

T.C.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

**IMPACT OF MICROPLASTICS AT CONCENTRATIONS
FOUND IN GREY WATER ON SOIL PROPERTIES AND PLANT
GROWTH**

BETÜL BALCI
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF ENVIRONMENTAL ENGINEERING

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SUMMARY

Grey water is domestic wastewater produced from bathroom, laundry and kitchen. Reusing grey water can help water management and decrease the freshwater requirement for irrigation. Furthermore, grey water can promote plant growth when used for irrigation by its nutrient content. However, irrigation with grey water can adversely affect soil properties and plant growth. Particularly, microplastics have become an important component of grey water due to release from laundry and personal care products recently. In the literature it is observed that microplastics can negatively affect soil's characteristics and plant growth when mixed directly and homogeneously with soil. However, effects of microplastics on plants and soil was not studied when they are at concentrations found in grey water and transferred into soil in inhomogeneously through irrigation. Therefore, the goal of this study is to investigate how synthetic water containing microplastics affects soil bulk density, water stable aggregate weight, and water holding capacity of soil, in addition to plant growth parameters like length and weight of plant's roots and shoots. For this purpose, pots filled with soil samples and planted with spring onions or grass were irrigated with synthetic water containing microplastics at the concentrations determined in grey water. Results indicate that presence of microplastics at concentrations relevant to grey water can affect soil properties and plant growth depending on microplastic concentration, plant type and soil characteristics. This study can contribute to research on grey water reuse potential in circular city and risk assessment of microplastics in grey water in case of irrigation.

Key Words: Grey water, Microplastic, Water Reuse, Water Stable Aggregation, Water Holding Capacity, Plant Growth.

ÖZET

Gri su, tuvalet atıkları dışında genellikle banyo, çamaşırhane ve mutfaktan üretilen evsel bir atık su kategorisi olarak tanımlanmaktadır. Gri suyun yeniden kullanılması, su yönetimine yardımcı olabilir ve sulama için gereken tatlı su miktarını azaltabilir. Ayrıca gri su, içerdiği besinler nedeniyle sulama suyu olarak kullanıldığında bitki büyümesini destekleme potansiyeline sahiptir. Bununla birlikte, sulama için gri su kullanılması, potansiyeline rağmen toprak özelliklerini ve bitki büyümesini olumsuz yönde etkileyebilir. Özellikle son yıllarda çevresel etkileri anlaşılmaya başlanan mikroplastikler, çamaşır ve kişisel bakım ürünlerinden salınması nedeniyle gri suyun önemli bir bileşeni haline gelmiştir. Literatürde mikroplastiklerin toprakla doğrudan ve homojen bir şekilde karıştırılması durumunda mikroplastiklerin toprağın özelliklerini ve bitki büyümesini olumsuz yönde etkileyebileceği görülmektedir. Ancak mikroplastiklerin gri sularda bulunan konsantrasyonlarda ve sulama yoluyla homojen bir şekilde toprağa geçtiğinde bitki ve toprak üzerindeki etkileri konusunda herhangi bir çalışma yapılmamıştır. Bu nedenle, bu çalışmanın amacı, bitki köklerinin ve sürgünlerinin uzunluğu ve kuru ağırlığı gibi bitki büyüme parametrelerine ek olarak, mikroplastik içeren sentetik suyun toprak kütle yoğunluğunu, suya dayanıklı agrega ağırlığı ve toprağın su tutma kapasitesini nasıl etkilediğini araştırmaktır. Bu amaçla, toprak örnekleri ile doldurulan ve taze soğan veya çim ekilen saksılar, gri suda belirlenen konsantrasyonlarda mikroplastik içeren sentetik su ile sulanmıştır. Sonuçlar, gri su ile ilgili konsantrasyonlarda mikroplastiklerin varlığının, mikroplastik konsantrasyonuna, bitki tipine ve toprak özelliklerine bağlı olarak toprak özelliklerini ve bitki büyümesini etkileyebileceğini göstermektedir. Bu çalışmanın, döngüsel şehirlerde gri suyun yeniden kullanım potansiyeli ve gri su ile sulama durumunda gri sudaki mikroplastiklerin risk değerlendirmesi üzerine araştırmalara katkıda bulunması beklenmektedir.

Anahtar Kelimeler: Gri Su, Mikroplastik, Su Yeniden Kullanımı, Suya Dayanıklı Agreg, Su Tutma Kapasitesi, Bitki Büyümesi.

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LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and Acronyms</u>	<u>Explanations</u>
cm	: Centimeter
m	: Meter
µm	: Micrometer
L	: Liter
mm	: Millimeter
Bio	: Starch-based biodegradable plastic
BOD	: Biological oxygen demand
COD	: Chemical oxygen demand
EPS	: Expanded polystyrene
HDPE	: Conventional high-density polyethylene
LDPE	: Low-density polyethylene
MP	: Microplastic
PA	: Polyamide
PC	: Polycarbonate
PE	: Polyethylene
PES	: Polyester
PET	: Polyethylene terephthalate
PLA	: Biodegradable polylactic acid
PP	: Polypropylene
PS	: Polystyrene
PU	: Polyurethane
PVC	: Polyvinyl chloride
TN	: Total nitrogen
TP	: Total phosphorus
TSS	: Total suspended solids

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1. INTRODUCTION

Water that has been contaminated due to usage in residential structures including homes, sites, dwellings, and hotels is known as domestic wastewater. Oils, organic compounds, pathogenic microorganisms, and detergents constitute a large part of the pollutants found in domestic wastewaters [Bingül and Altıkat, 2017] whereas many emerging contaminants and micropollutants such as surfactants (nonylphenols), pharmaceuticals (diclofenac), hormones (estrone), personal care products (ethylparaben, triclosan) or household chemicals (dimethyl phthalate) have also been detected recently [Margot et al., 2015]. Based on the characteristics and potential end use, segregation of domestic wastewater as yellow water, brown water, and grey water are described as residential wastewater fractions [Baykal and Allar, 2007].

Wastewater segregation has become one of the hot topics since it has the potential to address water scarcity and food security through use of grey water, as well as waste valorization through nutrient recovery from yellow water. Grey water which has the highest volume fraction and lighter pollutant load among domestic wastewater fractions is considered as one the best options for water reuse [Baykal, 2019]. Increasing stress on resources because of climate change drives the need for more sustainable solutions and need to design circular cities where a waste such as grey water can be utilized as a source rather than as a waste to dispose of. More specifically, it is suggested that grey water, which is rich in organic matter, can be reused and contribute to the water cycle through irrigation and groundwater recharge followed by necessary treatments. On the other side, yellow water which consists of human urine collected by separation at the source, contains close to 90% of the nitrogen in domestic wastewater and about half of the phosphorus and potassium can be reused as fertilizer in agriculture. Brown water consisting of separately collected human feces in addition to being rich in organic matter, potassium and pathogenic microorganisms is expected to be used in biogas production or as a soil conditioner after a series of treatment processes [Baykal and Allar, 2007].

Grey water is defined as wastewater discharged from bathroom, laundry, and kitchen, excluding toilet waste [Mourad et al., 2011], [Rodda et al., 2011a], [Rodda et al., 2011b], [Shaikh and Ahammed, 2020]. It is typically split into two categories as light grey water and dark grey water depending on the pollutant concentration in the

source. Light grey water contains wastewater from baths, showers, tubs, sinks and bathroom sinks, while wastewater from laundry, rinsing, kitchen, dishwashers, and washing machines constitute dark grey water. Compared to dark grey water, light grey water has a lower contamination load especially in terms of TSS, BOD and COD. Total grey water is often referred to as mixed grey water, which includes grey water from sources that produce both light and dark grey water [Shaikh and Ahammed, 2020].

Bathroom grey water refers to grey water from bathroom sinks, showers, and tubs, whereas sink grey water refers to grey water from hand basin (including those provide in a bathroom or toilet). Grey water from the kitchen and dishwashers is included in kitchen grey water, whereas grey water from the laundry and washing machines is included in laundry grey water (both wash and rinse water). [Shaikh and Ahammed, 2020].

1.1. Composition of Grey Water

The composition of grey water is determined by several factors, including the source and installation of the infrastructure like the quality and kind of water supply, home activities (lifestyle, custom, and chemical product usage), geographic area, demographics, and occupancy levels, as well as the device from which grey water is drawn (kitchen sink, bathroom, hand basin, or laundry wash).

Shampoo, soap, toothpaste lint, urine traces, body care products, hair, skin, sand/clay particles, and body lipids are components of bathroom grey water. As urine and feces are not usually present in bathroom grey water, phosphorus and nitrogen levels are lower. However, the potential contamination is increased if household animals are washed and dangerous materials including paint, pesticide, and herbicide residues are disposed. [Shaikh and Ahammed, 2020]. Grey water from hand basins contains a variety of substances, including soaps, personal care products, shaving debris, hairs, and skin cells.

In kitchen grey water, cleaning chemicals and detergents are among the alkaline chemical pollutants found. It also includes materials like oil and fats, food residue from washing raw meat, fruit or vegetables, tea or coffee, sand and clay particles, and traces of food preservatives. Additionally, food particles, odor, salts, oil and grease, bacteria, organic matter, soaps, and suspended solids all come from the automated dishwasher.

Kitchen grey water promotes the growth of microorganisms due to the presence of food residues, a significant amount of oil and fats, and a higher temperature than typical grey water [Shaikh and Ahammed, 2020].

Laundry grey water as another type of grey water is made up of chemicals from detergents, oils, solvents, bleaches, paints, and non-biodegradable clothing fibers. Washing dirty garments and baby diapers is linked to the spread of bacteria and viruses. When compared to grey water from the shower, laundry grey water has a higher concentration of heavy metals and phosphates, which reduces its biodegradability [Shaikh and Ahammed, 2020].

As summarized [Shaikh and Ahammed, 2020] in Table 1.1, the important parameters that characterize grey water include total suspended solids (TSS), ammonium (NH_4^+), surfactants, total phosphorus (TP), boron (B), fecal coliform (FC), chemical oxygen demand (COD), and biological oxygen demand (BOD) [Pinto et al., 2010], [R. Kulabako, 2011], [Wiel-Shafran et al., 2006] and oil and grease (O and G) [Al-Hamaiedeh and Bino, 2010], [Pinto et al., 2010], [Travis et al., 2008]. Although having various characteristics, most of the grey water components can biodegrade easily. Laundry grey water, for instance, has lower nitrogen and phosphorus levels than other forms of grey water. Furthermore, laundry grey water differs from other forms of grey water in that it has a higher pH due to the usage of chemical products (usually in the 5-10 range). Depending on the source, grey water has different COD and BOD values. For example, laundry water has COD and BOD values 58-4155 mg/l and 44-3330 mg/l, respectively. Bathroom grey water has BOD and COD values of around 20-673 and 64-903 mg/l. Nitrogen concentration is 2.7-148 mg/L in bathroom grey water, 0.5-65 mg/L in kitchen grey water, and 2.8-31 mg/L in laundry grey water.

Table 1.1: Quality parameters of grey water from different sources.

Parameter	Bathroom	Hand basin	Kitchen	Laundry	Light grey water	Dark grey water
Temperature (°C)	25.8–29.0	–	24.4–30.9	22.4–35.0	13.4–29.0	22.4–35.0
Turbidity (NTU)	19-375	35-164	210-357	34-510	13-375	34-510
Total suspended solids (mg/L)	19-793	25-181	11-3934	33-4564	7-793	11-4564
pH	5.94–8.40	6.72–9.82	5.58–10.00	5.00–10.33	4.90–8.53	5.00–10.33
BOD (mg/L)	20-673	33-305	185-2460	44-3330	20-673	44-3330
COD (mg/L)	64–903	47-587	411-8071	58-4155	23-1489	58-8071
Total nitrogen (mg/L)	2.7–148.0	2.5-10.4	0.5-65.0	2.8-31.0	1.3-148.0	0.5-65.0
Total phosphorous (mg/L)	0.1–60.0	0.3-2.6	2.7-187.0	0.2-51.6	0.1-60.0	0.2-187.0

When these sources are compared, the total nitrogen concentration is highest in the bath grey water. This is due to personal care products. phosphorous concentration is 0.1-60 mg/L in bathroom grey water, 2.7-187 mg/L in kitchen grey water, and 0.2-51.6 mg/L in laundry grey water. when these sources are compared, the total phosphorous concentration is highest in kitchen grey water (Table 1.1).

1.2. Quantity of Grey Water

In term of water consumption, a person's daily water consumption can range from 15–55 L up to 90–120 liters [Jabornig and Favero, 2013]. Moreover, it has been established that in households with several taps, daily water consumption might exceed 180-300 liters per person [Rodda, et al., 2011b]. Showers account for 40% of total freshwater consumption, followed by the kitchen (12%), laundry (13%), and the toilet reservoirs (25%) [Zhu et al., 2018]. The average grey water production per capita varies by country where it ranges between 90 and 120 liters per day, based on factors such as age, sex, environment, habits, way of life, and amount of available water [Mourad et al., 2011]. Showers, baths, sinks, washing machines, and grey water from the kitchen make up a significant amount of residential wastewater, accounting for up to 80% of the total domestic wastewater. For example, in South Africa, it is estimated that 65 to 85 percent of all household water use is made up of grey water [Rodda, et al., 2011b]. The rate of grey water generation in a hot country like Oman is reported about 151 litres per capita per day (Lpcd), accounting for around 82 percent of total freshwater usage, with showers accounting for 56 %, kitchens for 28-33 %, laundry for 6-9 %, and sinks for 5-7 % [Mourad et al., 2011]. In Turkey, with a share of 75%, grey water makes up the majority of domestic wastewater by volume [Kutlu et al., 2017].

Grey water is from 50 to 80% of total wastewater in different countries as illustrated in Table 1.2 [Noutsopoulos et al., 2018]. The percentage of grey water is the lowest in USA (52.89% of the total wastewater), while it is the highest in Australia (79.60% of the total wastewater).

Table 1.2: Quantitative distribution from various domestic wastewater sources (all values in L/pe/d).

Sources	Greece	Israel	Holland	India	Australia	USA	Denmark	Sweeden	China
Hand basin	11.3 ± 1.1	18	4	17	66	88.2	43		
Bath/ shower	37.5 ± 4.3	39.2	47	30					
Kitchen	29.7 ± 5.0	26.6	7	37	47	26.5	25		
Laundry	19.2 ± 5.0	16.6	27	33	47	81.8	17		
Dish washer	0.6 ± 0.4	–	1		–	–			
Total grey water	98.4 ± 11	100.4	86	117	160	196.4	85	66	90
Other	–	–		23	–	81	7		
Toilet flushing	37.2 ± 2.5	37.7	39	25	41	101.1	27		
Total wastewater	135.6 ± 11.5	138.1	134	160	201	378.5	119	93	124
Percent of grey water (%)	72.57	72.70	64.18	73.13	79.60	51.89	71.43	70.97	72.58

Additionally, among grey water components, wastewater from bath and shower is the highest contributor in Greece, Israel, and Holland. Besides, kitchen grey water constitutes the highest amount in India whereas hand basin greywater has the highest ratio in Australia, USA and Denmark.

1.3. Grey Water Reuse

Reusing grey water can help controlling water demand and lessen the need for fresh water in irrigation systems. It lowers overall procurement prices and environmental pollution [Al-Hamaiedeh and Bino, 2010], [Al-Zou'by et al., 2017]. Environmental issues like preventing resource depletion, coping with climate change, and the desire for a sustainable lifestyle are driving forces for the growth of grey water use [Eriksson et al., 2010]. Grey water reuse can assist water-scarce countries by conserving drinking water [Mourad et al., 2011]. The need for drinking water is reduced by avoiding use of high-quality water for purposes other than drinking. By using treated grey water for end uses except drinking, natural water resources could be preserved. Consequently, the volume of drinking water treatment systems that have investment and operation cost will decrease. Since grey water recovery systems reduce drinking water usage rates, they will also reduce water distribution costs. Grey water is considered as a valuable source of water for garden irrigation and growing plants, especially in arid regions [Kutlu et al., 2017]. Some nations have developed grey water reuse techniques as a result of the freshwater scarcity. For instance, reusing grey water for irrigation has resulted in annual freshwater savings of approximately 12-65% in the city of Los Angeles [Mourad et al., 2011]. Toilet flushing which is the most well-known use for grey water reuse can reduce residential water usage by up to 30% [De Gisi et al., 2016].

Moreover, grey water recovery benefits include reduced load on the septic tanks or wastewater treatment plants, since the amount of wastewater discharged to the sewerage will decrease due to grey water treatment and use on-site. Consequently, the volume of the treatment systems and associated high investment costs could decrease. As another benefit of reduced treatment requirement, energy and chemical use will be avoided. Furthermore, grey water has the potential to contribute to plant growth due to the nutrients, organic matter, and minerals it has. Additionally, unlike rainwater,

grey water recovery is not dependent on the season and precipitation changes, so it can be regarded as a reliable water source. This results in a smaller storage volume for the grey water collection systems compared to the rainwater collection systems [Kutlu et al., 2017].

Flushing toilets, watering of green spaces like parks, schoolyards, cemeteries, golf courses, washing cars, firefighting, using as boiler feed water or concrete production are among the most prevalent uses of recycled grey water in metropolitan settings [Al-Hamaiedeh and Bino, 2010], [Al-Zou'by et al., 2017], [De Gisi et al., 2016], [Kutlu et al., 2017]. In some countries, reuse rate of treated grey water has reached 80% [Kutlu et al., 2017]. In China, grey water reuse is divided into urban, environmental, industrial, agricultural, and groundwater recharge categories. A third of the total grey water recovered is used in agriculture in semi-arid regions where industrial reuse of recovered grey water is 10-15%. Additionally, about 50% of the reclaimed grey water in Japan is used for urban reuse, 27% is used for landscape irrigation, 18% is used for melting snow, and 3.5% is used for toilet flushing. Urban reuse incorporates use for heat exchange systems, vehicle washes, and maintenance of golf courses [Zhu et al., 2018].

Water stress could be alleviated by using grey water for irrigation in small-scale agriculture and gardens on a long-term basis. Grey water, which includes nitrogen and phosphorus, can be used as a nutrition source for plant growth. Especially, farmers who are unable to purchase fertilizers will benefit from use of grey water. Similarly, grey water with its soapy form can act as a pest repellent under certain circumstances and plays a crucial role for farmers who lack access to pesticides. Moreover, the use of grey water to supply irrigation water is appealing due to seasonal water restrictions in many areas all over the world and continued poverty in low-income settlements [Rodda, et al., 2011a].

Grey water reuse has been a common practice for a long time. For instance, plant irrigation has been implemented using grey water from the bathroom directly. Additionally, in Australia, Syria, South Africa, and Israel, grey water is applied directly to the gardens and landscapes as well as to fruit trees for irrigation. However, when grey water is used directly for irrigation over an extended period, salts, surfactants, oil, and grease can build up in the soil. In this context, grey water application affects plant health and soil structure negatively and causes groundwater pollution. Besides, grey water that is used untreated causes stains the toilet bowl which

results in using more toilet cleaner [Üstün and Tırpancı, 2015]. Thus, it is strongly suggested to treat grey water before usage. Even though direct use of grey water saves water and prevents grey water storage issues, the storage duration of grey water should not be long if it is used without being treated. For example, grey water from the bathroom can be used for irrigation in the garden upon cooling [Üstün and Tırpancı, 2015].

1.4. Grey Water Treatment

Depending on the quality of grey water, physical, chemical, or biological treatment technologies can be utilized to achieve the appropriate requirements [de Gisi et al., 2016], [Üstün and Tırpancı, 2015]. The best technique depends on a variety of parameters, including the size of the business, the ultimate use of the water, socio-economic factors affecting water costs, and regional norms and practices [De Gisi et al., 2016]. Physical treatment methods such as precipitation and filtration are often used as pretreatment processes [Kutlu et al., 2017], [Üstün and Tırpancı, 2015]. Filtration is a commonly employed step before the biological or chemical treatment units. Additionally, some organic compounds and pathogens can be eliminated during the filtration process by employing a sand filter, coarse filter, or membrane filter as a pre-treatment. Physical treatment technologies are incapable of removing nutrients. As a result, chemical treatment procedures that remove both particles and nutrients are used in many studies [Üstün and Tırpancı, 2015]. Physical treatments are not sufficient alone to adequately reduce the concentrations of organic matter, nutrients, and surfactants. As a result, reuse of grey water after physical treatment is not advised [Li et al., 2009].

Chemical treatment technologies include electrocoagulation, photocatalytic oxidation, ion exchangers, and granular activated carbon. Biological treatment systems, on the other hand, are recommended in grey water treatment to reduce chemical consumption because chemical treatment methods heavily depend on chemicals and increase chemical substance consumption [Kutlu et al., 2017], [Üstün and Tırpancı, 2015]. Chemical treatment can be effective to remove suspended particles, organic matter, and surfactants from low strength grey water [Li et al., 2009]. Possible biological treatment systems include the biological contactor (RBC),

sequential batch reactor (SBR), upstream horizontal flow anaerobic sludge bed reactors (UASB), membrane bioreactors (MBR), and built wetlands (CW) [Oteng-Peprah et al., 2018], [Yoonus and Al-Ghamdi, 2020]. Anaerobic treatment methods are not advised due to their ineffectiveness in removing both organics and surfactants from grey water [Li et al., 2009]. Filtration or precipitation for pre-treatment, and UV or chlorine disinfection as final treatment may help grey water to satisfy recovery criteria by biological processes other than the membrane bioreactor [Üstün and Tırpancı, 2015]. Aerobic biological processes such as the rotating biological contactor and the sequential batch reactor can be used to treat grey water of medium and high strength. Grey water reuse that combines the aerobic biological process with physical filtration and disinfection is determined as the most practical and affordable approach available. For medium- to high-strength grey water recovery, membrane bioreactors appear to be a desirable option, especially in large urban residential structures servicing populations of more than 500 [Li et al., 2009].

pH, electrical conductivity, suspended particles, heavy metals, *Escherichia coli*, dissolved oxygen, biological and chemical oxygen demand, total nitrogen, and total phosphorus are significant factors to take into account for the suitability of grey water for reuse. Recommended levels for irrigation, cleaning, and flushing toilets are 6-9 for pH, 2–20 NTU for turbidity, 15 mg/L for TN, 0.5 mg/L for TP, 2.2–100 CFU/mL for *T. coli*, 2–100 CFU/mL for *E. coli*, >5-45 mg/L for BOD, >20-200 mg/L for COD and 3–30 mg/L for TSS [Prajapati et al., 2019]. Reusing greywater with a pH over 8 may increase soil pH and lower the availability of some micronutrients for plants, which may have an impact on plant growth [Pinto et al., 2010].

Katukiza et al. (2014) and Laaffat et al. (2019) consider pH appropriate for irrigation without treatment, but according to Travis et al. (2008) treatment is required for a suitable pH. Since the required turbidity (2-20 NTU) is lower than the usually measured turbidity of grey water (20-375 NTU) grey water requires pretreatment which can decrease the turbidity levels down to 0.4-8 NTU. BOD and COD also present grey water's organic matter content which is vital for microbial growth. Table 1.3 shows that BOD and COD values are in the range of 44-3330 mg/L for BOD and 72-6563 mg/L for COD indicating that grey water should not be used without treatment since BOD and COD are above the maximum permitted limits (<45 mg/L for BOD and <200 mg/L for COD). N and P are nutrients and significant in terms of fertilizing the crops. However, excess amounts of N and P could cause algae growth

in storage tanks. Grey water can be used as irrigation water without treatment in the cases where TN is lower than 15 mg/L but treatment is compulsory if TN is higher as reported by some studies (above 226 mg/L). However, P is a more critical nutrient since TP values are reported between 0.35 and 24 mg/L and frequently higher than 0.5 mg/L. So, grey water has to be treated for P management. Table 1.3 summarizes the parameters before and after treatment more in detail based on the literature. The most frequent threats linked with irrigation are those brought by high pH, salinity, and Boron in grey water, as well as the possible buildup of pathogens, metals, and organic compounds in soils. The first three components primarily affect soil characteristics, whereas the latter three factors, particularly irrigation of edible crops, may have an impact on human health [Finley et al., 2009].

1.5. Negative Impacts of Grey Water Reuse

Despite its many potential benefits, using grey water for irrigation might have a negative impact on soil qualities and plant growth. Irrigation water at poor quality degrades the physical quality of the soil and reduces agricultural performance [Al-Zou'by et al., 2017]. For instance, detergents used for laundry contain several chemicals such as surfactants, builders, bleaching agents and auxiliaries or additives which are known to affect soil properties.

Nutrients can be beneficial to plants since a balanced nutrient content is necessary to prevent nutrient insufficiency or toxicity. However, the possibility of salt and boron accumulation in soil as a result of grey water irrigation could have a negative impact on soil characteristics and plant growth [Misra et al., 2010]. Thus, the concentrations of plant macronutrients, particularly nitrogen and phosphorus, as well as salts, are the variables of highest importance in plants and soil.

Table 1.3: Quality parameters of grey water before and after treatment.

Before Treatment											
Parameter	Unit	1	2	3	4	5	6	7	8	9 ¹	
										Indonesia	Thailand
Temperature	°C	23.5 ± 2.2	-	-	-		13.4-29.0		-		
pH	–	7.6 ± 1.5	8.1	7.6	10.5	6.3-8.1	4.9-8.53	8.1-9.8	8.5	6.5–8.6 (7.2 ± 0.8)	7.2 ± 0.4
Turbidity	NTU	-	57.87	65	-	29-375	13-375		20		
EC	µS cm ⁻¹	2150 ± 147	982	580	1358	-		267 ± 30	-		
COD	mg L ⁻¹	6563 ± 1864	72.16	77.2	-	100-700	23-1489	280-310	-	232–780 (418 ± 219)	160 ± 64
BOD ₅	mg L ⁻¹	1395 ± 466	-	44.2	-	47-466	44-3330		230	125–401 (225 ± 96)	90 ± 44
TOC	mg L ⁻¹	940 ± 161	-	-	-	-			-		
DOC	mg L ⁻¹	568 ± 102	-	-	-	-			-		

¹ 1. [Katukiza et al., 2014], 2. [Prajapati et al., 2019], 3. [Laaffat et al., 2019], 4. [Pinto et al., 2010], 5. [Li et al., 2009], 6. [Shaikh and Ahammed, 2020], 7.[Rodda, et al., 2011b], 8. [Sudarsan et al., 2021], 9. [Widyarani et al., 2022]

Table 1.3: Continued.

TSS	mg L ⁻¹	2828 ± 735	34	8		25-183	11-4564	750	77–382 (189 ± 115)	69 ± 31
Oil and grease	mg L ⁻¹	26.0 ± 7.1	-	-	-	-		-	24–87 (49 ± 33)	-
NH⁺₄ - N	mg L ⁻¹	29.5 ± 7.9	-	-	-	-		-		
NO⁻₃ - N	mg L ⁻¹	2.7 ± 0.5	-	-	-	-				
TN	mg L ⁻¹	-	7.02	7.1	0.2	1.7-34.3	1.3-148	206 ± 5.8	59–226 (142 ± 119)	-
TKN	mg L ⁻¹	34.8 ± 9.2	-	-	-	-		206 ± 2.7		
Total-P	mg L ⁻¹	6.2 ± 0.6	0.35	0.8	4.4	0.11-22.8	0.1-60	69 ± 0.6	24	1.5 ± 0.9
Ortho-P	mg L ⁻¹	3.9 ± 1.4	-	-	-	-		40 ± 7		
Total coliforms	cfu.(100 mL) ⁻¹	8.72×10^7 ($\pm 1.8 \times 10^7$) 1.1x10 ⁹	3×10^7 -	5×10^5	-	0.1-1.5x10 ⁸	2.0x10 ² -			
Fecal coliforms	cfu.(100 mL) ⁻¹	-	-	-	-	0.1-1.5x10 ⁸	3.2x10 ¹ -1.7x10 ⁵	-	2.4×10^3 – 1.2×10^9 (4.0 ± 6.9 × 10 ⁸)	
Escherichia coli	cfu.(100 mL) ⁻¹	3.70×10^6 ($\pm 3.8 \times 10^6$)	165.6	3×10^3	-	-	1.0x10 ¹ -1.1x10 ⁶	-		

Table 1.3. Continued.

Parameter	Unit	After Treatment			
		1	2	3	4 ²
Temperature	°C	24.0 ± 1.5	-	-	-
pH	-	7.1 ± 2.3	8.07	7.8	7.4 ± 0.1
Turbidity	NTU	-	0.4	8	-
EC	µS cm ⁻¹	1979 ± 281	1098	540	1.20 ± 0.06
COD	mg L ⁻¹	3194 ± 785	9.63	8.5	99 ± 20
BOD5	mg L ⁻¹	1025 ± 625	-	5.74	9 ± 2
TOC	mg L ⁻¹	892 ± 124	-	-	-
TSS	mg L ⁻¹	1145 ± 437	3.3	0.8	19 ± 4
Oil and grease	mg L ⁻¹	6.7 ± 2.1	-	-	4.3 ± 2.7
NH+4 - N	mg L ⁻¹	26.7 ± 6.3	-	-	1.26 ± 0.8
NO-3 - N	mg L ⁻¹	3.3 ± 0.3	-	-	-
TKN	mg L ⁻¹	29.5 ± 4.7	-	-	-
TN	mg L ⁻¹	-	4.96	4.12	6.3 ± 0.4
Total-P	mg L ⁻¹	5.8 ± 0.3	2 x 10 ⁷	0.4	2.9 ± 0.4
Ortho-P	mg L ⁻¹	3.4 ± 1.5	-	-	-
Total coliforms	cfu.(100 mL) ⁻¹	8.98 × 10 ⁷ (±1.5 × 10 ⁷)	507.1	-	-

² 1. [Katukiza et al., 2014], 2. [Prajapati et al., 2019], 3. [Laaffat et al., 2019], 4. [Travis et al., 2008]

Nutrients can help plants to grow and in case they are not present sufficiently in soil, grey water has the potential to serve as a low-cost fertilizer for low-income gardeners who can't purchase fertilizer. Sodium, on the other hand, is hazardous to plants due to direct absorption through roots and leaves, as well as its effects on soil water intake. It has the potential to produce soil salinization and the formation of sodic conditions, resulting in progressive deterioration of soil structure and plant growth ability [Rodda, et al., 2011b]. Furthermore, high salinity and boron buildup in the soil due to surfactants has negative consequences for crops, such as limiting growth [Wiel-Shafran et al., 2006].

Surfactants are a particular class of synthetic material that are frequently found in grey water as residues from laundry detergents and other household and personal care products. Although there are cases where surfactants are used as soil conditioners in agriculture and horticulture to enhance soil structure, infiltration, and control erosion, it has been also demonstrated that the presence of surfactants in irrigation water could significantly alter the hydraulic conductivity of soils. It has also been suggested that a significant buildup of surfactants in soils may eventually result in soils that are water repellent, which would have a detrimental effect on agricultural productivity and environmental sustainability [Misra et al., 2010].

When grey water is utilized for irrigation, salt and metals can build up in the soil [Al-Hamaiedeh and Bino, 2010]. Additionally, the deposition of oil and grease from grey water raises the soil-water contact angle, reducing water absorption into the soil substantially [Travis et al., 2008]. Lastly, reusing grey water with a pH more than 8 can result in decreased availability of some micronutrients because they affect plants' growth [Pinto et al., 2010].

Analysis was performed for growth of algae and willow trees to evaluate the phytotoxicity of grey water from different sources. Kitchen grey water was found to be toxic to both algae and willow trees likely due to presence of bleach, while bath grey water was found to be toxic only to algae. It was reported that there is a significant decrease in the transpiration rate of willow trees when the pH of the grey water was above 9.0. Willow trees showed insensitivity to pollutants, while algae showed significant growth reduction if the contaminant concentrations are increasing in grey water. A slight decreasing trend in shoot and root biomass was found as a result of irrigating chards with 100% grey water [Pinto et al., 2010]. However, it was shown

that no apparent detrimental effect on plant growth was observed for any of the grey water irrigation cases.

Irrigation with treated grey water resulted in significant increases in corn and tomato length compared to irrigation with tap water (control group), whereas irrigation with raw grey water also boosted plant heights but at a rate less than observed with tap water or treated grey water. In other words, the highest growth factor (GF) was found for corn watered with treated grey water, while the lowest GF was found in corn irrigated with raw grey water. Moreover, irrigation with untreated grey water caused mortality of maize and tomato seedlings after 4 weeks but irrigation with tap water and treated grey water kept the plants healthy and alive. In addition, untreated grey water irrigation was determined to cause serious root system damage [Qrenawi et al., 2022].

1.6. Microplastics in Grey Water

In addition to conventional pollutants, grey water contains microplastics which have recently been identified as having environmental impacts [Huang et al., 2022], [Kalčíková et al., 2017], [Praveena et al., 2018], [Raju et al., 2018]. Microplastics are heterogeneously mixed polymers with a size of less than 5 mm that comprise plastic fibers, granules, or shards [Guo et al., 2020].

The MP compositions generally include a high molecular polymer as the main body, and plasticizer, stabilizer, and coloring agent as auxiliary materials. The polymer types detected in the soil environment include many commonly used plastics, such as PE, polypropylene (PP), polyamide (PA), polyvinyl chloride (PVC), polystyrene (PS), polyethylene tere-phthalate (PET), styrene-butadiene rubber (SBR), polyurethane (PU) and expanded polystyrene (EPS) [Wang et al., 2022].

In grey water, microplastics can originate from personal care products and contribute to a pollution load [Kalčíková et al., 2017], [Lei et al., 2017], [Mon and Nakata, 2020], [Praveena et al., 2018], [Raju et al., 2018]. Personal care products are generally classified as facial cleanser, toothpaste, and shower gel. The sizes of microbeads originating from facial cleanser usually range from 10 to 1020 μm [Kalčíková et al., 2017], [Lei et al., 2017], [Mon and Nakata, 2020], [Praveena et al., 2018] whereas the size of the microbeads from the shower gel range from 110 to 970 μm [Lei et al., 2017]. Toothpaste as another personal care product contribute to

microbead release within the size of 63-250 μm and 3-145 μm [Mon and Nakata, 2020], [Praveena et al., 2018]. It was reported that a single application of toothpaste (1.6 g) can contain up to 4000 pieces of polyethylene [Raju et al., 2018]. As a result, the amount of microplastics emitted every year from shower gels is estimated as 39 tons [Lei et al., 2017]. Raju et al. (2018) estimated that 270 tons of microbeads from personal care products is released into the sewer system. Moreover, the amount of microbeads from personal care products released into the sewer system is reported as 15.2 mg per person per day [Kalčíková et al., 2017], while another other study indicated that only facial cleansers can release microbeads at an average daily rate of 15.2 mg per person [Raju et al., 2018]. The first source was personal care products that are rinsed off, which contributed with approximately 500 kg/year. personal care products can be used in both households and enterprises, but the large majority came from households. the dominant MP type from personal care products was polyethylene with a load in the range of 179.05 – 198.86 kg/year [Fältström et al., 2021].

The other source of microplastics in grey water is discharge from washing machine. The release of microfibers into wastewater during the washing of synthetic clothes using various washing techniques has been the subject of numerous researches. It was estimated that thousands of microfibers can be released in a single wash of 5–6 kg laundry at home [de Falco et al., 2020]. According to Browne et al. (2011), polyester clothing (blankets, fleece, and shirts) can lose more than 1900 fibers per wash where 180 % more fibers were sourced from fleece. Compared to other fabric types of wool and microwool synthetic fabrics shed the highest number of fibers up to 7360 per m^2 area of fabric per liter ($\text{fiber}/\text{m}^2/\text{L}$) in a single wash, according to Carney Almroth et al. (2018)'s study which used a laboratory scale washing machine. This finding suggests that fabric structure is a key factor in the potential of the fabrics to shed fiber. Similar findings were observed by Napper and Thompson (2016), who found that 6 kg of synthetic fabric (polyester, polyester-cotton blend, and acrylic fabrics) can release 140.000-700.000 fibers per wash in standard home washing machines. Another study demonstrated that more than 0.3% of the mass of unwashed clothes made up of microfibers can be lost during washing laundry. The lost mass can reach up to 2 g of mass per garment for coats or sweaters made of polyester, wool, or non-woven polyester nylon [Hartline et al., 2016]. The majority of the microplastics entering the Sjölanda wastewater treatment plant came from the laundry, which accounted for 40–87% of the overall load. Despite the fact that laundry is an industrial

activity as well, homes are thought to be responsible for 96–97% of the total contribution. The dominant MP type from washing machine was polyester with a load in the range of 230.73-3775.72 kg/year [Fältström et al., 2021].

Hernandez et al. (2017), in their study on polyester fabrics, showed that the use of detergents in home laundry had the greatest effect on microfiber release. It has been determined that approximately 75% more microfiber is released when detergent is used regardless of the type (liquid, powder, or surfactant) and dosage of the detergent. 0.025-0.1 mg fiber/g textile was released when washed with or without detergent, respectively. Similar trends were also seen in polyester and polypropylene knitted and woven fabrics. According to De Falco et al. (2018) powder detergent caused more microfiber emission than liquid detergent, and generally, commercial washing products had a higher influence on microplastic release than household washing detergents. They concluded that the use of detergents encourages the increase of microfibers released from fabrics during laundering [De Falco et al., 2018]. When the impact of using fabric softener was investigated, it was determined that fabric softener use decreased microfiber shedding by 35%. [De Falco et al., 2018], [Hernandez et al., 2017], [Napper and Thompson, 2016], [Pirc et al., 2016].

When washing machines of different sorts are examined, it was found that top-loading machines produce higher microfiber mass than front-loading machines [Hartline et al., 2016]. Furthermore, mechanically aged fabrics released more fiber than new fabrics, according to Hartline et al. (2016). Studies on the impact of sequential washing, on the other hand, revealed that the number of microfibers generated during washing decreases with increased wash cycles until it reaches a constant level [Carney Almroth et al., 2018], [Napper and Thompson, 2016], [Pirc et al., 2016], [Sillanpää and Sainio, 2017]. Sillanpää and Sainio (2017) reported polyester and cotton fabrics with different structures caused release of 0.12-0.33% (w/w) per laundered fabric and 2.1×10^5 - 1.3×10^7 fiber per kg fabric washed. The study's findings indicate that cotton fabrics have a stronger tendency to shrink than polyester fabrics. However, because of the variations in fabric structures, it is challenging to establish a firm inference from these results.

In conclusion, all fiber types release considerable amounts of microfibers after laundry, although cellulose-based fabrics release more microfibers than polyester fabrics with similar fabric structures. The physicochemical qualities of the fiber and yarn have a significant impact in microfiber production. Fabrics with higher abrasion

resistance, lower hairiness, and higher yarn breaking strength have a lesser tendency to release microfibers when washed mechanically. The use of detergent increases the formation of microfibers because the surfactant improves the mobilization of fibers from the cloth to the washing solution. In general, the results of accelerated laundry studies matched those of home laundry experiments, although accelerated laundry experiments were easier to regulate and had higher sensitivity and repeatability [Zambrano et al., 2019].

1.7. Impact of Microplastics on Soil Properties and Plant Growth

Microplastics in grey water have been found to have a deleterious impact on plant development and soil biophysical structure [Boots et al., 2019]. The effects of microplastics on soil vary depending on the type of microplastic and the plant-soil interaction involved [Lozano and Rillig, 2020]. Microplastics have the potential to affect the physical structure of soil, including bulk density, water holding capacity, and water-stable aggregates. The bulk density of the soil is one of the most essential soil parameters [Haddad et al., 2018] which describes the mass of solids per unit volume of soil [Eftene et al., 2020]. This property is a physical property that allows the determination of soil hydraulic potential, root growth and especially the amount of soil organic carbon (SOC) stock. Decrease or increase in bulk density can affect the movement of atmospheric CO₂ in soil and the evacuation of greenhouse gases [Haddad et al., 2018]. Bulk density is also a critical parameter for characterizing the unsaturated zone, measuring water, solute, and carbon transport and storage, and modeling water and solute transitions from the atmosphere to the hydrosphere via the lithosphere [Lu et al., 2021], [Tian et al., 2020]. The thermal, hydraulic, and mechanical properties of the soil are all influenced by the bulk density of the soil. Furthermore, soil bulk density has a substantial impact on the gas, water, and heat transport mechanisms in the soil [Tian et al., 2020]. Compacted soil has a high bulk density and low porosity, resulting in lower oxygen and water content, forming a physical barrier that prevents root growth; hence, lowers plant water and nutrient uptake [Zoz et al., 2021]. For example, it was determined that increasing bulk density inhibits the growth of plant roots, as observed in tobacco, tomato, chestnut-leaved oak and Cappadocia maple. This is due to a decrease in soil porosity, aeration, and water infiltration, as well as an increase in

mechanical impedance. Plants have access to a smaller soil volume due to limited root growth in compacted soils, which reduces availability of water and nutrients. When the plant is grown in a water-stressed environment, these effects are amplified. There are negative consequences of soil bulk density stress, such as reduced crop growth, development, and yield [Zoz et al., 2021]. Soil organic matter can be raised to have lower soil bulk density and higher nutrient cycle aiming to lessen the negative impacts of soil bulk density [Kurmi et al., 2020].

The other parameter that is affected by the presence of microplastics in soils is water-stable aggregates. Aggregates are created by the assembly of soil particles adhering to each other due to strong adhesive forces. Micro aggregates created from organic molecules are joined with clay and cations during the soil aggregation process, and then connected to other micro aggregates to form macro aggregates according to the hierarchical arrangements. Such an aggregation process physically preserves soil organic matter by isolating it from decomposers; thus, preventing decay and prolonging the average residence time of soil organic carbon [Kurmi et al., 2020]. Microorganisms and plant residues both promote the development of water-stable aggregates. The development of water-stable soil macroaggregates (>250mm) has an impact on a number of physical and biogeochemical processes, including carbon sequestration, erosion control, soil biodiversity, biological activity, plant growth, and others. The physical, chemical, and microbiological characteristics of soils, plant roots, and environmental conditions can all influence the production and stability of soil aggregates. Therefore, the presence of stable aggregates help to maintain soil fertility by minimizing erosion and improving the physical, chemical and biological quality of the soil [Cheng et al., 2020]. To promote the stability of the water-stable aggregates, the surface soil is suggested to be protected mechanically. For example, mulching causes an increase in aggregate size as well as a minor drop in soil bulk density, which reduces soil compaction [Steinmetz et al., 2016].

Water holding capacity is the third parameter that is important to determine the amount of available water for plants. The available water holding capacity of the soil is between the area capacity and the permanent wilting point [Blaschek et al., 2019]. It is known that the soil's ability to hold water affects plant distributions, as well as microbial activity, nutrient cycling, carbon allocation, and the rate of photosynthesis [Cianfrani et al., 2019].

Table 1.4 summarizes the studies in the literature which investigated the impact of microplastics on soil properties. Although polyester fibers may cause soil particles to be held in a clump, they are not involved in aggregate formation in sandy soils [Khalid et al., 2020]. But 0.1-0.3% (w/w) polyester microfiber was reported to increase water-stable macroaggregates (>2 mm) in a soil composed of 4% sand, 41% silt and 35% clay [Zhang et al., 2019]. On the other side, De Souza MacHado et al. (2019) observed a decrease in water stable aggregates with polyester (0.2%) and polyacrylic (2%) in loamy sand soil planted with grass. When compared to soil without the microplastics, micro-aggregates in soils exposed to 1 g (%0.1 w/w) HDPE and PLA were dramatically reduced in sandy clay loam soil with grass [Boots et al., 2019]. While microplastic films, particularly polyethylene (LDPE) foams, displayed the reverse trend as concentration increased, soil aggregate stability was lowered by microplastic films. At lower concentrations, PET and PP fragments decreased soil aggregation; however, at the maximum concentration (0.4%), this effect was neutralized.

The concentration effect of PC pieces was nonlinear, with the strongest decline in soil aggregate stability occurring at the highest concentration in sandy loam soil with carota [Lozano et al., 2021]. De Souza MacHado et al. (2018), found that decreased water stable aggregates when polyacrylic and PES at a concentration of 0.05, 0.1, 0.2 and 0.4% and PA and PE at a concentration of 0.25, 0.5, 1 and 2 % directly were added the loamy sand soil, grass seeds were planted in a 0.5 L pot and exposed to microplastics for 35 days (Table 1.4). Boots et al. (2019) planted grass seeds exposed to microplastics for 30 days in their experimental setup.

Table 1.4: Literature review of studies about impact of microplastics on soil properties and plant growth.

Type of MP ³	MP Concentration (w/w %) ⁴	Soil texture	Plant	Duration (day)	Scale of the study	Irrigation plan	Effect	Ref. ⁵
synthetic fibers (acrylic and nylon mixture) PLA HDPE	0.1 0.001	sandy clay loam soil (1060 g of soil)	Grass seeds	30	in a 1.3 L pot experiment	10 mL of tap water per day	decrease seeds germinated decrease in shoot height with biodegradable polylactic acid decrease micro-aggregates	1
PES	0.1 0.3	Nitisol soil (40% clay 42% silt 18% sand) 4% sand, 41% silt 35% clay	-	72 365	in PVC tube (8 cm height and 15 cm diameter) field experiment	-	cannot alter soil bulk density increase water-stable large macroaggregates (>2 mm)	2

Table 1.4: Continued.

Polyacrylic PES PA PE	0.05 0.1 0.2 0.40 0.25 0.50 1.00 2.00	loamy sand soil (70g of soil)	grass (mostly <i>Elymus repens</i>)	35	PP pots (5.5 cm height and 5.7 cm diameter)		Increase water holding capacity of the soil Decrease water stable aggregates Decrease bulk density	3
PES PA PE polyester terephthalate PP PS	0.05 0.1 0.2 0.4	Loamy sand soil (200 g soil)	spring onion seeds	45	200 mL glass beaker	every 2 days to 60% of water holding capacity	Decrease soil bulk density Decrease water stable aggregates, Increase water holding capacity	4

Table 1.4: Continued.

(LDPE) starch-based biodegradable plastic (Bio)	1	sandy soil (87% sand, 12% slit 1% clay)	wheat seeds	70	2 L pot	weekly with tap water and the soil moisture was kept at around 12% to 18% with respect to weight.	Decrease wheat growth Decrease Total plant biomass	5
PP PE PVC, PET	0.02 0.1 0.2	silty clayey soil	<i>C. pepo</i> plants seeds	28	dark coated glass pots (5.5 cm diameter x 6 cm depth)	two times a week with 8.5 mL	Polyethylene did not affect fresh weight at all the tested concentrations. In root fresh weight 0.1% concentration of polyethylene terephthalate and polyvinyl chloride decrease On both root and shoot fresh weight polypropylene decrease	6

Table 1.4: Continued.

LDPE biodegradable plastic (Bio- MPs)	0.5 1.0 1.5 2.0 2.5	sandy soil (6 kg)	bean (<i>Phaseolus vulgaris L</i>)	120	7 L PP pot	twice a week until the water content of the pots is 10(±1) %	LDPE-MPs showed no impact on all parameters Increase specific root length in 2.5% LDPE-MPs treatment	7
PES PA PP LDPE PET PU PS PC	0.1 0.2 0.3 0.4	sandy loam soil (190 g of soil)	<i>Daucus carota</i>	30	200 ml pot	50 ml of distilled water	Increase shoot mass Increase root biomass Decrease Soil aggregation	8

³ PLA; biodegradable polylactic acid, HDPE; conventional high-density polyethylene, LDPE; low-density polyethylene, Bio; starch-based biodegradable plastic, PP; polypropylene, PE; polyethylene, PVC; polyvinyl chloride, PET; polyethylene terephthalate, LDPE-MPs; low-density polyethylene, Bio-MPs; biodegradable plastic, PES; polyester, PS; polystyrene, PA; polyamide, PU; polyurethane, PC; polycarbonate

⁴ MP are added into soil and mixed thoroughly.

⁵ 1. [Boots et al., 2019], 2. [Zhang et al., 2019], 3. [de Souza MacHado et al., 2018], 4. [de Souza Machado et al., 2019], 5. [Qi et al., 2018], 6. [Colzi et al., 2022], 7. [Meng et al., 2021], 8. [Lozano et al., 2021]

What they found is that micro-aggregates were reduced when synthetic fibers (acrylic and nylon mixture), PLA and HDPE at a concentration of 0.1 and 0.001% directly were added the soil. They also found decreased soil aggregation when PES, PA, PP, LDPE, PET, PU, PS, and PC at a concentration of 0.1, 0.2, 0.3, and 0.4% directly were added the sandy loam soil.

When the studies about water holding capacity were reviewed it was realized that polyester (0.05, 0.1, 0.2, and 0.40) increased the water holding capacity whereas polyacrylic (0.05, 0.1, 0.2, and 0.40%) and polyethylene (0.25, 0.50, 1.00, and 2.00%) had no clear impact in loamy sand soil with grass [de Souza MacHado et al., 2018].

This increase in water holding capacity of the soil was attributed to the potential of polyester fibers to help aggregate formation. However, beads, flakes or other microplastic particles such as polyacrylic fibers were not found effective to change soil aggregation or water holding capacity [Khalid et al., 2020]. de Souza MacHado et al. (2018), found that increased water holding capacity of the soil, when polyacrylic and PES at a concentration of 0.05, 0.1, 0.2 and 0.4% and PA and PE at a concentration of 0.25, 0.5, 1 and 2 % directly were added the loamy sand soil, grass seeds were planted in a 0.5 L pot and exposed to microplastics for 35 days (Table 1.4).

Although polyester microfibers (0.1 and 0.3%) did not alter soil bulk density in some studies conducted with clay loam soil [Zhang et al., 2019], there were contradictory ones which reported that polyester (0.2%), PP (2%), HDPE (2%), PS (2%) and polyethylene (2%) decreased but PA (2%) no significant effect the bulk density of loamy sand soil with spring onion seedlings [de Souza MacHado et al., 2018]. De Souza MacHado et al. (2018), found that decreased bulk density when polyacrylic and PES at a concentration of 0.05, 0.1, 0.2 and 0.4% and PA and PE at a concentration of 0.25, 0.5, 1 and 2 % directly were added the loamy sand soil, grass seeds were planted in a 0.5 L pot and exposed to microplastics for 35 days (Table 1.4).

In addition to soil properties, microplastics have an effect on plant growth performance [Lozano and Rillig, 2020]. Microplastics in the soil affect root and shoot lengths of the plants, as well as root and shoot dry weights. The roots and the shoots are dependent on one another. This functional balance changes depending on the species; the shoot provi the roots carbohydrates, and the roots give the shoots water and nutrients. A relatively small root system may be advantageous for maximizing plant production if it can supply enough water and nutrients. However, under rapidly

altering environmental conditions, a strong root system can minimize risks [Sheng and Hunt, 1991].

The overall root length rose considerably with all examined microplastics in 0.05-0.4 % (w/w). When compared to PA, exposure to PES, PET, and PP, as well as exposure to PEHD and PS, significantly enhanced the ratio of root to leaf dry biomass for spring onion [de Souza Machado et al., 2019]. Also, (LDPE) and starch-based biodegradable plastic (Bio) added into sandy soil at a concentration of 1% considerably decreased the total plant biomass and growth of the wheat [Qi et al., 2018]. It was determined that 0.1% concentration of polyethylene terephthalate and polyvinyl chloride decreased root fresh weight of *Cucurbita pepo* L. Besides, it was observed that both of the root and shoot fresh weight of *Cucurbita pepo* L. decreased in case of polypropylene (0.02, 0.1 and 0.2%) presence. However, polyethylene did not affect *Cucurbita pepo* L. fresh weight at all the tested concentrations (0.02, 0.1 and 0.2%) [Colzi et al., 2022]. Colzi et al., (2022) found that polyethylene did not affect fresh weight at all the tested concentrations, in root fresh weight 0.1% concentration of polyethylene terephthalate and polyvinyl chloride decreased, and on both root and shoot fresh weight polypropylene decreased when PP, PE, PVC, and PET at a concentration of 0.02, 0.1 and 0.2% directly were added the silty clayey soil, watered two times a week with 8.5 mL C. pepo plants. seeds were planted in a 0.5 L pot and exposed to microplastics for 28 days. Another study pointed out LDPE-MPs (0.5, 1.0, 1.5, 2.0 and 2.5%) showed no impact on most parameters (shoot, root and fruit biomass) except specific root length which increased at 2.5% LDPE-MPs concentration. Also, biodegradable plastic (Bio-MPs) at a concentration of 1.5, 2, and 2.5% lowered shoot and root lengths of bean seeds significantly in the sandy soil [Meng et al., 2021]. As reported by Lozano et al. (2021) *Daucus carota* shoot mass was increased if fibers, films, foams, and fragments were found in soil in comparison to the control without microplastics other than PA and PS. In contrast to fibers, which produced a *Daucus carota* root biomass similar to the control, foams, films, and fragments all enhanced the biomass of the root. Boots et al. (2019) planted grass seeds exposed to microplastics for 30 days in their experimental setup. What they found is a decrease in seed germination (6 and 7%) and shoot height due to biodegradable polylactic acid (0.1%) or fibers. Lozano et al., (2021) found increased shoot mass and root biomass when PES, PA, PP, LDPE, PET, PU, PS, and PC at a concentration of 0.1, 0.2, 0.3, and 0.4% directly were added the sandy loam soil, watered 50 ml of

distilled water the plant *Daucus carota* were planted in a 200 mL pot and exposed to microplastics for 30 days.

In summary, literature about impacts of microplastics on soil properties and plant growth is limited to microplastic addition into soil directly and homogeneously. Moreover, there is no consensus about how characteristics of soils and growth of the plants was affected. Instead, impact depends on the type and concentration of the microplastic, in addition to type of the plant and soil. As a result, it is very challenging to compare results between studies and infer how microplastics found at concentration in grey water could influence soil and plants in case of grey water use for irrigation.

1.8. Aim and Scope of the Study

As summarized in the introduction, grey water could be utilized as a valuable water and nutrient source for irrigation to reduce the need of freshwater resources and synthetic fertilizers. However, there are some concerns about the negative impacts of grey water components on soil and plant health. Especially microplastics as a recently emerging pollutant group in grey water have not received sufficient attention yet.

Studies in the literature quantified microplastics in grey water from different sources. However, in studies that investigated impacts of microplastics on soil properties and plant growth experiments were designed where microplastics were mixed into soil homogeneously. In other words, microplastic entrance into soil through grey water irrigation was not considered. Additionally, literature review revealed that microplastic concentrations added into soils were much higher than microplastic concentrations detected in grey water. Therefore, there is a need to understand how microplastics at concentrations found in grey water can affect soil and plants in case microplastics are transferred into soil inhomogeneously through irrigation. Within the scope of the study, grey water originated from laundry hand basin and bathtub were considered. As literature review revealed that polyesters from laundry and polyethylene from personal care products are the major MP sources, synthetic water containing polyester and polyethylene at concentrations determined in grey water was prepared and used for irrigation. Additionally, another MP concentration from literature was inferred at which soil properties are reported to change. In order to

determine the effect of plant type, spring onion to represent edible crops and grass to represent common vegetation in green areas were used.

Goals of this study have become:

- Determination of the impact of microplastics concentration on soil properties such as bulk density, water stable aggregates and water holding capacity.
- Determination of the impact of plant presence and type on soil properties such as bulk density, water stable aggregates and water holding capacity in case of irrigation with synthetic water containing microplastics.
- Determination of the impact of microplastics concentration on plant growth parameters like root and shoot length as well as root and shoot dry weight.
- Determination of the impact of plant presence and type on plant growth parameters like root and shoot length as well as root and shoot dry weight in case of irrigation with synthetic water containing microplastics.

This study aims to answer if reuse of grey water for irrigation poses a threat on soil and plant health in terms of its microplastic content. Results of the study will enable risk assessment of microplastics in grey water and serve to further study the potential of grey water to contribute circularity in cities.

2. MATERIALS AND METHODS

2.1. Plant Characteristics

Commercial grass (Green Space genus, Gürçim) and spring onions (Arzuman) seeds were purchased.

2.2. Soil Properties

Garden soil was obtained from GMP Landscaping at two different times as 2 sets. Various properties of soil such as texture, moisture content, and organic content were determined. For the purpose of determining the soil's texture, sieve analysis was used. Initially, a precision balance was used to weigh 300 g of soils in 1 L beakers. It was then dried at 105 °C for 24 hours in the oven. The soils removed from the oven after 24 hours were cooled at room temperature and put through sieves with opening sizes 2 mm and 0.05 mm. The weight of the soils passed through the sieves were measured and percentage of the each size class is calculated. As illustrated in Table 2.1, while 1. set soil can be defined as silty loam soil, 2. set soil can be defined as loam soil.

Table 2.1: Soil characterization results.

	I. soil	II. soil
Moisture content (%)	13.4	8.82
Organic matter content (%)	2.8	26.1
Soil particle size >2 mm (%)	43	38.43
Soil particle size 0.05-2 mm (%)	56.3	42.61
Soil particle size <0.05 mm (%)	0.7	18.95

Soil moisture content was determined by weighing 300 g soil and drying at 105° C for 24 hours in an oven. It was allowed to cool at room temperature upon removal from the oven. Cooled soil was weighed using a precision balance. To determine soil organic matter, 56 g of soil was weighed and combusted at 550° C for

4 hours. Following that, the soil was weighed again, and the organic matter content was calculated from the weight lost during combustion.

2.3. Preparation of Synthetic Water with Microplastics

As indicated in the literature, PE from personal care products and PES from textile products were the most dominant microplastics found in grey water (Fältström et al., 2021). PE (density 0.86-0.90 g/cm³) was supplied and used in powder form with a size greater than 100 micrometers. Textile polyesters (density 1.38 g/cm³) were cut into fragments with a size smaller than 5 mm.

Synthetic water with microplastics was prepared based on the microplastic concentrations reported by Fältström et al. (2021). According to this study, the dominant MP type from washing machine was polyester with a load in the range of 230.73-3775.72 kg/year, whereas the dominant MP type from personal care products was polyethylene with a load in the range of 179.05 – 198.86 kg/year. Using these loads and the reported wastewater flow rate as 40x10⁶ m³/year, the dominant MP species and their concentrations in grey water were calculated as 0.1 – 0.025 mg/L (Table 2.2). However, the calculated amount of polyethylene could not be weighed accurately by the precision balance to prepare the synthetic water, so the concentration of polyethylene was adjusted to 0.1 mg/L same to polyester.

As mentioned previously, in the literature studies, impact of microplastics on soil properties and plant growth was investigated through adding microplastics directly into the soil [Boots et al., 2019], [Colzi et al., 2022], [de Souza MacHado et al., 2018], [de Souza Machado et al., 2019], [Lozano et al., 2021], [Meng et al., 2021], [Zhang et al., 2019]. However, the goal of this study is to determine the impact of microplastics when they were added through irrigation with grey water.

Table 2.2: Microplastic concentrations calculated based on microplastic load and flow rate of grey water.

Dominant MP	Source	Min load (kg/year)	Max load (kg/year)	Wastewater flow rate (m³/year)	Max MP concentration (mg/L)
Polyester	Washing machine	230.73	3775.72	40.000.000	0.1
PE	Personal care products	179.05	198.86	40.000.000	0.025

In order to compare the impact of microplastic concentrations found in greywater (Table 2.3) and used in the literature, another set of concentration adapted from literature was used. In this adaptation, concentration was based on the effective microplastic percent on weight basis in soil. It was reported that 0.1% polyester and 0.05% PE presence on weight basis in soil affected the soil and plant properties. This means 1 g PES and 0.5 g PE is required to be in the pots where 1 kg soil was placed (see Section 2.4 for Pot Experiments) to observe impact on soil and plant. So, 1 g PES and 0.5 g PE was added into 1 L water which is volume of water used for irrigation during experimental setups (see Section 2.4 for Pot Experiments).

Table 2.3: The concentration of microplastics in synthetic water for different sets.

	Microplastic Concentrations* (mg/L)	
	PES	PE
Based on microplastic concentration in grey water	0.1	0.1
Based on effective microplastic weight in soil	1000	500

*Concentration of MP added through grey water into pots containing 1 kg of soil during 2 weeks of experimental duration.

2.4. Pot Experiments

2.2 L brown polypropylene pots with a diameter of 18.5 cm were used for the experiments. There are 4 holes at the bottom of the pots (~1 cm in diameter) which provided water drainage. For each replicate, 1 kg of soil was added into the pots.

In the study, plant-free control was used to investigate the effect of plant presence on soil properties. Additionally, in order to determine the impact of microplastics in synthetic water on different plants, grass and spring onions were used as two different species. The amount of onion was adjusted according to de Souza Machado et al. (2019). 850 g of soil was placed into the pot. Then, 18 onion seeds were distributed on the soil surface and covered with 150 g of topsoil. The amount of grass was based on the study of Boots et al. (2019). 0.72 g of grass seeds were distributed on the 850 g of soil which was previously put into the pot. After that, 150 g of soil was added on the seeds.

In the literature, it was suggested to water the plants at 60% of its water holding capacity [de Souza Machado et al., 2019]. As water holding capacities of the two sets of soils used in this study were determined as 68% and 60%, the amount of irrigation water was calculated as 400 mL and 360 mL, respectively. However, trials demonstrated that 400 and 360 mL caused over watering. Further attempts to optimize water quantity revealed that irrigation with 200 ml in every other day during the first week and in every three days during the second week was suitable. So, pots were irrigated with a total of 1 L of water over a two-week period. Excess water drained from the pot through the holes at the bottom prevented rotting of plant roots and further muddying of the soil. To assure addition of microplastics at a homogenous concentration into the pots, synthetic water with microplastics was prepared daily and used fresh for each pot. Each beaker was mixed with a glass rod before daily irrigation. The experiments were repeated three times, where no results were obtained for spring onions in the first run due to rotting of the onions. After removal from the pot, soils were stored at 105 °C for 24 hours before analyzes.

2.5. Laboratory Analysis

2.5.1. Water Stable Aggregate Analysis

70 g of air-dried soil sieved to a size of 8 mm to 12 mm was prepared. 2 mm (macroaggregate) sieve was immersed into water up to 2 cm above the sieve and the soil sample was poured onto the immersed sieve. It was shaken for 5 minutes. Then the sieve was slowly dipped into and removed from the water 50 times in 2 minutes. Then, a rinse bottle was used to wash the sides of the sieve and any organic matter residues or small clay particles. After this process, the remaining aggregates on the sieve were poured onto a new, dry filter paper to be filtered. Aggregates drained through the sieve and passed into water were transferred on another sieve to determine aggregates with a size of 250 microns (microaggregate). Filters allowed to dry up to 105 °C to measure the weight of the aggregates.

2.5.2. Bulk Density

The bulk density was determined by core method. The soil sample was placed in the core (R=6.5 cm h= 5 cm) and then dried at 105°C for 24 hours. After that, the dried sample was weighed, and the bulk density was calculated using the dry weight of the soil and volume of the core based on the following equation.

$$\rho = \frac{W_d}{V} \quad (2.1)$$

Here;

- ρ = soil bulk density (g/cm³)
- W_d = dry soil weight (g)
- V = volume of soil (cm³)

2.5.3. Water Holding Capacity

First, a funnel and filter paper were placed in a soil grade measuring tape. Then, a pre-weighed 25 gram soil sample was poured onto the filter paper. Then 50 mL of water was poured over the soil sample, and the excess water was allowed to drain for 24 hours until it stops dripping. Then the water holding capacity is calculated with the following equation:

$$\text{Percent water holding capacity} = \frac{(V_1 - V_2)}{W} \times 100 \quad (2.2)$$

Here;

- W: weight of sample (g)
- V₁: volume of water poured through (mL)
- V₂: volume of water collected in cylinder (mL)
- V₁-V₂: volume of water retained by soil (mL)

2.5.4. Growth of Plants

The root and shoot lengths of the plants, which has been exposed to microplastic for a certain period of time, were measured with the help of a tape measure. Roots and shoot dry weight were analyzed by drying at 60 °C for 3 days.

3. RESULTS

3.1. Water-Stable Aggregate Weight

In the first set, where plants were planted, the water-stable 2 mm aggregate (macroaggregate) weight was 0.1 ± 0.03 grams in the control pot irrigated with tap water, while this value increased slightly to 0.31 ± 0.15 grams in pots irrigated with synthetic water containing 0.2 mg/L microplastic and 0.39 ± 0.06 grams in pots irrigated with synthetic water containing 1.5 g/L microplastic (Figure 3.1.a). In grass-grown soil, the water-stable macroaggregate weight was 0.52 ± 0.25 grams in case of irrigation with water without microplastics, while it is 0.52 ± 0.38 grams in case of irrigation with synthetic water containing 0.2 mg/L microplastics and decreased to 0.3 ± 0.14 grams in case of irrigation with synthetic water containing 1.5 g/L microplastic. That is, the presence of 0.2 mg/L microplastic in the irrigation water did not change the weight of macroaggregate in pots with grass plants, unlike in the no-plant condition.

On the contrary to what was observed in the pot without plants, an increase in microplastic concentration from 0.2 mg/L to 1.5 g/L in the grass-planted condition decreased the macroaggregate weight. In case of spring onion presence, it was observed that the water-stable macroaggregate weight in microplastic-free soil was almost the same (0.1 ± 0.05 grams) in plant-free pots irrigated with microplastic-free water. That is, in the absence of microplastics, the presence of the onion plant did not affect the weight of the water-stable aggregate unlike the grass. However, in pots planted with onions and watered with synthetic water containing 0.2 mg/L microplastic, the macroaggregate weight increased (0.14 ± 0.07 grams), although not as much as in pots without plants. In addition, as a result of irrigation of the onion planted soil with synthetic water containing 1.5 g/L microplastic, the macroaggregate weight increased to 0.18 ± 0.15 grams (Figure 3.1.a). This increase observed with the increase in microplastic concentration is almost at the same level as the increase in pots without plants (21%).

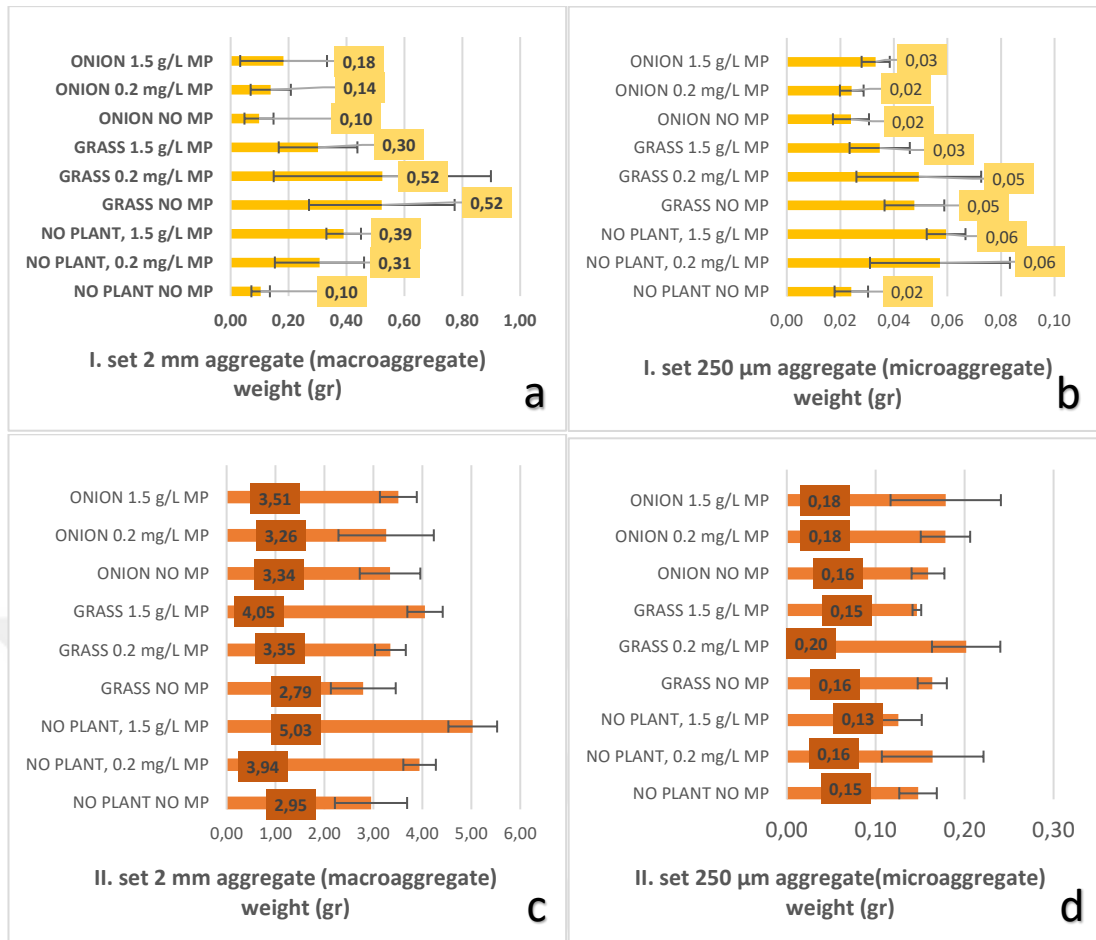


Figure 3. 1: Water stable aggregate weights a) I. set 2 mm aggregate b) I. set 250 µm aggregate c) II. set 2 mm aggregate d) II. set 250 µm aggregate.

As a result, it can be concluded that the amount of water-stable macroaggregate increased when the grass was irrigated with synthetic water containing 0.2 mg/L microplastic, compared to the experiments without plants and green onions. In other words, it can be said that the grass plant acts as a buffer against the effects of microplastics when the microplastics concentration (0.2 mg/L) was calculated based on the load in the grey water. On the other hand, when irrigated with synthetic water containing microplastics at a concentration based on the effective mass in soil (1.5 g/L), grass caused a decrease in macroaggregate compared to trials containing green onions or no plants. Decreased aggregate weight could result in deterioration of soil properties such as loss of pore continuity, lower hydraulic conductivity, increased runoff, and soil erosion and nutrient loss [Zhang et al., 2019]. Statistical difference between results was investigated through ANOVA by One-Way Analysis at significance level of 0.05. A statistical increase in water-stable macroaggregates was

observed only in the presence of grass plant when there was no microplastic in the irrigation water (Table 3.1). So increase in water stable aggregate was due to grass rather than microplastics. Also, it appeared that in the absence of plants, higher microplastic concentration caused higher aggregation whereas this impact disappeared when plants were present.

Table 3.1: Statistical analysis to evaluate impact of microplastics and plant type on water-stable aggregates.

	I.Set Soil		II.Set Soil	
	Impact on macro aggregate	Impact micro aggregate	Impact on macro aggregate	Impact on micro aggregate
Effect of the plant in the absence of MP	Yes	Yes	No	No
Effect of the plant when 0.2 mg/L MP	No	No	No	No
Effect of the plant when 1.5 g/L MP	No	Yes	Yes	No
Effect of MP concentration in the absence of plants	Yes	No	Yes	No
Effect of MP concentration in case of grass	No	No	Yes	No
Effect of MP concentration in case of onion	No	Yes	No	No

In the first set, 250 μ m aggregate (microaggregate) weight was 0.02 ± 0.01 grams in the control pot irrigated with tap water only, while it was 0.06 ± 0.03 and 0.06 ± 0.01 grams when irrigated with synthetic water containing 0.2 mg/L and 1.5 g/L microplastics, respectively. The fact that increasing microplastic concentration did not change the microaggregate weight did not match the situation observed for macroaggregates. In grass-planted soil, the microaggregate weight remained approximately the same (0.05 ± 0.01 and 0.05 ± 0.02 grams) in the microplastic-free condition and in the case of irrigation with synthetic water containing 0.2 mg/L microplastic. But synthetic water containing 1.5 g/L microplastic reduced the

microaggregate weight down to 0.03 ± 0.01 grams. These results observed in grass-planted soil are similar to the results obtained for macroaggregates. For pots with spring onions, the microaggregate weight was determined as 0.02 ± 0.01 grams in microplastic-free soil. This result was also similar to the case for macroaggregates because in both case this value is very close to that of the plant-free and microplastic-free soil. Moreover, in the onion planted setup, the increase in the microplastic concentration in the synthetic water increased the microaggregate weight to 0.03 ± 0.01 grams, as did the macroaggregates. (Fig. 1b). However, in pots planted with onions and watered with synthetic water containing 0.2 mg/L microplastic, microaggregate weight did not increase, unlike macroaggregates, and remained almost constant as 0.02 ± 0.01 grams.

As a result, it can be concluded that the weight of both microaggregate and macroaggregate were increased in case of grass presence compared to the experiments with green onions or no plants. However, it can be said that there was a lower weight of microaggregate in the setup with green onions compared to the setups with grass and without plants. When the results were evaluated statistically, it was seen that the grass plant significantly increased the weight of microaggregate in the absence of microplastics similar to what was observed for macroaggregates. In addition, the results were significantly different in the case of irrigation with synthetic water containing 1.5 g/L microplastic. Furthermore, the weight of microaggregate in the onion planted pots increased with the microplastic concentration.

In the second set, in the control pot with no plant irrigated with tap water, the water-stable macroaggregate weight was 2.95 ± 0.74 grams, while this value increased to 3.94 ± 0.33 grams in pots irrigated with synthetic water containing 0.2 mg/L microplastic and 5.03 ± 0.5 grams in pots irrigated with water containing 1.5 g/L microplastic. This increase observed in no-plant pots with the increase in microplastic concentration was almost at the same level as the increase in the first set (22%). In grass-planted soil, the macroaggregate weight was determined as 2.79 ± 0.66 grams, 3.35 ± 0.32 grams and 4.05 ± 0.36 grams in the case of microplastic-free condition, irrigation with synthetic water containing 0.2 mg/L microplastic, and irrigation with synthetic water containing 1.5 g/L microplastic, respectively. Contrary to the situation observed with the soil used in the first set, it was determined that the presence of microplastic increased the macroaggregate weight as its concentration increased. In addition, it was determined that the macroaggregate weight difference between pots

with grass and without plant was not as high as the difference observed in the first set. This supported the fact that the soil used in the 2nd set has a different structure compared to the first set and reveals that this different structure affects the aggregate weight both in the absence and presence of microplastics. For the pot planted with spring onion and irrigated with microplastic-free water, 3.34 ± 0.62 grams of macroaggregate weight were determined. This value was higher than the no-plant and grass-planted pots watered with microplastic-free water. Irrigation by synthetic water containing 0.2 mg/L microplastic decreased the weight of macroaggregate to 3.26 ± 0.97 grams in onion-planted pots. This is in contrast to the increase observed with the soil used in the first set under the same conditions. On the other hand, the weight of macroaggregate increased slightly (7%) as a result of irrigation of the onion planted soil with synthetic water at 1.5 g/L microplastic (3.51 ± 0.38 grams) (Figure 3.1.c). However, if this increase was compared with the result obtained with the soil in the first set under the same conditions (approximately 21% increase), it was seen that the increase was lower. Statistical evaluation demonstrated that microplastic concentration affected macroaggregate stability significantly in the absence of plants and in the presence of grass. Also, plant presence could change the macroaggregate stability at high concentrations (1.5 g/L) microplastics.

While the microaggregate weight in the 2nd set was 0.15 ± 0.02 grams in the control pot that had no plant and irrigated with tap water only, this value increased slightly to 0.16 ± 0.06 grams in pots irrigated with synthetic water containing 0.2 mg/L microplastic. This increase observed in non-vegetated soil was the lowest among the increases determined for macro and microaggregate in the soil used in the first set and for macroaggregates for this soil. Contrary to all previous results, in the pots without plants irrigated with water containing 1.5 g/L microplastic, the microaggregate weight decreased slightly to 0.12 ± 0.03 grams. In grass-planted soil, microaggregate weight was measured as 0.16 ± 0.02 (in case of no microplastics), 0.20 ± 0.04 grams (in case of irrigation with synthetic water containing 0.2 mg/L microplastic) and 0.15 ± 0.005 grams (in case of irrigation with synthetic water containing 1.5 g/L microplastic). That is, the microaggregate weight decreased with increasing microplastic concentration, as in the soil in the other set and in the macroaggregate weight. Again, for the onion planted setup, the microaggregate weight produced similar results with the plant-free setup, as in the previous set and macroaggregate weight. Similarly, the microaggregate weight was increased 0.16 ± 0.02 grams to 0.18 ± 0 due to presence of 0.2 mg/L

microplastic concentration in the synthetic water. However, further increase in the microplastic concentration in synthetic water to 1.5 g/L did not affect the microaggregate weight (0.18 ± 0.06 grams) in the onion planted soil (Figure 3.1.d). Results for water stable microaggregate weight were not found statistically different at all unlike the previous ones. In other words, neither plant presence, plant type nor microplastic concentration affected microaggregates in the soil used in the second set.

As a result, if an evaluation was made between the two sets, it was noteworthy that the aggregates in the II. set were higher. The most important reason for this was thought to be the soil structure. Soil microorganisms and unstable organic carbon have positive effects on the formation and stabilization of soil aggregates. Soil mineralogy and clay content are important determinants for the formation of soil aggregates [Zhang et al., 2019]. Clay and organic matter content of the soil used in I. Set (0.7% and 2.8%, respectively), were much lower than the clay and organic matter content of the soil used in the II. set (18.95% and 26%, respectively). Therefore, it can be concluded that the high organic matter and clay content in the second set increased the aggregate weight.

Overall, results obtained in this study indicated statistically considerable increase in macro and micro aggregates upon addition of polyester and polyethylene microplastics through irrigation. Higher water stable aggregates actually could be considered as a sign of better erosion control, carbon content and soil fertility [Huang et al., 2022]. So, microplastics at concentrations found in grey water might be unrisky in terms of water stable aggregates. Such an increase due to microplastics was also observed by Zhang et al. (2019) when polyester was added into plant-free soil composed of 4% sand, 41% silt and 35% clay. However, in the literature there were other studies with different results when microplastics (PLA, HDPE, polyacrylic, PES, PA, PE, PP, LDPE, PET, PU, PS, and PC) with a concentration range of 0.05-2% (w/w) were added into sandy clay loam, loamy sand or sandy loam type soils [Boots et al., 2019], [de Souza MacHado et al., 2018], [de Souza Machado et al., 2019], [Lozano et al., 2021]. In this study, the amount of microplastics transferred to the soil as a result of irrigation with 1 liter synthetic water at 0.2 mg/L microplastic concentration is 0.2 mg. On the other hand, in the studies in the literature, it was determined that soil properties were affected if the soil contains at least 0.05% polyethylene and 0.1% polyester by weight. If these ratios in the literature were applied to the soil weights in this study, however, soil properties were expected to change

when it contains 1.5 g of microplastics. The different results observed in the literature can be explained by the fact that the amount of microplastics in the soil was at least 7000 times lower in this study. In addition, the fact that different results were obtained in the experiments carried out with 2 soils with different characteristics in this study showed that the soil type also significantly determined the effect of the presence of microplastics.

3.2. Bulk Density

Looking at Figure 3.2.a, irrigation with synthetic water containing 0.2 mg/L of microplastics increased the bulk density in the plant-free assembly in the first set (from 1.33 ± 0.04 g/cm³ to 1.37 ± 0.05 g/cm³). Also, it was observed that irrigation onion-planted soil with synthetic water containing 0.2 mg/L of microplastics increased the bulk density from 1.37 ± 0.09 g/cm³ to 1.46 ± 0.02 g/cm³. Since the bulk density of grass-planted soil irrigated with microplastic-free water was high compared to experiments with no plants or with onion and did not increase when watered with synthetic water containing 0.2 mg/L of microplastics, this could be interpreted as the high bulk density observed in grass-planted soil (1.48 ± 0.06 g/cm³) was due to the presence of grass, regardless of microplastics. For onion-planted soils, it was found that the bulk density increased from 1.37 ± 0.09 to 1.42 ± 0.02 g/cm³ with the addition of synthetic water containing 0.2 mg/L of microplastics.

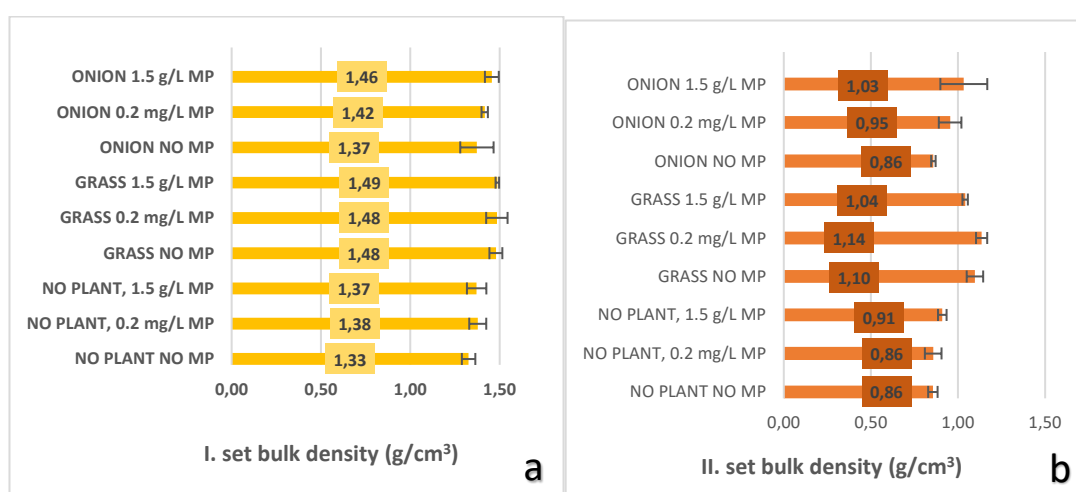


Figure 3. 2: Bulk density analysis results.

Overall, increasing the microplastic concentration in synthetic water from 0.2 mg/L to 1.5 g/L led to a higher bulk density of $1.49 \pm 0.01 \text{ g/cm}^3$ and $1.46 \pm 0.04 \text{ g/cm}^3$, respectively, in pots planted with grass or onions. Statistical comparisons showed that plant presence significantly increased bulk density only in case of irrigation with synthetic water containing 1.5 g/L microplastics (Table 3.2).

Table 3.2: Statistical analysis to evaluate impact of microplastics and plant type on bulk density.

	Impact on Bulk Density	
	I.Set Soil	II.Set Soil
The effect of the plant in the absence of MP	No	Yes
Effect of the plant when 0.2 mg/L MP	No	Yes
Effect of the plant when 1.5 g/L MP	Yes	No
Effect of MP concentration in the absence of plants	No	No
Effect of MP concentration in case of grass	No	Yes
Effect of MP concentration in case of onion	No	No

Looking at Figure 3.2.b, irrigation with synthetic water containing 0.2 mg/L microplastics did not change the bulk density, while irrigation with water containing 1.5 g/L microplastics raised the bulk density from $0.86 \pm 0.03 \text{ g/cm}^3$ to $0.91 \pm 0.02 \text{ g/cm}^3$ in the plantless setup with the soil used in 2nd set. The bulk density was found to be $1.10 \pm 0.05 \text{ g/cm}^3$ when the grass planted soil did not contain microplastics. Similar to the results obtained in Set 1, the bulk density of grass-planted soil was higher than that of no-plant or onion-grown soils.

However, unlike the 1st set, the addition of synthetic water containing 0.2 mg/L microplastic increased the bulk density of the grass-planted soil slightly to $1.14 \pm 0.03 \text{ g/cm}^3$. Moreover, it was observed that the bulk density decreased ($1.04 \pm 0.02 \text{ g/cm}^3$) as a result of irrigation with synthetic water where the microplastic concentration was higher (1.5 g/L). For onion-planted soil, it was determined that irrigation with increasing concentrations of microplastics in synthetic water increased the bulk density from $0.86 \pm 0.01 \text{ g/cm}^3$ to $0.95\text{-}1.03 \text{ g/cm}^3$, respectively. In general, when the 2nd set is compared among themselves, it was concluded that both the presence of plants and the presence of microplastics affect the bulk density. As summarized in Table 3.2, statistical evaluation demonstrated that plant presence and type affected the results in case of absence of microplastics and in case of microplastic concentration of

0.2 mg/L. Additionally, higher microplastic concentration (1.5 g/L) in case of grass planted soil decreased the bulk density in the second set of soil.

If the results obtained in the 1st and 2nd set were compared, it was revealed that the bulk density of the soil used in the first set was higher, and the bulk density of the soil used in the second set is lower. The soil in the second set planted with grass behaved differently than the soil in the first set. For example, contrary to the results obtained with the soil in the first set, irrigation with synthetic water containing 0.2 mg/L microplastic of grass-planted soil increased the bulk density, while irrigation with synthetic water containing higher concentration of microplastics decreased the bulk density.

In conclusion, in this study, the presence of MP increased the bulk density. This might be explained by the presence of polyester which has a considerably high density. High bulk density was known to reduce water and oxygen transfer, water infiltration and root development. So, higher bulk density as a result of microplastic introduction into soil could damage soil environment. Zhang et al., (2019) reported that the presence of PES in grass soils had no effect on bulk density of the soil (4% sand, 41% silt and 35% clay). But unlike this study, Souza Machado et al. (2019) observed that the presence of PES in onion planted soils reduced bulk density of loamy sand soil. A lower soil bulk density typically results in a higher soil macro-porosity and aeration, which may help roots penetrate the soil more easily, increase the abundance of aerobic microorganisms, and speed up anaerobic processes like nitrification and the mineralization of organic matter in the soil [Wang et al., 2022]. Therefore, lower bulk density could be beneficial to the soil on the contrary to what was observed in this study. Comparing with the literature was not possible due to the different amounts of microplastics in the soil, as mentioned in the water-stable aggregate weight section.

3.3. Water Holding Capacity

In the first set, the water holding capacity of the soil without plant and irrigated only with tap water was $84.67 \pm 1.15\%$, while irrigation with synthetic water containing 0.2 mg/L microplastics reduced the water holding capacity of the soil to $83.33 \pm 4.16\%$. Synthetic water containing 1.5 g/L microplastic further reduced this value to $78.67 \pm 4.62\%$. The water holding capacity of the grass-planted pots irrigated only with

tap water was lower than the plant-less setup ($66.67\% \pm 2.31\%$). Synthetic water containing 0.2 mg/L microplastic also reduced the water holding capacity of grass planted soil ($62.67 \pm 2.31\%$) as in the plant-free setup. However, irrigation with synthetic water containing a higher concentration of microplastics (1.5 g/L) increased the water holding capacity of the grass planted pot to $78 \pm 5.29\%$. When green onions were irrigated with microplastic-free water, the water holding capacity was found to be almost the same as the water holding capacity of the plant-free soil ($86.67 \pm 2.31\%$). Irrigation of onions with synthetic water containing microplastics decreased the water holding capacity to $81.33 \pm 4.62\%$ and $81.33 \pm 8.33\%$ (Figure 3.3.a). So, not much difference in water holding capacity of onion-planted soil was observed as a result of increasing microplastic concentration in synthetic water.

Consequently, the ones with the lowest water holding capacity were the grass planted setups. However, it was not entirely clear whether this was due to the presence of grass or the presence of microplastics. In addition, it was determined that the presence and increasing concentration of microplastics mostly reduced the water holding capacity.

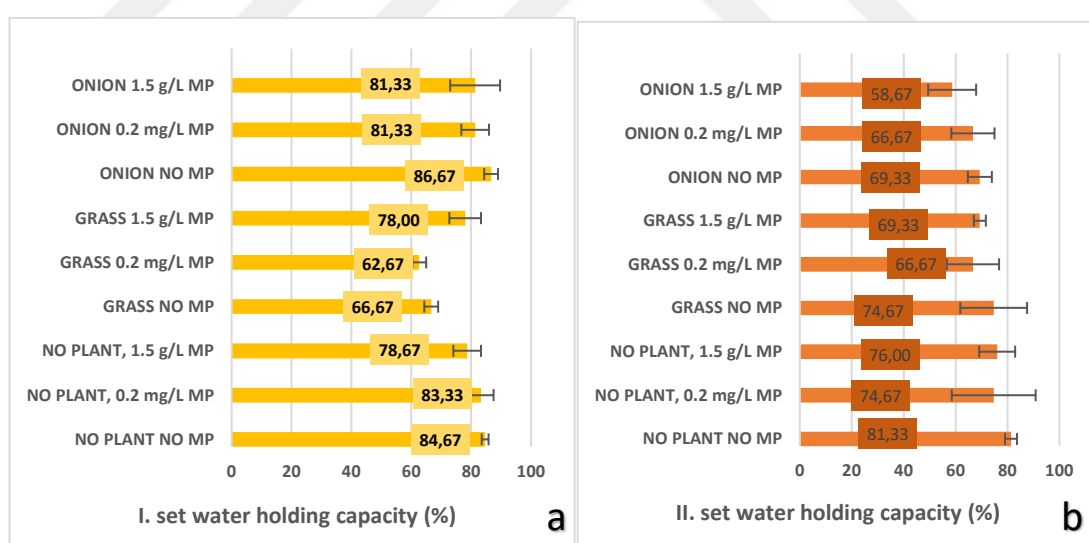


Figure 3.3: Water holding capacity analysis results.

In the absence of microplastic and in the case where the microplastic concentration in synthetic water is 0.2 mg/L, it was determined that the presence of plants statistically changed the results of water holding capacity. While the microplastic concentration did not significantly change the water holding capacity in

the onion planted pots, the water holding capacity in the grass planted pots changed with the microplastic concentration (Table 3.3). 0.2 mg/L microplastic concentration in the irrigation water decreased water holding capacity but elevation of microplastic concentration to 1.5 g/L increased the water holding capacity.

Table 3.3: Statistical analysis to evaluate impact of microplastics and plant type on water holding capacity.

	Impact on Water Holding Capacity	
	I.Set Soil	II.Set Soil
The effect of the plant in the absence of MP	Yes	No
Effect of the plant when 0.2 mg/L MP	Yes	No
Effect of the plant when 1.5 g/L MP	No	No
Effect of MP concentration in the absence of plants	No	No
Effect of MP concentration in case of grass	Yes	No
Effect of MP concentration in case of onion	No	No

In the 2nd set, while the water holding capacity was $81.33 \pm 2.31\%$ in the pots without plants and irrigated only with tap water, the water holding capacity decreased to $74.67 \pm 16.17\%$ as a result of irrigation with synthetic water containing 0.2 mg/L microplastic. Contrary to what was observed in the 1st set, when the microplastic concentration in synthetic water increased to 1.5 g/L, the water holding capacity increased slightly to $76 \pm 6.93\%$. The same results were observed in pots with grass, and this trend was in line with that observed with soil in set 1. The water holding capacity of the grass-planted pots irrigated only with tap water was lower ($74.67 \pm 12.86\%$) compared to the plant-less setup ($81.33 \pm 2.31\%$). This situation was also similar to the result obtained in Set 1. Again, similar to the 1st set, while water holding capacity decreased to $66.67 \pm 10.07\%$ as a result of watering grass with synthetic water containing 0.2 mg/L of microplastics, the water retention capacity increased slightly ($69.33 \pm 2.31\%$) with an increase in concentration of microplastics in the irrigation water (1.5 g/L). Contrary to the experiments without plant and with grass, it was observed that the water holding capacity of the soils with green onion decreased gradually. Contrary to the first set, the lowest water holding capacity was observed in green onion cultivated soils. The water holding capacity of green onions, which was $69 \pm 4.62\%$ when irrigated with microplastic-free water, decreased to $66.67 \pm 8.33\%$ and $58.67 \pm 9.24\%$, respectively, as a result of irrigation with synthetic waters containing

microplastics in increasing concentration. The fact that the increased microplastic concentration affected and decreased the water holding capacity was a different result compared to that obtained in the first set (Figure 3.3.b). Statistical assessment of results revealed that non of the factors (plant presence, plant type, microplastic concentration) affected the results significantly in the soil used in the second set unlike the soil used in the first set.

It is concluded that microplastics could decrease water holding capacity of the soils used in this study compared to no-microplastic controls. The only statistically different result was observed for grass planted soil irrigated with synthetic water containing 0.2 mg/L microplastics. Increase in water holding capacity was also reported by De Souza MacHado et al. (2018) and De Souza Machado et al. (2019) who found that in the presence of grass and green onion plants in loamy sandy soil, polyester type microplastics added directly to the soil at a rate of 0.4% w/w increased the water holding capacity. When the water holding capacity is increased, significant increases in yield can be achieved as the plant roots will receive sufficient water in arid conditions. The water holding capacity strongly depends on the texture of the soil and determines the irrigation interval. It also enhances microbial activity and maintain nutrient cycle. So, PE and polyester type microplastics transferred into soil due to irrigation with grey water have the possibility to contribute water holding capacity and attributed benefits.

3.4. Plant Root and Shoot Length and Weight

As seen in Figure 3.4.a in the first set, irrigation with synthetic water with microplastics increased the minimum root length of the grass plant from 1.03 ± 0.07 cm to 1.10 ± 0.56 cm and the minimum root length of the onion plant from 1.17 ± 1.15 cm to 1.23 ± 1.12 cm. With the further increase in the concentration of microplastic to 1.5 g/L, the minimum onion root length increased to 1.70 ± 0.67 cm, while the minimum root length of the grass decreased to 0.80 ± 0.2 cm. Maximum root lengths were also increased for both plants with microplastic concentration (Figure 3.4.b) except for the case where grass is irrigated with synthetic water containing 1.5 g/L microplastics.

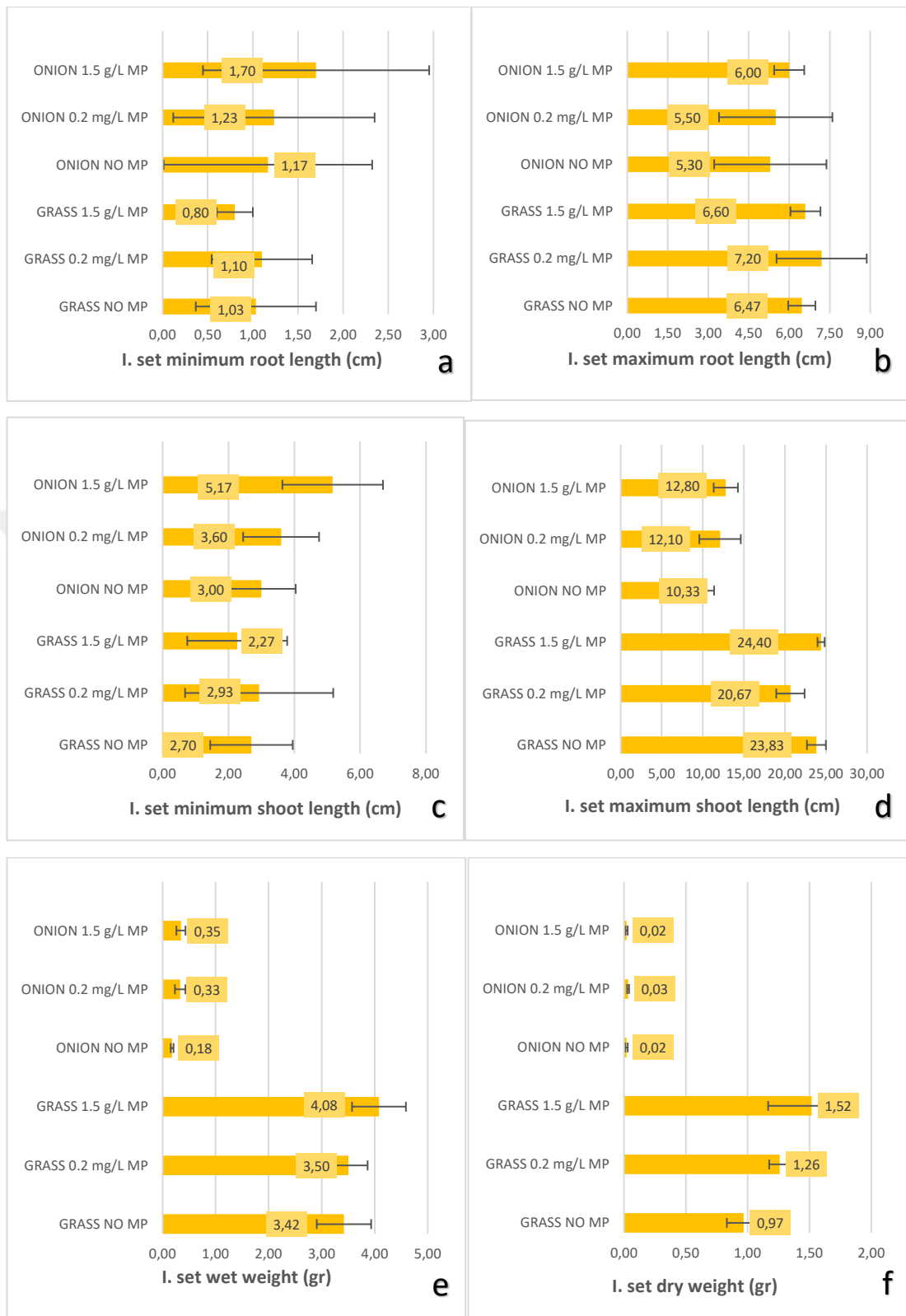


Figure 3. 4: a) Minimum root length, b) Maximum root length, c) Minimum body length, d) Maximum body length, e) Wet weight, f) Dry weight.

As can be seen in Figure 3.4.c, irrigation with synthetic water including microplastics increased the minimum shoot lengths of grass and onion plants (2.70 ± 1.25 cm to 2.93 ± 2.25 cm and 3.00 ± 1.04 cm to 3.60 ± 1.15 cm, respectively).

However, with the increase of microplastic concentration to 1.5 g/L, the minimum shoot length of the onion continued to increase (5.17 ± 1.53 cm) whereas the minimum shoot length of the grass decreased (2.27 ± 1.52 cm). With increasing concentration of microplastics in synthetic irrigation water, the maximum shoot length of the onion increased (10.33 ± 1.04 cm, 12.10 ± 2.52 cm and 12.80 ± 1.47 cm, respectively). On the other hand, the maximum shoot length of the grass was reduced from 23.83 ± 15 cm to 20.67 ± 1.72 cm due to irrigation with synthetic water at 0.2 mg/L microplastic concentration, but with the increase of microplastic concentration to 1.5 g/L, the maximum shoot length of the grass increased (24.40 ± 0.44 cm) (Figure 3.4.d). The effect of the addition of microplastics through irrigation with synthetic water containing microplastic on the wet and dry weights of grass and onion plants were also shown in Figures 3.4.e and 3.4.f, and it was determined that the wet and dry weights of both plants increased in the presence of microplastics at increasing microplastic concentrations. However, this increase was very minimal in case of dry weight of onion.

As seen in Figure 3.5.a, in the 2nd set, irrigation with synthetic water with microplastics increased the minimum root length of the grass plant from 0.57 ± 0.12 cm to 1.40 ± 1.01 cm, and the minimum root length of the onion plant from 0.83 ± 0.21 cm to 1.23 ± 0.23 cm. With the increase in the concentration of microplastics, the minimum onion root length was reduced to 0.90 ± 0.53 cm, while the minimum root length of the grass decreased to 1.23 ± 1.10 cm. Grass maximum root length decreased with the increasing microplastic concentration (from 7.33 ± 1.26 cm to 7 ± 0.5 cm). The maximum root length of onion was 4.37 ± 0.51 cm and 4.10 ± 1.35 cm, respectively, when irrigated with microplastic-free water and water containing 0.2 mg/L microplastic. However, a large increase in maximum root length was measured when microplastic concentration reached 1.5 g/L (6.07 ± 0.31 cm) (Figure 3.5.b). Minimum shoot length of grass increased while the microplastic concentration increased (from 1.53 ± 0.75 cm to 2.20 ± 0.69 cm). On the other side, minimum shoot length of onion increased in case of irrigation with water containing 0.2 mg/L microplastic (from 3.50 to 3.93 cm) yet decreased in case of irrigation with water containing 1.5 g/L to 3.57 cm (Figure 3.5.c). As seen in Figure 3.5.d, with increasing microplastic concentration

from 0.2 mg/L to 1.5 g/L, the maximum shoot length of both onion plant (from 11.47 cm to 11.67 cm) and grass plant (from 19.13±1 cm to 20.60±2.25 cm) increased slightly.

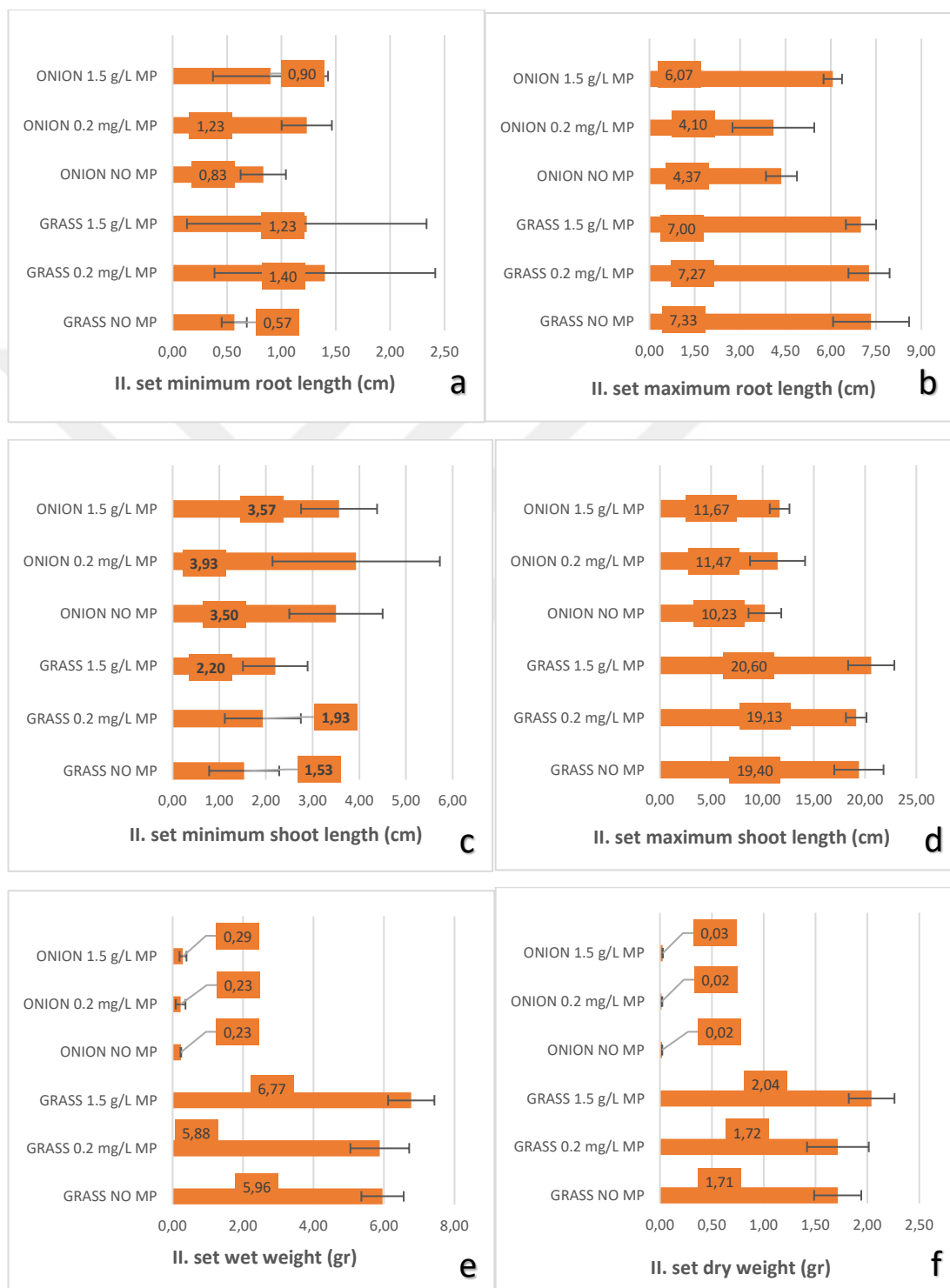


Figure 3.5: g) Minimum root length, h) Maximum root length, i) Minimum body length, j) Maximum body length, k) Wet weight, l) Dry weight.

As can be seen in Figure 3.5.e and 3.5.f, wet weight decreased (from 5.96 ± 0.60 g to 5.88 ± 0.83 g) as a result of irrigation of the grass with synthetic water containing 0.2 mg/L microplastic, while wet weight of grass increased if irrigation was done with synthetic water containing 1.5 g/L microplastic (6.77 ± 0.66 g). Additionally, grass dry weight increased with increasing microplastic concentration (1.71 ± 0.23 g, 1.72 ± 0.30 g and 2.04 ± 0.22 g).

Onion wet weight showed almost the same effect as a result of irrigation with microplastic-free water and synthetic water with 0.2 mg/L microplastic (0.23 ± 0.01 g and 0.23 ± 0.14). However, with the increase in the concentration of microplastic, the wet weight slightly increased (0.29 ± 0.10 g). Similar to wet weight of grass, onion dry weight was affected (0.02 ± 0.002 g and 0.02 ± 0.01 g) as a result of irrigation with microplastic-free water and synthetic water with 0.2 mg/L microplastic. Similar to wet weight, the dry weight increased with the increase of microplastic concentration (0.03 ± 0.002 g).

Although Boots et al., (2019) observed that the root and shoot length of grass plant decreased with the presence of microplastic, it was observed in this study that these parameters increased with the presence of microplastics. On the other hand, root and shoot length of onion plant, De Souza Machado et al. (2019) increased in parallel with the study. In this study, dry weights of grass and onion plants with the addition of microplastics were increased similar to determined by De Souza Machado et al. (2019). However, Boots et al. (2019) found that the dry weight of spring onions decreased with the addition of microplastics. Therefore, the effects of microplastics on plants have not yet been clearly answered in the current literature.

4. DISCUSSIONS

Grey water is defined as wastewater from sinks, except toilet waste. It is regarded as an important fraction of domestic wastewater which can play a critical role in circular cities to respond to water scarcity. Among end uses, irrigation is suggested as a proper way to reuse grey water owing to its nutrient content. However, risks posed to plant, soil and environmental health by reuse of grey water for irrigation have to be evaluated. This study specifically focuses on the threats originated from microplastics present in grey water.

In the studies conducted in the literature to understand the impacts of microplastics on soil and plants, microplastics were added directly to the soil, not by irrigation. Moreover, it has been determined that the amount of microplastics added to the soil in the literature is much higher than the amount of microplastics measured in grey water. In order to analyze the impact of microplastics at concentrations relevant to grey water, the transition of microplastics by irrigation with a synthetic water containing microplastics at concentrations found in grey water into soil was simulated in this study. So, the effects of microplastics in the concentrations found in grey water on plant and soil properties were studied.

Water- stable aggregate, bulk density and water holding capacity of two different types of soils in the absence of plants and in the presence of two different plants (grass and spring onion) were determined. Synthetic water containing polyester and polyethylene microplastics led to statistically higher macro and micro aggregate weight. As higher water stable aggregates mean preservation of soil carbon and nutrients as well as resistance to erosion, it can be concluded that microplastics at concentrations found in grey water might not threaten soil health. Furthermore, the presence of MPs was found to elevate the bulk density which might be attributed to polyester which has a considerably high density. High bulk density could result in limitations in water and oxygen transfer, water infiltration and root development and interrupt soil functions. So, higher bulk density as a result of microplastic introduction into soil could damage soil environment. On the other side, water holding capacity of soils irrigated with synthetic water containing microplastics was reduced compared to no-microplastic controls. Reduction in water holding capacity also indicates impairment in soil since lower water holding capacity causes decrease in available water for plants and for microbial

activity. However, in some cases, increase in water holding capacity was determined which demonstrated irrigation with grey water have the possibility to contribute water holding capacity and attributed benefits. In terms of impact on plants, microplastics at concentrations relevant to grey water were generally able to enhance growth parameters such as root length, shoot length, wet and dry weight whereas further increase in microplastic concentration (to 1.5 g/L) adversely affected plant growth by limiting growth parameters. So, in case of irrigation with grey water, microplastic concentrations are not expected to be at toxic levels. As a result, microplastic load in grey water can be considered safe to soil properties and plant growth.

The differences between this study and the literature can be explained by both the migration of microplastics inhomogeneously into the soil through irrigation in this study, the low concentration of microplastics (7000 times less) transferred to the soil with grey water in this study, and the difference in soil types used. As a continuation of this study, the effects of other components such as oil and surfactant contained in grey water on soil and plants should be investigated. In addition, although microplastics have no effect on soil and plant growth at environmental concentration ranges, their toxicity and effects on soil organisms were not studied in this study but should be identified as an important environmental risk.

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