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Research Article

Effect of microplastics on dry matter content in *Lactuca sativa* L.

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Abstract

Plastics are commonly used in every field of life due to their ease of use and economics. If the plastic wastes are not disposed of properly, they form microplastics by breaking down into smaller particles with decomposition. Microplastics are chemical substances in polymer structure. They are difficult to dissolve in nature and require a long time. Microplastics, that spread to the environment in various ways, cause pollution and negatively affect organisms and ecosystems.

This study aimed to determine the effects of microplastics and the combined impact of microplastics and Cd on dry matter content (the ratio of dry mass to fresh mass of an organ), which is the one of the major plant traits for plant growth, in lettuce (*Lactuca sativa* L.). Five experiment sets were designed by using Simila variety lettuce seedlings, including (1) control, (2) 1.5 % microplastic (3) 2.5 % microplastic, (4) 1.5 % microplastic and 200 ppm Cd solution and (5) 2.5 % microplastic and 200 ppm Cd solution added. The polyethylene mulch pieces (2,5 mm x 4 mm) were used as microplastics. Results showed that the polyethylene microplastic addition to the soil significantly reduced aboveground and belowground DMCs of lettuce seedlings. Concentration of the microplastics in the soil was also effective on DMC. The Cd addition to the soils, which contain microplastics, a bit increased the DMC.

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Dry matter content,
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1. Introduction

The plastics are one of the major problems that threaten the health and wellness of living things and sustainability of environment. Plastics, that do not occur naturally on earth, are obtained by reacting monomers with a catalyst under a certain temperature and pressure. Plastics are used in every field of life and cause pollution through domestic, industrial, and medical wastes. According to the previous researches, it has been revealed that plastic pieces are found in many places from the poles to deserted islands and the deepest point of the world (Yurtsever, 2019). The plastics are polymers made from petroleum materials and difficult to dissolve in nature. They turn into microplastics in various types and shapes when decomposed. The microplastic term is firstly used by Thompson in 2004. Microplastics are generally called as plastic particles smaller than 5 mm in size (Bouwmeester et al., 2015). They were defined in more detail as "regular or irregularly shaped synthetic solid particles or polymeric matrices originating from primary or secondary production, insoluble in water, varying in size from 1 mm to 5 mm" (Frias and Nash, 2019, Arı and Öğüt, 2021). Microplastics have many characteristic physicochemical properties such as having a hydrophobic surface, transporting and absorbing pollutants, and thermooxidation. Most of the sources of microplastics are the synthetic textile fibers, microbeads found in fertilizers, drugs, detergents and toothpastes, bags, scraps, pieces, debris, particles from automobile tires, greenhouse nylons, plastic mulching materials, irrigation pipes.

Because the microplastics are small enough to be easily transported from one ecosystem to another, they have became a global problem as one of the major pollutants (Thompson et al., 2009). Microplastics are transported to ecosystems by various ways and cause pollution. Microplastic pollution leads to various damages due to their presence in the environment and accumulation in the bodies of living things. They not only threaten the extinction of living things by disrupting terrestrial and aquatic ecosystems, but also damage the natural order by changing the physical, chemical and biological properties of soil and water (Yurtsever, 2019). The microplastics, which are in very small sizes, enter the body of organisms through respiration, food and water, cause various disruptions in their metabolism. Because they can adsorb chemicals and heavy metals, microplastics are complex pollutants for living things (Rochman et al., 2013). Microplastics also affect the seed germination, uptake of nutrients and plant growth by changing the soil structure in terrestrial ecosystems. Studies on microplastics have been carried out mostly in aquatic ecosystems because of their easy detection. The effects of microplastics on the living organisms and soil in terrestrial ecosystems have not yet been fully understood due to the insufficient number of studies.

In the study of Wu et al. (2020), polystyrene microplastic (PSMP) stress was applied to *Oryza sativa* L. and after 21 days shoot biomass decreased based on low, medium and high dose applications as %13,1, %18,8 and %40,3, respectively. The amounts of 12 amino acids, 16 saccharides, 26 organic acids and 17 other components (lipids and polyols) in the leaves of 24 samples exposed to PSMP at doses of 50 mg L^{-1} and 250 mg L^{-1} with hydropoic culture decreased. Li et al. (2020) applied PVC microplastic particles with sizes between 100 nm and 18 µm at 0.5%, 1% and 2% concentrations to the root systems and leaves of lettuce. Microplastics did not cause changes in root activity; but the total length, surface area, volume and diameter of the roots increased. They reported that superoxide dismutase activity increased by 1% in lettuce leaves and electron transfer, light absorption, diffusion and capture decreased. Khalid et al. (2020) determined that microplastics directly affect plants by clogging the seed pores, limiting the uptake of water and soil compounds, as well as causing accumulation in leaves, roots and stems. Indirectly, they change the physicochemical structure of the soil by affecting the microbes and fauna in the soil.

Dry matter content, which is the ratio of dry mass to fresh mass of an organ, is a useful plant trait for understanding the plant status, production and life strategies. Since dry matter content provides information about plant production, research use and water content, it is commonly used as an ecophysiological parameter in plant studies (Garnier et al. 2001, Grime et al. 1997)

In this study, it was aimed to determine the effects of microplastics and the combined impact of microplastics and Cd on dry matter content in lettuce (*Lactuca sativa* L.). We hypothesized that the microplastic addition to the soil would be decrease DMCs and the combined effect of microplastics and Cd, which are the two of the major pollutants in most of the agricultural areas, may leads to lower DMC.

2. Materials and Methods

2.1 Experimental Design

In this study, Simila variety lettuce seedlings were used as plant material. Lettuce seedlings were produced from certified seeds by Alp Fide in Amasya, Turkey. To determine the effect of microplastics and the combined impact of microplastics and Cd on dry matter content in lettuce, fivefactorial experiment set was designed, including (1) control, (2) 1.5 % microplastic (3) 2.5 % microplastic, (4) 1.5 % microplastic and 200 ppm Cd solution and (5) 2.5 % microplastic and 200 ppm Cd solution added. Each experiment set was performed with six repetitions.

Figure 1. Experiment sets of lettuce

A total of 60 lettuce seedlings were studied. Because of its common usage in agriculture as a cover material for production, microplastics were obtained by cutting the polyethylene mulch into 2,5 mm x 4 mm pieces. The garden soil taken at a depth of 0-25 cm was mixed with the peat in 50 % volume. The microplastics were added to the soils in relevant experimental sets. All the pots were irrigated in an equal amount water on alternate day.

2.2 Dry Matter Content

After 28 days, the lettuce seedlings were harvested by carefully separating from the soil. The fresh weights of the aboveground and the underground parts of the lettuce seedlings were immediately measured with scales. Then, they were oven-dried in paper bags at 65 ^oC until the constant weight reached and the dry weights of the lettuce seedlings were measured.

Figure 2. Harvested lettuce seedlings

The dry matter contents of aboveground and underground parts of the lettuce seedlings were calculated as dry mass per unit fresh mass (mg g^{-1}). The statistical analyses were performed by SPSS 20.0 statistical software. The differences in means among the treatments were determined by one-way ANOVA.

3. Results and Discussion

Results showed that both the aboveground and the belowground DMCs of the lettuce seedlings significantly varied based on microplastic and microplastic+Cd additions. The maximum aboveground and belowground DMCs were found in the control groups. The minimum aboveground and belowground DMCs were found in the lettuce seedlings grown in 1.5% microplastic added pots. DMC significantly decreased based on microplastic addition. Amount of the microplastics also changed the DMC in both aboveground parts and belowground of the lettuce seedlings. However, Cd addition to the soils, which also contain microplastics, altered the DMC. The combined applications of the microplastics and the Cd solution led to a bit increase in DMC in the aboveground parts and the belowground of the lettuce seedlings. The belowground DMCs (> 50 mg g^{-1}) of the lettuce seedlings were higher than the aboveground DMCs (<50 mg g^{-1}).

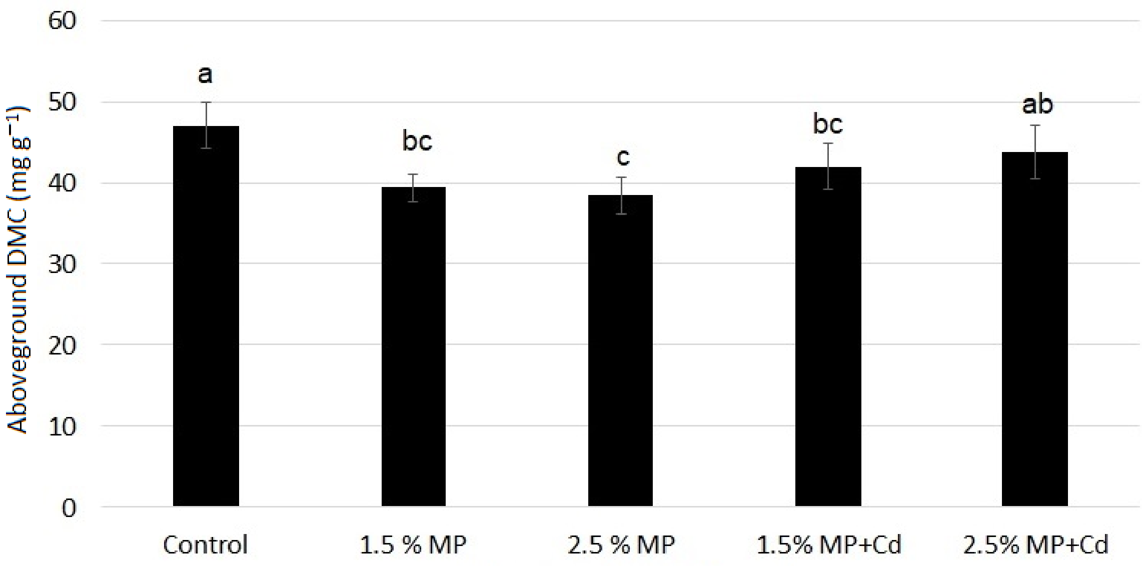

Figure 3. Variation in the aboveground DMCs (mg g^{-1}) of the lettuce seedlings (MP: Microplastic)

Table 1. Significance of variation in the aboveground DMC (mg g^{-1}) among the experiment sets

Aboveground DMC (mg g^{-1})	Sum of Squares	df	Mean Square	F	P
	289.574	4	72.393	10.676	0.000

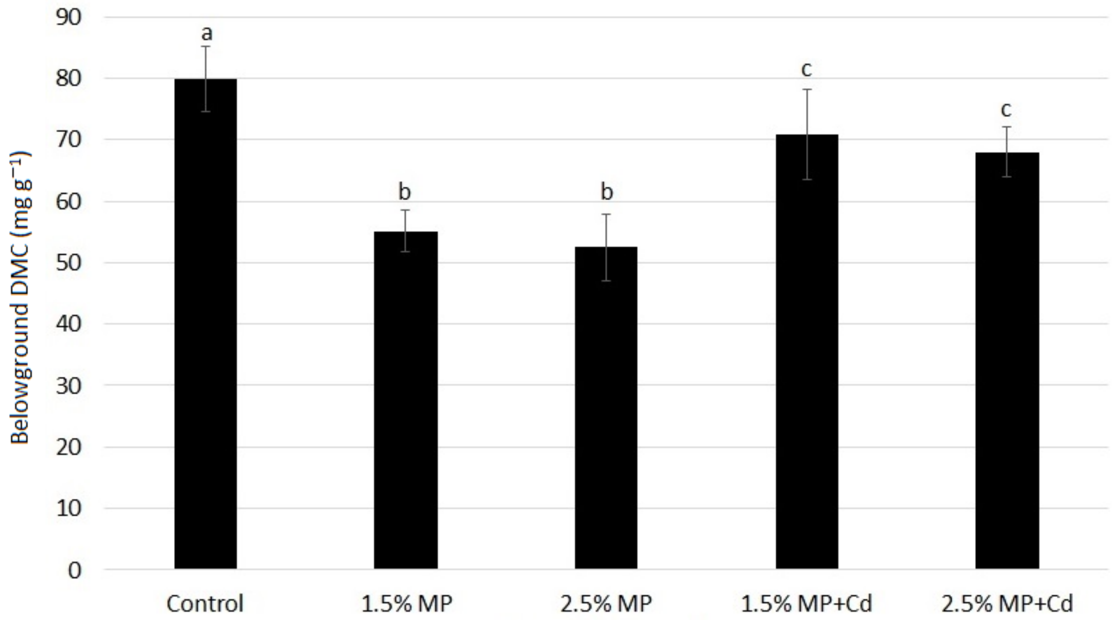

Figure 4. Variation in the belowground DMCs (mg g^{-1}) of the lettuce seedlings (MP: Microplastic)

Table 2. Significance of variation in the belowground DMC (mg g^{-1}) among the experiment sets

Belowground DMC (mg g^{-1})	Sum of Squares	df	Mean Square	F	P
	3119.155	4	779.789	28.246	0.000

Similar results were obtained in the some of the previous studies. In the study of Wang et al. (2021), while 0,1% MPs+Cd and 1% MPs+Cd additions did not cause any variation, the co-addition of Cd and 10% MPs decreased the plant biomass in lettuce. More than 25.9% biomass reduction was determined in crops exposed to polystyrene microplastic particles (PSMP) 142 days after planting by Wu et al. (2020). In the study of Enyoh et al. (2020), the root biomass of *Citrus aurantium* L. was negatively affected by the plastic addition. Additionally, they found that the microplastics exhibited greater negative effects than macroplastics. Qi et al. (2018) reported that wheat (*Triticum aestivum* L.) plant performance parameters such as leaf area decreased with the addition of biodegradable plastic mulch residue. Furthermore, significantly lower shoot biomass was determined in wheat plants grown in starch-based biodegradable microplastic added soils while no significant variation was found in those grown in low-density polyethylene microplastics. Total plant biomass of wheat was significantly decreased due to the addition of plastics to the soil. They emphasized that aboveground and the belowground parts of the wheat plants negatively affected by the microplastic addition. Our results coincided with the previous studies that reported the negative effects of the microplastics to the plant growth.

As a conclusion, polyethylene microplastic addition to the soil significantly reduced the aboveground and the belowground DMCs of the lettuce seedlings. The concentration of the microplastics in the soil was also effective on DMC. The Cd addition to the soils which also contain the microplastics a bit increased the DMC.

In recent years, plastic pollution became a basic problem in the world. One of the major sources of the plastic pollution is the intensive usage of plastic mulching in agriculture. Since they are difficult to dissolve in nature and easily transportable, microplastics are important pollutants in both aquatic and terrestrial ecosystems. They change the features of soil and water and cause damages in organisms. Because of their importance for health of organisms and ecosystems, future detailed studies are required to understand the effects of microplastics on plant traits and growth performance.

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