Journal of Elementology ISSN 1644-2296

Koçak, M.Z. (2023)
'Effects of copper stress on morphological traits and copper compartmentalization and microstructure of flax (*Linum usitatissimum* L.) cultivars',
Journal of Elementology, 28(3), 649-669,
https://dx.doi.org/10.5601/jelem.2023.28.2.2415

RECEIVED: 23 May 2023
ACCEPTED: 12 August 2023

ORIGINAL PAPER

Effects of copper stress on morphological traits and copper compartmentalization and microstructure of flax (*Linum usitatissimum* L.) cultivars

Mehmet Zeki Koçak

Department of Herbal and Animal Production, Vocational School of Technical Sciences Igdir University, Igdir, Türkiye

Abstract

Flax (*Linum usitatissimum* L.) is a crop plant that has long been used for various purposes, including production of fiber, oil, and food. In addition, it has more recently been studied for its phytoremediation potential to remove and detoxify environmental contaminants. Copper is a heavy metal that can be toxic to plants. In recent years, flax has begun to be used as a phytoremediation tool for phytoextraction of different heavy metals, especially when grown in metal-contaminated soils. The objective of the study was to conduct the efficiency of flax as a phytoremediation plant grown on the soil contaminated with different doses of copper (Cu) (control, 100 and 200 mg L^{-1}) metal. The results revealed that a high concentrations of Cu in the soil negatively affected plant growth and development by reducing plant height (30.17-22.33 cm), technical stem length (25.83-18.33 cm) and root length (7.50-4.00 cm) compared with the control (30.17-25.17 cm). The content of Cu in the leaves, stems and roots of flax cultivars was higher at 200 mg L^{-1} Cu concentration in the Mures and Erkendorfi cultivars. In addition, the two Cu concentrations (100 and 200 mg L^{-1}) were highly destructive to the plant, according to the SEM images, and the maximum stem damage was reported. Thus, the soil Cu content should be carefully controlled in order to avoid adverse effects on plant growth and development. Consequently, future research is needed for gaining better understanding of the physiology, biochemistry, anatomy, and molecular biology of flax in order to increase its pollutant removal efficiency.

Keywords: phytotoxicity, heavy metal, *Linum usitatissimum* l., flax, bioaccumulation

Mehmet Zeki Koçak, PhD, Department of Herbal and Animal Production, Vocational School of Technical Sciences, Igdir University, Igdir, Türkiye, e-mail: mehmetzekikocak@gmail.com

INTRODUCTION

Plants are frequently exposed to environmental stresses, which include both abiotic and biotic factors. These stresses typically reduce crops production and yields by 50-70% (Atkinson, Urwin 2012, Ghori et al. 2019). Ultraviolet radiations of different wavelengths, as well as salinity, drought, air and soil-borne pollutants, and exposure to various pathogens, represent some of the frequent stress factors for plants (Dutta et al. 2018). Plants use the trace elements they need for their basic metabolic activities. However, the supply of these metals in high amounts is dangerous and toxic. When heavy metal stress occurs, it causes many negative effects on seed germination, plant development and growth, physiological processes, and finally the yield of plants (Ali et al. 2015, Zainab et al. 2020). Heavy metals are known as the most dangerous pollutants in ecotoxicology due to their high toxicity and their significant release into the environment because of natural and anthropogenic processes.

In this context, many agricultural soils have been mildly to moderately contaminated with metals, such as (Cu), iron (Fe), cadmium (Cd), manganese (Mn), nickel (Ni), zinc (Zn), mercury (Hg), cobalt (Co), arsenic (As) due to anthropogenic activities, industrial waste and sewage disposal (Yadav 2010, Di Ferdinando et al. 2014). Heavy metals are significant environmental pollutants, and their toxicity is of great concern for economic, environmental, nutritional and evolutionary reasons. However, Cu is also a significant micronutrient needed by living organisms (Rehman et al. 2019, Saleem et al. 2020*a*). Copper has an important role in many biochemical and physiological processes, such as photosynthesis, mineral nutrition, chlorophyll biosynthesis, and in the formation of carbohydrates, proteins, lipids, and nucleic acids (Adrees et al. 2015, Ahmed, Slima 2018). The Cu concentration in higher plants is considered to range from 2-30 mg kg^{-1}; on the other hand, above this range, the metal is toxic to plants (Keller et al. 2015, Rizwan et al. 2016, Koçak et al. 2022). Toxic heavy metals released to agricultural lands because of anthropogenic activities, which include mining, discharge of wastewater, and application of pesticides, are the main cause of global heavy metal pollution (Saleem et al. 2020*a*). Additionally, metal pollution has been recognized as a serious threat to the environment and food security due to increased globalization, industrialization, and the operation of coal-fired power plants (Rafaj et al. 2018, Hopewell 2019, Saleem et al. 2020*b*). Generally, cultivation of agricultural products in areas contaminated with heavy metals due to various industrial activities can illuminate how heavy metals are taken up by plants and in which parts they accumulate. In addition, it is necessary to pay attention to the ability of crops to absorb and accumulate heavy metals, and to prefer more resistant cultivars in agricultural production (Angelova et al. 2004, Souri et al. 2019). Herewith, it has been reported that the uptake of heavy metals by plants depends on soil and plant factors, and the plant genotype is considered to be the most important among these factors (Li et al. 1997, Hocking, McLaughlin 2000). Generally, plants are sensitive to high Cu levels, and manifest toxic effects at above-optimal levels. Many plant species, including flax, have been reported to be used for phytoremediation of different heavy metals, such as Cu, Zn, or Hg (Saleem et al. 2020c).

Flax (*L. usitatissimum* L.) is an annual, herbaceous, and self-pollinating plant with 22 genera and 300 species, which belongs to the Linaceace family. Flax is known as an important industrial plant all over the world. It is also a diploid plant with (2*n*=30) chromosomes, and is distributed in temperate regions (Moghaddam et al. 2018). The above plant is widely cultivated for oil, fiber, and food purposes. *Linum usitatissimum*, an alternative oil plant containing 35-65% oil in its structure, is used in different industrial branches. In addition to being rich in fatty compounds, such as α-linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid, flax contains high levels of lignans, protein, and fiber (Wang et al. 2017, Zhang et al. 2018). The morpho-physiological and oxidative defense responses of flax plants against different Cu levels have been investigated (Hosman et al. 2017). In this context, a pot experiment has been conducted, aimed to determine the phytoremediation potential of flax varieties irrigated with Cu solutions at different doses, in order to determine the morphological characteristics of the crop and the copper accumulation rate. Although these features are not expressed much in the flax plant, one of the objectives of this study has been to assess the phytoremediation potential of the plant at different Cu application doses, and to determine the Cu accumulation in the stems, roots and leaves of flax.

MATERIAL AND METHODS

Plant material and details of the experiment

For obtaining the plant material, the flax cultivars Dakota, Omega, Mures and Erkendorfi were grown in the experiment. The study was carried out in a greenhouses of the Agricultural Research and Application Centre at 2023 (Igdir University, Türkiye), using a factorial experimental design (Figure 1). Before sowing, the seeds were first surface-sterilized using 1% (v/v) hypochlorite for 2-4 min, and then rinsed with distilled water to remove the residues of the disinfectant. Following sterilization, the seeds were dried with tissue paper at room temperature. Afterwards, the seeds were sown in 2 L-plastic pots containing peat and grown in a greenhouses with a 14/10 h photoperiod, 26-30°C/day and 16-20°C/night; relative humidity: 60% (Kosem et al. 2022). Additionally, the soil had the following properties: pH 5.5-6.00, 13 ppm Cu, 2195 ppm Fe, 32 ppm Zn, 38 ppm Mn; basic macroelements: % 0.215 P, % 1.186 total N, 1800 ppm K, 1620 ppm Mg, 2800 ppm

Fig. 1. Morphological characteristics of flax cultivars

Ca, 200 ppm Na. The study was based on three replicates and each replicate corresponded to twenty plants. Three Cu concentrations were tested: 0, 100 and 200 mg L^{-1}. Once the plants reached 5-10 cm height, they were subjected to Cu stress. The plants were irrigated with an amount of 200 ml solutions/ distilled water every 4 days.

Agro-morphological traits

Plants were harvested 45 days after irrigation with copper. For morphological traits, plant height (cm), technical stem length (cm), technical stem weight, root length (cm), root weight, and leaf fresh weight were recorded (Figure 1).

Quantification of copper using Atomic Absorption Spectrophotometry (AAS)

For an Atomic Absorption Spectrophotometry assay, 10 seedlings were collected from each pot, and the seedlings were separated into leaves, stems and roots. The leaf, root and stem parts of flax were prepared for analysis of elemental levels after drying. Dry samples of 9-15 flax plants (leaf, root and stem) from each treatment (control, 100 and 200 mg L^{-1} Cu) were washed to remove residual perlite and vermiculite particles. Subsequently, approximately 1-1.5 g dried samples were placed in Teflon cells of a Milestone brand Ethos One model microwave; 6 mL nitric acid (HNO_3) and 2 mL hydrogen peroxide (H_2O_2) were added to each solid sample, which were kept afterwards in the fume hood for 10 min to completely remove the gas outlet inside. Subsequently, the Teflon cells were closed and microwaved for 40 min at 1800 watts at 250°C. After the Teflon apparatus was cooled, the total volume was replenished by adding distilled water to 25 ml. The amount of copper (Cu) heavy metal in the plant samples was determined in mg kg^{-1} (Peronico, Raposo 2016). The analysis was performed on a Agilent brand 240FS model Atomic Absorption Spectrophotometer – AAS (Agilent Technologies®, Santa Clara, CA, USA).

Microstructure analysis of flax using a Scanning Electron Microscope (SEM)

To observe the morphology of the stem and leaf samples, a Field Emission Scanning Electron Microscope (ZEİSS Sigma 300) with an accelerating voltage of 10 kV (Yavuzer, Akinay 2021) was used. This part of the experiment was carried out at the Van Yüzüncü Yıl University Science Application and Research Center (Van, Türkiye). Before the analysis with FE-SEM, since herbal products are not electrically conductive, the surface of the samples was coated with a Qurom gold-plating device to provide electron scattering from the surface. A secondary electron (SE) detector was used to view the morphological information from the samples.

Experimental design and statistical analysis

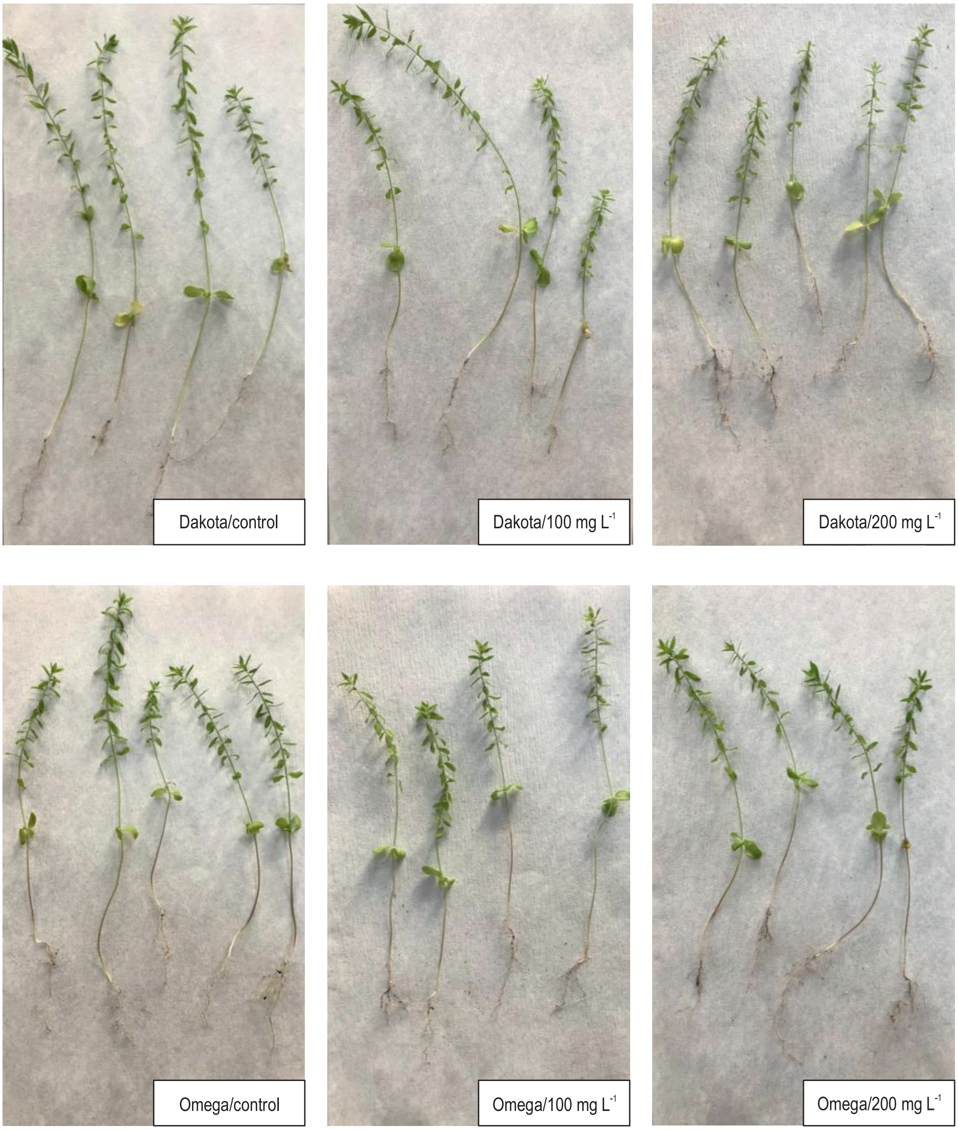
Three replications were used for each treatment, and each replicate included ten plants. Data were expressed as means. The means were compared with a one-way variance analysis followed by the Duncan's multiple *post-hoc* tests. The differences between individual means were considered as significant at $p<0.05$ (SPSS). In addition, heat map clustering (ClustVis online) and principal component analysis (JAMOVI) were carried out to reduce the dimension and to associate variables.

RESULTS

Effects of copper on morphological properties and biomass and correlation analysis of some flax cultivars

The results indicated that the morphological traits and biomass were significantly different in Cu-contaminated peat flax compared to the control

(Table 1). Plants suffering from Cu stress achieved shorter plant height and lower biomass in comparison to the control ($p \leq 0.05$). As regards the Cu concentrations, the high concentration caused more damage to the parameters considered for analysis (Figure 2). It was also determined that there was an increase in the Dakota and Omega cultivars (root weight, leaf fresh weight), but a decrease ($p \leq 0.05$) in the Mures and Erkendorfi cultivars (Table 1). Furthermore, a decrease in root length (7.50-6.50, 5.66 and 6.33 cm) was observed in the respective flax cultivars (Dakota, Omega, Mures and Erken-



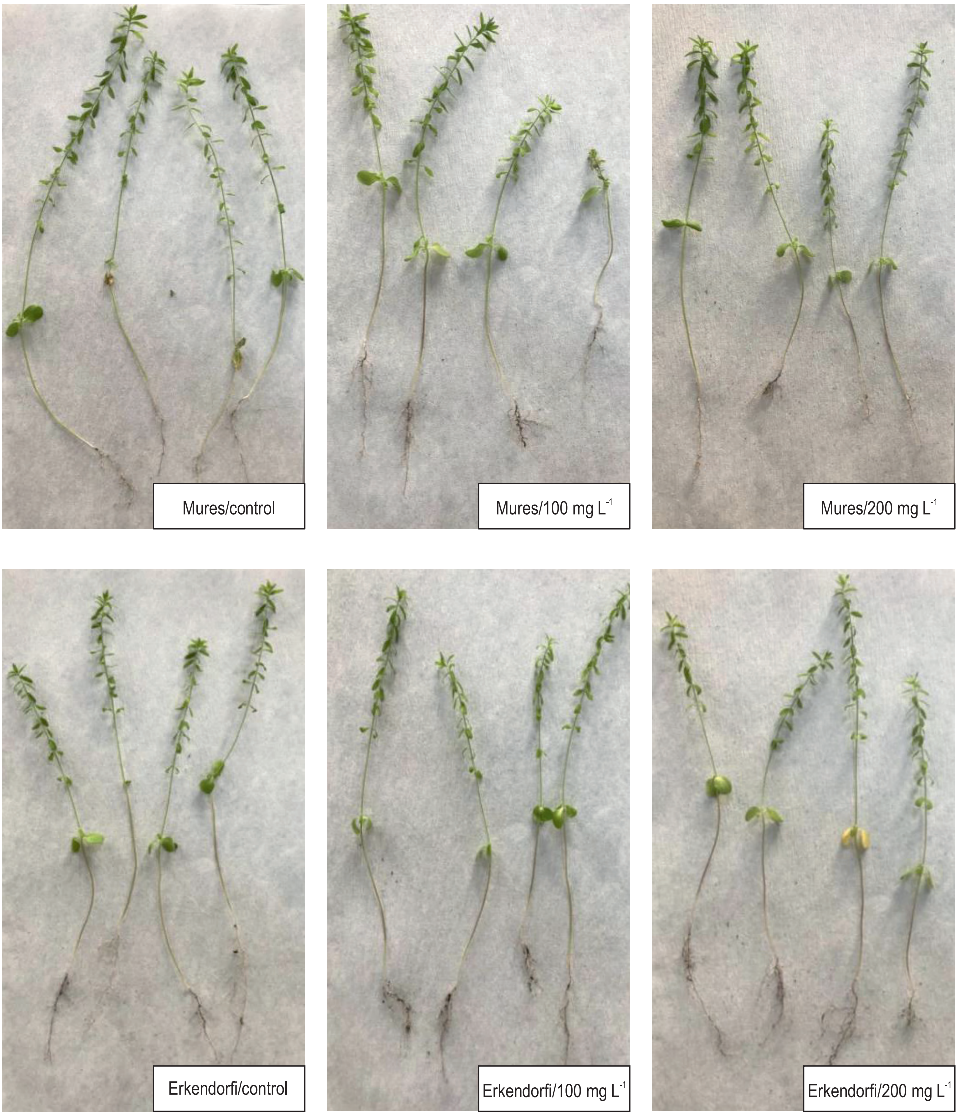


Fig. 2. Measurement and drying of morphological characteristics of flax varieties

dorfi) at both Cu concentrations (100 mg L^{-1}, 200 mg L^{-1}) compared to the control.

However, it was determined that the root weight ratios of the same cultivars increased in Cu treatments (100 mg L^{-1}, 200 mg L^{-1}) – Table 1. Flax is a multi-purpose crop, i.e. grown for oil and fiber, and therefore the plant height is an especially important morphological feature. In this context, it was determined that the plant height decreased gradually at 100 and 200 mg L^{-1} doses of Cu relative to the control values of the same cultivars

Table 1

Effects of copper (Cu) levels (control, 100 and 200 mg L^{-1}) on some agronomic attributes of flax (*Linum usitatissimum* L.)

Cultivars	Cu doses (mg L^{-1})	Plant height (cm)	Technical stem length (cm)	Technical stem weight (g)	Root length (cm)	Root weight(g)	Leaf fresh weight (g)
Dakota	0	25.17±2.094cd	20.66±1.027cd	0.48±0.124bc	7.50±0.816a	0.34±0.327b	0.51±0.126bc
	100	25.83±1.027bc	22.00±0.408bc	0.60±0.082a-c	3.66±1.027e	0.09±0.021bc	0.64±0.197bc
	200	23.66±1.247cd	16.33±1.929e	0.51±0.021bc	4.50±1.080de	0.09±0.008bc	0.64±0.012bc
Omega	0	25.83±1.312b-d	17.66±1.433de	0.36±0.096c	6.50±0.408ab	0.70±0.163a	0.53±0.122bc
	100	23.50±1.080cd	18.17±0.623de	0.50±0.210bc	5.66±0.849b-c	0.12±0.038bc	0.73±0.069ab
	200	23.66±1.699cd	17.67±0.623de	0.65±0.057ab	4.83±0.623c-e	0.14±0.008bc	0.94±0.012a
Mures	0	30.17±2.094a	25.83±1.699a	0.83±0.242a	5.66±0.623b-d	0.09±0.008bc	0.92±0.213a
	100	28.66±1.699ab	24.83±1.433ab	0.62±0.032a-c	5.00±0.408b-e	0.04±0.008c	0.53±0.026bc
	200	22.33±1.247d	18.33±2.248de	0.47±0.020bc	4.16±0.235de	0.08±0.012c	0.53±0.008bc
Erkendorfi	0	26.83±1.027bc	19.50±1.080c-e	0.48±0.077bc	6.33±0.849a-c	0.08±0.020c	0.60±0.020bc
	100	25.00±1.471cd	20.67±1.247cd	0.44±0.012bc	4.33±0.623de	0.06±0.012c	0.41±0.028c
	200	22.66±1.649d	18.33±2.460de	0.51±0.016bc	4.00±0.408de	0.12±0.012bc	0.63±0.012bc
Mean±SD		25.25±2.79	20.00±3.23	0.54±0.16	5.18±1.34	0.16±0.21	0.63±0.18
p-value^{cultivars}		5.030	7.869	2.428	5.394	6.030	5.070
p-value^{Cu}		<0.000	<0.000	<0.000	<0.000	<0.000	<0.000

Different letters in the same column indicate significant differences according to the Duncan's multiple range test ($p<0.05$).

(Table 1). Additionally, among the cultivars, the cv. Mures obtained a significant difference (control 30.17 cm). However, it was determined that the maximum plant height was achieved at the level of 200 mg L^{-1} Cu (22.33 cm). Similarly, the technical stem length, which is an important feature, especially in fibrous varieties, was 25.83 cm for the control Mures and 18.33 cm in the 200 mg L^{-1} Cu treatment. The correlation analysis revealed that the plant height was positively correlated with the technical stem length (r=0.690; p<0.05). The technical stem length was positively correlated with the leaf fresh weight (r=0.630; p<0.05). In addition, positive correlation coefficients were determined for the technical stem weight with plant height (r=0,380) and root weight; as for the positive correlation with the root (r=0.420), the coefficients were not significant (Figure 3).

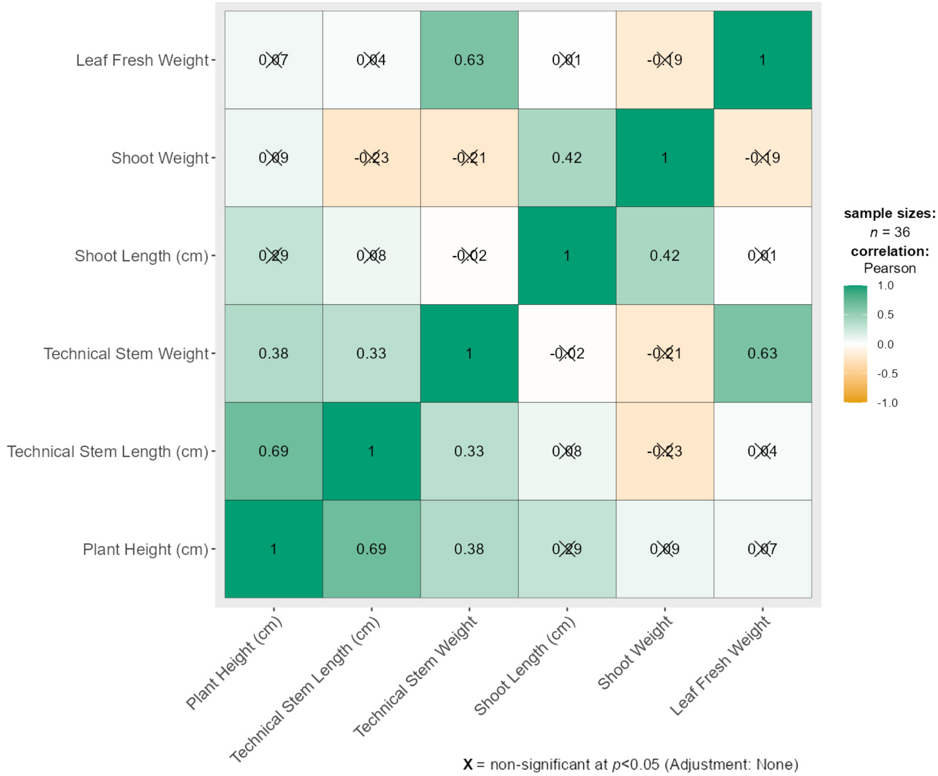


Fig. 3. Correlation analysis of the morphological measurements corresponding to the treatments

Relationships between the copper content and various growth parameters (leaf, stem, and root) of flax

The content of copper in leaves, roots and stems of the flax cultivars was collectively presented in Figure 4. Unsurprisingly, the higher content was observed at 200 mg L^{-1} Cu concentration. Concerning the Cu-content

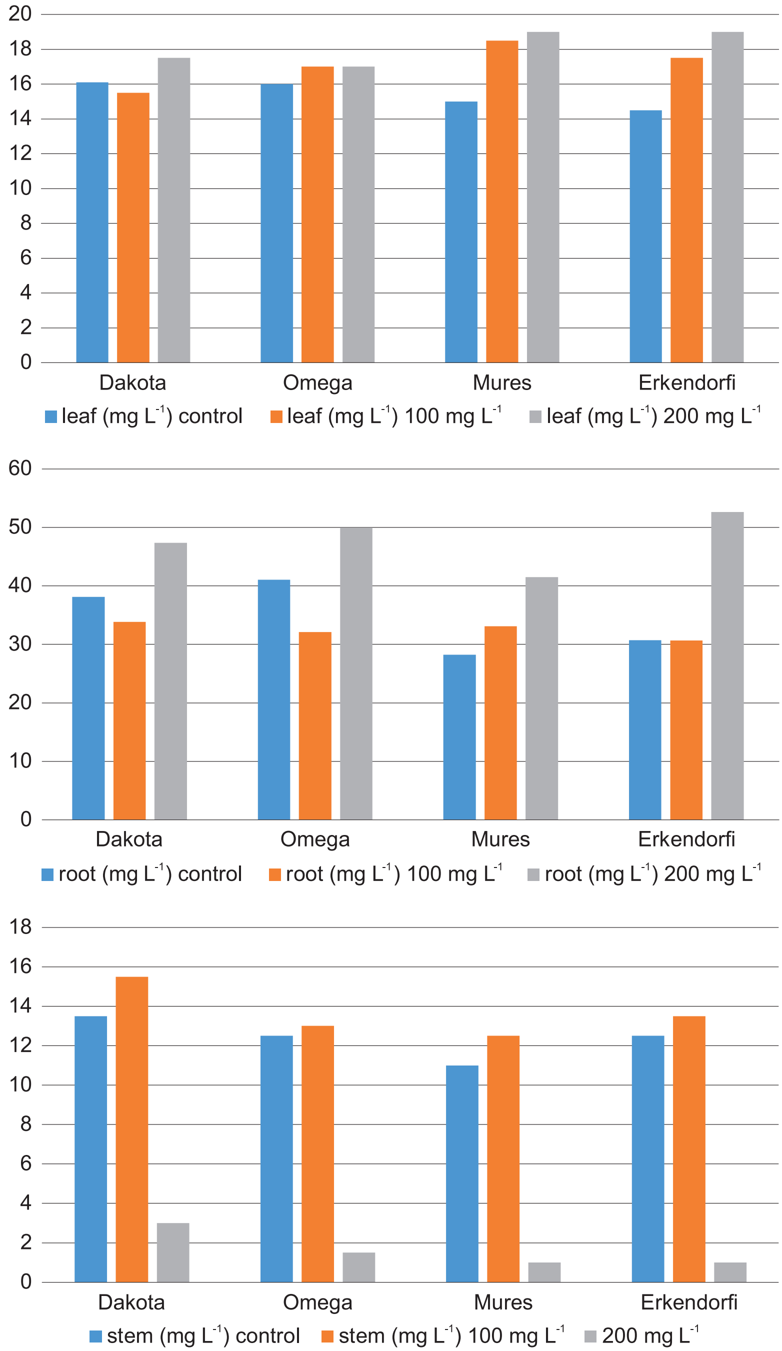


Fig. 4. Content of copper (Cu) doses (control, 100 and 200 mg L^{-1}) and copper content in some organs (leaf, root and stem) of flax

of the leaf, 15%, 18.5%, and 19% concentrations were recorded under control, 100 mg L^{-1} and 200 mg L^{-1}, respectively, in the Mures cultivar. The respective results for the Erkendorfi cultivars were 14.5%, 17.5%, and 19%. When the roots of the plant were examined, it was seen that the copper accumulation in all cultivars was the highest at the highest rate of Cu pollution, i.e. 200 mg L^{-1}. In addition, cv. Erkendorfi (52.61%) achieved the highest value among the cultivars; interestingly, it was found to be almost the same in the control and 100 mg L^{-1} treatment. While copper accumulation in the root structure of the in Dakota and Omega cultivars of the test plant was 38.10% and 41.06%, respectively, at the control Cu dose, it achieved 33.87% and 32.13%, respectively, in the 100 mg L^{-1} treatment. In the present study, it was observed that the copper (Cu) doses applied to the tested flax cultivars increased only at 100 mg L^{-1} in the stem part of the plant. In addition, it was determined that there the accumulation was very low as compared to 100 mg L^{-1} (Figure 4). The results showed a close link between copper (Cu) accumulation and different growth characteristics of flax.

Heatmap clustering and Principal Component Analysis (PCA) of flax cultivar's growth and biomass production traits

To visualize, correlate and clarify the morphological traits considered for analysis, a heatmap (Figure 5) and PCA (Figure 6) were performed. In this context, the heatmap clustering revealed two major clusters. The first cluster was comprised of the leaf fresh weight and technical stem weight. And the second major cluster was composed of the plant height, technical stem

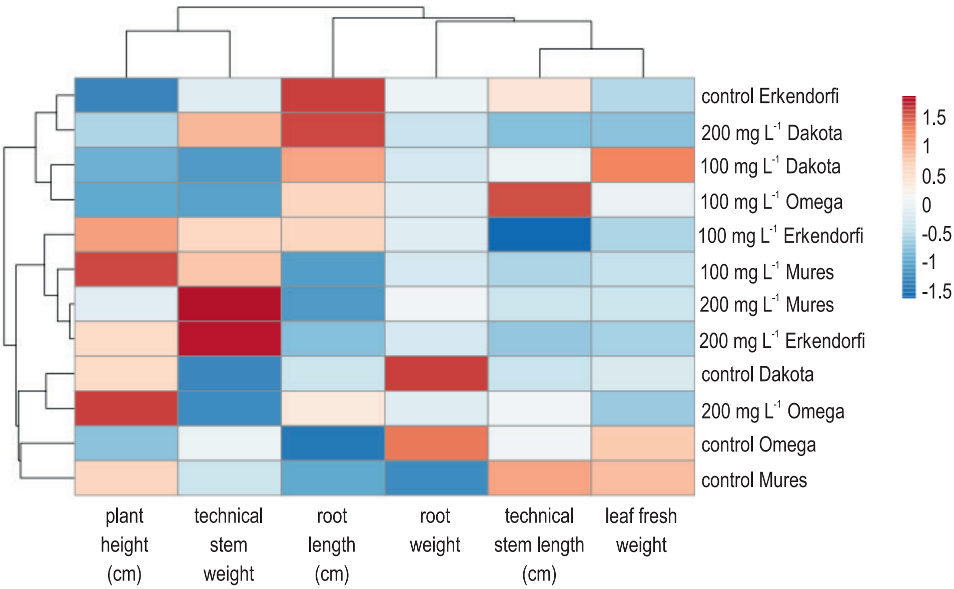


Fig. 5. Heatmap clustering of morphological features after treatments

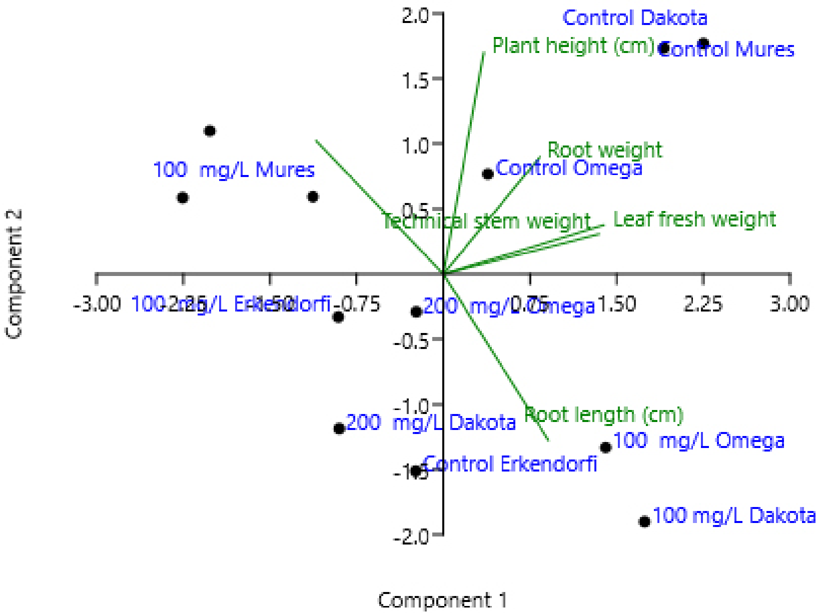


Fig. 6. Principal component analysis (PCA) of morphological attributes corresponding to the treatments

length, root length, and root weight of the flax (Figure 5). Furthermore, to explain the percentage of variation, PCA was performed in order to identify the type of correlation and the level of differentiation between the cultivars and related parameters (Figure 6). Therefore, based on the correlations, heatmap-like scattering was also observed for PCA. For instance, such attributes as the plant height, technical stem length and root weight were separated from the other morphological parameters. Accordingly, two components with Eigen values over 1 were observed. These two components (F_1 45.2% and F_2 29.1%) explained the total variation of 74.3%. Such a high explanation ratio might be significant for the discrimination of cultivars.

Heatmap clustering of flax cultivars based on AAS and PCA results

The quantitative analysis of the attributes of the flax was supplemented by AAS of the attributes corresponding to the treatments of the flax cultivars. Four flax cultivars and three levels of Cu, control, 100 and 200 mg L^{-1}, were analyzed. The AAS analysis revealed the level of copper uptake and various growth parameters (leaf, root and stem) of flax (Figure 4). During the Cu percentage of the growth parameters (leaf, root and stem) was examined at the 100 mg L^{-1} level compared to the control, 100 mg L^{-1} Mures (18.5-33.09-12.5 mg L^{-1}) were founded to be increased respectively, among the cultivars. In addition, cv. Erkendorfi (19-52.61 mg L^{-1}) was determined in Cu percentage at the 200 mg L^{-1} level of growth parameters (leaf, root) respectively (Figure 4). Compared to the control results, it was determined that there was Cu accumulated more in the leaf and stem than in the root of the flax. According to the heatmap clustering, both the cultivars and the examined results of the AAS analysis revealed that the level of copper uptake and various growth parameters (leaf, root and stem) of flax divided into two major clusters. Regarding the growth parameters, the leaf and the root of flax formed one cluster, whereas the stem fell into the other main cluster (Figure 7). Furthermore, the PCA was performed (Figure 8), where 2 components (principal factors) with eigenvalues above 1> were identified. The two of them (F_1 77% and F_2 17.4%) explained 94.4% of the total variation.

Microstructure analysis of stem and leaf using SEM

The surface morphology of the stems and leaves of the untreated flax was visualized under a SEM (Figures 9-10). In addition, a method demonstrated by Mir et al. (2021) was used to perform SEM imaging of leaf stomata and stems (Figure 9). It was determined that the structures of numerous stomata on the flax leaves surface were different in response to the applied Cu. Copper contamination considerably reduced the stomatal aperture as

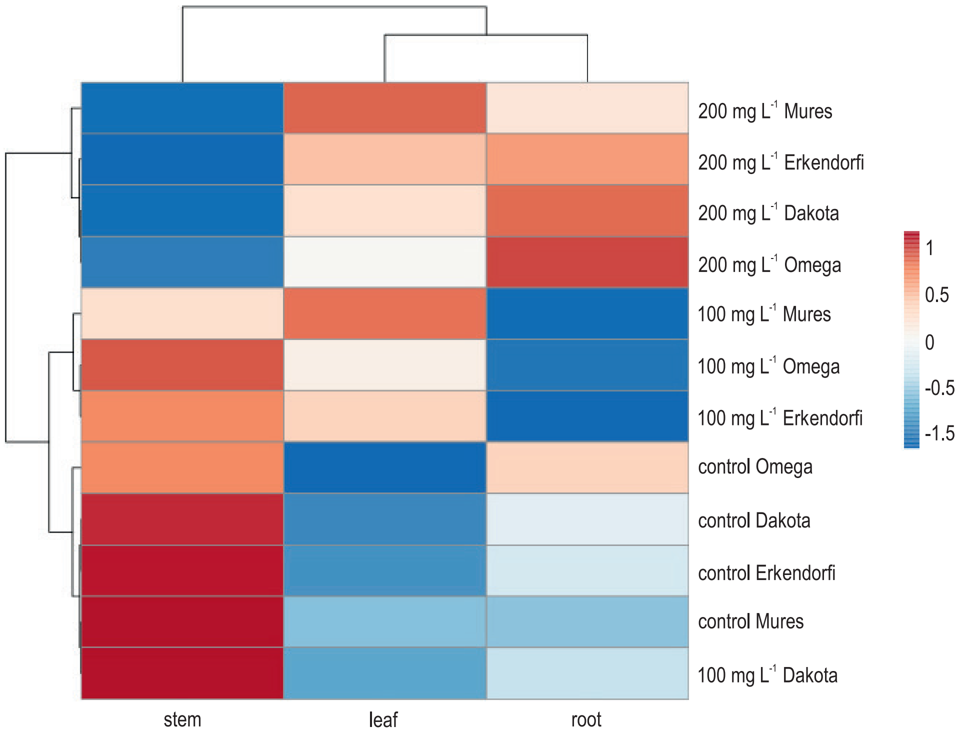


Fig. 7. Heatmap clustering of Atomic Absorption Spectrophotometer (AAS) attributes corresponding to the treatments

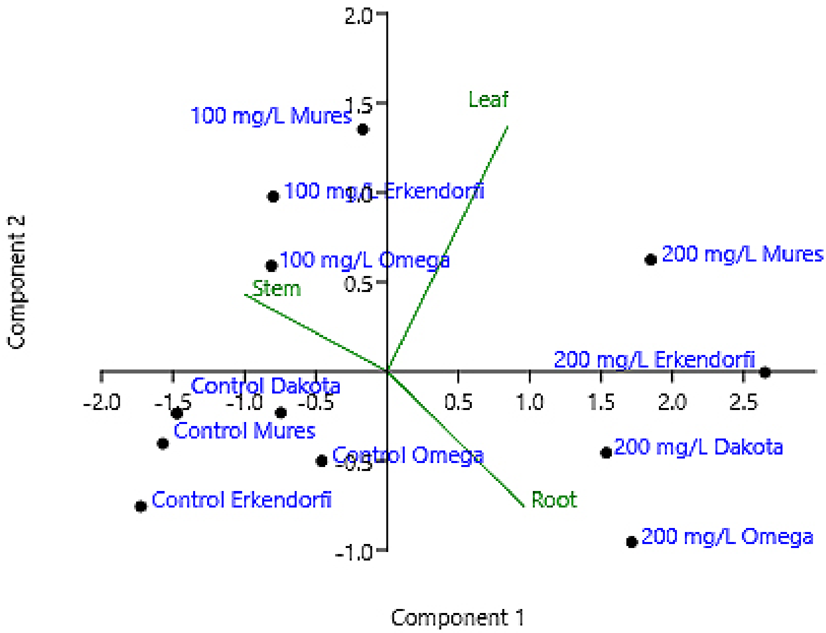


Fig. 8. Principal Component Analysis [PCA] of Atomic Absorption Spectrophotometer (AAS) attributes corresponding to the treatments

compared to the control plants. The copper concentrations (100 and 200 mg L^{-1}) and doses (200 mg L^{-1}) were found to be more destructive to the plant in terms of reduced stomatal size. In addition to these results, the Cu concentrations were found to have resulted in a higher dose (200 mg L^{-1}) in the leaf (Figure 4). As evident from Figure 10, the surface of the untreated flax stems had rough surface with multiple grooves. It was observed that the structure of the surface of many flax stems were different depending on the Cu applied. In addition, copper contamination greatly reduced the binding structures between the flax stem (fibers) as compared to the control plants. The various Cu concentration doses proved severely destructive to the plant structure, and the maximum stem destruction was reported.

DISCUSSION

Soils contaminated with heavy metals are detrimental to plant growth and development, causing various phytotoxicities at very low doses, although some heavy metals, such as Cu, are also reported to be essential for the normal plant growth and development (Li et al. 2008, Briffa et al. 2020, Saleem et al. 2020*a*, Saxena et al. 2020). In addition, soil pollution with heavy metals continues to be a serious threat to humans and animals due to the

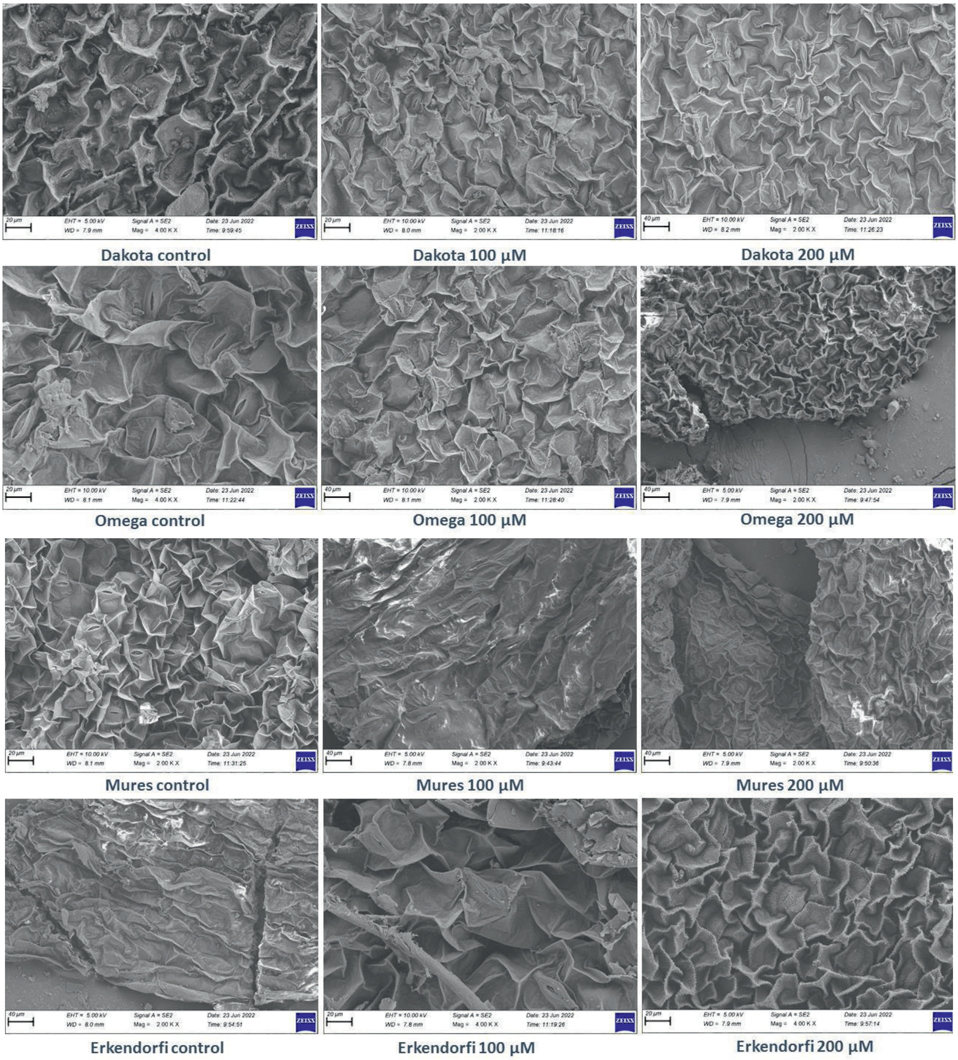


Fig. 9. Scanning Electron Microscope (SEM) images of the stomata: the effect of copper on the flax parts – leaf (μM)

contamination of food by heavy metals (Zwolak et al. 2019). High heavy metal concentrations in soil reduce yields of crops (Khan et al. 2015, Osman et al. 2017). In this context, the use of plant species tolerant to toxic heavy metals can be an effective way of purifying contaminated agricultural soils (Sarwar et al. 2017). Flax (*L. usitatissimum* L.) can be grown in heavy metal contaminated agricultural areas because it has an economic value as well as a phytoremediation potential, being able to accumulate high metal content (Cleophas et al. 2022). In addition, although copper (Cu) is a potentially toxic heavy metal, it is also essential for plant growth and development.

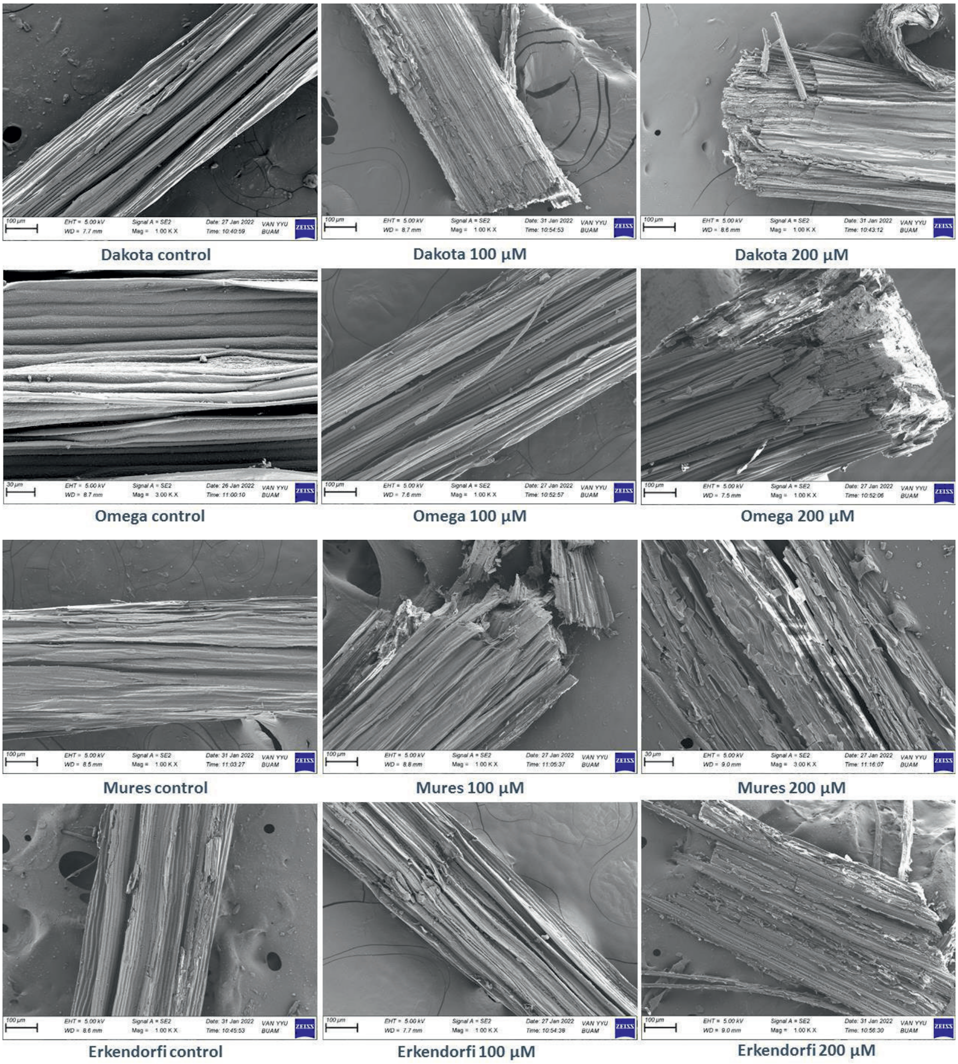


Fig. 10. Scanning Electron Microscope (SEM) images of stem the effect of copper on the flax parts – stem (µM)

However, serious problems arise due to excess copper amounts. Additionally, heavy metal toxicity varies across plant species, specific metal, its chemical form and concentration as well as the soil composition and pH (Yruela 2005, Ayangbenro and Babalola 2017, Kumar et al. 2021).

After the contamination of soil with copper (0, 100 and 200 mg L^{-1}) in our experiment, were significant effects on the plant were observed. Additionally, morphological measurements showed that flax cultivars can tolerate up to 100 mg L^{-1} Cu compared to the control. At the same time, an increase in the root weight, fresh leaf weight of the Dakota and Omega cultivars, with oily properties, and a decrease in the Mures and Erkendorfi cultivars, with fibrous properties, were observed in this study. Moreover, the stem part of the fibers is longer in the latter group of cultivars than in the oily cultivars. Previous studies reported significant reductions in the biomass of copper--treated plants of *Brassica napus* (Zehra et al. 2023). Increasing copper concentration has been reported to reduce both the stem and root growth in *Arabidopsis thaliana* (Kolbert et al. 2012), in addition to supporting our current findings (Liu et al. 2015). A significantly increased Cu and Si content in roots and stems has also been reported due to increasing copper concentrations (100 and 200 mg L^{-1}) compared to control plants (El-Beltagi et al. 2020). Additionally, it was determined that following Cu application the plant height of the cultivars decreased gradually at 100 and 200 mg L^{-1} doses compared to the control. El-Beltagi et al. (2020) reported reductions in plant height occurring gradually at 100 mg L^{-1} (67.1 cm) and 200 mg L^{-1} (63.8 cm) compared to the control (76.5 cm). In addition, the technical body length gradually decreased at 100 mg L^{-1} (53.1 cm) and at 200 mg L^{-1} (50.4 cm) compared to the control (62.2 cm) The results obtained in soil contaminated with different levels of Cu were T1; 0, 9.8 cm; T2; 1, 12.1 cm; T3; 2, 14.9 cm; T4; 4, 18.4 cm, and were lower compared to the control – 25 cm (T: treatment) – Saleem et al. (2020*b*). In light of the data obtained, the accumulation of copper in the root and leaf parts of flax increases as the dose of Cu increases (Figure 4). In addition, the highest value detected in roots and leaves was at 200 mg L^{-1} (52.61 mg kg^{-1} and 19 mg kg^{-1}, respectively). The SEM analysis performed on leaf images showed that some stomatal structures were deformed as a result of the Cu accumulation compared to the control, and some were completely closed (Figure 9). In addition, it was determined that there were significant deformations in the stem part of the plant (Figure 10). According to Mir et al. (2021), Cu contamination reported (90 mg kg^{-1}) greatly reduced stomatal patency compared to control plants. Previously reported findings showed that the flax plant at different lead (Pb), cadmium (Cd) and zinc (Zn) concentrations has the ability to collected high concentrations of heavy metals from the soil (Hosman et al. 2017), hence flax can be used as a phytoremediation plant for heavy metals (Saleem et al. 2020*c*).

CONCLUSIONS

Several methods should be used for the rehabilitation of soils contaminated with toxic metals. Primarily, studies showed that flax is suitable for the phytoremediation of soil and wastewater. The results of this study showed that flax could tolerate a certain level of copper contamination and effectively remove copper from the soil. However, excessive copper doses had negative effects on the morpho-physiological characteristics of flax, including reduced plant height (cm), technical stem length (cm) and root length (cm). In addition, via SEM analysis, it was determined that copper doses (control, 100 and 200 mg L^{-1}) had a significant effect on some structures of flax (leaves, stem and root). Furthermore, when the phytoremediation potential of the cultivars used in the study was examined, the Erkendorfi cultivar, came to the fore as the most tolerant one. In conclusion, flax can be used as an effective phytoremediation plant for copper contaminated soil, but the soil content of the heavy metal should be carefully controlled to prevent negative impacts on plant growth and development. Furthermore, the potential of flax for the remediation of soils polluted with heavy metals should be tested under field conditions.

Author contribution

The author confirms that the text, figures, and tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ACKNOWLEDGEMENTS

For analysis; Atomic Absorption Spectrophotometer (AAS); Igdir University Research Laboratory Application and Research Center (ALUM) and SEM (Scanning Electron Microscope); Van Yüzüncü Yıl University Science Application and Research Center. I would like to thank the employees. Additionally, I would like to thank Muhittin Kulak and Mustafa Güven Kaysim for the morphological measurements and statistical analysis of the plants.

REFERENCES

Adrees M., Ali S., Rizwan M., Ibrahim M., Abbas F., Farid M., Bharwana S.A. 2015. *The effect of excess copper on growth and physiology of important food crops: a review*. Environ. Sci. Pollut. Res., 22(11): 8148-8162. https://doi.org/10.1007/s11356-015-4496-5

Ahmed D.A., Slima D.F. 2018. *Heavy metal accumulation by Corchorus olitorius* L. *irrigated with wastewater*. Environ. Sci. Pollut. Res., 25: 14996-15005. https://doi.org/10.1007/s11356-018-1675-1

Ali N., Masood S., Mukhtar T., Kamran M.A., Rafique M., Munis M., Chaudhary H.J. 2015. *Differential effects of cadmium and chromium on growth, photosynthetic activity, and metal uptake of Linum usitatissimum in association with Glomus intraradices*. Environ. Monit. Assess., 187(6): 1-11. https://doi.org/10.1007/s10661-015-4557-8

Angelova V., Ivanova R., Delibaltova V., Ivanov K. 2004. *Bio-accumulation and distribution of heavy metals in fibre crops (flax, cotton and hemp).* Ind. Crops Prod., 19(3): 197-205. https://doi.org/10.1016/j.indcrop.2003.10.001

Atkinson N.J., Urwin P.E. 2012. *The interaction of plant biotic and abiotic stresses: from genes to the field.* J. Exp. Bot., 63(10): 3523-3543. https://doi.org/10.1093/jxb/ers100

Ayangbenro A.S., Babalola O.O. 2017. *A new strategy for heavy metal polluted environments: a review of microbial biosorbents.* Int. J. Environ. Health Res., 14(1): 94. https://doi.org/10.3390/ijerph14010094

Briffa J., Sinagra E., Blundell R. 2020. *Heavy metal pollution in the environment and their toxicological effects on humans.* Heliyon, 6(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691

Cleophas F.N., Zahari N.Z., Murugayah P., Rahim S.A., Mohd Yatim A.N. 2022. *Phytoremediation: A novel approach of bast fiber plants (hemp, kenaf, jute and flax) for heavy metals decontamination in soil.* Toxics, 11(1),5. https://doi.org/10.3390/toxics11010005

Di Ferdinando M., Brunetti C., Agati G.,Tattini M. 2014. *Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas.* Environ. Exp. Bot., 103: 107-116. https://doi.org/10.1016/j.envexpbot.2013.09.012

Dutta S., Mitra M., Agarwal P., Mahapatra K., De S., Sett U., Roy S. 2018. *Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability.* Plant Signal. Behav., 13(8), e1460048. https://doi.org/10.1080/15592324.2018.1460048

El-Beltagi H.S., Sofy M.R., Aldaej M.I., Mohamed H.I. 2020. *Silicon alleviates copper toxicity in flax plants by up-regulating antioxidant defense and secondary metabolites and decreasing oxidative damage.* Sustainability, 12(11): 4732. https://doi.org/10.3390/su12114732

Ghori N.H., Ghori T., Hayat M.Q., Imadi S.R., Gul A., Altay V., Ozturk M. 2019. *Heavy metal stress and responses in plants.* Int. J. Environ. Sci. Technol., 16(3): 1807-1828. https://doi.org/10.1007/s13762-019-02215-8

Hocking P.J., McLaughlin M.J., 2000. *Genotypic variation in cadmium accumulation by seed of linseed and comparison with seeds of some other crop species.* Aust. J. Agric. Res. 51: 427-433. https://doi.org/10.1071/AR99124

Hopewell K. 2019. How rising powers create governance gaps: *The case of export credit and the environment.* Glob Environ Polit., 19(1): 34-52. https://doi.org/10.1162/glep_a_00490

Hosman M.E., El-Feky S.S., Elshahawy M.I., Shaker E.M., 2017. *Mechanism of phytoremediation potential of flax (Linum usitatissimum* L.*) to Pb, Cd and Zn.* Asian J. Plant Sci. Res, 7(4): 30-40.

Keller C., Rizwan M., Davidian J.C., Pokrovsky O.S., Bovet N., Chaurand P., Meunier J.D. 2015. *Effect of silicon on wheat seedlings (Triticum turgidum* L.*) grown in hydroponics and exposed to 0 to 30 μM Cu.* Planta, 241(4): 847-860. https://doi.org/10.1007/s00425-014-2220-1

Khan A., Khan S., Khan M.A., Qamar Z., Waqas M. 2015. *The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review.* Environ. Sci. Pollut. Res., 22: 13772-13799. https://doi.org/10.1007/s11356-015-4881-0

Koçak M.Z., Göre M., Kurt O. 2022. *The effect of different salinity levels on germination development of some flax (Linum usitatissimum* L.*) varieties.* TURJFAS, 10(4): 657-662. https://doi.org/10.24925/turjaf.v10i4.657-662.4758

Kolbert Z., Pető A., Lehotai N., Feigl G., Erdei L. 2012. *Long-term copper (Cu 2^+) exposure impacts on auxin, nitric oxide (NO) metabolism and morphology of Arabidopsis thaliana* L. Plant Growth Regul., 68: 151-159. https://doi.org/10.1007/s10725-012-9701-7

Kosem H., Kocak M.Z., Kaysim M.G., Celikcan F., Kulak M. 2022. *Liquid leachate produced from vermicompost effects on some agronomic attributes and secondary metabolites* *of sweet basil (Ocimum basilicum* L.*) exposed to severe water stress conditions*. Horticulturae, 8(12): 1190. https://doi.org/10.3390/horticulturae8121190

Kumar V., Pandita S., Sidhu G.P.S., Sharma A., Khanna K., Kaur P., Setia R. 2021. *Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review*. Chemosphere, 262, 127810. https://doi.org/10.1016/j.chemosphere.2020.127810

Li G., Niu J. Sang N. 2008. *Assessing the phytotoxicity of different particle-size aged refuse using Zea mays* L. *bioassay*. Chemosphere, 74(1): 106-111. https://doi.org/10.1016/j.chemosphere.2008.09.010

Li Y.M., Chaney R.I., Schneiter A.A., Miller J.F., Elias E.M., Hammond J.J. 1997. *Screening for low grain cadmium genotypes in sunflower, durum wheat and flax*. Euphytica, 94: 23-30. https://doi.org/10.1023/A:1002996405463

Liu Q., Zheng L., He F., Zhao F.J., Shen Z., Zheng L. 2015. *Transcriptional and physiological analyses identify a regulatory role for hydrogen peroxide in the lignin biosynthesis of copper -stressed rice roots*. Plant Soil., 387: 323-336. https://doi.org/10.1007/s11104-014-2290-7

Mir A.R., Alam P., Hayat S. 2021. *Effect of different levels of soil applied copper on the morphophysiological, photochemical, and antioxidant system of Brassica juncea*. J. Soil Sci. Plant Nutr., 21: 3477-3492. https://doi.org/10.1007/s42729-021-00621-x

Moghaddam M., Babaei K., Saeedi Pooya E. 2018. *Germination and growth response of flax (Linum usitatissimum) to salinity stress by different salt types and concentrations*. J. Plant Nutr., 41(5): 563-573. https://doi.org/10.1080/01904167.2017.1392573

Osman M.E.H., El-Feky S.S., Elshahawy M.I., Shaker E.M. 2017. *Efficiency of flax (Linum usitatissimum* L.*) as a phytoremediator plant for the contaminated soils with heavy metals*. Int J Agric Environ Res, 3(4): 3577-3600. ISSN: 2455-6939

Peronico V.C.D., Raposo Jr J.L., 2016. *Ultrasound-assisted extraction for the determination of Cu, Mn, Ca, and Mg in alternative oilseed crops using flame atomic absorption spectrometry*. Food Chem, 196: 1287-1292. https://doi.org/10.1016/j.foodchem.2015.10.080

Rafaj P., Kiesewetter G., Gül T., Schöpp W., Cofala J., Klimont Z., Cozzi L. 2018. *Outlook for clean air in the context of sustainable development goals*. Glob. Environ. Change., 53: 1-11. https://doi.org/10.1016/j.gloenvcha.2018.08.008

Rehman M., Liu L., Wang Q., Saleem M.H., Bashir S., Ullah S., Peng D. 2019. *Copper environmental toxicology, recent advances, and future outlook: a review*. Environ. Sci. Pollut. Res., 26(18): 18003-18016. https://doi.org/10.1007/s11356-019-05073-6

Rizwan M., Meunier J.D., Davidian J.C., Pokrovsky O.S., Bovet N., Keller C., 2016. *Silicon alleviates Cd stress of wheat seedlings (Triticum turgidum* L. *cv. Claudio) grown in hydroponics*. Environ. Sci. Pollut. Res., 23: 1414-1427. https://doi.org/10.1007/s11356-015-5351-4

Saleem M.H., Fahad S., Khan S.U., Ahmar S., Khan M.H.U., Rehman M., Liu L., 2020*a*. *Morpho-physiological traits, gaseous exchange attributes, and phytoremediation potential of jute (Corchorus capsularis* L.*) grown in different concentrations of copper-contaminated soil*. Ecotoxicol. Environ. Saf., 189, 109915. https://doi.org/10.1016/j.ecoenv.2019.109915

Saleem M.H., Kamran M., Zhou Y., Parveen A., Rehman M., Ahmar S., Liu L. 2020*b*. *Appraising growth, oxidative stress and copper phytoextraction potential of flax (Linum usitatissimum* L.*) grown in soil differentially spiked with copper*. J. Environ. Manage., 257, 109994. https://doi.org/10.1016/j.jenvman.2019.109994

Saleem M.H., Rehman M., Kamran M., Afzal J., Noushahi H.A., Liu L. 2020*c*. *Investigating the potential of different jute varieties for phytoremediation of copper-contaminated soil. Environ*. Sci. Pollut. Res., 27(24): 30367-30377. https://doi.org/10.1007/s11356-020-09232-y

Sarwar N., Imran M., Shaheen M.R., Ishaque W., Kamran M.A., Matloob A., Hussain S. 2017. *Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives*. Chemosphere, 171: 710-721. https://doi.org/10.1016/j.chemosphere.2016.12.116

Saxena G., Purchase D., Mulla S.I., Saratale G.D., Bharagava R.N. 2020. *Phytoremediation of heavy metal-contaminated sites: eco-environmental concerns, field studies, sustainability issues, and future prospects*. Rev Environ Contam Toxicol, 249: 71-131. https://doi.org/10.1007/398_2019_24

Souri M.K., Hatamian M., Tesfamariam T. 2019. *Plant growth stage influences heavy metal accumulation in leafy vegetables of garden cress and sweet basil*. Chem. Biol. Technol. Agric., 6(1): 1-7. https://doi.org/10.1186/s40538-019-0170-3

Wang H., Wang J., Qiu C., Ye Y., Guo X., Chen G., Liu R.H. 2017. *Comparison of phytochemical profiles and health benefits in fiber and oil flaxseeds (Linum usitatissimum* L.*)*. Food Chem., 214: 227-233. https://doi.org/10.1016/j.foodchem.2016.07.075

Yadav S.K. (2010). *Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants.* S. Afr. J. Bot., 76(2): 167-179. https://doi.org/10.1016/j.sajb.2009.10.007

Yavuzer K., Akinay Y. 2021. *Microstructure and chemical analysis in pseudoexfoliation syndrome.* Curr. Eye Res, 46(4): 490-495. https://doi.org/10.1080/02713683.2020.1812088

Yruela I. 2005. *Copper in plants.* Braz. J. Plant Physiol., 17: 145-156.

Zainab N., Din B.U., Javed M.T., Afridi M.S., Mukhtar T., Kamran M.A., Chaudhary H.J. 2020. *Deciphering metal toxicity responses of flax (Linum usitatissimum* L.*) with exopolysaccharide and ACC-deaminase producing bacteria in industrially contaminated soils*. Plant Physiol. Biochem., 152: 90-99. https://doi.org/10.1016/j.plaphy.2020.04.039

Zehra A., Wani K.I., Choudhary S., Naeem M., Khan M.M.A., Aftab T. 2023. *Involvement of abscisic acid in silicon-mediated enhancement of copper stress tolerance in Artemisia annua*. Plant Physiol. Biochem., 195: 37-46. https://doi.org/10.1016/j.plaphy.2022.12.026

Zhang Z.S., Liu Y.L., Che, L.M. 2018. *Characterization of a new a-linolenic acid-rich oil: Eucommia ulmoides seed oil.* J Food Sci, 83(3).

Zwolak A., Sarzyńska M., Szpyrka E., Stawarczyk K. 2019. *Sources of soil pollution by heavy metals and their accumulation in vegetables: A review*. Water Air Soil Pollut, 230: 1-9. https://doi.org/10.1007/s11270-019-4221-y