

Effects of microplastic and copper applications on chlorophyll and carotenoid contents in kale and tomato

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Abstract: Microplastics (MP) are important pollutants observed almost everywhere in the ecosphere and threaten agricultural ecosystems due to modern agricultural practices. The MPs change the soil structure and affect plant growth. This study investigated individual and combined effects of MP and copper (Cu) on chlorophyll and carotenoid contents of tomato (*Lycopersicon esculentum* Mill.) and kale (*Brassica oleracea* var. *acephala* DC) plants. Experimental groups were treated with 100 ppm and 500 ppm CuSO₄ and 0.5%, 1.5% and 2.5% MP concentrations. The MPs were obtained by cutting polyethylene films. Chlorophyll a, b, total chlorophyll and carotenoid contents were measured spectrophotometrically and analyzed using one-way ANOVA. In tomato plants, 1.5% MP application and the combination of 500 ppm Cu and 1.5% MP decreased the chlorophyll a content by 3% compared to the control ($p<0.05$), while the combination of 100 ppm Cu and 0.5% MP increased it by 2.5%. In tomato, 100 ppm Cu and 0.5% MP application decreased the chlorophyll b and total chlorophyll contents by 29% and 14%, respectively, while other applications caused an increase ($p<0.05$). There was no significant difference in the carotenoid contents compared to the control group. In kale, chlorophyll a, total chlorophyll and carotenoid contents did not significantly vary compared to the control group, while 500 ppm Cu application increased the amount of chlorophyll b by 15% ($p<0.05$). The results showed that tomato was more sensitive to Cu and MP applications than kale. The response in plants varied depending on whether Cu and MP were applied individually or combined.

Keywords: Copper, kale, microplastic, mulch, tomato, stress.

Mikroplastik ve bakır uygulamalarının karalahana ve domatesteki klorofil ve karotenoid üzerine etkileri

Öz: Mikroplastikler (MP) ekosferin hemen her yerinde gözlenen önemli kirleticilerdir ve modern tarım uygulamaları nedeniyle tarımsal ekosistemleri de tehdit etmektedirler. MP'ler toprağın yapısını değiştirir ve bitki gelişimi etkiler. Bu çalışmada, mikroplastik (MP) ve bakır (Cu) uygulamalarının domates (*Lycopersicon esculentum* Mill.) ve karalahana (*Brassica oleracea* var. *acephala* DC) bitkilerinin klorofil ve karotenoid içerikleri üzerine ayrı ayrı ve kombin etkileri araştırılmıştır. Deney grupları 100 ppm ve 500 ppm CuSO₄ ve %0.5, %1.5 ve %2.5 konsantrasyonlarında MP ile muamele edilmiştir. mikroplastikler polietilen malç naylonunun kesilmesiyle elde edilmiştir. Klorofil a, b, toplam klorofil ve karotenoid içerikleri spektrofotometrik olarak ölçülmüş ve tek yönlü ANOVA kullanılarak analiz edilmiştir. Domates bitkilerinde %1,5 MP uygulaması ile 500 ppm Cu ve %1,5 MP kombinasyonu klorofil a içeriğini kontrole kıyasla %3 oranında azaltırken ($p<0,05$), 100 ppm Cu ve %0,5 MP kombinasyonu %2,5 oranında artırmıştır. Domatesteki 100 ppm Cu ve %0,5 MP uygulaması klorofil b ve toplam klorofil içeriklerini sırasıyla %29 ve %14 oranlarında azaltırken, diğer uygulamalar artışa neden olmuştur ($p<0,05$). Karotenoid miktarında ise kontrol grubuna kıyasla önemli bir farklılık olmamıştır. Karalahana klorofil a, toplam klorofil ve karotenoid miktarları kontrol grubuna kıyasla önemli bir farklılık göstermezken, 500 ppm Cu uygulaması klorofil b miktarını %15 oranında artırmıştır ($p<0,05$). Sonuçlar, domatesin, karalahana göre Cu ve MP uygulamalarına karşı daha duyarlı olduğu göstermektedir. Bitkilerde oluşan yanıt, Cu ve MP'in ayrı ayrı ya da birlikte uygulanmasına bağlı olarak değişmiştir.

Anahtar kelimeler: Bakır, domates, karalahana, malç, mikroplastik, stres

1. Introduction

Plastics can break down into smaller particles because of various activities. These tiny plastic particles are called microplastics. Microplastics refer to plastic particles <5 mm size, that result from the breakdown of plastics (Yurtsever, 2015). Microplastic pollution is now observed in almost every part of the ecosphere, which threatens agricultural ecosystems due to modern agricultural practices. The main factors that cause microplastic pollution in agro-ecosystems are pesticides, synthetic fertilizers, agricultural machinery, and mulch nylon used as cover material (Okeke et al., 2022). Microplastics (MPs) change the structure of the soil, the quality of the water, and the metabolic processes of organisms by creating a hydrophobic surface, carrying pollutants on them, absorbing organic pollutants, and creating a habitat for harmful microorganisms, and causing deterioration (Yurtsever, 2015; Prabhu et al., 2022). The MPs change soil properties and affect root structure, nutrient uptake, germination rates, chlorophyll content, seedling height, gene expression, and other processes in plants (Yu et al., 2020). The beneficial or harmful effects are related to the size and concentration of MPs (Zhang et al., 2019; Mondol et al., 2024). It has been reported that the size and concentration of MPs affect the adsorption and adsorption potential of other pollutants in soil (Huang et al., 2022). Copper (Cu), which is an essential element for plant growth and plays a role in lipid and carbohydrate metabolism, DNA and RNA production, enzyme activities, and resistance to diseases and pests, has a toxic effect at high concentrations (Vatansever et al., 2017). High concentrations of Cu may lead to tissue damage, pigment deficiency, decrease in leaf width, deterioration, and ion loss in the roots, DNA damage due to deterioration of the plant's membrane permeability, and negative effects on the photosynthesis reaction. Due to the toxic effects, it causes the disruption of the stomatal movements, photosynthesis, germination, protein synthesis, the hormonal and enzymatic balance (Yıldız et al., 2011; Yıldırım, 2022). Some studies reported that Cu increased the amount of chlorophyll a, chlorophyll b and total chlorophyll in plants (Syuhada et al., 2014; Sevgi, 2023), while others stated that they decreased it (Zong et al., 2021).

Studies showed that MPs pollution influences carotenoids and chlorophylls, which perform absorption of light energy during photosynthesis

(Okeke et al., 2022; Zang et al., 2022). Chlorophyll content in leaves is considered an important eco physiological feature in research as it gives an idea about the photosynthesis, above-ground biomass, and plant growth (Talebzadeh & Valeo, 2022). Chlorophyll content is affected and controlled by ecological factors in the surrounding environment such as climate conditions, soil characteristics, environmental pollution, and stress factors (Talebzadeh & Valeo, 2022). There are two different types of chlorophyll in plants and green algae, i.e., chlorophyll a and chlorophyll b that which both function as photoreceptors in photosynthesis. Chlorophyll a is found in higher amounts in plants, with a ratio of chlorophyll a to b of about 3:1 (Rajalakshmi & Banu, 2015). Carotenoids, which protect chlorophyll from intense sunlight, are another type of pigment in plants (Strzałka et al., 2003). The chlorophyll and carotenoid contents are a reliable indicator of the response of plants to stress levels (Talebzadeh & Valeo, 2022). It has been reported that chlorophyll content in plants decreases faster than carotenoid content under stress conditions (Talebzadeh & Valeo, 2022; Abbasi et al., 2023). Some of the studies reported that MPs pollution in soil increases or does not affect the chlorophyll and carotenoid contents (Zong et al., 2021; Naziri et al., 2023) while others stated that it decreases them (Abbasi et al., 2023).

The study aims to examine the impacts of MPs and Cu on the chlorophyll and the carotenoid contents in tomato (*Lycopersicon esculentum* Mill.) and kale (*Brassica oleracea* L. var. *acephala* DC.) plants. Tomato is an economically important crop used as food in all countries and widely cultivated worldwide (Peralta & Spooner, 2007). Tomato is a nutrient and lycopene rich summer vegetable and belongs to the Solanaceae family. According to FAO 2022 data, 186 million tons of tomatoes were produced in the world (Özmaya et al., 2025). Turkey is one of the leading countries in the world with 14.7 million tons of tomato production (Özmaya et al., 2025). Kale is a winter vegetable that is rich in vitamins and antioxidants and belongs to the Brassicaceae family. It is widely grown and consumed in the world and in Turkey because it has many positive effects on health. It was thought that the responses of the plants to the MPs and copper may be different when applied together or combined.

This study has attempted to fill the following scientific gap. Although numerous studies have investigated the

individual effects of MPs or heavy metals such as Cu on plant physiology, limited research has focused on their combined effects, particularly on photosynthetic pigments such as chlorophyll and carotenoids. Furthermore, comparative studies evaluating how different plant species (especially those with distinct seasonal and physiological characteristics like tomato and kale) respond to simultaneous MP and Cu exposure are scarce. This study aims to address this gap by analyzing how MPs and Cu, applied individually and together, influence chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in tomato and kale plants, thereby contributing valuable insight into species-specific responses to emerging soil pollutants. The results of the study are expected to provide valuable data for future studies, agriculture professionals, and scientists.

2. Materials and Methods

Tomatoes and kale have different ecological requirements and developmental stages, so they have been grown in different ways at different periods. Rio Grande tomato seeds and Black Sea leaf kale seeds were used to grow the tomato and kale seedlings. Polyethylene mulch was cut into 4×2.5 mm pieces to produce MPs (Xiao et al., 2024). The amount of MPs to be mixed into the soil was determined according to the literature (Wu et al., 2019; Meng et al., 2021). Seedlings were grown in a soil-peat mixture (1:1). The MPs were added to the soil-peat mixture at rates of 0%, 0.5%, 1.5%, and 2.5%. 12 different experimental sets were designed (Control, 100 ppm CuSO₄, 500 ppm CuSO₄, 100 ppm CuSO₄+0.5% MP, 100 ppm CuSO₄+1.5% MP, 100 ppm CuSO₄+2.5% MP, 500 ppm CuSO₄+0.5% MP, 500 ppm CuSO₄+1.5% MP, 500 ppm CuSO₄+2.5% MP) for each plant species. The experiment was set up according to a split plot design with 12 replicates. The pots were incubated for one week (Zhang et al., 2020). To sterilize, the seeds were soaked in a 20% Sodium hypochlorite (NaClO) solution for about 10 minutes, then rinsed with distilled water (Pinto et al., 2020). The sterilized seeds were sown into the pots and grown in the greenhouse. Tomato seedlings were watered with tap water during the germination phase. 20 ml of nitrogenous liquid organomineral fertilizer (containing 34% total organic matter, 8% total nitrogen, 3.2% nitrate nitrogen, 3.8% urea nitrogen, 1% organic nitrogen, 3% water-soluble calcium oxide (CaO), 2% water-soluble magnesium oxide (MgO), 0.5% water-

soluble boron (B), 5% free amino acid) was diluted with 10 L of water and used. The 100 and 500 ppm CuSO₄ solutions were applied to the relevant experimental sets after one month from germination. CuSO₄ solution was applied 4 times during the experiment. Tomato plants were harvested after 43 days. Kale seeds were germinated in viols and then transplanted into pots. Preparation of vials and pots, and planting of seeds were done as in tomatoes. Until the germination, each viol was watered with 5 ml of tap water every day. Lotufert Hector brand nitrogenous liquid organomineral fertilizer was used. After two weeks, the seedlings were placed in the pots. The kale seedlings were grown under standard room conditions for 3 weeks, then the pots were taken out to grow outdoors. The plants were protected from wind and rain by a device made of polyethylene greenhouse nylon. 100 and 500 ppm CuSO₄ solutions were applied four times to the kale plants during the growth period. After 110 days, the kale plants were harvested. Six of the 12 kale and tomato plants in each experimental setup were randomly selected to be used in chlorophyll analysis. 0.20 g of fresh tomato and kale leaves were weighed and turned into a solution by pounding in a mortar containing 2 ml of 96% acetone. The homogeneous solution, which was completed to 10 ml by adding 8 ml of acetone, was filtered through a blue band filter paper, and the absorbance values (A: 662, 652, 645, and 470 nm) were measured with the spectrophotometer. The chlorophyll a, b, total chlorophyll, and carotenoid contents were calculated according to Lichtenthaler & Wellburn (1983).

The IBM SPSS Statistic-20 statistical program was used to examine the data. Differences were determined by One-way Analysis of Variance (One-way ANOVA) as $P < 0.05$. The Levene test was applied to the dataset for homogeneity of variances. Post-hoc Tukey HSD test was applied to variables with homogeneous variances, and Thamhane's T2 test was applied to variables with non-homogeneous variances.

3. Results

3.1. Chlorophyll a content

Chlorophyll a content in tomato plants decreased gradually with the increase in Cu concentration, but this trend was not statistically significant (Figure 1). The MPs addition decreased the chlorophyll a content compared to the control group. While 1.5% MPs addition significantly decreased the chlorophyll a

($p \leq 0.05$), the decrease in chlorophyll a observed in 0.5% and 2.5% MPs additions was statistically non-significant. In the 100 ppm Cu + 0.5% MPs treatment, chlorophyll a content increased significantly compared to the control group. The amount of chlorophyll a decreased as MPs concentration increased. Chlorophyll a content decreased significantly in 500 ppm Cu + 1.5% MPs application, while 500 ppm Cu + 0.5% and 2.5% MPs applications showed similar trends to the control group. In tomato plants, 1.5% MPs application and the

combination of 500 ppm Cu and 1.5% MPs decreased the chlorophyll a content by 3% compared to the control ($p < 0.05$), while the combination of 100 ppm Cu and 0.5% MP increased it by 2.5%. In kale plants, although there was a slight decrease in chlorophyll a due to the increase in MPs concentration, chlorophyll a showed a similar trend in all experimental sets (Figure 2). The variation in chlorophyll a was not statistically significant.

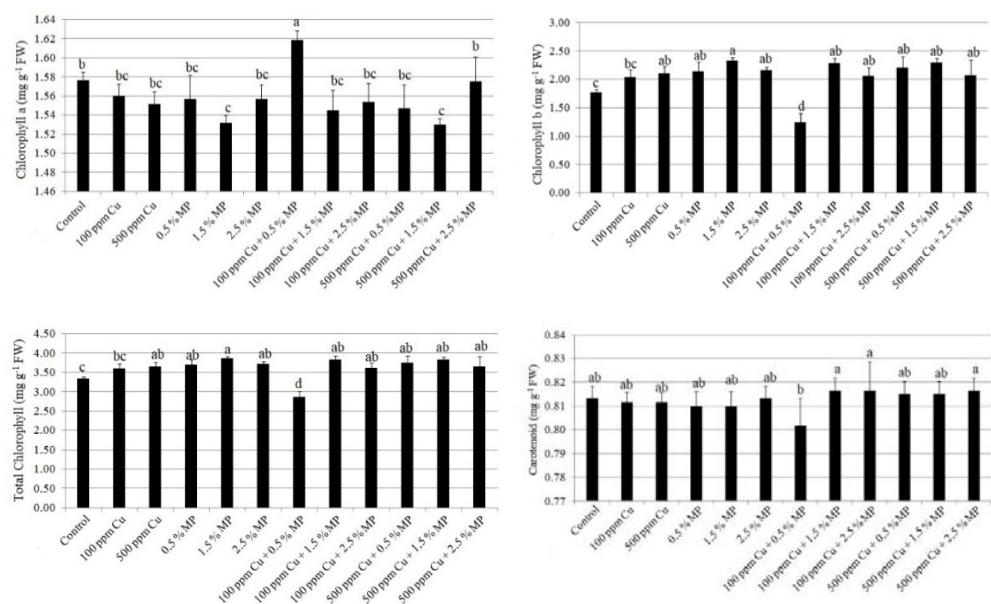


Figure 1. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents in tomato.

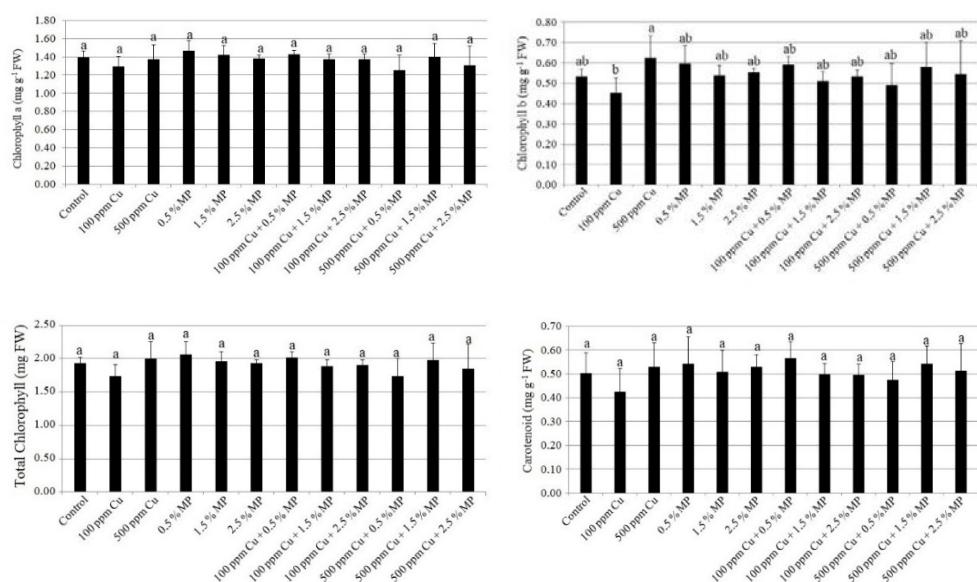


Figure 2. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents in kale

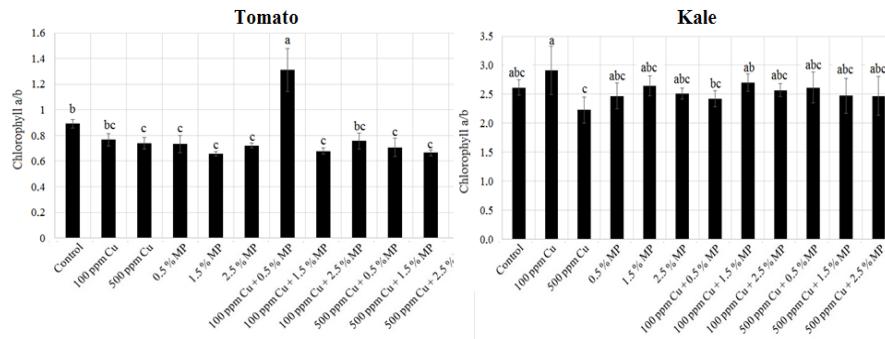


Figure 3. Chlorophyll a/b ratio in tomato and kale

3.2. Chlorophyll b content

The Cu applications significantly increased chlorophyll b content in tomato plants, whereas MPs applications increased chlorophyll b content compared to the control group (Figure 1). The increase in MPs concentration did not lead to a significant difference in chlorophyll b. The lowest chlorophyll b value (1.25 ± 0.15) was observed in 100 ppm Cu + 0.5% MPs applications, and the highest value (2.33 ± 0.05) was in 1.5% MPs applications. In 100 ppm Cu application, the increase in MP concentration caused an increase in chlorophyll b content. The amount of chlorophyll b increased compared to the control group, but the increase in MPs concentration did not cause any change in 500 ppm Cu applications. In tomato, 100 ppm Cu and 0.5% MPs application decreased the chlorophyll b contents by 29% ($p < 0.05$). In kale plants, chlorophyll b content increased with increased Cu concentration (Figure 2). Although there was a slight decrease in chlorophyll b content depending on MPs concentration, it was not statistically significant. In the experimental sets where 100 and 500 ppm Cu and MPs were applied together, no significant decrease or increase trend was observed in chlorophyll b content, and similar results were obtained with the control group. 500 ppm Cu application increased the amount of chlorophyll b by 15% ($p < 0.05$) in kale plants. Chlorophyll a/b ratios differed significantly among the experimental sets in both species (Figure 3).

3.3. Total chlorophyll content

Total chlorophyll content in tomato plants increased gradually with the increase in Cu concentration. The MPs applications increased the total chlorophyll content compared to the control group. The lowest total chlorophyll content (2.87 ± 0.14) was measured in the 100 ppm Cu + 0.5 MP applications. The increase in MPs concentration in 100 ppm Cu applications increased the

total chlorophyll content. In 500 ppm Cu + MP applications, total chlorophyll content increased compared to the control group, but the increase in MPs concentration did not cause a significant difference. The MPs and Cu stresses led to similar responses in tomato plants. In tomato, 100 ppm Cu and 0.5% MP application decreased the total chlorophyll contents 14% ($p < 0.05$). Non-significant variation was observed in total chlorophyll content in kale plants due to either MPs or Cu applications. Total chlorophyll content showed similar trends in all experimental sets. The lowest total chlorophyll content (1.74 ± 0.18) was observed in 100 ppm Cu applications, and the highest total chlorophyll content (2.07 ± 0.19) was observed in 0.5% MP applications. The increase in applied Cu caused an increasing trend in total chlorophyll content, while the increase in MPs concentration caused a decreasing trend. There was no significant difference in the combined Cu and MPs applications compared to the separate applications.

3.4. Carotenoid content

In tomato plants, Cu and MPs applications did not cause any significant variation in carotenoid content. The lowest carotenoid content (0.80 ± 0.01) was measured in the 100 ppm Cu + 0.5 MPs applications, and the highest carotenoid content (0.82 ± 0.01) was measured in the 100 ppm Cu + 2.5 MPs applications. In the 100 ppm Cu applications, the carotenoid content increased as the MPs concentration increased. In the 500 ppm copper applications, the carotenoid content was similar to that of the control group but increased as MP concentration increased. MP and Cu applications produced similar responses in tomato plants. The lowest carotenoid content in kale plants (0.43 ± 0.10) was measured in 100 ppm Cu application, while the highest carotenoid content (0.57 ± 0.07) was measured in 100 ppm Cu + 0.5% MPs application. The MPs

applications did not create a significant trend in carotenoid content. Cu and MP applications led to similar responses in kale plants.

4. Discussion

The results of the study showed that MPs and Cu applications are more effective on tomato plants compared with kale. The reason for choosing tomato and kale as research material was to obtain preliminary information on whether there could be a difference in the response to MP and Cu applications between summer and winter crops. The MPs and Cu applications did not cause any significant change in chlorophyll a in kale but caused significant variation in tomato. The Cu applications tended to reduce chlorophyll a content in tomato plants, whereas MPs addition altered this response. Similar to the results obtained from Cu applications in tomato, Yıldırım (2022) determined that the amount of chlorophyll a decreased as the Cu concentration increased in safflower. Yerli et al. (2020) reported that Cu has a toxic effect at high concentrations, disrupts the chloroplast structure, and causes a decrease in the amount of chlorophyll. In this case, chlorosis is observed in plant tissues, especially in leaves, due to the decreased amount of chlorophyll. The MPs applications alone reduced the amount of chlorophyll a. Low-dose MPs and Cu applications (100 ppm Cu + 0.5 % MPs) significantly increased the amount of chlorophyll a. It is thought that this increase may be due to the potential of MPs to improve soil properties to some extent, and Cu being a plant-available micronutrient element. The highest decrease was observed in the experimental sets where 1.5% MPs was applied. It is thought that this dose may be the threshold value concerning soil porosity and therefore has a more negative effect. While increasing the Cu concentration from 100 ppm to 500 ppm in 0.5% MPs applications caused a decrease in chlorophyll a content in tomato plants, it did not create a significant difference in other MPs doses. It was expressed that MPs lead to a reduction in plant growth and chlorophyll content in several plants (Okeke et al., 2022). The results showed that MP applications decreased the chlorophyll a content, increased the chlorophyll b and total chlorophyll content in tomato, while it did not cause any change in chlorophyll a, b and total chlorophyll in kale. In the study conducted by Zhang et al. (2022), which investigated the effect of MPs applications on chlorophyll content in tobacco plants,

the change trend in chlorophyll a, chlorophyll b and total chlorophyll contents were approximately in accordance with the results we obtained in tomato and kale. The MPs and Cu applications caused significant changes in chlorophyll b contents in both species. As Cu concentration increased in tomato plants, chlorophyll b content also increased gradually. The MPs applications also caused an increase in chlorophyll b. The increase in chlorophyll b due to MPs applications was greater than that in Cu applications. In combined MPs and Cu applications, chlorophyll b decreased significantly with 100 ppm Cu + 0.5% MP application, while the increase in both MP and Cu concentrations improved the chlorophyll b contents. Chlorophyll b content in kale plants increased with increasing Cu concentration, while the increase in MPs concentration caused a decreasing trend. However, the variations that emerged were not statistically significant. Non-significant change was observed in chlorophyll b in kale plants due to MPs applications, which is consistent with the results of Zang et al. (2022). Similar with to the results we obtained in tomato and kale, in the study of Boots et al. (2019), neither chlorophyll a nor chlorophyll b changed significantly due to MPs application. Combined MPs and Cu applications did not lead to significant variation or a notable trend in chlorophyll b of kale plants. The variation in total chlorophyll due to MP applications corresponded to the results reported by Zang et al. (2022). Although this coherence was broken due to the decrease in total chlorophyll based on MPs concentration in kale, the variation was not found to be statistically significant in both species as in the results of Zang et al (2022). While Cu and MPs applications significantly increased the total chlorophyll content in tomato, they did not cause a significant change in kale. While there was no significant change in the total chlorophyll content in kale in combined Cu and MPs applications, 100 ppm Cu + 0.5% MP caused a significant decrease in tomato. In addition, the total chlorophyll content increased due to the increase in Cu and MPs concentration. Except for the 100 ppm Cu + 0.5% MPs experimental set in tomato, the application of Cu and MPs separately or together did not cause any significant differences in total chlorophyll content in both species. The results suggest that the plant response may not be different even if the pollutant concentration increases after the limit value, which causes the response in the plant, is exceeded. Yıldırım (2022) reported that high concentrations of Cu caused

a decrease in the carotenoid content in safflower plants. In the study conducted by Zhuang et al. (2023) in cucumber (*Cucumis sativus L.*), PVC and PS significantly increased the chlorophyll a, chlorophyll b, and carotenoid contents. The increase observed in chlorophyll b due to the increase in MP concentration in tomato supports these findings. Similar to the results in kale, low polyethylene MPs applications did not cause any significant difference in chlorophyll a, chlorophyll b, and carotenoid contents in cucumber compared to the control group. When MPs concentration increased, carotenoid content also increased, but there was no significant change in other pigments. In this study conducted with different MPs types in cucumber, it was determined that polyethylene MPs were less effective on photosynthetic pigments. Similarly, Lian et al. (2021) determined that the application of polystyrene MPs decreased chlorophyll a (9.1%), chlorophyll b (8.7%), and carotenoids (12.5%) in lettuce (*Lactuca sativa L.*). These findings are supported only by our results for chlorophyll a in tomato. The difference between the results of the studies may be caused by the type and concentration of MPs as well as the plant type (Jia et al., 2023). The chlorophyll a/b ratio is a key parameter for photosynthetic activity, which is widely used as an indicator of stress in plants (Boots et al., 2019). In this study, Cu and MP applications did not cause a significant change in the chlorophyll a/b ratio in kale plants compared to the control group. In tomato, it was determined that as Cu concentration increased, the chlorophyll a/b ratio decreased, and MPs also caused a decrease, but the increase in MPs concentration did not create a significant difference. Mondal et al. (2022) suggested that a higher chlorophyll (a/b) ratio occurs due to inhibition of chlorophyll b synthesis under MPs stress, and the decrease in photosynthesis may be due to decreased chlorophyll b production (Jia et al., 2023). It was stated that the decrease observed in chlorophyll a/b ratios in experiments conducted with wheat in stress conditions was due to the increase in chlorophyll b ratio in the structure of the chlorophyll complex, which increased photosynthetic activity (Trukhachev et al., 2022). Similar results to Cu and MPs applications in tomato, where chlorophyll a decreased while chlorophyll b increased and therefore low chlorophyll a/b ratios were detected, were also observed in some wheat varieties under stress by Trukhachev et al. (2022). This suggests that when chlorophyll a

decreases due to stress, photosynthesis is tried to be balanced by increasing chlorophyll b. Studies showed that Cu and MPs applications to the soil usually lead to stress (Jia et al., 2023). Some of the studies expressed that MPs may enhance soil conditions for plants by providing more soil pores, water, and oxygen (Mondol et al., 2024). However, it has also been reported that excessive amounts of microplastics can block soil pores and cause water stress and since MPs are hydrophobic, they prevent water from being retained in the soil (Wang et al., 2024). Previous studies have reported that when the plant is exposed to stress such as heavy metal stress, chlorophyll synthesis is disrupted due to the inhibition of enzymes that play a role in these processes, and chlorophyll and carotenoid production are negatively affected (Pignattelli et al., 2020). Jia et al. (2023) reported that MPs stress reduced chlorophyll by causing the conversion of chlorophyll to phytol (Xu et al., 2022) and high exfoliation of the oxygen evolution complex. In agricultural areas where plastic mulch was used for 32 years, 2.2–3.7 million microplastic particles per kilogram were detected in the soil (Li et al., 2022). The most detected polymer was polyethylene, and these particles spread to a depth of 0–100 cm (Zhang and Liu, 2018)

5. Conclusion

The results indicated that both Cu and MPs applications were effective on photosynthetic pigments in tomato and kale plants. However, the responses of these two plants to Cu and MPs applications were different. Significant variations were determined in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in tomato and only chlorophyll b contents in kale among the experimental sets, depending on Cu and MPs applications. Chlorophyll a/b ratios differed significantly among the experimental sets in both species. Although Cu and MPs stresses caused similar effects, tomato plants were more sensitive to Cu and MP applications than kale plants. The response of the plants varied depending on whether Cu and MP were applied separately or together. As more comprehensive studies are conducted with different plant species in different ecological conditions, more definitive conclusions will be reached.

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Conflict of interest

The authors declare no conflicts of interest.

Authorship contribution statement

B.Ç.: Data collection, laboratory work, article writing.
N.K.: Planning, editing, article writing.

References

Abbasi, S., Amiranipour, S., Karimi, J., Mohsenzadeh, S., & Turner, A. (2023). Impacts of polyethylene microplastics on the microalga, *Spirulina (Arthrosphaera platensis)*. *Environmental Pollution*, 327, 121611. <https://doi.org/10.1016/j.envpol.2023.121611>

Boots, B., Russell, C. W., & Green, D. S. (2019). Effects of microplastics in soil ecosystems: above and below ground. *Environmental Science & Technology*, 53(19), 11496-11506. <https://doi.org/10.1021/acs.est.9b03304>

Huang, D., Wang, X., Yin, L., Chen, S., Tao, J., Zhou, W., ... & Xiao, R. (2022). Research progress of microplastics in soil-plant system: ecological effects and potential risks. *Science of the Total Environment*, 812, 151487. <https://doi.org/10.1016/j.scitotenv.2021.151487>

Jia, L., Liu, L., Zhang, Y., Fu, W., Liu, X., Wang, Q., Tanveer, M., & Huang, L. (2023). Microplastic stress in plants: effects on plant growth and their remediations. *Frontiers in Plant Science*, 14, 1226484. <https://doi.org/10.3389/fpls.2023.1226484>

Li, C., Wang, F., Zhang, W., Liu, Y., & Wang, H. (2022). Long-term plastic film mulching causes a significant accumulation of microplastics in agricultural soils. *Science of the Total Environment*, 806, 150500. <https://pubmed.ncbi.nlm.nih.gov/35122919>

Lian, J., Liu, W., Meng, L., Wu, J., Chao, L., Zeb, A., & Sun, Y. (2021). Foliar-applied polystyrene nanoplastics (PSNPs) reduce the growth and nutritional quality of lettuce (*Lactuca sativa* L.). *Environmental Pollution*, 280, 116978. <https://doi.org/10.1016/j.envpol.2021.116978>

Lichtenthaler K, Welburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans* 11:591-592.

Meng, F., Yang, X., Riksen, M., Xu, M., & Geissen, V. (2021). Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. *Science of the Total Environment*, 755(2), 142516. <https://doi.org/10.1016/j.scitotenv.2020.142516>

Mondal, N. K., Kundu, S., Debnath, P., Mondal, A., & Sen, K. (2022). Effects of polyethylene terephthalate microplastic on germination, biochemistry and phytotoxicity of *Cicer arietinum* L. and cytotoxicity study on *Allium cepa* L. *Environ. Toxicol. Pharmacol.* 94, 103908. <https://doi.org/10.1016/j.etap.2022.103908>

Mondol, M., Angon, P. B., & Roy, A. (2024). Effects of microplastics on soil physical, chemical and biological properties. *Nat. Hazards Res.*, 5(1), 14-20. <https://doi.org/10.1016/j.nhres.2024.02.002>

Naziri, A., Mina, T., Manoli, K., Beretsou, V. G., Christou, A., Michael, C., Agathokleous, E., & Fatta-Kassinos, D. (2023). Looking into the effects of co-contamination by micro (nano) plastics in the presence of other pollutants on irrigated edible plants. *Science of The Total Environment*, 892, 164618. <https://doi.org/10.1016/j.scitotenv.2023.164618>

Okeke, E. S., Okoye, C. O., Atakpa, E. O., Ita, R. E., Nyaruaba, R., Mgbechidinma, C. L., & Akan, O. D. (2022). Microplastics in agroecosystems-impacts on ecosystem functions and food chain. *Resources, Conservation and Recycling*, 177, 105961. <https://doi.org/10.1016/j.resconrec.2021.105961>

Özmayra, M., Paksoy, M., Kayak, N., & Mutlu, A. (2025). Türkiye ve Kırgızistan'dan derlenen bazı yerel soframış domates genotiplerinin morfolojik olarak incelenmesi. *Anadolu Journal of Agricultural Sciences/Anadolu Tarım Bilimleri Dergisi*, 40(1), 73-85.

Pignattelli, S., Broccoli, A., & Renzi, M. (2020). Physiological responses of garden cress (*L. sativum*) to different types of microplastics. *Science of the Total Environment*, 727, 138609. <https://doi.org/10.1016/j.scitotenv.2020.138609>

Pinto, A. C. R., Demattê, M. E. S. P., Creste, S., & Barbosa, J. C. (2011, September). Seed and seedling surface-sterilization for in vitro culture of *Tillandsia gardneri* (Bromeliaceae). In VII International Symposium on In Vitro Culture and Horticultural Breeding 961 (pp. 383-389). <https://doi.org/10.17660/ActaHortic.2012.961.50>

Prabhu, P. P., Pan, K., Krishnan, J. N. (2022). Microplastics: Global occurrence, impact, characteristics and sorting. *Frontiers in Marine Science*, 9, 893641. <https://doi.org/10.3389/fmars.2022.893641>

Rajalakshmi, K., & Banu, N. (2015). Extraction and estimation of chlorophyll from medicinal plants. *International Journal of Science and Research*, 4(11), 209-212.

Sarica, V. and Özbay, G. (2023). Karadeniz Bölgesi'nin yenilebilir otlarının fonksiyonel gıdalar kapsamında değerlendirilmesi. *Adiyaman Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*, (44), 759-798. <https://doi.org/10.14520/adyusbd.1179582>

Sevgi, K. (2023). Kurşun ve bakır uygulamasının *Cucurbita moschata* Duch.'nın bazı ekofizyolojik parametreleri ve antioksidan savunma sistemi üzerine etkileri [Yayınlanmamış yüksek lisans tezi]. *Bilecik Şeyh Edebali Üniversitesi*.

Strzalka, K., Kostecka-Gugała, A., & Latowski, D. (2003). Carotenoids and environmental stress in plants: significance of carotenoid-mediated modulation of membrane physical properties. *Russian Journal of Plant Physiology*, 50, 168-173. <https://doi.org/10.1023/A:1022960828050>

Syuhada, N., Jahan, M. S., Khandaker, M. M., Nashriyah, M., Khairi, M., Nozulaidi, M., & Razali, M. B. (2014). Application of

copper increased corn yield through enhancing physiological functions. *Aust J Basic Appl Sci*, 8(16), 282-6.

Talebzadeh, F., & Valeo, C. (2022). Evaluating the effects of environmental stress on leaf chlorophyll content as an index for tree health. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1006, No. 1, p. 012007). IOP Publishing.

Trukhachev, V. I., Seregina, I. I., Belopukhov, S. L., Dmitrevskaya, I. I., Fomina, T. I., Zharkikh, O. A., & Akhmetzhanov, D. M. (2022). The effect of stressful ecological conditions on chlorophyll content in the leaves of spring wheat plants. In *IOP Conference Series: Earth and Environmental Science* (Vol. 981, No. 3, p. 032093). IOP Publishing.

Vatansever, R., Ozyigit, I. I., & Filiz, E. (2017). Genome-Wide Identification and Comparative Analysis of Copper Transporter Genes in Plants. *Interdisciplinary Sciences: Computational Life Sciences*, 9, 278-291. <https://doi.org/10.1007/s12539-016-0150-2>

Wu, Y., Guo, P., Zhang, X., Zhang, Y., Xie, S., & Deng, J. (2019). Effect of microplastics exposure on the photosynthesis system of freshwater algae. *Journal of Hazardous Materials*, 374, 219-227. <https://doi.org/10.1016/j.jhazmat.2019.04.039>

Xiao, X., Sallach, J. B., & Hodson, M. E. (2024). Microplastics and metals: Microplastics generated from biodegradable polylactic acid mulch reduce bioaccumulation of cadmium in earthworms compared to those generated from polyethylene. *Ecotoxicology and environmental safety*, 282, 116746. <https://doi.org/10.1016/j.ecoenv.2024.116746>

Xu, Z., Zhang, Y., Lin, L., Wang, L., Sun, W., Liu, C., Yu, G., Yu, J., Lv, Y., Chen, J., Chen, X., Fu, L., & Wang, Y. (2022). Toxic effects of microplastics in plants depend more by their surface functional groups than just accumulation contents. *Sci. Total Environ.* 833, 155097. <https://doi.org/10.1016/j.scitotenv.2022.155097>

Yerli, C., Çakmakçı, T., Sahin, U., & Tüfenkçi, Ş. (2020). The Effects of Heavy Metals on Soil, Plant, Water and Human Health. *Tr. J. Nature Sci.*, 9(special issue), 103-114.

<https://doi.org/10.46810/tdfd.718449>

Yıldırım, N. (2022). Possible effects of heavy metal (Cu) and simulated acid rain stresses on mRNA expression levels of FAD2 gene responsible for linoleic acid transduction of oleic acid and some ecophysiological traits in safflower (*Carthamus tinctorius* L.) [Unpublished master's thesis]. Amasya University.

Yıldız, M., Terzi, H., & Uruşak, B. (2011). Bitkilerde Krom Toksisitesi ve Hücresel Cevaplar. *Erciyes Üniversitesi, Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi*, 27(2):163-176.

Yu, H., Hou, J., Dang, Q., Cui, D., Xi, B., & Tan, W. (2020). Decrease in bioavailability of soil heavy metals caused by the presence of microplastics varies across aggregate levels. *J. Hazard. Mater.*, 395, 122690. <https://doi.org/10.1016/j.jhazmat.2020.122690>

Yurtseven, M. (2015). Mikroplastikler'e Genel Bir Bakış. *Dokuz Eylül Üniversitesi Mühendislik Fakültesi Fen ve Mühendislik Dergisi*, 17(50), 68-83.

Zhang, G. S., & Liu, Y. F. (2018). The distribution of microplastics in soil aggregate fractions in southwestern China. *Science of The Total Environment*, 642, 12-20. <https://doi.org/10.1016/j.scitotenv.2018.06.004>

Zhang, S., Gao, W., Cai, K., Liu, T., & Wang, X. (2022). Effects of Microplastics on Growth and Physiological Characteristics of Tobacco (*Nicotiana tabacum* L.). *Agronomy*, 12(11), 2692. <https://doi.org/10.3390/agronomy12112692>

Zhuang, H., Liu, X., Ma, H., Li, R., Liu, B., Lin, Z., & Li, Z. (2023). Growth and physiological–biochemical characteristics of cucumber (*Cucumis sativus* L.) in the presence of different microplastics. *Arabian Journal of Geosciences*, 16, 194. <https://doi.org/10.1007/s12517-023-11273-9>

Zong, X., Zhang, J., Zhu, J., Zhang, L., Jiang, L., Yin, Y., & Guo, H. (2021). Effects of polystyrene microplastic on uptake and toxicity of copper and cadmium in hydroponic wheat seedlings (*Triticum aestivum* L.). *Ecotoxicology and Environmental Safety*, 217, 112217. <https://doi.org/10.1016/j.ecoenv.2021.112217>