

Commagene Journal of Biology

Çil & Karavin, (2025) *Comm. J. Biol.* 9(2), 179-186. DOI: 10.31594/commagene.1658960

e-ISSN 2602-456X

Research Article/Araştırma Makalesi

The Effects of Microplastic and Copper Treatments on the Number of Leaves, Stem, and Root Diameters in Tomato and Kale

Büşra ÇİL¹, Neslihan KARAVİN*²

¹Amasya University, Institute of Science, Amasya, TÜRKIYE ²Amasya University, Arts and Science Faculty, Amasya, TÜRKIYE

ORCID ID: Büşra ÇİL: https://orcid.org/0000-0003-0593-6820; Neslihan KARAVİN: https://orcid.org/0000-0002-7603-3832

Received: 16.03.2025 **Accepted:** 09.09.2025 **Published online:** 30.09.2025 **Issue published:** 31.12.2025

Abstract: Microplastics and heavy metals, which originated from irrigation water, fertilizers, pesticides, vehicles, and agricultural processes such as cover material treatments, are significant pollutants in agricultural ecosystems. Their harmful effects pose a threat to the health of organisms and ecosystems. This study aimed to determine the effects of microplastic and copper treatments on the number of leaves stem and root diameter in tomato *Lycopersicum esculentum* L.) and kale (*Brassica oleraceae* L. var. *acephala* DC.) plants. Microplastics were obtained by cutting agricultural mulch, used as a cover material, into small pieces (2.5 mm-4 mm) using scissors. Twelve experimental sets were created using microplastic concentrations of 0%, 0.5%, 1.5%, and 2.5%, along with copper sulphate (CuSO4) at concentrations of 100 ppm and 500 ppm. The number of leaves, the stem and root diameters of the plants were measured. While the number of leaves, stem and root diameters in tomato plants, as well as the stem and root diameters in kale plants, did not significantly vary with microplastic and copper (Cu) treatments, significant differences were observed in the number of leaves in kale plants. The maximum and minimum leaf numbers were observed in the control group and the experimental set where 500 ppm CuSO4 + 0.5% microplastic was applied, respectively. Different results emerged when microplastics and Cu were applied separately and together. These findings highlight the need for future studies that explore the long-term effects of microplastics and heavy metals on various plant species under different environmental conditions and using multiple physiological and biochemical parameters.

Keywords: Brassica oleraceae L. var. acephala DC, crop, Lycopersicum esculentum L., pollution, soil.

Mikroplastik ve Bakır Uygulamalarının Domates ve Karalahanada Yaprak Sayısı, Gövde ve Kök Çapı Üzerine Etkileri

Öz: Sulama suyu, gübreler, pestisitler, araçlar ve örtü materyali uygulamaları gibi tarımsal işlemlerden kaynaklanan mikroplastikler ve ağır metaller, tarımsal ekosistemlerde önemli kirleticilerdir. Zararlı etkileri organizmaların ve ekosistemlerin sağlığı için tehdit oluşturmaktadır. Bu çalışmada, mikroplastik ve bakır uygulamalarının domates (*Lycopersicum esculentum* L.) ve lahana (*Brassica oleraceae* L. var. *acephala* DC.) bitkilerinde yaprak sayısı gövde vekök çapı üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Mikroplastikler, örtü malzemesi olarak kullanılan tarımsal malçın makas kullanılarak küçük parçalara (2.5 mm-4 mm) kesilmesiyle elde edilmiştir. %0, %0.5, %1.5 ve %2.5 mikroplastik ile 100 ppm ve 500 ppm konsantrasyonlarında bakır sülfat (CuSO4) kullanılarak on iki deney seti oluşturulmuştur. Domates ve lahana bitkilerinin yaprak sayısı ve gövde/kök çapları ölçülmüştür. Bulgular, mikroplastik ve bakır (Cu) uygulamalarının domates ve lahana bitkilerinde gövde çapı ve kök çapı üzerinde önemli bir değişikliğe neden olmadığını göstermiştir. Bununla birlikte, yaprak sayısı mikroplastik ve bakır (Cu) uygulamalarına bağlı olarak lahana bitkisinde önemli ölçüde değişirken, domates bitkisinde önemli bir değişim olmamıştır. Lahana bitkisinde en fazla yaprak kontrol grubunda, en az yaprak 500 ppm CuSO4 + %0.5 mikroplastik uygulanan deney setinde tespit edilmiştir. Mikroplastik ve Cu ayrı ayrı ve birlikte uygulandığında elde edilen bulgular farklılık göstermiştir. Sonuçlar, mikroplastik ve ağır metallerin bitkiler üzerindeki etkilerini farklı koşullarda araştıracak kapsamlı çalışmalara gereksinim olduğunu ortaya koymaktadır.

Anahtar kelimeler: Brassica oleraceae L. var. acephala DC, mahsul, Lycopersicum esculentum L., kirlilik, toprak.

1. Introduction

In recent years, the health of living organisms and ecosystems has been threatened due to the increasing severity of environmental pollution derived from anthropogenic activities. Intensive industrial activities based on technological developments and population growth have greatly increased the diversity and concentration of environmental pollutants (Ofoezie et al., 2022). Microplastics (MPs) and heavy metals are important pollutants whose harmful effects are better understood thanks to the studies conducted in recent years (Zhou et al., 2019; Kajal & Thakur, 2024).

Plastics, whose use is increasing daily due to their practical and economical nature, cause permanent

pollution because of their long decomposition process in nature. Through transformation processes, biotic and abiotic weathering, plastics break down into small particles and create invisible pollution in all ecosystems (Lin et al., 2022; Megha et al., 2025). Plastic particles smaller than 5 mm are called MP (Koelmans et al., 2022; Guo et al., 2025). Due to their small size and light weight, they can easily be transported and dispersed in any environment. In the hadal sediments of the Mariana Trench, known as the deepest place in the world's ocean, 200-2200 MPs per litter were detected (Peng et al., 2018). At an altitude of 8.440 miles on Mount Everest, the highest point in the world, Napper et al. (2020) estimated 1-30 MPs per litter, likely originating from the climbers' clothing. Allen et al. (2019) determined that MPs can be transported

up to 95 km and thus reach even isolated settlements through atmospheric transport. MPs facilitate the transport of environmentally harmful substances, including organic pollutants, heavy metals, and human pathogens. For this reason, in the United Nations Environment Programme (UNEP), it was reported that MPs are among the top 10 contributors to environmental pollution (Desai, 2015).

MPs are classified into two groups based on their formation (Rezaie & Forghani, 2022). Microbead-shaped MPs found in cosmetic products are called primary MPs (Waldman & Rillig, 2020). Secondary MPs are generated when plastics undergo physical, chemical, or biological degradation into smaller pieces due to factors such as pH changes, heat, and especially sunlight (Nasseri & Azizi, 2022). Since secondary MPs result from the breakdown of macroplastics, they are more abundant in nature than primary MPs (Waldman & Rillig, 2020).

MPs also pose a threat as pollutants in agricultural ecosystems. Irrigation water containing plastic, pesticides, fertilizers, and cover material (mulch) treatments are the main factors that cause MP pollution in agricultural ecosystems (Wang et al., 2019; Jin et al., 2022). The mulch treatments used in the cultivation of crops such as tomato, strawberry, maize, sunflower, and watermelons have significantly increased microplastic pollution in agricultural soils in recent years (Adamczewska-Sowińska et al., 2025). Research highlighting the potential issues arising from soil contamination with microplastics in agricultural areas (Jin et al., 2022; Yu et al., 2022) emphasizes the need for further studies on this topic.

Studies on MPs have mostly focused on aquatic ecosystems because they are easier to study (Bakir et al., 2012; Rillig, 2012; Sun et al., 2019). There are few studies on the effects of MPs in terrestrial ecosystems and therefore, their impact is poorly understood (Azeem et al., 2021; Chia et al., 2021; Colzi et al., 2022). Plant growth and production are also affected by MP pollution because of the contamination of soils by MPs.

For example, Wu et al. (2020) exposed *Oryza sativa* L. to polystyrene MP stress and after 21 days, shoot biomass decreased by 13.1%, 18.8%, and 40.3% in low, medium, and high-concentrations treatments, respectively. Li et al. (2020a) applied polyvinyl chloride (PVC) MP particles with sizes ranging from 100 nm to 18 µm at concentrations of 0.5%, 1%, and 2% to the root systems and leaves of lettuce. MPs did not cause any changes in root activity. However, the total length, surface area, volume, and diameter of the roots increased. Khalid et al. (2020) found that MPs directly affect plants by clogging seed pores, limiting nutrient uptake in water and soil, and causing plastic accumulation in leaves, roots, and stems. de Souza Machado et al. (2019) investigated the effects of six different MPs on spring onion (Allium fistulosum L.) and observed significant changes in plant biomass, tissue element composition, and root properties.

Ullah et al. (2021) found that MP accumulation in soil harms agricultural production by negatively affecting plants. It was observed that the number of leaves, leaf area, and stem diameter changed in response to MP treatments in wheat and grass (Qi et al., 2018; Boots et al., 2019).

Heavy metals such as Pb, Cu, Mn, Fe, and Cd are classified as a group of metals with a density greater than 5 g/cm³ and an atomic number greater than 20 (Apaydın, 2005; Yücel Tartan, 2023). The concentrations of heavy metals in water and soil are typically low but they increase due to human activities such as mineral extraction, energy production, domestic and industrial waste, agricultural processes as well as geological and volcanic activities, erosion, and fires (Akyıldız & Karataş, 2018). While heavy metals found in nature are essential for living organisms in small amounts, they can become toxic at higher concentrations (Sharma & Agrawal, 2005).

Copper (Cu) is a micronutrient required by plants for chlorophyll production, protein synthesis, respiration, DNA and RNA regulation as well as the functioning of respiration, photosynthesis, cell wall metabolism, and the activation of various oxidase enzymes (Okcu et al., 2009; Bolat & Kara, 2017). High copper concentrations in plants can lead to tissue damage, pigment deficiency, reduced leaf width, and deterioration of plant roots. Negative effects on photosynthesis may occur due to ion loss and DNA damage in the roots, resulting from the deterioration of the plant's membrane permeability (Yıldırım, 2022). Toxic levels of copper disrupt anabolic processes in plants such as stomatal movement, photosynthesis, germination, protein synthesis, and the hormonal and enzymatic balance (Yruela, 2005; Yıldırım, 2022). Cai et al. (2014) reported that high concentrations of Cu lead to changes in root morphology. Marques et al. (2018) explained that elevated copper concentrations negatively affected the stem and root diameters of Hymenaea courbaril L. plants and that there was a decrease in the vascular cylinder area and cortex thickness in terms of root anatomy.

This study aimed to determine the effects of MPs and Cu treatments on the number of leaves, stem and root diameters in tomato (*L. esculentum*) and kale (*B. oleraceae L.* var. *acephala* DC.), two commonly produced vegetables worldwide. Tomato is a summer vegetable from the Solanaceae family, widely cultivated both worldwide and in Türkiye due to its taste, nutritional value, and diverse uses (Peralta & Spooner, 2007). Tomato is a rich source of lycopene and, therefore, an excellent antioxidant (Durmuş et al., 2018). Kale is a cold-climate winter vegetable from the Brassicaceae family, rich in chlorophyll and betacarotene and abundant in antioxidants, vitamins, and minerals (Ayaz et al., 2008; Alibas & Okursoy, 2012).

MPs and copper sulphate (CuSO₄) were applied to the plants both separately and together. It was hypothesized that plant responses to MPs and copper might differ when applied together compared to when applied separately. Therefore, the effects of MP pollution, especially from mulch treatment and copper, which is found in many pesticides and accumulates to high concentrations in soil, were assessed based on the number of leaves, stem and root diameters, which are indicators of plant development. The data obtained from this study is expected to provide valuable information for professionals in the fields of agriculture, ecology, and botany.

2. Material and Method

2.1. Materials

Tomato seedlings were produced from Rio Grande tomato

seeds obtained from the Yalova Atatürk Horticultural Central Research Institute. Black Sea leaf cabbage kale seeds (TARMEN Tohumculuk, Balıkesir, Türkiye) were used to grow the kale seedlings. MPs were obtained by cutting the mulch (0.4 μm thick polyethylene), which is used as a cover material in agriculture, into small pieces $2.5\ to\ 4\ mm$ in size by scissors (Ren et al., 2021). The MP rates applied to the soil were determined based on a literature review (de Souza Machado et al., 2018; Wu et al., 2019; Li et al., 2020b; Meng et al., 2021). Four different concentrations of MPs (0%, 0.5%, 1.5%, and 2.5%) were added to the growing environment of the plants. The 1:1 soil-peat mixture was used as a growing environment for the plants. The soil was taken from the field at a depth of 0-25 cm, air-dried, and sieved through a 4 mm sieve. Peat was added to the field soil at a volume of 1:1. According to the determined MP concentrations, MPs were added to the soil-peat mixture.

2.2. Experimental sets

This study was conducted to determine the effects of microplastic (MP) and copper (Cu) treatments on the number of leaves, stem and root diameters in tomato and kale plants, 12 different experimental sets were designed (Table 1) for each plant species. The experimental sets were arranged according to a strip plot design. For each experimental set, 12 plants were used (replicated), totalling 144 tomato and 144 kale plants.

Table 1. Experiment sets

Control
100 ppm CuSO ₄
500 ppm CuSO ₄
0.5% MP
1.5% MP
2.5% MP
100 ppm CuSO ₄ + 0.5% MP
100 ppm CuSO ₄ + 1.5% MP
100 ppm CuSO ₄ + 2.5% MP
500 ppm CuSO ₄ + 0.5% MP
500 ppm CuSO ₄ + 1.5% MP
500 ppm CuSO ₄ + 2.5% MP

2.3. Cultivation of plants

The cultivation of the tomato plants was carried out in the greenhouse located in the garden of Amasya University Suluova Vocational School. The pots were watered with tap water, considering the field capacity, and left to incubate for one week (Zhang et al., 2020). The surface sterilization of the seeds was done by being soaked in a 20% NaClO (Sodium hypochlorite) solution for 10 minutes, then washed with distilled water 5 times (Pinto et al., 2011). Tomato and kale seeds were sown in pots. All pots were watered daily with 10 mL of tap water until germination was completed. After germination, tomato seedlings were watered with 100 ml of tap water every other day. When plant growth slowed down, the pots were watered with a solution prepared with 20 mL nitrogenous liquid organomineral fertilizer (Lotufert Hector, Lotus Tohumculuk ve Gübre San. A.Ş., Antalya, Türkiye) for 10 litters of water. After the seedlings reached the desired size (1 month after germination), 100 and 500 ppm CuSO₄

solutions started to be applied to the relevant experimental sets. The control pots and the pots containing only MPs were watered with tap water and the other pots were watered with the determined concentrations of CuSO₄ solution. CuSO₄ solution was applied 4 times during the experiment. Tomato plants were harvested 43 days after sowing seeds.

To increase the survival rate of kale plants, kale seeds were germinated in viols and then transplanted into pots. For this purpose, field soil and peat were mixed in a volumetric ratio of 1:1. MPs were added to soil-peat mixtures at 0%, 0.5%, 1.5%, and 2.5% rates. The viols and the pots were filled with soil-peat or soil-peat-MP mixtures according to the experiment sets. Each of the viols was watered with 20 mL of tap water and incubated for one week. Then, the surface sterilization of the kale seeds was done, and the seeds were sown to the viols. Each viol was watered with 5 mL of tap water every day until germination. After 8 days, the kale seeds had germinated, and the seedlings were watered with tap water for approximately 10 days. Then, the seedlings were watered consistently every day with a solution of Lotufert Hector brand nitrogenous liquid organomineral fertilizer, once with tap water and once with 30 ml of fertilized water. After the seedlings matured (2 weeks later), they were placed in the pots, which were watered with 400 mL of tap water and incubated for one week before. The kale seedlings were grown under standard room conditions. After 3 weeks, the pots were taken out to grow outdoors. The side facing the outside is covered with a transparent nylon cover to prevent them from being affected by rain. According to the experimental sets, 4 concentrations of 100 and 500 ppm CuSO₄ solution were applied to the kale plants. The kale plants were harvested 110 days after sowing seeds.

Fungicides and insecticides used in tomato and kale cultivation to prevent diseases and herbivore attacks were applied to each experimental set and plant individual at equal concentrations and times to ensure they did not interfere with the effects of microplastic and Cu treatments.

2.4. Measurements of plants

The number of leaves of the plants was determined by counting the leaves emerging from the main branch. The stem diameters of the plants were measured using millimetric paper based on 1 cm above the point of emergence from the soil. Plant root diameters were calculated by rotating the first part of the plants underground around the root with millimetric paper.

2.5. Statistical Analysis

The data were tried to be determined by calculating the standard deviation values, mean values, and statistical significance levels (*P*≤0.05) using the IBM SPSS Statistic-20 statistical program. Differences between the experimental groups were determined by using the One-way ANOVA test, Tukey and Tamhane post-hoc tests. Relationships between parameters were examined by Pearson's Correlation analysis.

3. Results and Discussion

Supplying healthy and sufficient food is one of the most

important threats of today and the future. The increase in environmental pollution in recent years has required serious focus on this problem. Revealing the effects of environmental pollution through studies guides solution suggestions. In this study, artificial pollution was created by applying MP and Cu to the soil and it was tried to determine how the number of leaves, root and stem diameter of tomato and kale plants were affected. Since leaves are basic photosynthetic organs, they provide key information both in terms of production and the general development status of the plant. The stem and root diameters are parameters that provide important information about the development status of the vascular bundles and the plant growth. The results of the study showed that the responses of the plants to the MP and Cu treatments for the examined parameters varied depending on the plant species, pollutant type, and separate or combined exposure to pollutants.

By adding MP and Cu to the growing medium of tomato and kale plants, the effects of these two factors on the number of leaves, stem and root diameter were tried to be determined separately and together. The results of the changes in the parameters examined in tomato and kale plants caused by MP and Cu treatments are presented under the following headings.

3.1. Number of Leaves

Results showed that although there were differences among the data sets, the number of leaves in tomatoes did not significantly vary due to MP and Cu treatments (Table 2). The Cu addition applied at different concentrations did not affect the mean number of leaves in a significant trend in the tomato plants. Similar trends were observed in the experimental sets where MPs were applied. While the number of leaves increased slightly with moderate MP addition (1.5%), the addition of 0.5% and 2.5% MP showed similar results to the control group in tomato plants (Fig. 1). The most effective MP rate was 1.5% for the number of leaves in tomato plants.

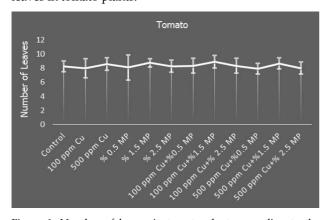


Figure 1. Number of leaves in tomato plants according to the experimental sets

Table 2. Differences in mean number of leaves, stem diameter, and root diameter of tomato plants

	Sum of Squares	df	Mean Square	F	Р
Number of Leaves	13.910	11	1.265	1.206	0.289
Stem Diameter (mm)	39.410	11	3.583	1.339	0.210
Root Diameter (mm)	37.354	11	3.396	1.172	0.313

In the kale plants, there was a significant variation in

the mean number of leaves (Table 3). The number of leaves of kale plants grown in 500 ppm $\text{CuSO}_4+0.5\%$ MP and 500 ppm $\text{CuSO}_4+1.5\%$ MP added soils differed from those of the control groups (Fig. 2). The maximum and the minimum leaf number were determined in the control group and the experimental set where 500 ppm $\text{CuSO}_4+0.5\%$ MP was applied, respectively. Although it was not statistically significant, the number of leaves increased in parallel with the increase in Cu concentration applied to the kale plants. Different concentrations of MP treatments resulted in different trends depending on the presence and concentration of Cu (Table 2).

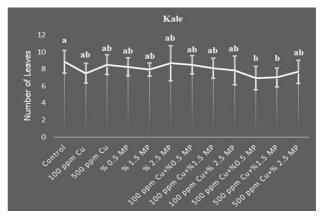


Figure 2. Number of leaves in kale plants according to the experimental sets

Table 3. Differences in mean number of leaves, stem diameter, and root diameter of kale plants

	Sum of Squares	df	Mean Square	F	P
Number of Leaves	51.222	11	4.657	2.665	0.004
Stem Diameter (mm)	18.910	11	1.719	0.852	0.589
Root Diameter (mm)	31.021	11	2.820	0.796	0.644

While the number of leaves significantly varied depending on MP and Cu treatments in kale, no statistically significant variation was observed in tomato plants. This difference may be because the plants are genetically, anatomically, physiologically, metabolically different from each other. In addition, it may also have resulted from climatic factors since they are vegetables produced in summer and winter conditions. While the number of leaves showed a similar trend among the experimental sets in tomato, it tended to decrease in cabbage due to the increase in MP concentration only in MP and 100 ppm Cu + MP applied plants and when the copper concentration was increased, it tended to increase in parallel with the increase in MP concentration. When high concentrations of copper (500 ppm) and MP combination were applied to the kale plants, a more significant negative effect was observed on the number of leaves and this negative effect appeared to decrease as the MP concentration increased. This may be due to the low sorption of Cu in the soil due to high concentrations of MPs.

While copper did not change the number of leaves at low concentrations, it significantly increased the number of leaves at high concentrations in Arabidopsis plants (Kolbert et al., 2012). Hasan and Jho (2023) found that the number of leaves decreased by 16.5% in lettuce plants exposed to 3% polyethylene MP fragments compared with

those of controls, while there was no significant variation in lettuce plants exposed to polyethylene MP fiber. Singh and Singh (2022) determined that compared with the controls, the number of leaves of *Trigonella foenum-graecum* L. treated with MPs did not differ after 20, 60, and 80 days. Colzi et al. (2022) reported that, except for polypropylene MPs, other MP types had no effect on the number of leaves in *Cucurbita pepo* L. Gao et al. (2019) indicated that the number of leaves of *Lactuca sativa* L. decreased with an increase in MP concentration. In the study of Ikhajiagbe et al. (2023), a reduction was determined in the number of leaves of *Celosia argentea* L. grown in soils polluted with the MPs. Although it varies depending on the plant species, applied MP size and type, the findings of previous studies approximately support the results of our study.

3.2. Stem and Root Diameter

The stem diameters of tomato and kale plants did not significantly vary due to MP and Cu treatments. The maximum stem diameter was measured in 500 ppm Cu applied tomato plants and in 1.5% MP applied kale plants, while the minimum stem diameters were measured in 100 ppm Cu applied tomato plants and in 100 ppm Cu + 2.5% MP applied in kale plants (Fig. 3). The increase in Cu concentration led to a slight increase in stem diameter in tomato plants but it was not statistically significant. While the increase in MP rate led to a decrease in stem diameter in tomato plants where MPs were applied single and combined with 500 ppm Cu, a slight increase was determined in tomato plants where MPs were applied combined with 100 ppm Cu. Although it was not statistically significant, the increase in Cu concentration led to a slight decrease in stem diameter in kale plants (Fig. 4). MP treatments caused the same trends in all experimental sets and 1.5% of MP treatments led to a bit increase in stem diameter in kale plants.

Similar to the stem diameters, the root diameters of tomato and kale plants did not significantly differ due to MP and Cu treatments. The maximum root diameter was measured in 500 ppm Cu + 1.5% MP applied tomato plants and in 500 ppm + 2.5% MP applied kale plants, while the minimum stem diameters were measured in 2.5% MP applied tomato plants and in 500 ppm Cu applied kale plants. The increase in Cu and MP concentrations led to a decrease in the root diameter of the tomato plants (Fig. 5). In 100 and 500 ppm Cu applied tomato plants, root diameters increased parallel with increased MP concentration. Similar to tomato, the increase in Cu concentrations decreased the root diameters of the kale plants. However, the increase in the MP concentrations increased the root diameters nearly in all experimental sets (Fig. 6). The MP and Cu treatments did not significantly change the stem and root diameters of the tomato and kale plants in this study. Pinto-Poblete et al. (2023) expressed that both single and combined treatments of Cu nanoparticles and MPs significantly decreased the stem diameter of strawberries. While the single treatment of MPs was more effective than other treatments 40 days after transplanting of strawberries, the effects of MPs, Cu and Cu + MPs treatments on stem diameter were similar on day 60. These results explained that plant age, in other words, measurement time also affects plant responses to MP and Cu treatments. Marques et al. (2018) observed that Cu negatively affected the stem diameter at the highest concentration (800 mg kg⁻¹) in Hymenaea courbaril L.

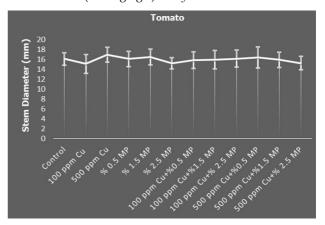


Figure 3. Mean stem diameters in tomato plants according to the experimental sets

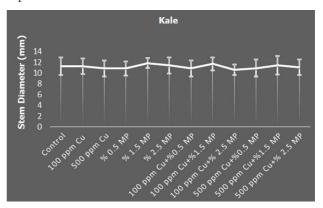


Figure 4. Mean stem diameters in kale plants according to the experimental sets

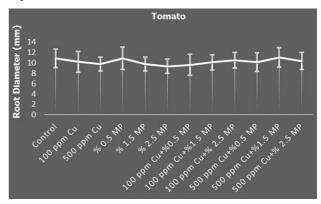


Figure 5. Mean root diameters in tomato plants according to the experimental sets

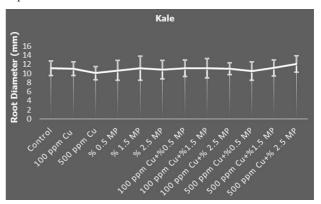


Figure 6. Mean root diameters in kale plants according to the experimental sets

Qi et al. (2018) reported that a 1.5% macro and MP mixture in the sandy soil did not significantly affect the stem diameter of Triticum aestivum L. No significant difference was found in the stem diameter between the control and the MP-treated groups in T. foenum-graecum (Singh & Singh, 2022). In the studies of Boots et al. (2019), Bosker et al. (2019), de Souza Machado et al. (2019), and many others, it was reported that MPs affect the plant physiological growth parameters and effects of MPs change depending on several factors such as plant species, the type of MPs, and soil and ecological conditions (Rillig et al., 2019; Singh & Singh, 2022). The Cu treatment significantly influenced the mean root diameter in Elsholtzia haichowensis Y.Z.Sun (Cai et al., 2014). The root diameter of Pinus pinaster Aiton exposed the Cu significantly increased while that of Pinus pinea L. did not increase (Arduini et al., 1995). The high concentrations of Cu increased the root diameter by altering the cell organization in the root apex and increasing the areas of cortex and vascular cylinder in Vitis labrusca L. (Ambrosini

de Souza Machado et al. (2019) observed an average reduction of approximately 5% in root diameter in spring onions exposed to polyester MPs. Li et al. (2023) determined that the root diameters of the Oryza sativa L. plants treated with MPs were higher than those of the control groups. Similar with our results, root diameters did not significantly vary based on different concentrations of MPs in Nicotiana tabacum L. (Zhang et al., 2022). In the study of Li et al. (2020a), an increase in PVC-a (<18 µm) concentrations reduced the root diameter of lettuce, while an increase in PVC-b (>18 µm) concentrations increased the root diameter. Additionally, the root diameters of the lettuce plants treated with 0.5% a MP were significantly larger than those of the control group. However, there was no significant difference in root diameter of the lettuce plants treated with the 1.5% and 2.5% MPs from those of the control group. These results indicated that different sizes and concentrations of the MPs led to different effects on the root diameter.

4. Conclusions

Our findings, along with results from the literature, indicate that MPs and high concentrations of metals such as Cu negatively impact crop development and pose a threat to agricultural ecosystems. The effects of MPs on growth parameters, such as the number of leaves, stem and root diameters, vary depending on factors like plant species, type, size, and concentration of MPs as well as harvest time (plant age) and environmental conditions. The variation in the number of leaves, stem and root diameters may have been influenced by many factors affecting plant development such as water stress, changes in nutrient uptake, MP, and copper stress. More detailed and comprehensive studies are needed to clarify these factors. Additionally, a broader range of growth parameters and plant species should be studied to better understand the effects of MPs on plant species.

Future studies should focus on understanding the interactive effects of microplastics and heavy metals on a broader range of crops and physiological parameters.

Acknowledgment: The authors also wish to thank the administrators and academicians of Suluova Vocational School,

whose laboratory facilities were used in this study, and Amasya University Scientific Research Projects Coordination Unit for its financial support to our study with the projects of FMB-BAP 21-0517 and FMB-BAP 22-0580.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – N.K.; Design – N.K.; Supervision – N.K.; Fund – N.K.; Materials – B.Ç.; Data Collection and Processing – B.Ç.; Analysis Interpretation – B.Ç.; Literature Review – N.K., B.Ç.; Writing – N.K., B.Ç.; Critical Review – N.K., B.C.

References

- Adamczewska-Sowińska, K., Bykowy, J., & Jaworska, J. (2025). Effect of Biodegradable Mulch and Different Synthetic Mulches on Growth and Yield of Field-Grown Small-Fruited Tomato (*Lycopersicon esculentum* Mill.). *Agriculture*, 15(2), 212. https://doi.org/10.3390/agriculture15020212
- Akyıldız, M. & Karataş, B. (2018). Adana şehir merkezindeki topraklarda ağır metal kirliliğinin araştırılması. *Çukurova Üniversitesi Mühendislik-Mimarlık Fakültesi Dergisi*, 33(2), 199-214. https://doi.org/10.21605/cukurovaummfd.509559
- Alibas, İ., & Okursoy, R. (2012). Karalahana (Brassica oleracea L. var. acephala), Pazı (Beta vulgaris L. var. cicla) ve Ispanak (Spinacia oleracea L.) yapraklarının bazı teknik özellikleri. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 26(1), 39-48.
- Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., ... & Galop, D. (2019). Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nature Geoscience*, 12(5), 339-344. https://doi.org/10.1038/s41561-019-0335-5
- Ambrosini, V.G., Rosa, D.J., Prado, J.P.C., Borghezan, M., de Melo, G.W.B., de Sousa Soares, C.R.F., ... & Brunetto, G. (2015). Reduction of copper phytotoxicity by liming: A study of the root anatomy of young vines (Vitis labrusca L.). Plant Physiology and Biochemistry, 96, 270-280. https://doi.org/10.1016/j.plaphy.2015.08.012
- Apaydın, A. (2005). Investigation of soil pollution orijinated from industries: Samsun-Tekkeköy Region. Retrieved from: https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp
- Arduini, I., Godbold, D.L., & Onnis, A. (1995). Influence of copper on root growth and morphology of *Pinus pinea* L. and *Pinus pinaster* Ait. seedlings. *Tree Physiology*, 15(6), 411-415. https://doi.org/10.1093/treephys/15.6.411
- Ayaz, F.A., Hayırlıoglu-Ayaz, S., Alpay-Karaoglu, S., Grúz, J., Valentová, K., Ulrichová, J., & Strnad, M. (2008). Phenolic acid contents of kale (*Brassica oleraceae* L. var. acephala DC.) extracts and their antioxidant and antibacterial activities. Food Chemistry, 107(1), 19-25. https://doi.org/10.1016/j.foodchem.2007.07.003
- Azeem, I., Adeel, M., Ahmad, M.A., Shakoor, N., Jiangcuo, G.D., Azeem, K., ... & Rui, Y. (2021). Uptake and accumulation of nano/microplastics in plants: a critical review. *Nanomaterials*, 11(11), 2935. https://doi.org/10.3390/nano11112935
- Bakir, A., Rowland, S.J., & Thompson, R.C. (2012). Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. *Marine Pollution Bulletin*, 64(12), 2782-2789. https://doi.org/10.1016/j.marpolbul.2012.09.010
- Bolat, İ., & Kara, Ö. (2017). Plant nutrients: sources, functions, deficiencies and redundancy. Bartın Orman Fakültesi Dergisi, 19(1), 218-228. http://doi.org/10.24011/barofd.251313
- 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
- Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., & Vijver, M.G. (2019).

 Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. Chemosphere, 226, 774-781.

 https://doi.org/10.1016/j.chemosphere.2019.03.163
- Cai, S., Xiong, Z., Li, L., Li, M., Zhang, L., Liu, C., & Xu, Z. (2014). Differential responses of root growth, acid invertase activity and transcript level to copper stress in two contrasting populations of

- Elsholtzia haichowensis. Ecotoxicology, 23, 76-91. https://doi.org/10.1007/s10646-013-1153-y
- Chia, R.W., Lee, J.Y., Kim, H., & Jang, J. (2021). Microplastic pollution in soil and groundwater: a review. Environmental Chemistry Letters, 19(6), 4211-4224. https://doi.org/10.1007/s10311-021-01297-6
- Colzi, I., Renna, L., Bianchi, E., Castellani, M.B., Coppi, A., Pignattelli, S., ... & Gonnelli, C. (2022). Impact of microplastics on growth, photosynthesis and essential elements in *Cucurbita pepo L. Journal of Hazardous Materials*, 423, 127238. https://doi.org/10.1016/j.jhazmat.2021.127238
- de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., & Rillig, M.C. (2018). Impacts of microplastics on the soil biophysical environment. *Environmental Science & Technology*, 52(17), 9656-9665. https://doi.org/10.1021/acs.est.8b02212
- de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J. B., Faltin, E., ... & Rillig, M.C. (2019). Microplastics can change soil properties and affect plant performance. *Environmental Science & Technology*, 53(10), 6044-6052. https://doi.org/10.1021/acs.est.9b01339
- Desai, B.H. (2015). 14. United Nations Environment Program (UNEP). https://doi.org/10.1093/yiel/yvw063
- Durmuş, M., Yetgin, Ö., Abed, M.M., Haji, E.K., & Akcay, K. (2018).
 Domates bitkisi, besin içeriği ve sağlık açısından değerlendirmesi.
 International Journal of Life Sciences and Biotechnology, 1(2), 59-74.
 https://doi.org/10.38001/ijlsb.482443
- Gao, M., Liu, Y., & Song, Z. (2019). Effects of polyethylene microplastic on the phytotoxicity of di-n-butyl phthalate in lettuce (*Lactuca sativa L. var. ramosa* Hort). *Chemosphere*, 237, 124482. https://doi.org/10.1016/j.chemosphere.2019.124482
- Guo, Y., Wu, R., Zhang, H., Guo, C., Wu, L., & Xu, J. (2025). Distribution of microplastics in the soils of a petrochemical industrial region in China: Ecological and Human Health Risks. *Environmental Geochemistry and Health*, 47(1), 1-16. https://doi.org/10.1007/s10653-024-02324-5
- Hasan, M.M. & Jho, E.H. (2023). Effect of different types and shapes of microplastics on the growth of lettuce. *Chemosphere*, 339, 139660. https://doi.org/10.1016/j.chemosphere.2023.139660
- Ikhajiagbe, B., Omoregie, G.O., Adama, S.O., & Esheya, K.U. (2023). Growth responses of *Celosia argentea* L. in soils polluted with microplastics. bioRxiv, 2023-01. https://doi.org/10.1101/2023.01.07.523084
- Jin, T., Tang, J., Lyu, H., Wang, L., Gillmore, A.B., & Schaeffer, S.M. (2022). Activities of microplastics (MPs) in agricultural soil: a review of MPs pollution from the perspective of agricultural ecosystems. *Journal of Agricultural and Food Chemistry*, 70(14), 4182-4201. https://doi.org/10.1021/acs.jafc.1c07849
- Kajal, S., & Thakur, S. (2024). Coexistence of microplastics and heavy metals in soil: Occurrence, transport, key interactions and effect on plants. *Environmental Research*, 119960. https://doi.org/10.1016/j.envres.2024.119960
- Khalid, N., Aqeel, M., & Noman, A. (2020). Microplastics could be a threat to plants in terrestrial systems directly or indirectly. *Environmental Pollution*, 267, 115653. https://doi.org/10.1016/j.envpol.2020.115653
- Koelmans, A.A., Redondo-Hasselerharm, P.E., Nor, N.H.M., de Ruijter, V.N., Mintenig, S.M., & Kooi, M. (2022). Risk assessment of microplastic particles. *Nature Reviews Materials*, 7(2), 138-152. https://doi.org/10.1038/s41578-021-00411-y
- 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 Regulation, 68, 151-159. https://doi.org/10.1007/s10725-012-9701-7
- Li, L., Luo, Y., Li, R., Zhou, Q., Peijnenburg, W.J., Yin, N., ... & Zhang, Y. (2020a). Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. *Nature Sustainability*, 3(11), 929-937. https://doi.org/10.1038/s41893-020-0567-9
- Li, Z., Li, Q., Li, R., Zhao, Y., Geng, J., & Wang, G. (2020b). Physiological responses of lettuce (*Lactuca sativa* L.) to microplastic pollution. *Environmental Science and Pollution Research*, 27, 30306-30314. https://doi.org/10.1007/s11356-020-09349-0
- Li, G., Zhao, X., Iqbal, B., Zhao, X., Liu, J., Javed, Q., & Du, D. (2023). The effect of soil microplastics on *Oryza sativa* L. root growth traits under alien plant invasion. *Frontiers in Ecology and Evolution*, 11, 1172093. https://doi.org/10.3389/fevo.2023.1172093
- Lin, Z., Jin, T., Zou, T., Xu, L., Xi, B., Xu, D., ... & Fei, J. (2022). Current progress on plastic/microplastic degradation: Fact influences and mechanism. *Environmental Pollution*, 304, 119159. https://doi.org/10.1016/j.envpol.2022.119159
- Marques, D.M., Veroneze Júnior, V., da Silva, A.B., Mantovani, J.R., Magalhães, P.C., & de Souza, T.C. (2018). Copper toxicity on

- photosynthetic responses and root morphology of *Hymenaea courbaril* L. (Caesalpinioideae). *Water, Air, & Soil Pollution,* 229, 1-14. https://doi.org/10.1007/s11270-018-3769-2
- Megha, K.B., Anvitha, D., Parvathi, S., Neeraj, A., Sonia, J., & Mohanan, P.V. (2025). Environmental impact of microplastics and potential health hazards. *Critical Reviews in Biotechnology*, 45(1), 97-127. https://doi.org/10.1080/07388551.2024.2344572
- 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, 142516. https://doi.org/10.1016/j.scitotenv.2020.142516
- Napper, I.E., Davies, B.F., Clifford, H., Elvin, S., Koldewey, H.J., Mayewski, P.A., ... & Thompson, R.C. (2020). Reaching new heights in plastic pollution-preliminary findings of microplastics on Mount Everest. *One Earth*, 3(5), 621-630. https://doi.org/10.1016/j.oneear.2020.10.020
- Nasseri, S., & Azizi, N. (2022). Occurrence and fate of microplastics in freshwater resources. In Microplastic Pollution: Environmental Occurrence and Treatment Technologies Cham: Springer International Publishing, 187-200. https://doi.org/10.1007/978-3-030-89220-3_9
- Ofoezie, E.I., Eludoyin, A.O., Udeh, E.B., Onanuga, M.Y., Salami, O.O., & Adebayo, A.A. (2022). Climate, urbanization and environmental pollution in West Africa. *Sustainability*, 14(23), 15602. https://doi.org/10.3390/su142315602
- Okcu, M., Tozlu, E., Metin Kumlay, A., & Pehluvan, M. (2009). Ağır metallerin bitkiler üzerine etkileri. Alınteri Journal of Agriculture Science, 17(2), 14-26.
- Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., ... & Bai, S. (2018).

 Microplastics contaminate the deepest part of the world's ocean.

 Geochemical Perspectives Letters, 9(1), 1-5.

 https://doi.org/10.7185/geochemlet.1829
- Peralta, I.E., & Spooner, D.M. (2007). History, origin and early cultivation of tomato (Solanaceae). In: Razdan MK, Mattoo AK (eds) Genetic improvement of solanaceous crops, vol 2. Tomato, Enfield, United States, Science Publishers, 1-27.
- Pinto, A.C.R., Demattè, M.E.S.P., Creste, S., & Barbosa, J.C. (2011). Seed and seedling surface-sterilization for in vitro culture of Tillandsia gardneri (Bromeliaceae). VII International Symposium on in Vitro Culture and Horticultural Breeding September 18-22, 2011, Ghent, Belgium, pp. 383-389. https://doi.org/10.17660/ActaHortic.2012.961.50
- Pinto-Poblete, A., Retamal-Salgado, J., Zapata, N., Sierra-Almeida, A., & Schoebitz, M. (2023). Impact of polyethylene microplastics and copper nanoparticles: Responses of soil microbiological properties and strawberry growth. *Applied Soil Ecology*, 184, 104773. https://doi.org/10.1016/j.apsoil.2022.104773
- Qi, Y., Yang, X., Pelaez, A.M., Lwanga, E.H., Beriot, N., Gertsen, H., ... & Geissen, V. (2018). Macro-and micro-plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Science of the Total Environment, 645, 1048-1056. https://doi.org/10.1016/j.scitotenv.2018.07.229
- Ren, X., Tang, J., Wang, L., & Liu, Q. (2021). Microplastics in soil-plant system: effects of nano/microplastics on plant photosynthesis, rhizosphere microbes and soil properties in soil with different residues. Plant and Soil, 462, 561-576. https://doi.org/10.1007/s11104-021-04869-1
- Rezaie, A., & Forghani, S. (2022). A Novel Method for Removal of Hazardous Microplastics from Water Using Magnets. Journal of Environmental Health and Sustainable Development. 7(4), 1775-6. https://doi.org/10.18502/jehsd.v7i4.11428
- Rillig, M.C. (2012). Microplastic in terrestrial ecosystems and the soil? Environmental Science & Technology, 46(12), 6453-6454. https://doi.org/10.1021/es302011r
- Rillig, M.C., Lehmann, A., de Souza Machado, A.A., & Yang, G. (2019). Microplastic effects on plants. New Phytologist, 223(3), 1066-1070. https://doi.org/10.1111/nph.15794
- Sharma, R.K., & Agrawal, M. (2005). Biological effects of heavy metals: an overview. *Journal of Environmental Biology*, 26(2), 301-313.
- Singh, B., & Singh, K. (2022). Microplastics contamination in soil affects growth and root nodulation of fenugreek (*Trigonella foenum-graecum* L.) and 16 s rRNA sequencing of rhizosphere soil. *Journal of Hazardous Materials* Advances, 7, 100146. https://doi.org/10.1016/j.hazadv.2022.100146
- Sun, J., Dai, X., Wang, Q., Van Loosdrecht, M.C., & Ni, B.J. (2019). Microplastics in wastewater treatment plants: Detection, occurrence and removal. Water Research, 152, 21-37. https://doi.org/10.1016/j.watres.2018.12.050
- Ullah, R., Tsui, M.T.K., Chen, H., Chow, A., Williams, C., & Ligaba-Osena, A. (2021). Microplastics interaction with terrestrial plants and their

- impacts on agriculture. Journal of Environmental Quality, 50(5), 1024-1041. https://doi.org/10.1002/jeq2.20264
- Waldman, W.R., & Rillig, M.C. (2020). Microplastic research should embrace the complexity of secondary particles. Environmental Science & Technology, 54(13), 7751-7753. https://doi.org/10.1021/acs.est.0c02194
- Wang, X., Fan, J., Xing, Y., Xu, G., Wang, H., Deng, J., ... & Li, Z. (2019). The effects of mulch and nitrogen fertilizer on the soil environment of crop plants. *Advances in Agronomy*, 153, 121-173. https://doi.org/10.1016/bs.agron.2018.08.003
- Wu, P., Cai, Z., Jin, H., & Tang, Y. (2019). Adsorption mechanisms of five bisphenol analogues on PVC microplastics. Science of the Total Environment, 650, 671-678. https://doi.org/10.1016/j.scitotenv.2018.09.049
- Wu, X., Liu, Y., Yin, S., Xiao, K., Xiong, Q., Bian, S., ... & Yang, J. (2020). Metabolomics revealing the response of rice (*Oryza sativa* L.) exposed to polystyrene microplastics. *Environmental Pollution*, 266, 115159. https://doi.org/10.1016/j.envpol.2020.115159
- Yıldırım, N. (2022). Possible effects of heavy metal (Cu) and sitimulated acid rain stress on mRNA expression levels of *FAD2* gene responsible for linoleic acid transduction of oleic acid and some ecophysiological traits in safflower (*Carthamus tinctorius* L.)". Retrieved from: https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp
- Yruela, I. (2005). Copper in plants. Brazilian Journal of Plant Physiology, 17, 145-156. https://doi.org/10.1590/S1677-4202005000100012
- Yu, H., Zhang, Y., Tan, W., & Zhang, Z. (2022). Microplastics as an emerging environmental pollutant in agricultural soils: effects on ecosystems and human health. Frontiers in Environmental Science, 10, 855292. https://doi.org/10.3389/fenvs.2022.855292
- Yücel Tartan, G. (2023). The determination of the effect of microplastic pollution on plant growth and cadmium (Cd) accumulation in *Lactuca sativa* L. (lettuce). Retrieved from: https://tez.yok.gov.tr/UlusalTezMerkezi/giris.jsp
- Zhang, S., Han, B., Sun, Y., & Wang, F. (2020). Microplastics influence the adsorption and desorption characteristics of Cd in an agricultural soil. *Journal of Hazardous Materials*, 388, 121775. https://doi.org/10.1016/j.jhazmat.2019.121775
- 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
- Zhou, Y., Liu, X., & Wang, J. (2019). Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. Science of the Total Environment, 694, 133798. https://doi.org/10.1016/j.scitotenv.2019.133798