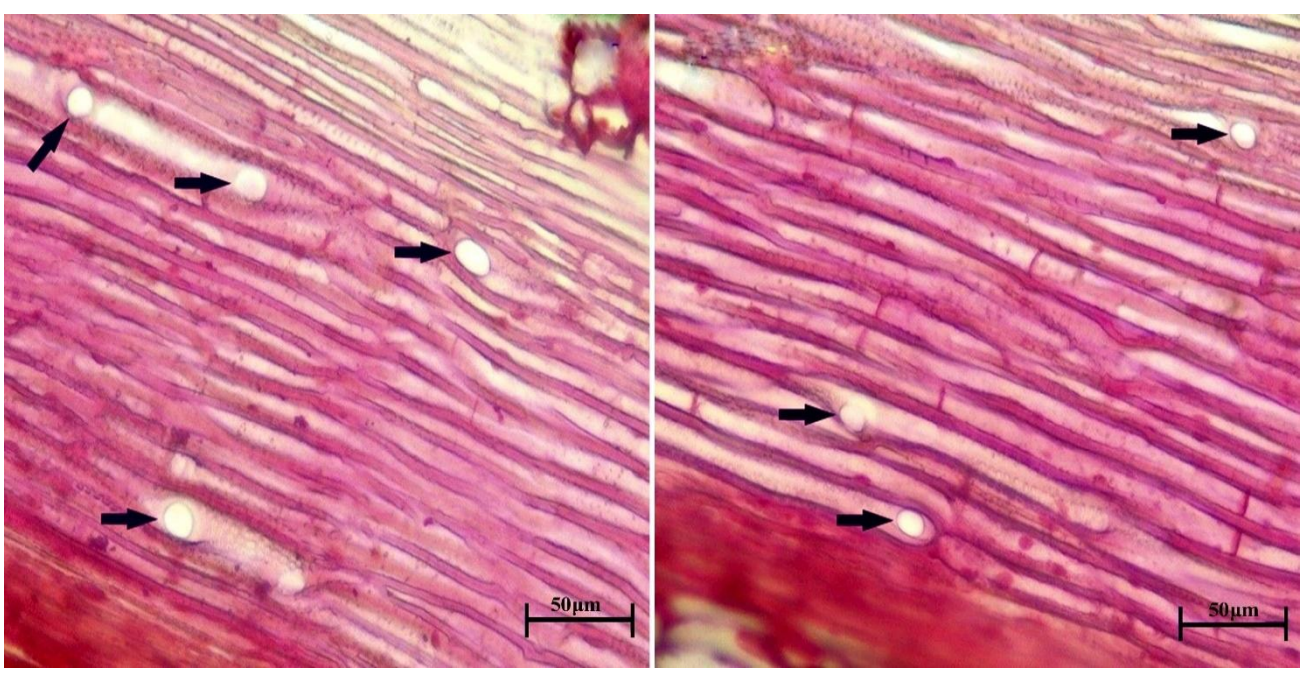

Fig. 7. View of simple tracheal perforation in root radial sections.

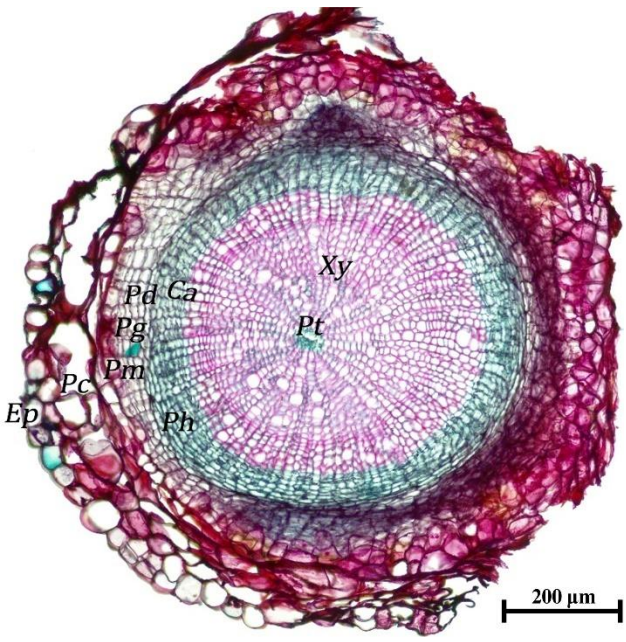

Fig. 8. General view of the root in cross section. Ep: Epidermis, Pc: Primary cortex, Pm: Phellem, Pg: Phellogen, Pd: Phelloderm, Ph: Phloem, Ca: Cambium, Xy: Xylem, Pt: Pith.

In the examinations of the sections taken, it was determined that the tracheae of *Mentha spicata* had simple perforation plates and that the cell walls were simply pitted (Figs. 6 and f7).

A general anatomical view of root cross sections, including epidermis, cortex, phloem, xylem, phellem, phellogen, phelloderm, cambium, and pith tissues is presented (Fig. 8). In addition, representative root cross sections of all treatment groups illustrate the progressive reduction in vascular tissues with increasing $HgCl_2$ concentration (Fig. 12). It was observed that mercury significantly decreased the root area with the increasing mercury concentration. The highest concentration of mercury (3 mg/L $HgCl_2$) reduced the root area by 70.30 %, compared to the control (Fig. 3, Table 3). This decrease occurred as a result of reduction in the central cylinder area (xylem and phloem areas). Xylem area and phloem area decreased by 88.57% and 88.69%, respectively, compared to the control at 3 mg/L mercury concentration (Fig. 9, Table 4). The difference between the reduction in root area and the reduction in central cylinder area was due to the disintegration and shedding of the epidermis and primary cortex as a result of early onset of cork cambium activity in the control and low mercury concentration groups. The decrease in xylem area occurred as a result of reductions in the total area of tracheal lumens, total area of tracheid lumens, and total wall area of xylem. The application of mercury (3 mg/L $HgCl_2$) reduced the total area of tracheal lumens, total area of tracheid lumens and total wall area of xylem by 91.75%, 80.95% and 91.76%, respectively, compared to the control (Figs. 9 and 10). In addition, trachea number, tracheid number and mean tracheal lumen area decreased by 89.75%, 82.11% and 27.00%, respectively, compared to the control at 3 mg/L mercury concentration (Figs. 10 and 11).

Root xylem area, root phloem area and total area of tracheal lumens: The results revealed that as the amount of applied mercury increased, xylem area, phloem area, and total area of tracheal lumens in the plant roots decreased significantly (Fig. 9; Table 4).

Root mean tracheal lumen area, total area of tracheid lumens and total wall area of root xylem: The results showed that as the amount of applied mercury increased, mean tracheal lumen area, total area of tracheid lumens, and total wall area of xylem in the plant roots decreased significantly (Fig. 10; Table 5).

Root trachea number and root tracheid number: The results demonstrated that as the amount of applied mercury increased, trachea number and tracheid number in the plant roots decreased significantly (Fig. 11; Table 6).

Table 4. Effect of different $HgCl_2$ concentrations on root xylem area, root phloem area and total area of tracheal lumens.

Concentration (mg/L)	XA		PA		TATL	
	mm²	RC (%)	mm²	RC (%)	mm²	RC (%)
0	0.39 ± 0.04^{a}	-	0.12 ± 0.01^{a}	-	0.10 ± 0.03^{a}	-
0.5	0.29 ± 0.04^{b}	-25.45	0.11 ± 0.01^{a}	-6.08	0.09 ± 0.03^{a}	-9.28
1	0.25 ± 0.04^{bc}	-34.54	0.10 ± 0.01^{a}	-16.52	0.03 ± 0.01^{b}	-64.95
1.5	0.18 ± 0.03^{c}	-52.20	0.06 ± 0.01^{b}	-46.08	0.03 ± 0.02^{b}	-64.95
2	0.17 ± 0.03^{c}	-54.80	0.07 ± 0.01^{b}	-41.74	0.04 ± 0.01^{b}	-57.73
2.5	0.08 ± 0.02^{d}	-78.96	0.03 ± 0.00^{c}	-76.52	0.01 ± 0.00^{b}	-87.63
3	0.04 ± 0.01^{d}	-88.57	0.01 ± 0.00^{c}	-88.69	0.01 ± 0.00^{b}	-91.75

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), XA= Xylem area, PA= Phloem area, TATL= Total area of tracheal lumens

Table 5. Effect of different $HgCl_2$ concentrations on root mean tracheal lumen area, total area of tracheid lumens and total wall area of xylem.

Concentration (mg/L)	MTLA		TATDL		TWAX	
	µm²	RC (%)	mm²	RC (%)	mm²	RC (%)
0	176.84 ± 10.67^{a}	-	0.11 ± 0.01^{a}	-	0.18 ± 0.02^{a}	-
0.5	159.20 ± 3.87^{ab}	-9.97	0.08 ± 0.01^{b}	-24.76	0.12 ± 0.02^{bc}	-34.61
1	158.19 ± 9.13^{ab}	-10.55	0.09 ± 0.01^{ab}	-19.05	0.13 ± 0.03^{b}	-27.47
1.5	138.42 ± 5.39^{bc}	-21.72	0.07 ± 0.01^{b}	-30.47	0.08 ± 0.01^{cd}	-58.24
2	137.84 ± 5.10^{bc}	-22.05	0.07 ± 0.01^{b}	-33.33	0.06 ± 0.01^{de}	-65.93
2.5	130.19 ± 10.23^{c}	-26.38	0.04 ± 0.01^{c}	-63.81	0.03 ± 0.01^{de}	-83.51
3	129.09 ± 9.95^{c}	-27.00	0.02 ± 0.00^{c}	-80.95	0.02 ± 0.00^{e}	-91.76

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), MTLA= Mean tracheal lumen area, TATDL= Total area of tracheid lumens, TWAX= Total wall area of xylem

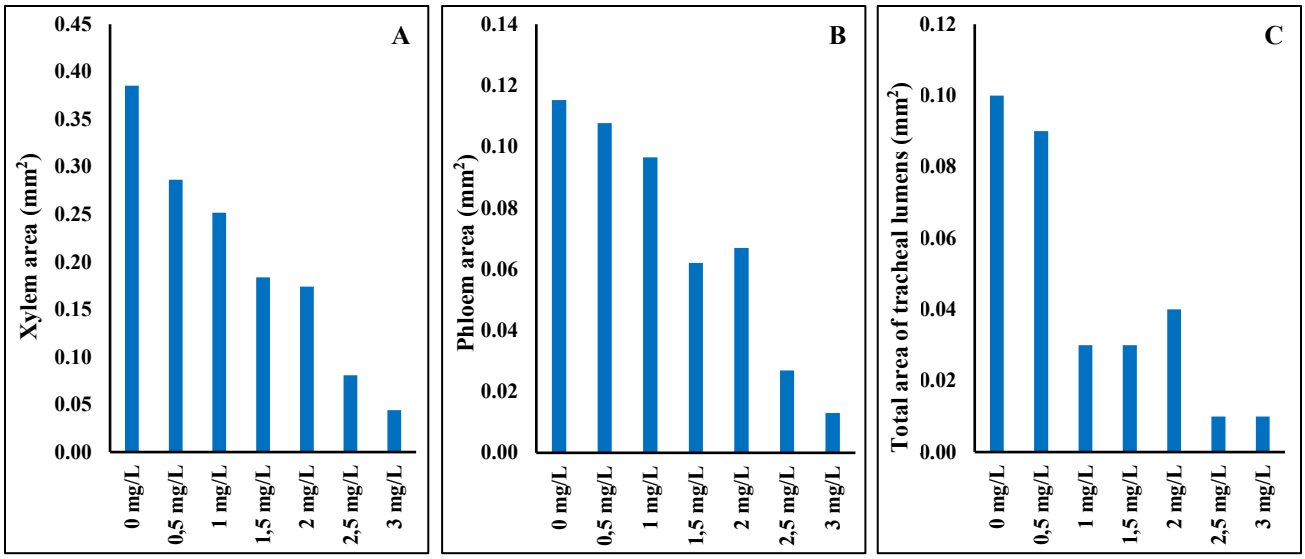

Fig. 9. Effect of different $HgCl_2$ concentrations on xylem area, phloem area and total area of tracheal lumens: A, Xylem area (mm^2); B, Phloem area (mm^2); C, Total area of tracheal lumens (mm^2).

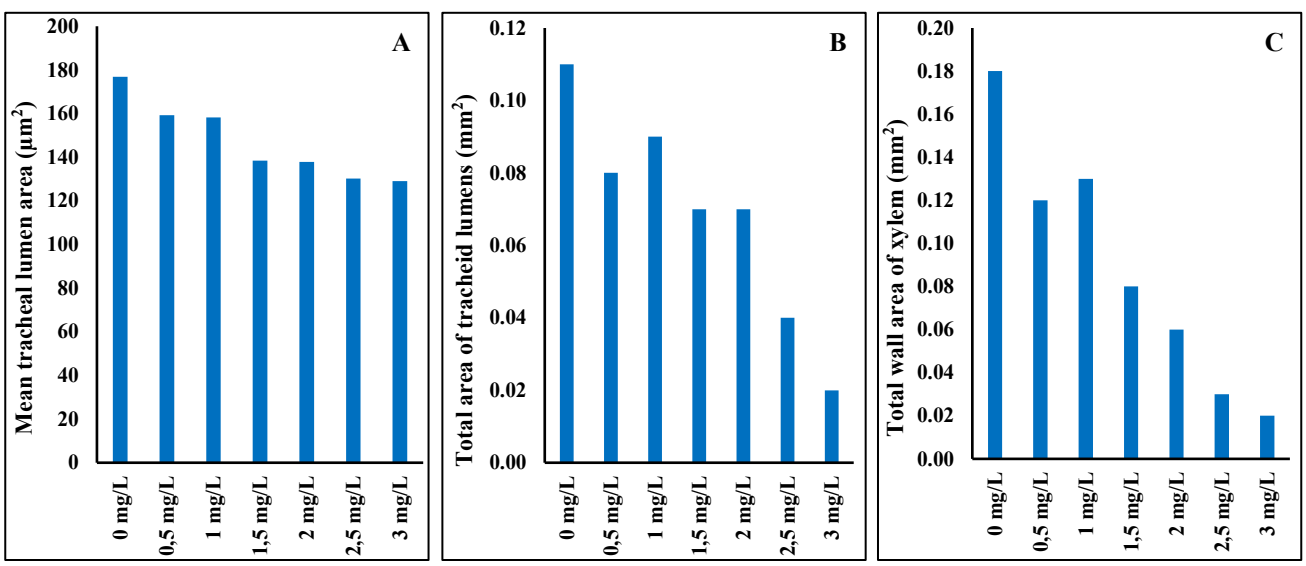

Fig. 10. Effect of different $HgCl_2$ concentrations on mean tracheal lumen area, total area of tracheid lumens and total area of tracheal lumens: A, Mean tracheal lumen area (µm²); B, Total area of tracheid lumens (mm^2); C, Total wall area of xylem (mm^2).

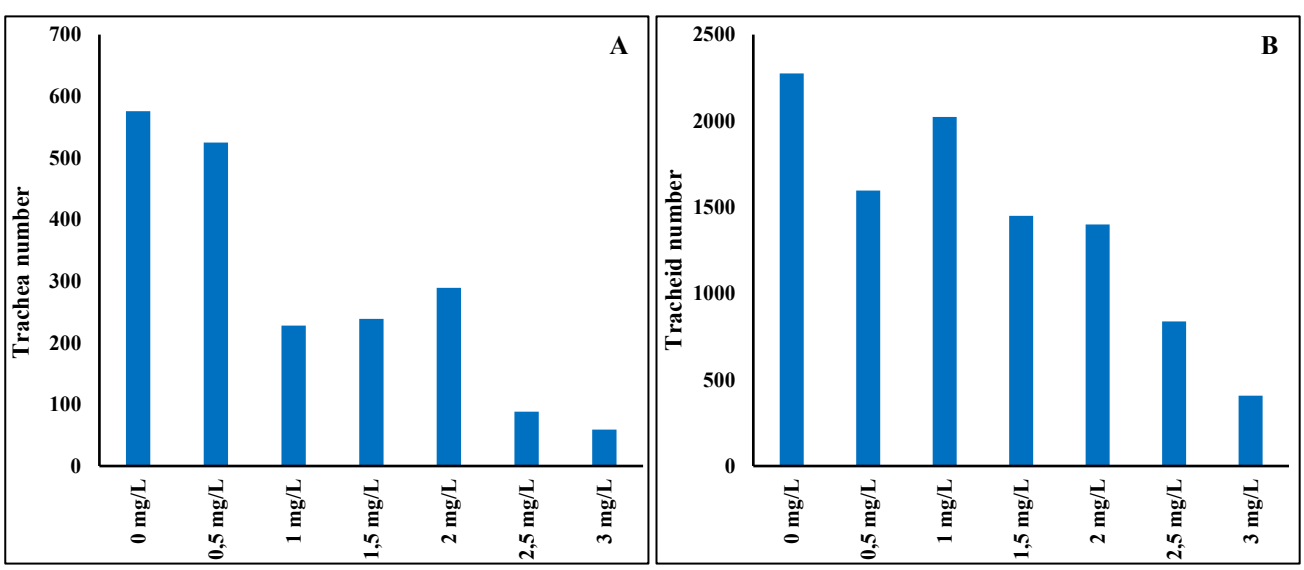

Fig. 11. Effect of different $HgCl_2$ concentrations on trachea number and tracheid number: A, Trachea number; B, Tracheid number.

Table 6. Effect of different $HgCl_2$ concentrations on root trachea number and tracheid number.

Concentration (mg/L)	TN		TDN	
	Number	RC (%)	Number	RC (%)
0	576 ± 168.10^{a}	-	2275 ± 137.32^{a}	-
0.5	525 ± 138.18^{ab}	-8.85	1597 ± 228.47^{bc}	-29.80
1	228 ± 59.38^{bc}	-60.42	2022 ± 348.63^{ab}	-11.12
1.5	239 ± 109.56^{bc}	-58.50	1449 ± 101.90^{bc}	-36.31
2	289 ± 87.00^{abc}	-49.82	1399 ± 185.48^{cd}	-38.50
2.5	88 ± 29.79^{c}	-84.72	838 ± 153.76^{de}	-63.16
3	59 ± 18.72^{c}	-89.75	407 ± 106.02^{e}	-82.11

Values with different superscript letters in a column differ significantly ($p<0.05$), RC (%) = Relative changes over control (%), TN= Trachea number, TDN= Tracheid number

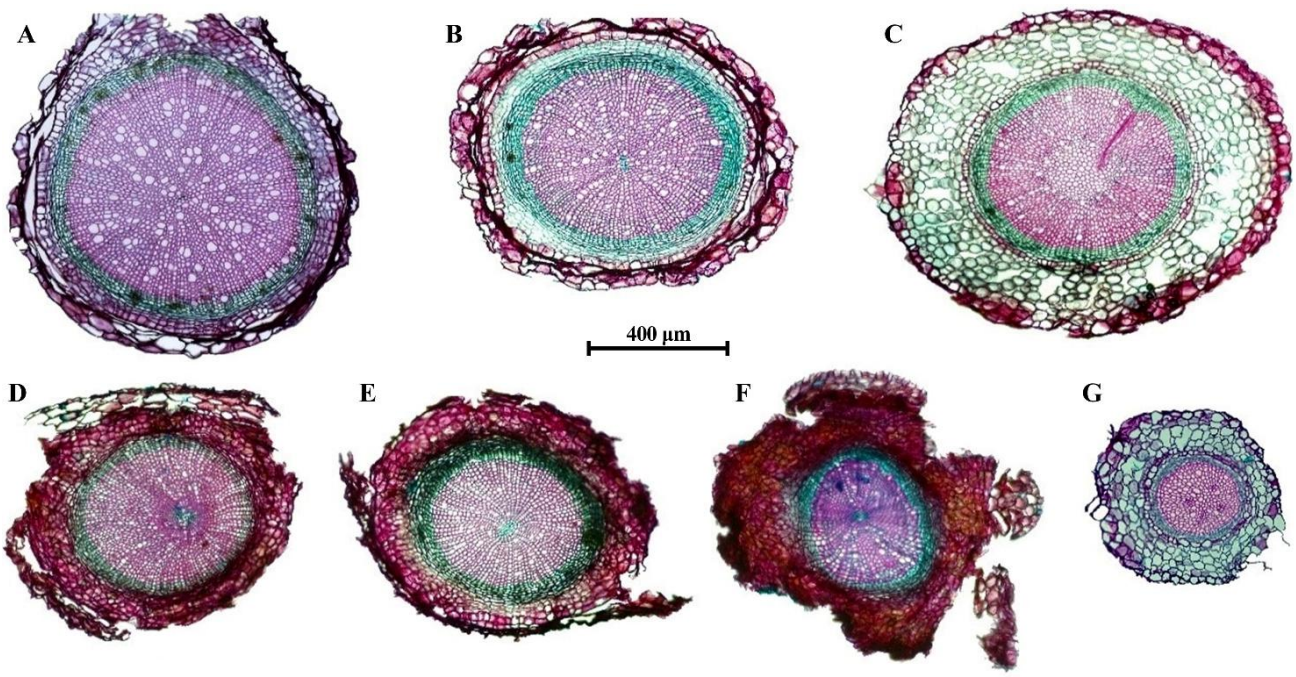

Fig. 12. General view of root cross sections: A, 0 mg $HgCl_2$ + 50% Hoagland; B, 0.5 mg $HgCl_2$ + 50% Hoagland; C, 1 mg $HgCl_2$ + 50% Hoagland; D, 1.5 mg $HgCl_2$ + 50% Hoagland; E, 2 mg $HgCl_2$ + 50% Hoagland; F, 2.5 mg $HgCl_2$ + 50% Hoagland; G, 3 mg $HgCl_2$ + 50% Hoagland.

Discussion

Germination rate and germination speed: Early seed germination contributes to seed and seedling performance, which is important for plant establishment in natural and agricultural ecosystems (Weitbrech *et al.*, 2011). It was determined that mercury significantly decreased the studied germination parameters with the increasing mercury concentration (Fig. 1). The results obtained in germination parameters in this study are consistent with previous research. Cardoso *et al.*, (2015) stated that mercury (10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M $HgCl_2$) application on *Plathymenia reticulata* Benth. seeds significantly reduced the germination rate and embryo axis water content at 10^{-3} M concentration, in addition to completely inhibited germination at 10^{-2} M concentration. Li *et al.*, (2005) found that the germination inhibition effect of various heavy metals applied to *Arabidopsis thaliana* L. Heynh. seeds was in the following order: $Hg^{+2}>Cd^{+2}>Pb^{+2}/Cu^{+2}$. Pugalvendhan *et al.*, (2009) stated that mercury (100 mg/kg^{-1}) applied to *Arachis hypogaea* L., seeds decreased the germination rate and germination speed by 51% and 67.82%, respectively. Mercury, as demonstrated in various studies, significantly reduced seed germination rate and speed by blocking water uptake in seeds. However, Rodríguez-Alonso *et al.*, (2019), suggests that certain plant species, like *Quercus ilex* L., may exhibit resistance to the adverse effects of mercury, indicating variability in species-specific responses to mercury contamination.

The reason why germination rate and germination speed are negatively affected by mercury application is that mercury blocks aquaporins and prevents the seed water uptake. In addition, mercury may primarily affect the embryo and secondarily the endosperm. Mercury strongly affects the -SH system in living cells by causing the formation of the -S-Hg-S- bridge. The normal -SH system disrupted by the influence of mercury may affect both germination and subsequent growth of the young embryo, since these tissues are rich in -SH groups. Mercury compounds prevent water permeability by binding to the sulfhydryl group of cysteine residues around water channels (Patra & Sharma, 2000; Javot & Maurel, 2002; Cardoso *et al.*, 2015).

However, Rodríguez-Alonso *et al.*, (2019) stated that mercury (5, 25 and 50 µM $HgCl_2$) applied to *Quercus ilex* L. seeds did not affect the germination rate and germination speed at any concentration. However, his findings cotradict with the results of other workers. This may be due to the resistance of aquaporins of *Quercus ilex* L. to mercury compounds.

These negative effects on germination highlight the risks to the continuity of plants in nature, as well as the risk of not achieving the desired yield in annually cultivated crops.

Root fresh and dry weight: Fresh weight change, a combination of growth and water status alterations in the plant, may be calculated from transpiration and water uptake (Van Leperen & Madery, 1994). It was determined that mercury significantly decreased the fresh and dry weight with the increasing mercury concentration (Fig. 2). The results obtained in the fresh weight parameter in this study are consistent with previous research. Xu *et al.*, (2020) stated that mercury (1.5, 3, 6 and 9 mg/kg^{-1} $HgCl_2$) application to *Zingiber officinale* Roscoe significantly reduced the root, stem and leaf fresh weights. Askari & Azmat (2013) reported that mercury (5, 10, 15, 20 and 25 ppm) applied to *Cicer arietinum* L. significantly reduced the plant fresh weight. Mei *et al.*, (2021) reported that root and shoot fresh weights of *Gossypium hirsutum* L. were significantly negatively affected by mercury application (1, 10, 50, 100 µM $HgCl_2$) at concentrations of 10 µM and above.

The reason of the decrease in fresh weight is the inhibition of water uptake from the root by mercury. It has been demonstrated by many researchers that mercury blocks aquaporins in cells and reduces water uptake. Previous studies have indicated that mercury reduces water uptake by 32% in *Opuntia acanthocarpa* var. *ganderi* C.B. Wolf, 47% in *Populus tremuloides* Michx., 57% in *Lycopersicon esculentum* L., 57-84% in *Allium cepa* L., 66% in *Triticum aestivum* L., 66% in *Capsicum annuum* L., 80% in *Cucumis melo* L. cv. Amarillo oro, 80% in *Beta vulgaris* L. and 90% in *Hordeum vulgare* L. (Maggio & Joly, 1995; Carvajal *et al.*, 1996; Tazawa *et al.*, 1997; Amodeo *et al.*, 1999; Wan & Zwiazek, 1999; Carvajal *et al.*, 1999; Carvajal *et al.*, 2000; Barrowclough *et al.*, 2000; Martre *et al.*, 2001; Javot & Maurel, 2002). Javot & Maurel (2002) reported that mercury compounds blocked aquaporins in most plants, and mercury compounds bind to the sulfhydryl group of cysteine residues around water channels and prevent water permeability. Hejnowicz & Sievers (1996) stated that the water uptake and water loss rates in the inner tissue segments of the hypocotyls of *Helianthus annuus* L. cv. Giganteum were sensitive to submillimolar $HgCl_2$ concentrations and turgor changes in the water channels were affected by $HgCl_2$. In addition, Swapna *et al.*, (2015) reported that mercury ($HgCl_2$) caused damage to the root hair layer of *Chromolaena odorata* L.

The results obtained in the dry weight parameter are consistent with previous research. Iqbal *et al.*, (2014) stated that mercury application (1, 3, 5 and 7 mM $HgCl_2$) to *Albizia lebbeck* L. decreased dry weight at 5 and 7 mM concentrations, and this reduction occurred by 42% at 7 mM concentration. Muhammad *et al.*, (2015) reported that mercury (1, 3, 5 and 7 mM $HgCl_2$) applied to *Vigna radiata* (L.) Wilczek reduced plant dry weight by 47% at 7 mM concentration. Marrugo-Negrete *et al.*, (2016) stated that mercury (II) nitrate (5, 10, 20, 40 and 80 µg/mL) applied to *Jatropha curcas* L. significantly reduced plant dry weight. Mei *et al.*, (2021) reported that mercury (1, 10, 50, 100 µM $HgCl_2$) application to *Gossypium hirsutum* L. significantly reduced root and shoot dry weights at concentrations of 10 µM and above.

This decrease in dry weight occurs primarily as a result of the decrease in the plant's organic matter production and inadequate mineral nutrition (Singh *et al.*, 2015). Marrugo-Negrete *et al.*, (2016) stated that mercury (II) nitrate application (5, 10, 20, 40 and 80 µg/mL) to *Jatropha curcas* L. significantly reduced the level of net photosynthesis. Küpper *et al.*, (1996) reported that mercury ($HgCl_2$) applied to *Elodea canadensis* Michx., *Stratiotes aloides* L., *Myriophyllum spicatum* L., *Ceratophyllum demersum* L., *Callitriche stagnalis* Scop., *Crassula helmsii* (Kirk) Cockayne, *Lemna trisulca* L. and *Lemna minor* L. plants negatively affected both the light and dark reactions of photosynthesis. They reported that mercury prevented photosynthetic light absorption in chlorophyll molecules by replacing Mg and caused cessation of photosynthesis. Godbold & Hüttermann (1988) reported that mercury (1, 10, 100 and 1000 nM $HgCl_2$ and CH_3HgCl) applied to *Picea abies* L. Karst. significantly reduced the net photosynthetic rate, chlorophyll amount, carbon dioxide uptake and caused an increase in the number of closed stomata. They also reported that 1000 nM $HgCl_2$ significantly reduced Ca, Zn, Mn and Mg uptake and that the resulting root damage led to a decrease in nutrient levels. Pugalvendhan *et al.*, (2009) stated that mercury (10, 25, 50, 75 and 100 mg/kg^{-1}) applied to *Arachis hypogaea* L. decreased the chlorophyll a, chlorophyll b, total chlorophyll amount by 46.53%, 51.66%, 49% at 100 mg/kg^{-1} mercury concentration, and also decreased the sugar, starch, amino acid and protein amounts in the root by 43.50%, 43.50%, 42.10% and 42.16%, respectively. Sahu *et al.*, (2012) found that the application of mercury (2.5, 5.0, 10 and 25 µM $HgCl_2$) to *Triticum aestivum* L. led to a significant reduction in the total chlorophyll content as well as in the total soluble protein levels in both the roots and leaves at the 10 and 25 µM concentrations. Additionally, they reported that these higher concentrations (10 and 25 µM) considerably diminished the uptake of Ca, K, and Mg by the plants.

The decrease in fresh and dry weight parameters, which are indicators of water uptake, mineral nutrition, and organic matter synthesis in plants, manifests as insufficient yield and reduced product quality, especially in food crops.

Root mercury accumulation and root length: It was observed that as the concentration of mercuric chloride applied increased, so did its accumulation in the roots (Fig.3). The results obtained in this study in the root mercury accumulation parameter are consistent with previous research. Cavallini *et al.*, (1999) reported that mercury(II) nitrate (50 µg Hg^{+2}/L) applied *Triticum durum* Desf. via the root accumulated mainly in the roots, then in the shoot meristem, and subsequently in the leaves. Israr *et al.*, (2006) indicated that the accumulation of mercury (10, 20, 30, 40, 50 and 100 mg/L^{-1} $HgCl_2$) applied to *Sesbania drummondii* Rydb. seedlings via the root increased with the increasing concentration, and that mercury accumulation in the roots was higher than in the shoots at each concentration. Rodríguez-Alonso *et al.*, (2019) stated that mercury (5, 25 and 50 µM $HgCl_2$) applied to *Quercus ilex* L. via the roots accumulated in the order of roots > leaves > stem. They also revealed that as the root system developed, the amount of mercury accumulated in the above-ground parts of the plant per unit time decreased. However, the increase in mercury concentration in the above-ground parts of the plant in parallel with the concentration increase indicates that the

root is unable to fully function as a barrier at high concentrations. This, in turn, can lead to increased accumulation in the above-ground parts, rendering them unsuitable for consumption as food.

The endodermis in the root acts as a barrier that blocks the apoplastic transport of water and dissolved substances. Instead transport actively occurs via the symplastic pathway. In this way, heavy metals entering the cell are converted into less toxic forms by binding with ligands and are transported to metabolically inactive parts of the cell (cell wall and vacuole) and retained (Emamverdian *et al.*, 2015; Ghori *et al.*, 2019; Yan *et al.*, 2020). Wang *et al.*, (2015) reported that mercury (0.5, 1.0 and 2.5 mg/L^{-1} $HgCl_2$) applied to *Oryza sativa* L. induced the development of a well-formed casparian strip.

The results obtained in root length parameter in this study are consistent with previous research. Mishra & Choudhari (1998) stated that mercury (10 µM $HgCl_2$), applied to two different varieties of rice (*Oryza sativa*), significantly inhibited root elongation. Munzuroglu & Geckil (2002) indicated that mercury application (0.1-1.7 mM) to *Triticum aestivum* L. and *Cucumis sativus* L. reduced root length more than other heavy metals (Cd, Cu, Pb, Co, Zn) used in the experiment. Mondal *et al.*, (2015) reported that mercury (0.1, 0.5, 1 and 1.5 mg/L $HgCl_2$) applied to *Vigna radiata* L. Wilczek inhibited root elongation at all concentrations. Puzon *et al.*, (2015) reported that mercury application (0.1 and 1.0 ppm $HgCl_2$) to *Euphorbia hirta* L. significantly reduced root length.

One of the main reasons for the reductions observed in the parameters investigated in these studies is the negative effect of mercury on cell division. Mercuric chloride exhibits an inhibitory effect on mitosis, leading to a decrease in the mitotic index, which in turn reduces root elongation. This reduction in mitotic activity results from mercury-induced chromosomal anomalies, including anaphase bridges, chromosome stickiness, micronucleus formation, chromosome fragmentation, and binucleated cells (Principal, 2010; Animasaun *et al.*, 2024). Further research is needed on the possibility that the differentiation process of the primary meristematic tissue in root tips into primary permanent tissues may slow down due to the effects of mercury.

Anatomical parameters: The anatomical findings in this study are consistent with previous research. Khan & Chaudhry (2005) reported that the mercury (50 and 100 ppm $HgCl_2$) application to *Lagenaria siceraria* (Mol) Standl. caused a reduction in the width of phloem region, width of metaxylem vessels, width of protoxylem elements and number of upper cambial layers parameters in both transverse and longitudinal planes of internode. Chaudhry & Khan (2007) stated that the mercury (50 and 100 ppm $HgCl_2$) application to *Cucumis sativus* L. caused a decrease in the width of phloem region, width of metaxylem vessels, width of protoxylem elements and number of upper cambial layers parameters in both transverse and longitudinal planes of internode. Swapna *et al.*, (2015) indicated that mercury (15 µM) applied to *Chromolaena odorata* L. caused narrowing in the root central cylinder region and damaged piliferous layer consisting of irregular and uneven cells.

The primary reason for the reduction at the permanent tissue level due to mercury exposure in these studies is the decrease in the mitotic activity of the cambium, which is a secondary meristem. Various studies have demonstrated that mercury reduces the mitotic index in primary meristems located at root tips (Principal, 2010; Animasaun *et al.*, 2024). Previous research has also reported that the number of cell rows in the cambial zone decreases along with the phloem area, protoxylem, and metaxylem width (Khan & Chaudhry, 2005; Chaudhry & Khan, 2007). In addition, not only the inhibition of cell division but also the slowing down of cell differentiation could be one of the possible factors. Studies comparing the number of cell rows in the cambial zone with the areas of xylem and phloem formed could provide more explanatory results on this matter.

Conclusion

In this study, the effects of mercuric chloride ($HgCl_2$) on the root anatomy, morphology, and germination parameters of *Mentha spicata* L. grown in a sand culture system were investigated. The results showed that increasing $HgCl_2$ concentrations had negative effects on germination rate, germination timing, root fresh and dry weight, root length, and various anatomical parameters. With the increase in mercury concentration, mercury accumulation in the roots was also increased, leading to developmental retardation in root tissues. The study revealed, for the first time, the effects of mercury on plant roots at the tissue level through parameters such as total area of tracheal lumens, root mean tracheal lumen area, total area of tracheid lumens, total wall area of root xylem, root trachea number, and root tracheid number.

Considering the literature, this developmental reduction is thought to be the result from the combined effects of a decrease in chlorophyll content, net photosynthetic rate, and carbon dioxide uptake, as well as a reduction in the division rate of meristematic cells and disruptions in the uptake of water and nutrients (Ca, K, Zn, Mn, and Mg).

Present findings align with its initial objectives by providing significant insights into the cytotoxic and physiological effects of mercury contamination on plants. The reduction in root length is primarily associated with the decreased mitotic activity of the primary meristem located at the root tip. Previous studies have reported that mercury inhibits root growth by reducing mitotic activity in meristematic cells (Principal, 2010; Animasaun *et al.*, 2024). Furthermore, the observed reductions in xylem and phloem areas indicate that mercury also inhibits mitotic activity in the secondary meristem, the cambium. The inhibition of mitotic activity in the cambium, along with the reduction in trachea and tracheid lumen areas, suggests that mercury negatively affects vascular development, impairing water and nutrient transport efficiency and disrupting long-term plant growth.

Although the detrimental effects of mercury on root structure and function have been clearly demonstrated, further research is needed to investigate the underlying molecular mechanisms driving these changes. Future studies should explore the role of antioxidant defense systems, gene expression related to mercury detoxification, and potential strategies to reduce mercury uptake in agricultural crops. Additionally, research is required on

plant nutrition strategies and soil composition modifications to prevent mercury uptake by roots and enhance their ability to retain absorbed mercury.

Particularly, the long-term effects of mercury-containing pesticides used in agricultural fields should be assessed, and preventive measures should be developed to minimize their potential harm to crop production. The use of alternative, environmentally friendly pesticides should be promoted, and stricter regulations should be implemented regarding the use of mercury-containing agricultural inputs. Furthermore, additional research is needed on phytoremediation techniques and biological soil remediation methods to reduce mercury accumulation in agricultural soils.

This study highlights the severe impact of mercury contamination on plant root development, emphasizing the risks posed by heavy metal pollution to agricultural production and ecosystem health. If phytoremediation techniques, biological soil amendments, and regulatory measures to mitigate mercury toxicity are not developed, mercury contamination inevitably become one of the most significant environmental challenges threatening agricultural sustainability.

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