MICROPLASTIC IN SOIL AND ITS EFFECT ON PLANT GROWTH

by

Mehmet Meriç TUNALI

B.S. in Environmental Engineering, Istanbul University, 2011

M.S. in Environmental Technology, Boğaziçi University, 2015

Submitted to the Institute of Environmental Sciences in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

in

Environmental Technology

Boğaziçi University

2023

Thanks to my thesis Supervisors;

Prof. Dr. Orhan YENİGÜN and Assoc. Prof. Dr. Başak GÜVEN

to whom I feel gratitude for their academic and personal support during my PhD.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the jury members; Prof. Dr. Güleda ENGİN, Prof. Dr. Nadim Kamel COPTY, Prof. Dr. Neslihan SEMERCİ, and Asst. Prof. Dr. İrem Daloğlu ÇETİNKAYA for their time to review and valuable feedback on this study. I am sincerely thankful for having the support of my colleagues and coworkers during this study; especially, Prof. Dr. Oleksiy MYRONYUK from Kyiv Polytechnic Institute, National Technical University of Ukraine and Ms. Filiz AYILMAZ for their contribution to this thesis, as well as Boğaziçi University, Department of Molecular Biology and Genetics; Efe Elbeyli, Ankara University, Nuclear Sciences Institute, Electron Microscope Laboratory; Dr. Elif Yıldız, and Prof. Dr. Nadim Copty from Bogazici University Institute of Environmental Sciences for the technical equipment support during the laboratory experiments.

I am grateful to my colleagues and friends; from whom I received very important contributions; Mr. Amin FOROOHANDEH, Mr. Sinan KILIÇASLAN, and Mr. Bahadır ERDEM.

Finally, my special appreciation to my beloved family for their everlasting support. My parents; Nurşen TUNALI, and Sencer TUNALI for both their invaluable moral, technical, and financial support, also my sister and colleague Dr. Merve TUNALI for her guidance and accompany. Without them, I would not have chosen the profession and concluded this study.

ABSTRACT

MICROPLASTIC IN SOIL AND ITS EFFECT ON PLANT GROWTH

Microplastics have been identified in various ecosystems including terrestrial environments. Once they enter the terrestrial systems, they may affect the soil properties as well as soil organisms. Even though the number of studies that investigate the abundance and the effects of MPs is increasing, there are still gaps regarding their abundance and effects, especially in soil systems. This research investigates the evaluation of MPs abundance in human-influenced soils to reveal the impact of different anthropogenic activities on soil MP contamination. For this purpose, three main categories as industrial, residential, and recreational areas were considered and samples were collected for each category from Istanbul, Turkey. Another subject of interest was the possible effects of different levels of MPs in soils on plant growth of two crop plant species; sunflower and sorghum. Additionally, the effect of MPs in the presence of *Glomus mosseae* (AMF species) and the effect of increased temperature was also assessed to stimulate a more real-environmental conditions. Results showed that the residential areas had the lowest MP content (mean = 3378 items/kg), while highest abundance was found in recreational zones (mean = 7956 items/kg). Surprisingly, the highest content was found in a forest area (Belgrade Forest) with 9332 items/kg. Effects on plant growth were observed as; low-level MPs (0.4% w:w) in soil enhanced overall plant growth while increasing amounts significantly hindered the process. *Glomus mosseae* supported the plant growth but the trend remained the same. Increased temperature hindered sunflower growth while promoting sorghum plants.

Keywords: Microplastic; Land Use; Soil; plant growth; AMF; *Glomus mosseae.*

ÖZET

TOPRAKTA BULUNAN MİKROPLASTİKLER VE BİTKİ GELİŞİMİ ÜZERİNDEKİ ETKİLERİ

Mikroplastikler, toprak dahil olmak üzere pek çok farklı sistemde tespit edilmişlerdir. Toprak bünyesine girdikten sonra mikroplastikler, toprak organizmalarının yanı sıra toprağın fiziksel özelliklerini de değiştirebilir. Mikroplastiklerin konsantrasyonu ve etkilerini araştıran çalışmaların sayısı son yıllarda artıyor olsa da, özellikle toprak ekosisteminde mikroplastiklerin varlığı ve etkileri konusunda literatürde eksiklikler bulunmaktadır. Bu araştırmada, farklı antropojenik aktivitelerin toprak mikroplastik seviyeleri üzerindeki etkisini ortaya çıkarmak için çeşitli alanlardan alınan toprak örneklerinde mikroplastik seviyesinin saptanması amaçlanmıştır. Bu doğrultuda, sanayi, konut ve rekreasyonel alanlar olmak üzere üç ana kategori ele alınmış ve her kategori için İstanbul'da farklı arazilerden örnekler toplanmıştır. Diğer bir araştırma konusu ise, topraklarda farklı seviyede bulunan mikroplastiklerin iki farklı bitki türü üzerinde (ayçiçeği ve sorgum) bitki büyümesi üzerindeki olası etkilerinin değerlendirilmesidir. Ek olarak, mikroplastiklerin *Glomus mosseae* varlığında etkisi ve benzer şekilde artan sıcaklığın etkisi de gerçek çevre koşullarına yaklaşmak için kapsama dahil edilmiştir. Sonuçlar yerleşim yerlerinin en düşük mikroplastik içeriğine (ortalama = 3378 öğe/kg) sahip olduğunu, en yüksek seviyenin ise rekreasyonel bölgelerde (ortalama = 7956 öğe/kg) bulunduğunu ortaya koymuştur. Şaşırtıcı bir şekilde, en yüksek içerik 9332 öğe/kg ile bir ormanlık alanda (Belgrad ormanı) saptanmıştır. Bitki büyümesi üzerindeki etkileri ise; topraktaki düşük seviyedeki mikroplastiklerin (%0,4 w:w) bitki büyümesini arttırırken, artan miktarlarda süreci önemli ölçüde yavaşlattığı gözlemlenmiştir. *Glomus mosseae* ise bitki büyümesini desteklemiş olmasına rağmen mikroplastik etkisinin eğilim olarak etkilenmediği görülmüştür. Yüksek sıcaklığın ise ayçiçeği büyümesini azaltırken, sorghum bitkisinde artırdığı gözlemlenmiştir.

Anahtar Sözcükler: Mikroplastik; Arazi kullanımı; Toprak; Bitki gelişimi; AMF; *Glomus mosseae*.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOL/ABBREVIATIONS

Abbreviation	Explanation
MPs	Microplastics
AMF	Arbuscular Mycorrhizal Fungi
FTIR	Fourier-Transform Infrared Spectroscopy
SEM	Scanning Electron Microscope
EDS	Energy Dispersive Spestroscopy
WHC	Water Holding Capacity
Low-Level MPs	0.4% MPs (w:w)
Mid-Level MPs	0.8% MPs (w:w)
High-Level MPs	1.6% MPs (w:w)

1. INTRODUCTION

There has been an increasing concern about microplastics (MPs); defined as tiny plastic fragments, fibers, and granule particles that are <5 mm in diameter (Thompson et al., 2004; Lusher et al., 2017) - especially over the past decade. Classification of MPs by their sources can be categorized in two groups:

i) Primary MPs: Plastic microbeads and nanoparticles directly used in industrial processes, and cosmetic products and their formulations.

ii) Secondary MPs: Originating from the degradation of large plastic products in situ; including weathering of agricultural plastic films, mulching, household waste, sewage and sludge, as well as vehicle emissions and atmospheric deposition.

Once the MPs enter an ecosystem whether it is aquatic or terrestrial, they may affect the organisms as well as the conditions of the environmental compartment. In terrestrial systems, MPs can damage the soil structure, interfere with soil organisms, effect microbial activities, and can change physical characteristics of soils.

Additionally, after entering the soil, plastics may absorb a variety of heavy metals or release organic pollutants into the soil, which may pose potential risks to soil biology and human health (Kasirajan and Ngouajio, 2012; Steinmetz et al., 2016; Wang et al., 2016). Another important aspect of MPs in the terrestrial environment is that the abundance is expected to be higher when compared to aquatic ecosystems (Horton et al., 2017a; Nizzetto et al., 2016b). MP pollution in terrestrial ecosystems is crucial due to its potential long-term impact especially on agroecosystem functions, food security and by extension; human health (Qi et al., 2020).

This research investigates the abundance of MPs in soil samples taken from different regions, along with their potential impact of MPs on plant growth. In detail, the effect of different levels of MPs on two crop plant species; sunflower and sorghum was investigated under Arbuscular Mycorrhizal Fungi (AMF) presence, and increased temperatures. These plants were selected as model species since they are commonly cultivated in Eurasian region, and native to Turkey. The effect of MPs in the presence AMF species; *Glomus mosseae* was selected based on its ability to enhance plant resistance/growth of sunflower and sorghum plants based on previous research (Tunali, 2015). In

addition, the motivation for including increased temperature is to mimic the multifactorial nature of global change.

The objectives of this research are:

(1) Evaluation of current MPs content in different areas of Istanbul that have different land uses (industrial/residential/recreational),
(2) Investigation of the effect of MPs pollution on plant growth for both root and stems of the plants,
(3) Contribution of specific microbe(s) – AMFs; through their interaction with host plant,
(4) Understanding the functional role of mycorrhizal fungi in the presence of MPs.
(5) Determine the effects of MPs when assisted with global climate change factor.

2. LITERATURE REVIEW

MPs have been identified and quantified in various ecosystems such as aquatic environments including oceans and seas (Kanhai et al., 2017; Lindeque et al., 2020, Çullu et al., 2021), sediments (Wessel et al., 2016; Yuan et al., 2016; Zbyszewski and Corcoran, 2011), as well as terrestrial systems (Rillig et al., 2020) including agricultural (Corradini et al., 2019; Piehl et al., 2020; Chen et al., 2020), residential/forest (Choi et al., 2020; Tunali et al., 2022), industrial (Fuller et al., 2016; Zhang et al., 2020), and coastal soils (Zhou et al., 2018; Şener et al., 2019). MPs can enter the ecosystems directly from their respective sources as they have been produced or indirectly from the fragmentation of macroplastics (e.g. plastic mulch films), atmospheric deposition, and vehicle emissions (Qi et al., 2020).

2.1. Soil Microplastics

Even though MPs are already acknowledged as emerging pollutants and more extensively detected in aquatic ecosystems, there is still a knowledge gap regarding MP pollution in terrestrial ecosystems. In their review article Qi et al. (2020) have analyzed publications related to MPs in the Scopus database by the keywords ‘‘microplastic or microplastics” combined with ‘‘terrestrial or soil” and ‘‘sediment, beach, or sludge” and ‘‘water, river, lake, sea, ocean, or marine.” Their findings support that; between 2004 and February 2019, 71% of the published articles concentrated on marine environments, 24% studied on sediments, and only 5% were devoted to terrestrial ecosystems. In the recent years, there has been an increasing trend on studies of MP contamination and its potential effects in soils (Deng et al., 2022; Ingraffia et al., 2022; Wang, 2021; Lozano et al., 2021; de Souza Machado et al., 2018; Horton et al., 2017; Nizzetto et al., 2016; Scheurer and Bigalke, 2018; Yang et al., 2018; Zhang and Liu, 2018).

The main sources of MPs in soils are littering, agricultural applications, street runoff, atmospheric deposition (Bläsing and Amelung, 2018). For agricultural applications; MPs can enter the soil by application of plastic mulch films (Huang et al., 2020), wastewater effluent irrigation (Bläsing and Amelung, 2018), and application of sewage sludge (Zubris and Richards, 2005), as well as compost. Another source of MPs in soils is the wearing of tires during transportation means, MPs formed this way is also called tire-wear particles (TWP) (Campanale et al., 2022). It can be stated that human presence and activities have major influence on MP levels in the terrestrial systems. Tunali et al. (2023) indicated that urban and industrial soils have a median value of 3600 items kg^{-1},

while agricultural and natural soils have 4400, and 1400 items kg^{-1} , respectively. It can be attributed to population, urbanization, cultural differences, and socio-economic level of the population as they are correlated with the consumption habits leading to more plastic litter circulation in the respective areas.

Considering the findings of relevant studies in the literature; the differences in location, respective applications on land, experimental methods used for extraction in laboratory makes it difficult to make comparison; however, data are still comparable and consistent across studies regarding different land uses. Natural forest areas with a mean MP abundance of 160 items kg^{-1} (Choi et al., 2020) and between 0 – 595 items kg^{-1} in natural soils in Swiss floodplain (Scheurer and Bigalke, 2018); on the other hand, human influenced soils tend to have more MP content. 3683 ± 363 items kg^{-1} (Wang et al., 2021) and range between $2.2*10^4$ and $6.9*10^5$ items kg^{-1} (Zhou et al., 2018) have been reported for forest/woodland areas. Residential areas can have a wide range of MP content related with the population and related factors, 500 items kg^{-1} (Choi et al., 2020), 4781 ± 1976 items kg^{-1} (Rafique et al., 2020) are some of the studies refer to MP content of residential areas. Choi et al. (2020) compared the MP concentration in forest, residential and agricultural areas. As stated previously, residential areas showed higher MP concentration than forestal area, but less MPs than agricultural areas. Rafique (2020) also assessed the MP concentration in recreational parks, industrial areas and agricultural areas together with residential areas. The abundance of MPs was reported as highest in recreational parks, followed by industrial, residential and agricultural areas. They also reported fibers are the most abundant shape of MPs among fragment, sheet, foam and beads. In an Australian industrial zone MP content was found to be 300 - 67500 items kg^{-1} (Fuller et al., 2016), similarly samples from industrial land located in Lahore had 5780 ± 3251 items kg^{-1} (Rafique, 2020). Additionally, agricultural lands and agroecosystems have MP abundance ranging from 62.5 to 5490 items kg^{-1}. (Chen et al., 2020; Wang et al., 2021). Chen et al. (2020) also reported the types and shapes of the MPs; stating that microbead and fibers are the most common shapes, followed by fragments and foams. Besides, they found that PA and PP are the main types of MPs found. Ding et al. (2020) reported 1430-3410 items kg^{-1} for an agricultural farmland in China and stated the most common shape was as fibers (49%). Zhang et al. (2020) showed the effect of sludge application on the MP content in soils. They reported the MP abundance is approximately 5 times higher in sludge applied soils compared to compost applied soils. The average abundances also suggest that the different use of the land is also significant for the amount of MPs associated with that particular area; comparison is detailed in Table 2.1. It has also been reported that total atmospheric fallout, which may be another source of the MPs, equates between 29 – 280 particles $m^{-2}d^{-1}$ in urban areas such as Paris (Dris et al., 2015).

2.2. Effects on Soil and Plant

Soil acts as a sink for MPs, and decomposition process of the plastic materials in soils are rather unknown and is assumed to accumulate over-time (Rillig, 2012). Physical movement of MPs through dry and wet cycles, vertical penetration (O'Connor et al., 2019), and transportation via soil organisms (Rillig et al., 2017) can introduce the pollutant to deeper layers of the soil. MPs may damage the structure of the soil aggregates (Huerta Lwanga et al., 2016; de Souza Machado et al., 2018), change the soil aeration rate, effect soil permeability (Zeng et al., 2013). MPs may also affect soil organisms such as nematodes (Kim et al., 2020), earthworms (Sobhani et al., 2021), plants (de Souza Machado et al. 2019; Rillig et al., 2019; Pignattelli et al. 2020; Lozano et al., 2020), decreased germination rate and reduced root length were observed by Bosker et al. (2019) in *Lepidium sativum* with the exposure to MPs, additionally MPs can affect soil organisms such as earthworms growth and reproduction (Cao et al., 2018; Lahive et al., 2019), as well as soil ecosystem multifunctionality (Lozano et al., 2021). Some examples include; Zhou et al. (2020) who reported the growth of *Eisenia fetida* was affected by the presence of fragment shaped PP type MPs. Sobhani et al. (2020) also worked with the same species and found that the reproduction of *Eisenia fetida* was effected under 0.1 g/kg MP. Pflugmacher et al. (2020) also reported that the mortality of *Enchytraeus crypticus* was affected by the presence of HDPE type MPs under 20 g/kg concentration.

When it comes to the effects on plants, there are several studies which focus on the effects of MPs on different plant species. In some researches MPs had no significant effect, while other studies demonstrated that the effects could increase or decrease the plant growth depending on species involved, MP levels, and environmental factors. Namely, de Souza Machado et al. (2019) used various types of polymers including PA, PES, HDPE, PP, PS, and PET to investigate the effects on *Allium fistulosum* and concluded that the plant growth either increased or decreased in response to MPs in soil, they stated that polyester fibers, together with polyamide beads resulted the most highest impacts on plant traits and functions Similarly, Lozano et al. (2021), observed effects on *Daucus carota* in the presence of PP, PE, PES, PET, PU, and PC MP fragments and fibers. Wang et al. (2020) observed no effect on *Zea mays* L. growth in 30 days under 100 g/kg MP (HDPE) particles. Whereas there were negative effects reported, for instance, spherical-shaped PA decreased the growth at 20 g/kg MP concentration, Pignattelli et al. (2021) reported only 0.2 g/kg of MP (PE) exposure to *Lepidium sativum* resulted in growth inhibition using 62.5 μm sized MP particles. Additionally, PES MP fibers (sized 3000 μm) in soil effected *Bidens bipinnata* growth over 30-day period (Deng et al, 2022) Therefore, positive or negative effects as well as its size and magnitude may vary on MP type, size, shape and, soil conditions as well as soil organisms. Researches are still ongoing and MPs

effect on terrestrial systems are under investigation, and currently there is no agreed specification of MPs to model the pathway or no consensus to predict the effects whether its positive or negative, or even the effect size on specific species of plants. Polymer type of MPs may be one of the driving factors for the possible effects of MPs in environmental systems. Recent study carried out by Tunali et al. (2023), evaluated the hazard and exposure assessment of MPs gathered from published articles until August 2021, and reported that the effects of polyester (PES) and polyamide (PA) were higher than other polymers in terms of toxicity. Additionally, according to the shapes of MPs in the system, MP fibers was found to have higher toxicity ratio compared to spherical particles and fragments. They also stated that no size relation was observed in response to toxicity in soil organisms. More data is needed to fully understand the effect including its size and magnitude due to MP presence in terrestrial ecosystems. On the other hand, MP particles generating from tires, referred as tire wear particles (TWP) is one of the main sources or MP in/around urban areas where transportation means are heavily utilized. However, tire production contains a complex mixture of polymers, rubbers, reinforcement agents and other additives separates them from other commonly utilized plastic polymers by their physical response in the environment such as sorption properties (Hüffer, 2019). Effect of the TWP (0.02 – 1.5 % w:w) in soil decreased the reproduction rate of *E. Crypticus*, but did not affect their survival, for *F. Candida*, and *P. Scaber* their reproduction and survival rate were not affected (Selonen, 2021). Fort et al. (2022) also assessed the effects of car tire on different organisms, including *Eisenia fetida* and *Lactuca sativa*. The mortality of *Eisenia fetida* was increased, and the growth of *Lactuca sativa* was decreased when exposed to 1000 g/kg car tire particles. However, Lehmann et al. (2021) didn't observe any effect on the reproduction of *Eisenia Andrei* under 1g/kg car tire particles. Even though the concentration of tire particles may be one of the factors that may have an effect, much more data is needed to make such a statement.

On the other side, soils host numerous microorganisms in addition to plants and macro-organisms such as earthworms. One of the universal and global symbiotic microorganisms such as azotobacters and arbuscular mycorrhizal fungi (AMF); who belongs into the *Glomerales order*, can form symbiotic relationships with roots of 80~90% land plants as their host in both natural and agricultural ecosystems (Brundrett, 2002), including xerophytes, hydrophytes and halophytes (Khan, 2006). Mycorrhizal fungi are known to benefit to the plant nutrition, plant growth and their survival due to their greater exploitation of soil for nutrients (Khalvati, 2005). These symbiotic associations represent a key factor in the below ground networks including the rhizosphere area which influences the diversity and plant community structure (Zhanbei, 2008). The degree of benefit to each partner in any AMF-plant host interaction depends not only on the particular plant species and AMF species involved but also on the rhizobacteria and soil abiotic factors.

AMF activity can also increase the soil quality and stability through protein production as a result of their biological activity, such as Glomalin Related Soil Protein (GRSP). Interactions between MPs and AMF community in soil bodies have been confirmed but not studied in depth. In a study carried out by Wang; it was revealed that the AMF genera were affected by MPs presence (type and concentration) (Wang et al., 2020).

It's also been reported that MPs did not have significant effects on enzyme activities in soil without humic substances such as organic matter, however with addition of wheat straw or plantago enzyme activities decreased by 26.11 - 37.57% (Liang et al., 2021).

Table 2.1. Literature data on microplastic abundance in different areas.

Type of the soil	Tested range	Abundance	Type	Size	Shape	References
Vegetable farmlands, China	0.02-5 mm	62.50–78.00 items/kg	PA (32.5%), PP(28.8%), PS (16.9%), PE (4.2%), PVC (1.9%) *rest no plastic	< 0.2 mm (70%) 0.5–1 mm (13%), 0.2–0.5 mm (9%) 1.0–3.0 mm (7%)	Microbeads (48%), Fibers (37%), Fragments (15%), Foams (1%)	Chen et al., 2020.
Agricultural Fields, Chile (Sludge Application)	0.1 - 5 mm	2,3 - 19 items/5 g	Acrylic Polyester Nylon		Fibers (90%)	Corradini et al., 2019
Sludge		170 items/5 g	LDPE PVC		Fibers (97%)	
Agricultural farmland, China	0.45 µm-5 mm	1430 to 3410 items/kg	Fiber (PP, PET, PE), Film (HDPE, PE), Fragment (PVC, HDPE),Pellet (PS)	0–0.49 mm (81%),	Fiber (49%), Film (23.8), Fragment (21.9%) Pellet (5.07%)	Ding et al., 2020.
Agricultural Soils, China (Sludge Application)	0.45 µm- 5 mm	545.9 item/kg		Field A: < 0.2 mm (7.9%) 0.5–5mm (75%), 0.2–0.5 mm (17.1%)	Flakes (~50%), Fibers (~40%), Films (~10%)	Zhang et al., 2020.
Field A: 30 t/ha sludge compost			accounting respectively for 26.2%, 14.3%, 19.0%, 9.5%, 14.3%, 4.8%, and			
Field B: 15 t/ha sludge compost		87.6 items/kg	11.9%	Field B: 0.5–5mm (93.8%), 0.2–0.5 mm (6.2%)	Flakes (~70%), Fibers (~30%)	
Agricultural farmland, Germany	2-5 mm	0.34±0.36 items/kg	PE (62.5%), PP (25%), PS (12.5%)		Films (43.75%), Fragments (43.5%) Fibers (12.5%)	Piehl et al., 2020.
		items/kg				
Wheat Land	0.2 - 5 mm	3910 ± 1031	PVC (7.84%)			Wang et al., 2021
Paddy Land		5490 ± 573	PA (20.31%)	< 0.2 mm (54.29%)	Fragments (54.4%)	
Woodland		3683 ± 362	PP (10.82%)	0.2–0.5 mm (20.85%)	Fibers (26.88%)	
Orchard Land		3386 ± 593	PS (11.38%)	0.5–1 mm (8.13%)	Films (10.19%)	

Type of the soil	Tested range	Abundance	Type	Size	Shape	References
Mulch Film Soil		5386 ± 835	PE (20.88%)		Spheres (8.53%)	
Greenhouse Soil		5124 ± 632	Polyester (12.51%)			
Industrial Area (Former e-waste recycling)	0.05 - 5 mm	600 - 14200 items/kg	ABS (24%), PET (14%), PE (13%), PVC (13%), PP (12%), PA (6%), PBT (5%), PMMA (4%), PP/PE (3%), PC (3%)	0.05 - 5 mm		Zhang et al., 2021
Industrial Area		300 - 67500 items/kg	PE, PS, PVC			Fuller et al., 2016
Forest	0.45 µm-5 mm	160 items/kg	SBR, SIS	>5mm (22.1%) 0.5–5mm (10.86%), 0.2–0.5 mm (67%)		Choi et al., 2020
Residential		500 items/kg	PS, SBR			
Agricultural		664 items/ kg	EPS, PP			
Forest Buffer Zone	0.05 - 5 mm	8180 - 18.100 items/kg		>1mm (5%), 0.05 - 0.25mm (82%)	Fiber (92%)	Zhang et al., 2018
Woodland	0.45 µm-5 mm	9.6*10^4 – 6.9*10^5 items/kg	Polyethylene (36.1%),	<50 µm (46.1%), <100 µm (81.3%), <500 µm (99.8%),	Fragments (50.5 - 55.4%), Fibers (10.4 - 20.8%)	Zhou et al., 2018
Vacant Land		4.3*10^4 – 6.2*10^5 items/kg	Polyamide (17.3%),			
Vegetable Plot		2.2*10^4 – 2*10^5 items/kg	Polypropylene (11.5%)			
Recreational Park	0.50 µm-5 mm	6250 ± 3776 MPs/kg			Fiber (64%), Sheet (28%), Fragment (7%), Foam (0.75%), Bead (0.25%)	Rafique et al., 2020
Residential		4781 ± 1976 MPs/kg,				
Industrial		5780 ± 3251 MPs/kg,				
Agricultural		3712 ± 2156 MPs/kg,				

3. MATERIAL AND METHODS

3.1. Determination of Microplastics in Soil Samples

In order to determine the current levels of MPs in soils, sites which had different land uses were selected in Istanbul. After sample collection and initial treatment, MP abundances were reported via laboratory analysis results. Throughout the experiment, samples were kept and proper measurements were implemented to ensure samples were not subjected to any further MPs contamination.

3.1.1. Sample Collection

The samples were taken from a mega city; Istanbul which is located in the North-Western part of Turkey and has a population of 15.46 million as of December 2020 (Turkish Statistical Institute,2021). Sample sites were selected as per their respective category and sampling was done from non-vegetative parts of the areas. Soil samples were chosen from close points of industrial, residential, and recreational areas of Istanbul (Figure 3.1). Dilovası organized industrial zone was also included as one of the industrial zones around İstanbul area to ensure the land use differentiation was carried out effectively in this study. Samples from industrial areas were gathered from soils from closest area to the production zone as factories are enclosed. A total of 27 soil samples were collected to glassware tubes by using a stainless-steel spoon. Three sub-samples were collected from topsoil (0-3 cm) at each site in a triangle shaped sampling area; consisting of 1 m edge. Sampling of all soil samples were done between May – June ’21, with no prior rainfall observed during sampling. Afterwards, collected soil samples from each site were mixed manually for further analysis. The details of the sample sources can be found below:

i) Industrial Areas;

a. Ikitelli Organized Industrial Area (N41°4′47.2764″, E28°47′40.9056″),
b. Dilovası Organized Industrial Area (N40°50'02.8",E 29°32'59.5),
c. Atatürk Automotive Industrial Area (N41°6′47.0268″, E29°1′18.2352″),

ii) Residential Areas;

a. Zekeriyaköy (N41°5′16.638″, E29°0′25.614″),
b. Levent (N41°5′16.638″, E29°0′25.614″),
c. Darüşşafaka (N41°06’56.9088", E29 °00’58.0788")

iii) Recreational Areas:

a. Boğaziçi University (Northern Camp.) (N41°05′09.9348″, E29°02′41.172″),
b. Belgrad Forest (N41°5′59.2848″, E28°59′30.1776″),
c. Yıldız Park Area (N41°5′16.638″, E29°0′25.614″).

Ikitelli Organized Industrial Area: Compact zone for small and medium sized industrial businesses with full infrastructural support, where also plastic manufacturers are actively present.

Dilovası Organized Industrial Area: Zone for medium and higher sized industrial businesses with infrastructural support, contains multiple big-scale factories.

Atatürk Automotive Industrial Area: Former industrial zone located in the center of the business district, for the last few decades most of the heavy industrial activity has been moved; yet automotive sector is still operational along with the headquarters of respected corporations.

Zekeriyaköy: Residential area located in the northern part of the city, a few nearby roads as well as the forestal area.

Levent: Residential area located in a busy district, with high traffic activity in nearby roads.

Darüşşafaka: Residential area located in northern part of the city, steadily increasing population over the last decade.

Boğaziçi University Campus (Northern Campus): University campus area where students can participate in recreational activities.

Belgrad Forest: Forest area in the northern part of the city, soil sample was taken from a place out of the walking line in a flat surface with a nearby water stream.

Yıldız Park Area: Located closer to one of the centers of the city, popular spot and hosts multiple recreational activities daily.

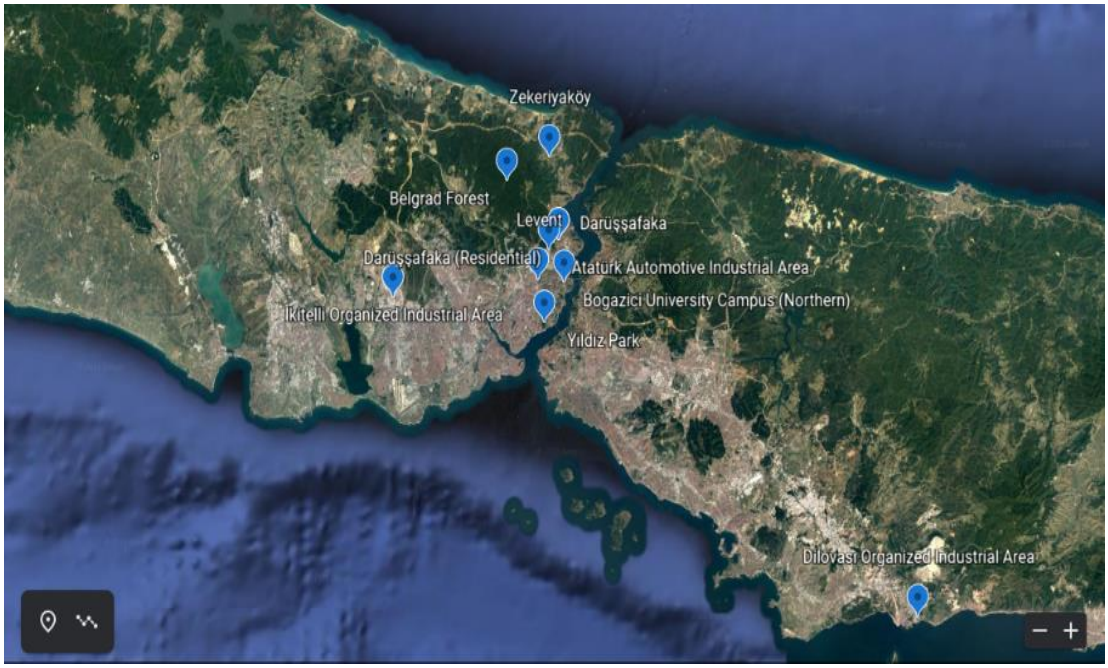

Figure 3.1. Sampling Locations.

3.1.2. Sample Treatment

Samples were first dried in an oven at 58 ºC (±2) for 48 hours in a glass petri-dish to reduce the moisture content without damaging the plastic particles present in the soil samples. After drying process, subsamples of 2.5 g of dry soils were taken in a pre-rinsed glass beaker along with respective control samples. All samples were always kept in closed containers and spaces.

3.1.3. Laboratory Analysis

Since there is no standardized method to investigate the MPs in soil samples, the methodology for this study was developed by considering different experimental procedures. Extraction and digestion procedures were combined from various studies in literature considering Mehdinia et al. (2020), Corradini et al. (2019), and Fakour et al. (2021) to optimize the extraction of MPs from the soil samples.

The samples were subject to sequenced digestion by using H_2O_2; %35 (Merck 108600.2500) and Fenton reagent; 0.05M – prepared with $FeSO_4.7H_2O$ (Sigma-Aldrich F8633). Fenton reagent was used for the digestion of organic materials in soil samples; soil samples were treated with Fenton reagent (15 ml) and H_2O_2 was slowly added until the digestion process has been completed. Final ratio of Fenton reagent: H_2O_2 was 1:1. This process is based on the research conducted by Hurley (2018) on method validation. Additionally, during the digestion process, a water bath was prepared with ice mixture and temperature was kept below 55°C to ensure the physical characteristics of plastic particles remain intact (Munno, 2018). Digestion process was followed by a two-step density separation stage. The first stage density separation was done using dH_2O as the agent. In order to release trapped particles between soil aggregates, the mixtures were stirred and then settled. In the second stage, prepared NaI solution (ρ=1,6 g/cm^3) was utilized following the same principle. After settling, supernatants were filtered by using differentiated filters for each sample (pore size < 20μ) (Whatman glass microfiber filters Grade GF/A).

The particles were then subjected to counting and imaging by microscope analyses using Microscope Axio Observer.Z1, with EC Plan-Neofluar 10x/0.3 objective, and AxioCam MR5 camera. Further analysis on MPs were carried out by Thermo Fisher Nicolet 380 FTIR Spectrometer Vertical FT-IR with a diamond ATR accessory (4000-600 cm-1 range) with random sampling method extensively for all 27 soil samples. 32 scans per spectrum to detect the available and used binding sites of the MPs, spectral changes were noted. Randomized samples from digestates (2 samples/area) were also subjected to Energy Dispersive Spestroscopy (EDS) with Scanning Electron Microscope (SEM) model ZEISS EVO 40 to determine the morphological surface structure and to obtain high-resolution images of present MP particles. Larger particles were placed on double-sided adhesive carbon bands manually, while suspended particles were drawn from the aliquot and transferred onto the adhesive band; afterwards samples were mounted on 9-mm stubs and coated with gold under 25 mA current, using a secondary electron detector at 20 kV potential difference and 52 pA probe current.

3.1.4. Preventation

In order to prevent plastic and fiber contamination, all materials in the laboratory, including the work bench surfaces were cleaned and treated with ethanol solution (70%) during sample preparation and handling. During the digestion and extraction processes, all instruments and equipment were rinsed carefully, and then filtered with distilled water under fume hood in the laboratory. In addition, sample containers were packed carefully for transportation in order to avoid the potential contamination during all stages of analysis. During the experimental work, blank experiments were also conducted. Control samples without any soil were prepared to check whether the lab material (e.g. centrifuge tubes) or airborne particles caused any plastic contamination or not. Second control group was prepared with sterile soil by using muffle furnace (550°C), same procedure carried out with all control groups and samples. There were no MPs observed in these control groups.

3.2. Plant Experiments

Sunflower (*Helianthus annuus L.*) - P64LC108 variation and Sorghum (*Sorghum bicolor L.*) were selected as the herbaceous plants which are native to Turkey and commonly cultivated plants in Eurasian region. Seeds of these plants were obtained from agricultural establishments located in Thracian region, and Ege region, respectively. *Glomus mosseae* was utilized as the primary AMF species in this study; based on its ability to enhance plant resistance/growth according to the previous research (Tunali, 2015).

3.2.1. Materials and Experimental Design

To investigate the effects of MPs in varying levels, pot experiments were conducted. Commercially available polyester ropes (Paraloc rope, Mamutec, Switzerland, product number: 8442171) that was obtained from Germany, were manually cut and sieved (<1 mm) to generate MP particles. Polyester was selected as the polymer type and shape in this study as it is widely utilized polymer in everyday life and commonly used in experimental studies (Lozano et al., 2020; de Souza Machado et al., 2019). Soil was obtained from a local botanical garden and was sterilized in an oven at 151°C prior to the experimental setup. Prepared MP particles were mixed with soil on 0.4% (w:w), 0.8% (w:w), and 1.6% (w:w) levels, and then mixed mechanically with varying stirring speeds to ensure the homogeneous distribution of MP in the soil body. Mixing was done individually for each pot. The MP concentrations were determined based on previous studies on the microplastic induced

effects such as de Souza Machado et al. (2018b), de Souza Machado et al.(2019) and Lehmann et al.(2020).

Experiments were conducted in 2x3 factorial design, covering following groups:

- Control group (without MPs, and AMF)
- AMF Control Group (without MPs, with AMF)
- MPs Group1 (with MPs, without AMF)
- MPs Group2 (with MPs, and AMF)

All of the groups were constructed with 3 parallels, equating to 24 per plant, 48 in total. In addition, to determine the effect of climate change; two different sets of plants were maintained at 26°C and 31°C, respectively (96 pots in total).

Differentiation on the plant growth was determined by the comparison of the respective control groups; first control group was maintained to observe the plant growth without any stress from MPs and AMF. Second control group was the AMF control group to identify the AMF influence without MP presence. Through MP groups 1 and 2 data on the effects of MPs on plant growth with/without AMF were obtained. Therefore, the experimental set and statistical analyses will be sufficient for explaining the cause of possible negative effects on plant growth; whether it's MPs effect on plant growth or MPs effect on AMF, or both.

3.2.2. Preliminary Germination Tests

Preliminary germination tests were conducted in petri dishes in order to determine the germination capacity of sunflower (*Helianthus annuus L.*) and sorghum (*Sorghum bicolor L.*). Sunflower and sorghum seeds were supplied from Turkish commercial farming business located in Tekirdağ and İzmir, respectively. Seeds were treated with distilled water and ethanol sequentially. Afterwards MPs were incorporated into petri dishes in accordance with relative weights of petri dishes and stirred with distilled water to ensure homogeneity within each petri dish prior to the placement of plant seeds. 3 different levels of MPs were monitored; 0.4% (w:w), 0.8% (w:w), and 1.6% (w:w), respectively.

Introduction of the Arbuscular Mycorrhizal Fungi; *Glomus mosseae*, into the germination system, was conducted as following procedure; 30.002 g of Glucose Hydrate ($C_6H_{14}O_7$) was dissolved in 100 ml distilled water. Afterwards, 1.8 g of AMF spores was added to the prepared solution. 5 ml

of this mixture was added to each petri dishes prior to the plantation of seeds. Initial concentration of the AMF spores was 100 mg mycorrhizal fragments including spores and hyphae per petri dish (Brundrett, 2002). Arbuscular Mycorrhizal Fungi (*Glomus mosseae*) along with proper control groups all treatment levels had 3 parallels. 48 sterile petri dishes were prepared to conduct the germination test. Planted numbers of seeds in petri dishes were 5 for sunflower, and 10 for sorghum seeds for each petri dish. Germination of seeds was monitored in the presence of MPs for 5 days and results were reported as cumulative sum for each group.

3.2.3. Plant Growth Experiments

Dark-Brown colored plastic pots with volume of 1.1 liters and 135 gr tare weight were used for the cultivation of sunflower and sorghum plants. Seed sterilization was carried out with following procedure; seeds treated with ethanol solution (70%), with contact time of 1 minute and afterwards seeds were rinsed with distilled water for 3 minutes. This process repeated for 3 times prior to plantation to soil body. Soil sanitation was carried out in oven under 151°C for 48 hours, and after the cooling period; all pots were filled with 100 g of stream sand and 500 g of soil.

The experiments consist of 3 factors; MPs - 3 different levels, AMF presence (yes or no), and increased temperature (yes or no). Considering climate change as a global change factor for soil studies the warming effect is used as +5°C based on IPCC (2014) and Rillig et al. (2019). Both plant species are cultivated in warmer temperatures (May-Aug) in Thracian region, thus, 26°C was utilized as base temperature and 26 + 5°C was set to simulate warming factor in this section.

The effect of the listed factors was assessed on two different plant species; sunflower and sorghum. MPs were provided on 3 different levels; 0.4% (w/w), 0.8% (w/w), and 1.6% (w/w) and Arbuscular Mycorrhizal Fungi (*Glomus mosseae*) along with proper control groups. Sunflower and sorghum seeds were sown and inoculated with *Glomus mosseae* added to the system for the AMF included groups. Seeds were planted into the plastic pots and kept in an air-conditioned laboratory; with day/night with a photoperiod light cycle of 16/8 h, 3 klx, relative humidity of ~50%, and a mean daily temperature of 26 ± 0.77 °C for 35 days. 3 seeds were grown per pot to increase the number of plants while each pot was wide enough to support individual growth of the plant species. We also confirmed during the harvest that none of the roots were in contact and rhizosphere areas were intact. Each pot has three parallels. For the increased temperature, infrared lamps were used and experiments were conducted at 31 ± 0.97 °C with connection to waterproof temperature sensors (Product number: DS18B20). Initial soil moisture content was adjusted to ~70% of the water holding capacity of the

soil, irrigation was maintained regularly with distilled water every 2-3 days in order to maintain soil moisture at intended levels. In order to provide enough nutrients for plant growth, nutrient solution based on Modified Strullu-Romand medium (Table 3.1) was prepared and added biweekly to each pot in addition to water irrigation for optimal plant growth. All treatment levels had 3 parallels, resulting 96 pots in total.

Table 3.1. Composition of MSR medium (Fortin et al., 2002).

Elements	Concentration, μM
$N(NO_3^-)$	3800
$N(NH_4^+)$	180
P	30
K	1650
Ca	1520
Mg	3000
S	3013
Cl	870
Na	20
Fe	20
Mn	11
Zn	1
B	30
Mo	0.22
Cu	0.96

In-situ measurements such as soil moisture content, pH, electrical conductivity, and temperature of the soil were monitored by using Combi 5000 - STEP Systems GmbH and automated system with probes during the experiments. Operating conditions of sets were detailed in Appendix B section (Tables B.1 - B.4)

At harvest, roots and shoots of each plant, and soil samples from the rhizosphere area were collected. Roots and shoots were cut during the harvest, and dried at 58 ºC (±2) for 3 days prior to weighting. One of the most important parameters in plant related studies is dry weight of the plant biomass and plant roots. These parameters are milestones to reliably determine the effects of the stressors on plant growth in different settings and conditions. Both parameters are directly related with plant growth rate and operational rate. Individual sampling from roots of each pot were collected separately during the harvest and stored in 70% ethanol solution at 4°C for the determination of mycorrhization.

3.2.4. Determination of Mycorrhization

After harvest, obtained root samples were preserved in 70% ethanol solution at 4°C prior to root staining process. Root staining was done in following order; each root sample was packed individually and kept in 10% KOH solution (w/v) for 4 hours in water bath at 60 ºC. Afterwards, roots were treated with 1% HCl solution 0.05% (w/v) and transferred into another container for contact with trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) mixture for 2 days. After the completion of the staining process all root samples were observed under microscope for the determination of mycorrhization. Microscope Axio Observer.Z1, with EC Plan-Neofluar 10x/0.3 objective, and AxioCam MR5 camera was used.

3.2.5. Glomalin Related Soil Protein (GRSP) Measurement

GRSP levels of the soil samples were evaluated by Bradford method. Extraction of reactive soil protein was determined by the procedure in accordance with Rillig, 2002. For this method, all samples were well-dried and sieved before this procedure. 2 g of soil samples from each pot were placed in a container (3 parallels). 50 mM Citrate Buffer (pH=7) was added and autoclaved at 121ºC for 30 minutes. Afterwards samples were subjected to centrifugation at 5000rpm for 15 minutes and supernatants were transferred into sterile falcon tubes and stored at 4ºC. After the extraction process a calibration curve was created with standard protein solution (Protein Standard, P0834-10x1ml; Sigma; 2mg/ml; Lot SLBS3852), Bradford Reagent (B6916 SIGMA**),** and distilled water (Ratio: 1:4) was prepared and 20 µl of the solution was added to each sample (Figure B.1). All samples, including calibration solutions were analyzed at 595 nm with UV-160A Spectrophotometer, Shimadzu.

3.2.6. Statistical Analysis

All data were analyzed by using Statistical Package for the Social Sciences (SPSS) version 22 and version 27. Multivariate Analysis of Variance (MANOVA) full factorial models were applied. $p<0.05$ and $p<0.001$ were set as the boundary for significance between samples from respective results to differentiate the parameters affecting plant growth parameters. Afterwards, post-hoc analysis (LSD) was utilized to reveal the parameter(s) responsible for the impact on plant growth parameters. Additionally, R (R Core Team, 2019) was used for the visualization where needed by using "xlxs" and "ggplot2" packages.

4. RESULTS AND DISCUSSION

4.1. Microplastic Abundance in Soil Samples

Determination of MP abundance of the soils from selected areas was carried out by microscopic evaluation followed by FT-IR spectrometry and SEM-EDS analyses.

4.1.1. Visual Determination

For visual determination of MPs frames were noted and photographed for each sample individually. Particles and fibers were categorized in terms of their shape and characteristics. Size range of the MPs in this study was between 20µ - 5mm. Examples of selected particles from microscopic determination were shown in Figure 4.1; while fibers were detailed in Figure 4.2.

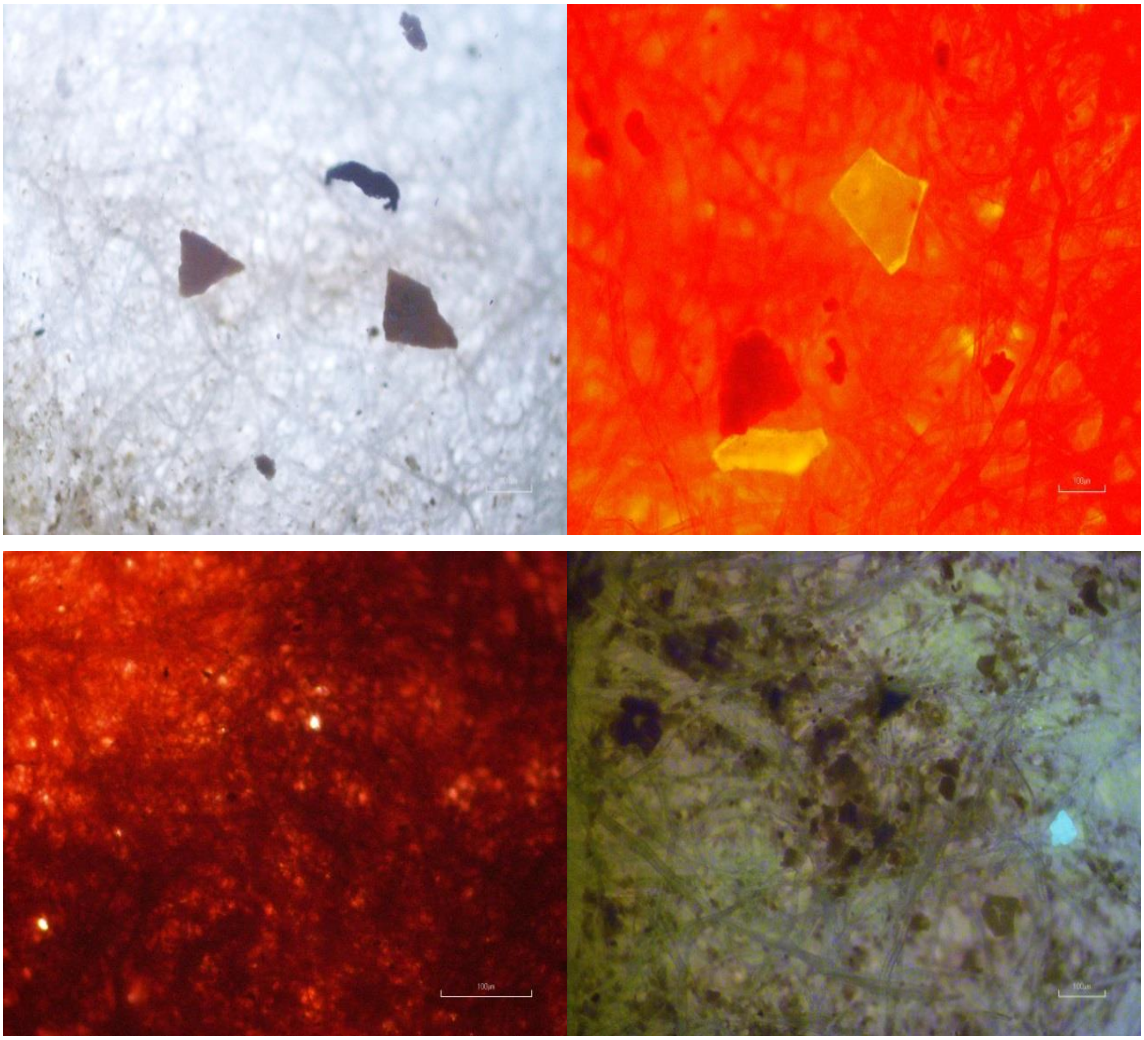

Figure 4.1. Microscopic images of microplastic particles.

Obtained data from the determination of the abundance of MPs particles in different land use areas in Istanbul showed that lowest MP counts are from the soil gathered from one residential area; Darüşşafaka with **1868 items kg^{-1}**, and one of the industrial zones; Atatürk Automotive Industrial Area with **2133.33 items kg^{-1}** (This area was a former industrial zone is considered to be the center of the business district nowadays). Here "items" is number of MP particles of all types.

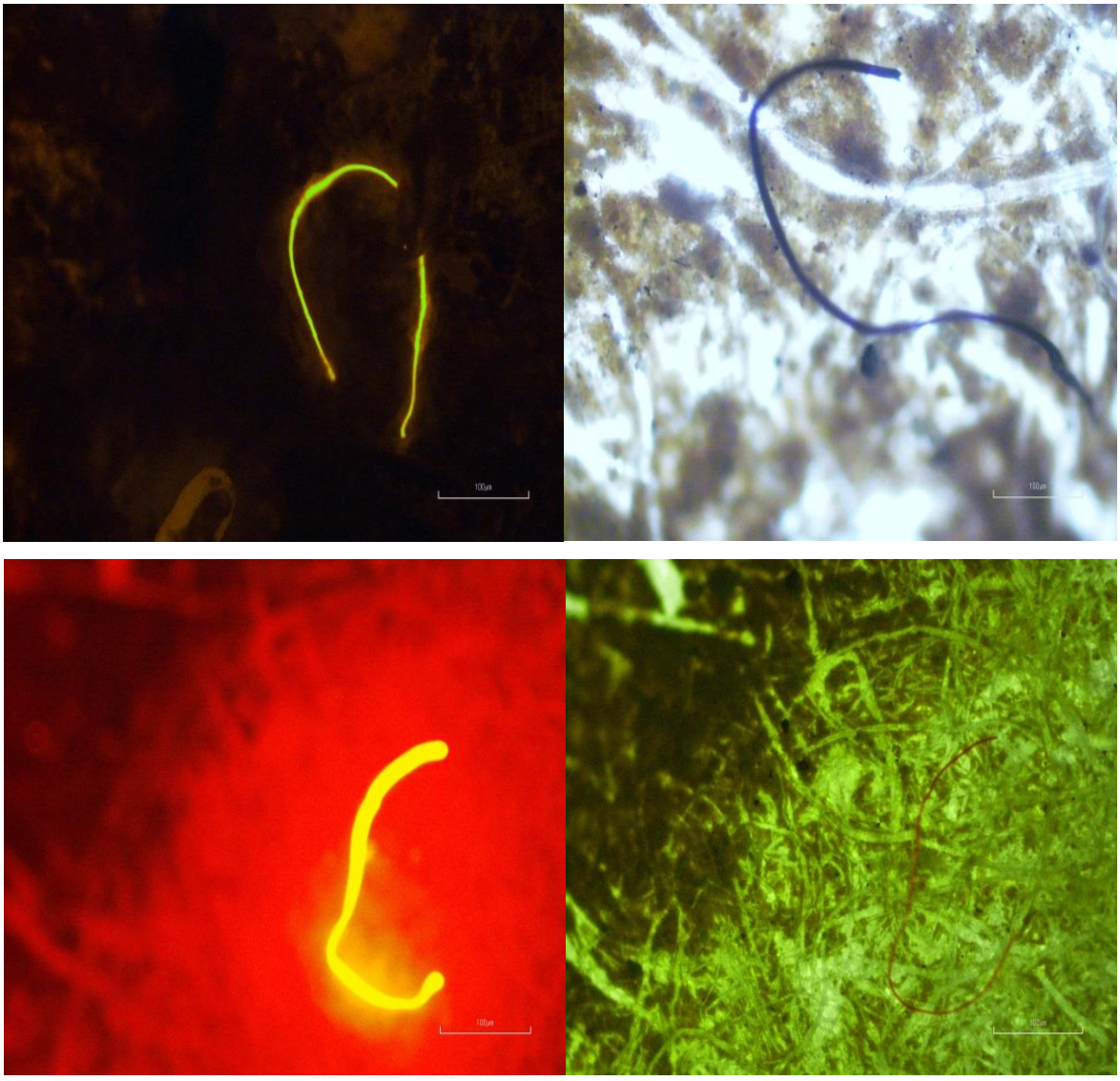

Figure 4.2. Microscopic images of microplastic fibers.

Residential areas had the lowest average in terms of their MP counts **3468, 4800, and 1868 items kg^{-1}** in Zekeriyaköy, Levent and Darüşşafaka, respectively. University campus had slightly higher amount equating around **6668 items kg^{-1}**, main difference between the university campus and residential areas is the circulation of people and involving recreational activities that were held on a frequent basis. Ikitelli Organized Industrial Area has small and medium sized industrial businesses and very active, MP found in this area averaged at **6400 items kg^{-1}** and it is on the higher end in terms

of MP concentration in this study, this may be related to the heavy industrial activity in that area as well as the heavy traffic surrounding the zone. Summary of the MPs abundance in these sites can be found in Figure 4.3, Figure 4.4, and breakdown of each site are detailed in Table 4.1.

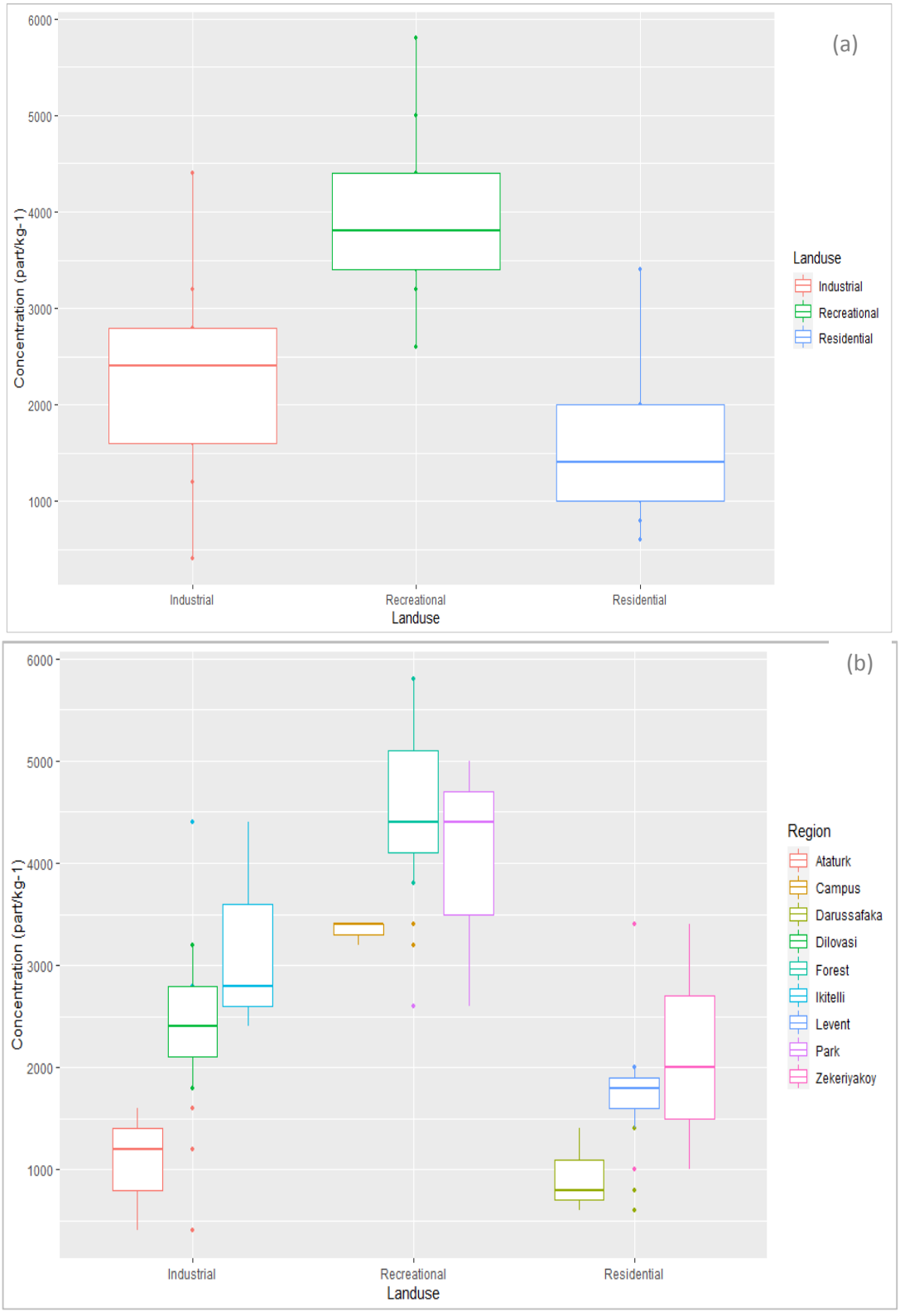

Figure 4.3. (a) MP concentration based on different land uses, (b) MP concentration of individual samples.

The abundance of MPs within different human-influenced soils significantly differs in relation to land use. According to the obtained results, Recreational Areas – Industrial Areas (p=0.003), and Recreational Areas – Residential Areas (p=0.0001) are significantly different in terms of their MPs content. Residential areas analyzed in this study were in the range of 1868 – 4800 items kg^{-1}. As for industrial areas, it should be noted that type of industry and operational capacity can heavily influence MP content in nearby area; however, results are still comparable to some extent. Reported MP content from literature and our study are on a similar range. Our results for recreational areas –including forest area were comparable with other studies by ranging between 8000 – 10933 items kg^{-1}. Interestingly, Industrial Areas – Residential Areas did not have significant difference in this aspect p=0.382 (Table A.1). It is also important to note that areas in each category did not have significant differences within their respective groups. Although there was no significant difference in between groups of land use; the mean difference between areas can be discussed. Ikitelli organized industrial area and Dilovasi organized industrial area have plastic manufacturers within their organizational structure while Atatürk Automotive Industrial Area do not have direct production of plastics but mainly specializes on automobile and tire repair. This may be the reason for lower numbers of MPs found in the area. Additionally, Atatürk Automotive Industrial Area has more fibrous MPs (25.01%) compared to Ikitelli organized industrial area and Dilovasi organized industrial area (10% and 16.33%, respectively). On the other hand, Ikitelli organized industrial area and Dilovasi organized industrial areas have more pellet structured particles compared to Atatürk Automotive Industrial Area (18.33%, 16.33%, and 10.72%, respectively) (Figure 6).

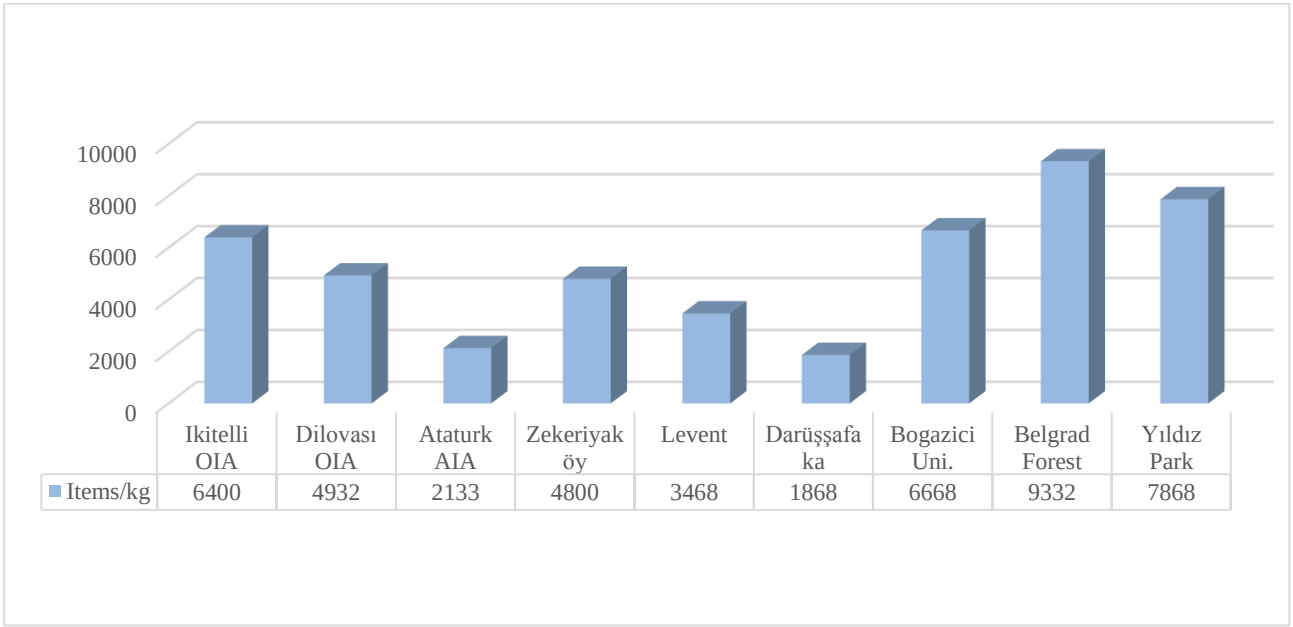

Figure 4.4. Microplastic abundance in different land use areas.

Belgrad forest is a part of a forested area close to human-influence while Yıldız Park is a popular park with various recreational activities. In this sense, high MP/microfiber count in the park area is understandable; however, samples from the forest area containing close to higher amounts of MPs, along with university campus' higher MP content, findings suggest that human-influence is prevalent and transport ratio may be relatively lower for MPs in recreational areas.

As for residential areas, Zekeriyaköy is a relatively newer region which gained popularity over the last few decades and Levent is closer to the city center and surrounded by heavy traffic. Darüşşafaka on the other hand, is more secluded with limited amount of traffic and had fewer constructions in the vicinity over the last few years. Therefore, it is quite understandable for Darüşşafaka having less amount of MP content compared to Zekeriyaköy and Levent; yet it is still alarming that even remote region soils with less expected inflow are still heavily influenced by human activity and leads to MP accumulation in soil bodies.

Table 4.1. Breakdown of the specifications of microplastic items found in this study.

MP	Ikıtelli OIA	Dilovası OIA	Ataturk AIA	Zekeriyaköy	Levent	Darüşşafaka	Bogazici Uni.	Belgrad	Yıldız Park
Shape	%			%			%		
Spherical	33.33	34.7	28.58	36.36	31.57	34.6	28.33	23.17	8.33
Rectangular	20	8.16	17.86	29.54	13.15	3.84	8.33	6.1	2.78
Pellet	18.33	16.33	10.72	6.82	0	3.84	8.33	2.44	4.17
Fiber	10	16.33	25.01	6.82	13.15	26.91	21.67	37.81	48.61
Fragment	18.33	24.49	17.86	20.45	42.09	30.76	33.33	30.49	36.11
Color	%			%			%		
Red	20	18.37	25.01	47.72	47.36	30.76	36.67	43.91	37.5
Blue	5	16.33	17.86	9.09	7.89	15.38	13.33	15.86	18.06
Yellow	23.33	24.49	32.15	6.82	7.89	26.91	1.67	7.32	16.67
White	23.33	10.21	17.86	13.63	10.52	3.84	13.33	3.66	9.72
Black	13.33	18.37	0	9.09	15.79	23.07	21.67	21.95	13.89
Grey	15	12.25	7.15	13.63	10.52	0	13.33	7.32	4.17

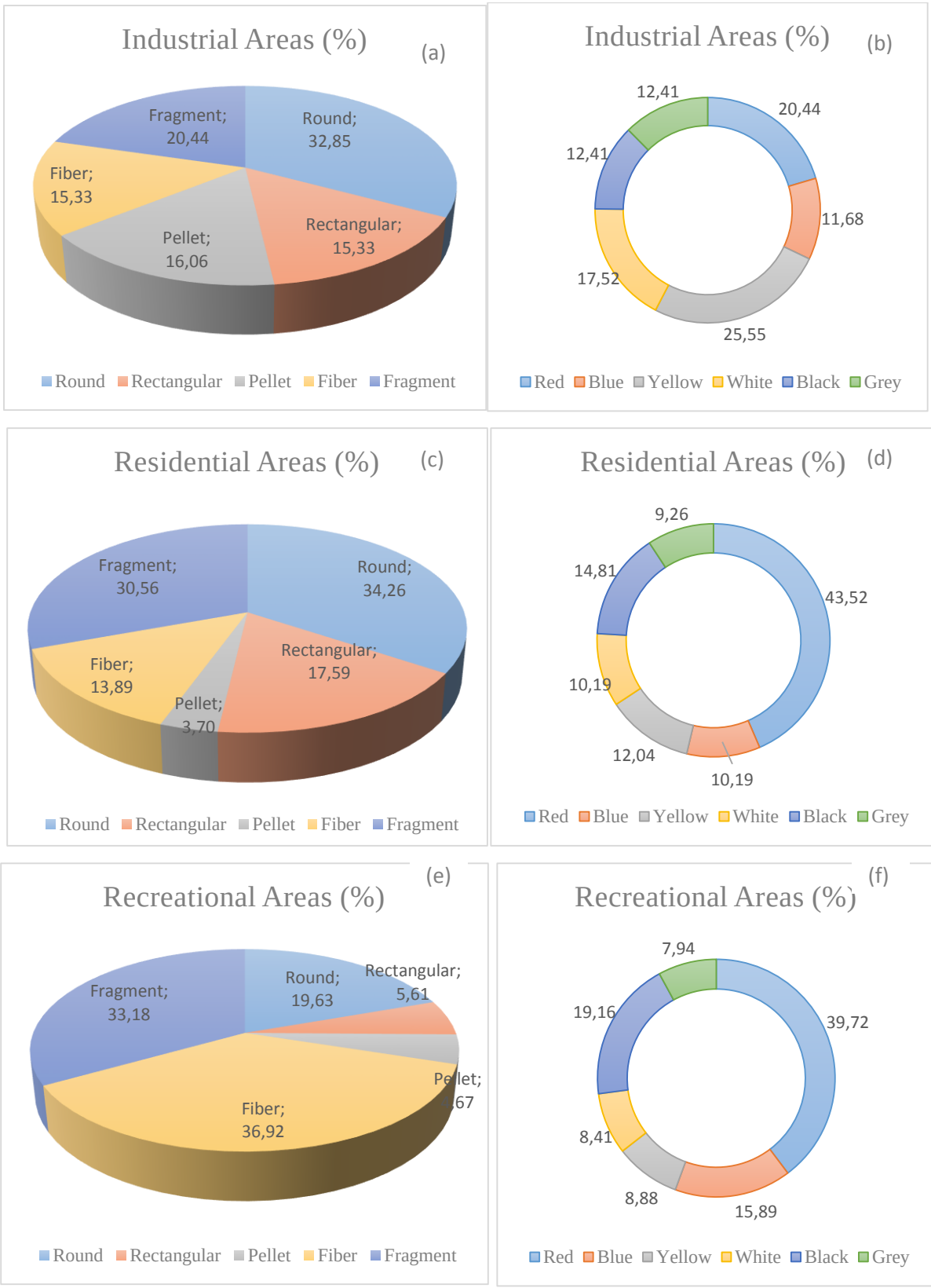

Figure 4.5. Shares of MP with respect to shape (a) Industrial; (c) Residential, (e) Recreational areas and color distribution (b) Industrial; (d) Residential; (f) Recreational Areas.

According to the breakdown of the MP abundance;

* Industrial areas; round/spherical **(34.18%),** rectangular/skewed (15.54%) cylindrical (3.55%), fragmented (30.10%), and fibers (15.63 %). While mostly red, grey, yellow, and while particles were commonly observed.
* Residential areas; round/spherical (31.57%), rectangular/skewed (13.15%) cylindrical (7.32%), fragmented **(32.32%)**, and fibers (9.76%). And color-wise particles were observed mostly shades of red and white.
* Recreational areas; round/spherical (19.48%), rectangular/skewed (5.74%) cylindrical (4.98%), fragmented (33.31%), and fibers **(36.49%).**

Ratios of the different shapes of MPs found in different land uses can be attributed to the activities held in respective zones, such as industrial areas had round/spherical MPs as dominant shape and those particles were observed to be intact with limited deterioration during microscopic evaluation. This may be related to relatively recent contamination considering industrial zones have primary point of production of materials. Whereas in residential areas, fragments and round/spherical particles were more common and can be explained by the fragmentation or weathering of primary MP sources. In recreational areas, most abundant MP shape observed was fibers and can be linked to the detachment from textile products during recreational activities. Red-colored particles and the highest rate among all soil samples in recreational areas; supports the contamination possibility by human interference and recreational activities. This may be related to plastic particles' original color (dye) in throw-away plastic items or originating from packaging material. Since all analyzed samples were gathered from topsoil (0-3 cm), where the MP introduction to soil systems is relatively new, deeper layers of the soil would still have MP content to some extent, but in relatively lower amounts since MP particles in deeper layers of the soil is the result of vertical penetration.

4.1.2. FT-IR

In order to determine the types of MPs in these areas; FT-IR spectrometry analysis with diamond ATR accessory (32 scans per spectrum) was utilized. Control samples were run and results background for comparison was presented on Figure A.1 as well as some selected examples' FT-IR results from different land uses are presented on Figures A.2 - A.5.

For the sample of one of the recreational areas; the spectrum contains two bands of asymmetric and symmetric C-H stretching (2916 and 2849 cm^{-1}) which are not overlapping. Bending of CH (1453

cm^{-1}) with other minor bands up to 1375 cm^{-1} along with the carbonyl area between 1700 and 1800 cm-1 as it stated in Almond et al., 2020 also contains minor peaks inside it. While the major peaks are absent, it can be determined that the originating type of plastic was not polyester, nor polyamide. Hence, it may be noted that in this sample contains a material probably of polyolefin type (the closest type of polypropylene), but due to the contamination with cellulose paper filter residue the spectrum also contains a wide 1027 cm^{-1} artefact band (Figure 4.6).

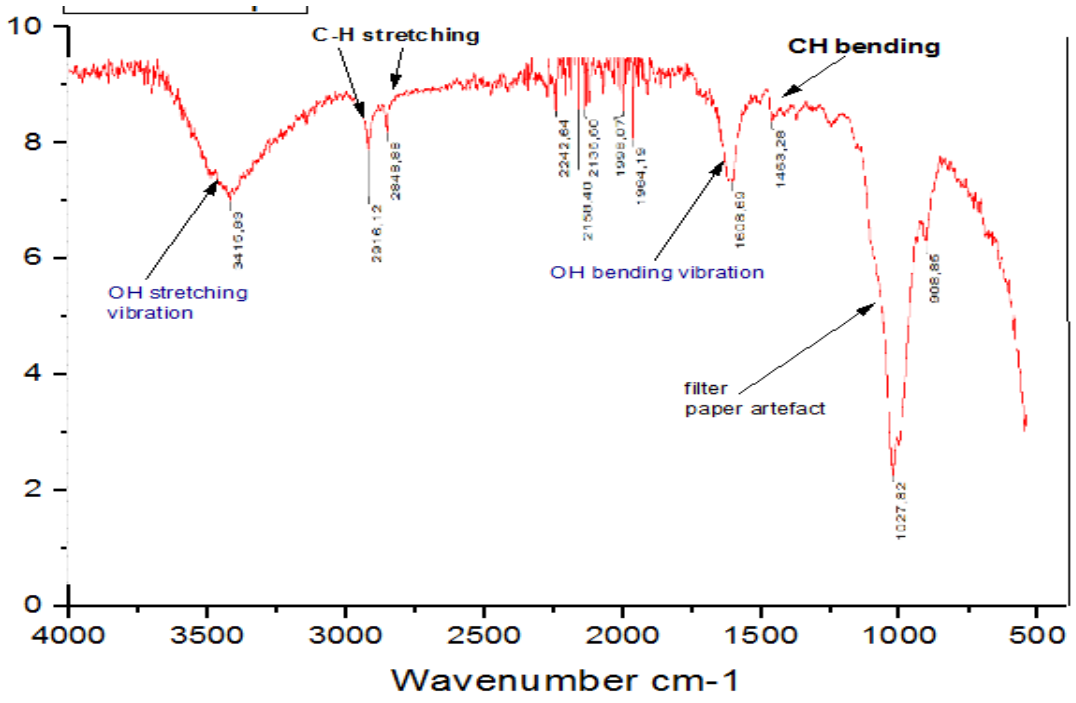

Figure 4.6. FT-IR spectra of the residential area.

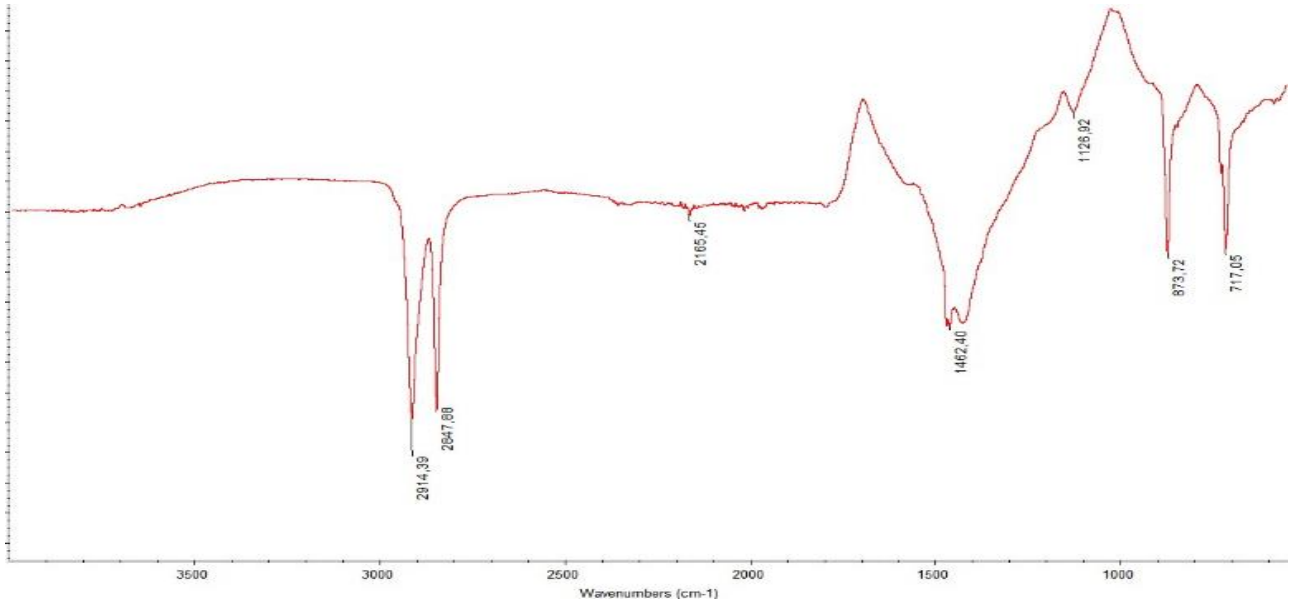

Figure 4.7. FT-IR spectra of the industrial area.

In the sample from Dilovası, the PE plastic remains almost unchanged by the weathering. Peaks at 2914 cm^{-1} and 2949 cm^{-1} corresponding to asymmetrical and symmetrical stretching of C-H bond in CH_2, CH_3 and CH groups. The doublet at 1462-1440 cm^{-1} is related to bending vibrations of the respective CH bond, which is typical for polyethylene. Spectra of this sample was shown in Figure 4.7, spectra for university campus and Belgrad forest were detailed in Figure 4.8 and Figure 4.9, respectively.

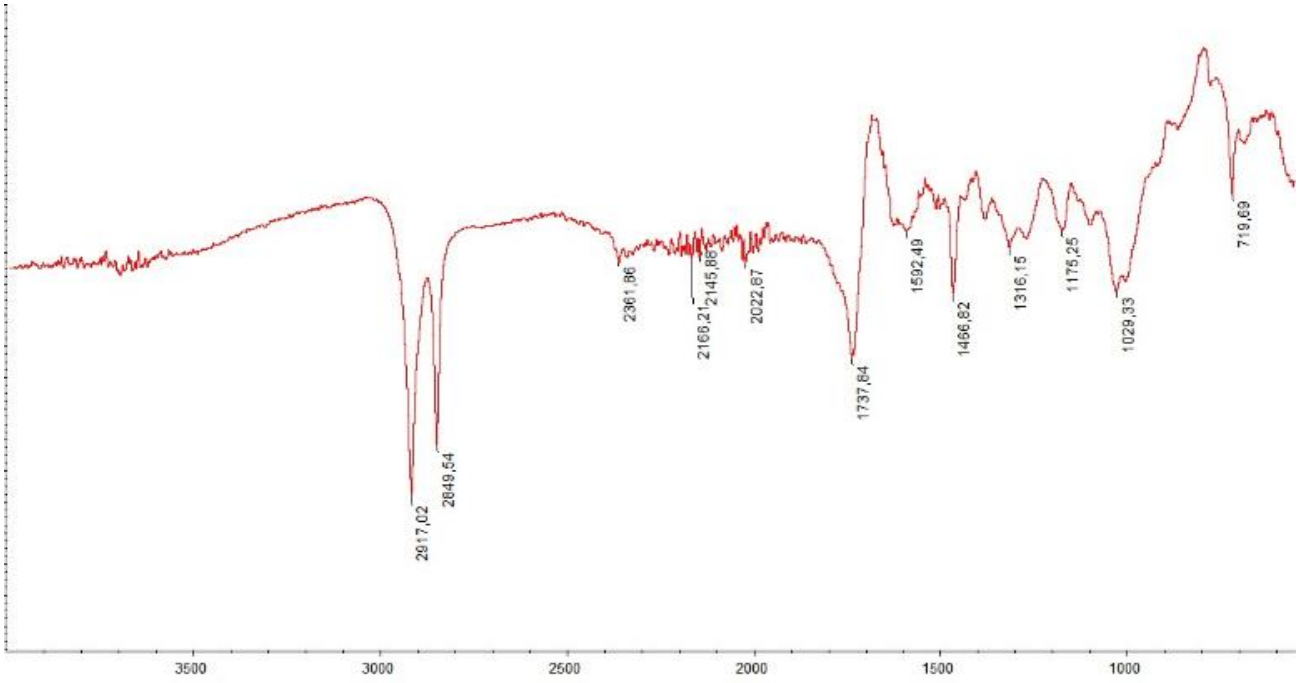

Figure 4.8. FT-IR spectra of the recreational area (University Campus).

Spectra obtained from the sample from the university campus showed the same configuration of aliphatic C-H bond stretching peaks (2917 and 2850 cm^{-1}), a sharp **PE-like** band at 1468 cm^{-1}, but significant carbonyl C=O stretching band at 1740 cm^{-1} with noticeable shoulder. Minor bands at 1316 cm^{-1} and nearby can be associated with the ether bond and be a product of the polymer transformations in contact with environment, as it is shown by Pagès, 2015.

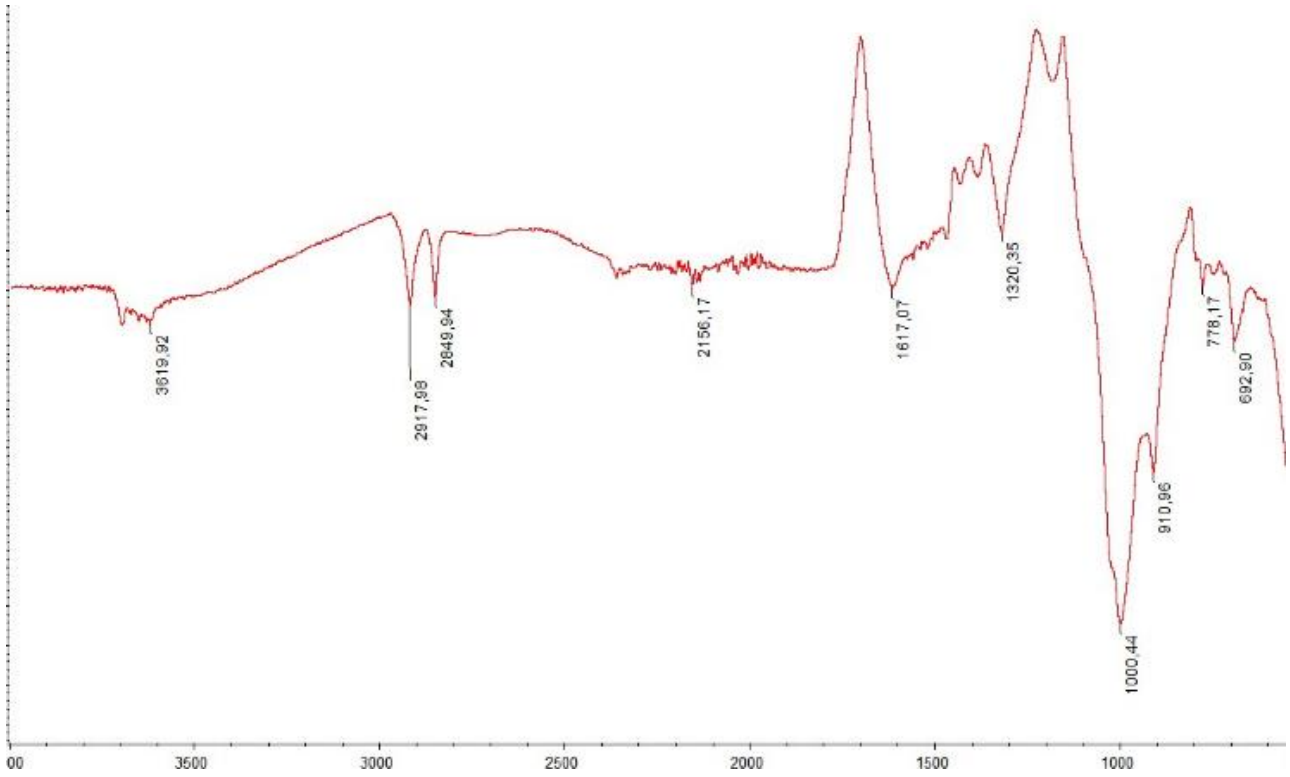

Figure 4.9. FT-IR spectra of the recreational area (Belgrad Forest).

Belgrad forest and Yıldız Park have shown similar attributions in terms of their spectra; the presence of organic matter may be detected by the bands of aliphatic CH groups at 2918 cm^{-1} and 2849 cm^{-1} for both samples. There are no signs of carboxylic band, indicating the fact that the material destruction has just started. However, two bands at 3675 and 3620 cm^{-1}, attributed to O-H bond stretching in materials structure may possibly point out on the hydrolytic way of the material destruction, which is usual for such plastics as **polyacetal, nylons,** and **polyethers** (Jansen, 2015).

In short, the group of materials found in recreational areas may be considered as small particles of the polymer on the beginning of destruction. The presence of OH groups (even after drying) indicates that this polymer undergoes the hydrolysis instead of UV or oxygen/ozone driven oxidation. Hence, it may be considered either as the initially C=O containing polymer (such as **acrylate**), that is frequently used in packaging or oxidized **polyolefin** (PE or PP). The material found in industrial zone are **PE** MP with still well-defined structure and relatively clean suggesting the MP contamination was recent.

4.1.3. SEM - EDS

In order to determine the morphological surface structure and to obtain high-resolution images of present MP particles, samples were analyzed with SEM. Along with this examination, larger particles of suspected MP were subjected to energy dispersive spectroscopy (EDS) to evaluate the elemental composition of related particles and to confirm if they are plastic particles or not. Some examples from microscopic images are given in Figure 4.10.

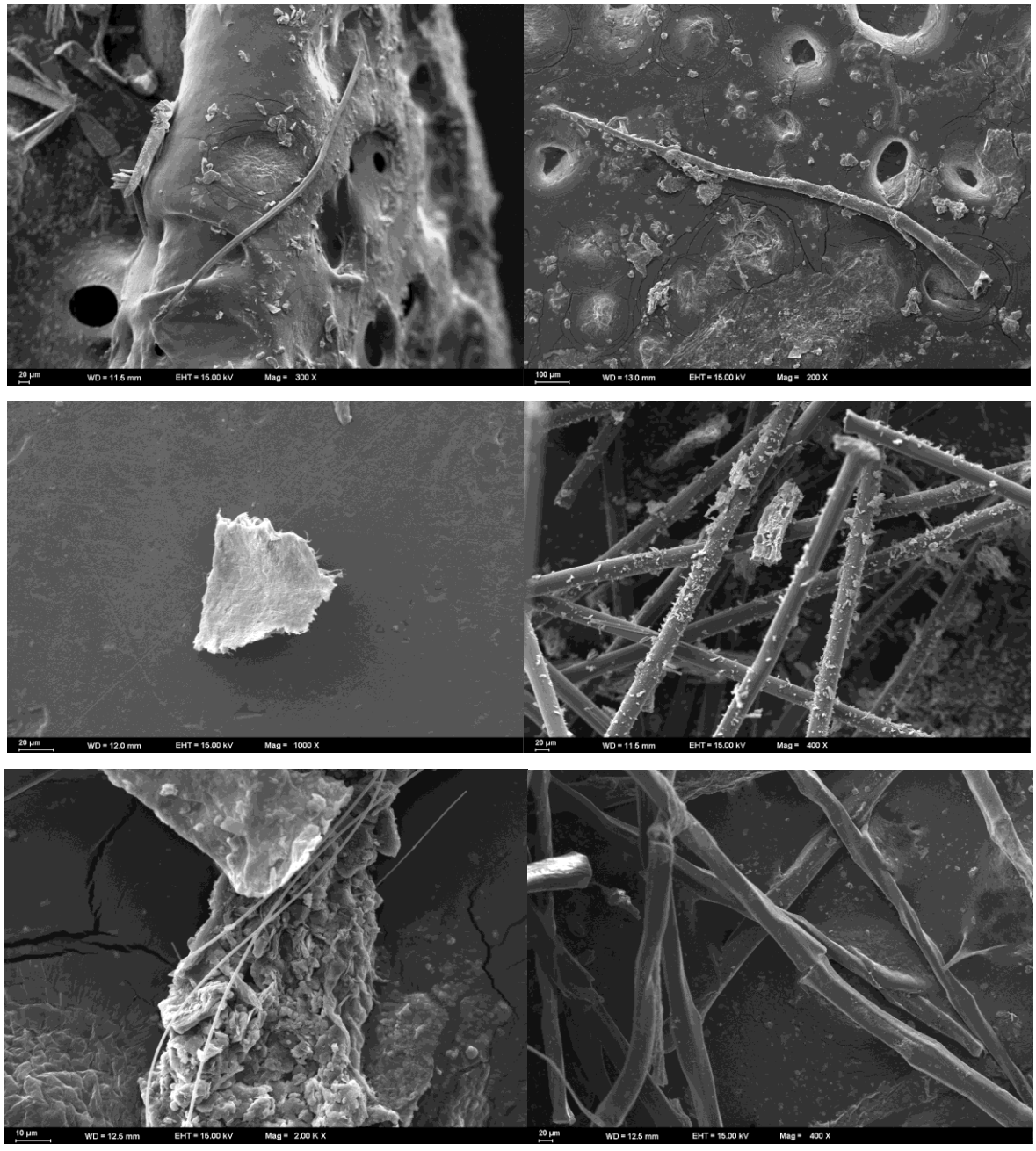

Figure 4.10. Selected SEM images of microplastic particles.

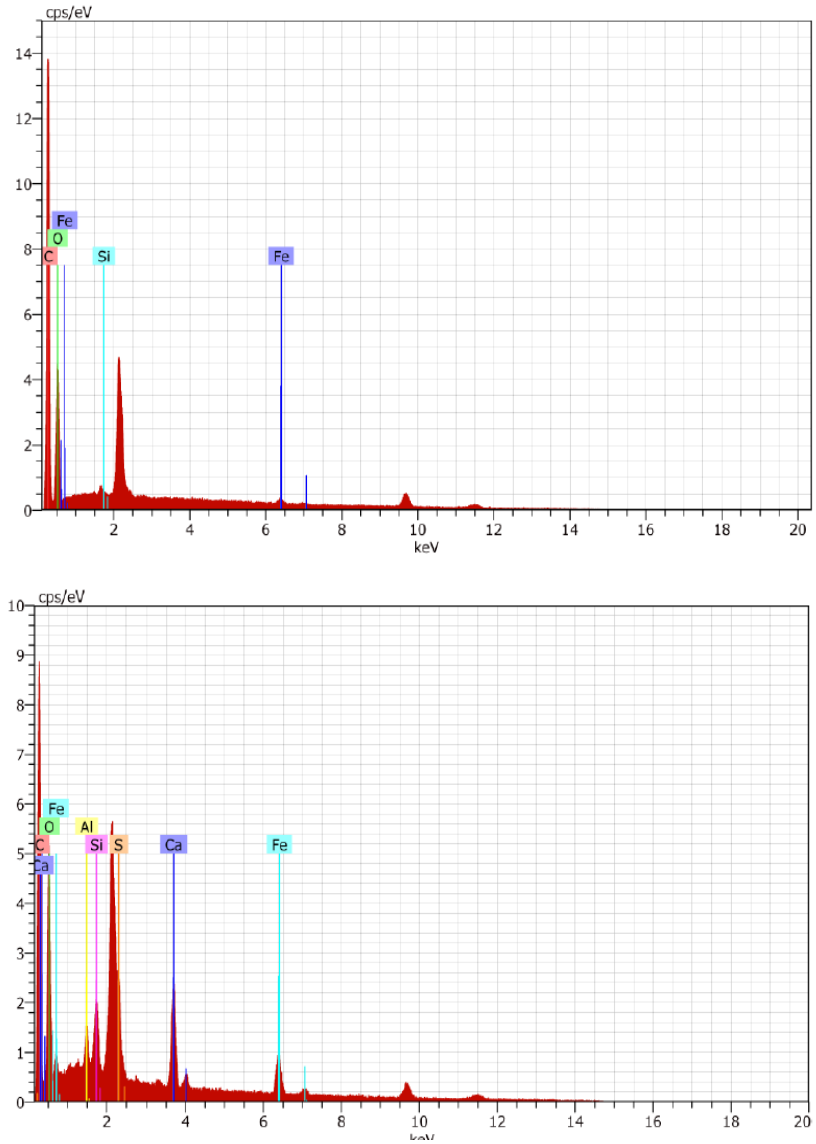

Figure 4.11. Sample from EDS analysis of microplastic particles.

Details of EDS analysis were given in Figure 4.11 and Table 4.2. Particles' elemental compositions were mainly consisted of Carbon (C) by 45 – 55% and Oxygen (O) 50-55%. Positively reinforcing that found particles were commonly, MP and microfiber particles.

The presence of Si and Ca may be attributed to inclusions of inorganic disperse particles inside plastic (fillers, pigments, heat stabilizers etc.; Bolgar, 2008), but the contamination of the sample with Fe3+ ions is probably caused by the absorptive properties of MP particles as it shown by Hildebrandt (2021).

Table 4.2. Elemental composition.

Spectrum: Acquisition

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error [%]
O	8	K-series	53.50	53.50	46.62	16.7
C	6	K-series	45.75	45.75	53.10	14.1
Fe	26	K-series	0.42	0.42	0.10	0.0
Si	14	K-series	0.34	0.34	0.17	0.0
		Total:	100.00	100.00	100.00	

4.2. Preliminary Germination Test

According to germination rates, sunflower had higher germination rate compared to sorghum variation, 91.1% and 63.33% for control groups, respectively. Germination rates over time are detailed in Table 4.3.

Germination study results were consistent within their own categories, it can be argued that MPs presence affects the germination capacity with high level of MPs. Only sunflower seeds treated with low amount of MP -0.4% had better germination rate compared to control group of the same category by 8%. With increasing MP levels number of germinated seeds have decreased for both plants. Sunflower seeds' germination went down by 6% for 0.8% MPs and 17% for 1.6 MPs, respectively. Whereas Sorghum seeds germination decreased by 10%, 11.11% and 24.55% for the same range of MP fibers present in the system.

Table 4.3. Germination ratio of different sunflower varieties.

Sunflower	Day 3	Day 4	Day 5	Sorghum	Day 3	Day 4	Day 5
Control	80%	80%	80%	Control	80%	80%	80%
	80%	100%	100%		50%	50%	50%
	100%	100%	100%		60%	60%	60%
0.4% MP	100%	100%	100%	0.4% MP	40%	40%	50%
	100%	100%	100%		60%	60%	60%
	100%	100%	100%		50%	60%	60%
0.8% MP	100%	100%	100%	0.8% MP	50%	50%	50%
	60%	80%	80%		60%	60%	60%
	80%	80%	80%		40%	50%	50%
1.6% MP	40%	60%	60%	1.6% MP	40%	40%	40%
	60%	100%	100%		30%	40%	40%
	80%	80%	80%		30%	30%	30%
Control + *G.mossaea*	80%	80%	80%	Control + *G.mossaea*	40%	40%	40%
	100%	100%	100%		50%	30%	50%
	80%	100%	100%		40%	40%	40%
0.4% MP + *G.mossaea*	100%	100%	100%	0.4% MP + *G.mossaea*	40%	40%	40%
	100%	100%	100%		30%	30%	30%
	100%	100%	100%		50%	50%	50%
0.8% MP + *G.mossaea*	80%	80%	80%	0.8% MP + *G.mossaea*	30%	30%	30%
	100%	100%	100%		50%	50%	50%
	80%	80%	80%		30%	30%	30%
1.6% MP + *G.mossaea*	100%	100%	100%	1.6% MP + *G.mossaea*	10%	10%	10%
	100%	100%	100%		50%	50%	50%
	80%	100%	100%		20%	20%	20%

Additionally, AMF introduction to the system seems to have helped with germination of seeds with relatively high-level of MPs especially for sunflower species. Sunflower control group with AMF inoculation (Control +) has 4.4% better germination rate compared to sunflower control group without inoculation; low-level MPs of 0.4% was not affected by AMF presence since all sunflower seeds were germinated in both sets; for MP levels of 0.8% and 1.6% the germination rate was increased by 2.22% and 26.66%, respectively.

Sorghum seeds have not benefited from AMF inoculation in terms of their germination rates. Overall germination rate decreased by 22% for control group and 13.3%, 15.6%, and 12.2% for increasing MP levels present in the system.

Germination of the seeds is the first step of plant growth and seedling properties are highly dependent on the plant species. Physical changes in the soil by MP presence can influence the growth behavior either positively/negatively or that may not be on significant levels depending on the

concentration of MPs. In a study carried out by Judy et al. (2019); HDPE, PET, PVC (<2mm) were utilized as MPs on 0.1 – 1% (w:w) and reported that no significant effect was observed on the seedling behavior of *Tricitum Aestivum* in the presence of mixed organic waste. On the other hand, delayed and decreased germination rate were observed as short-term impact by Bosker et al. (2019) in *Lepidium sativum* with the exposure to MPs. In this preliminary experiment, we also observed effects of MPs both positive and negative ends; however, it can't be concluded as significantly difference were observed based on preliminary results alone. It can be argued that they supplied a basic understanding of the outline for what might happen during plant growth phase as a baseline.

4.3. Plant Experiments

Two different main experimental sets were run under controlled environment. Average conditions of pH and temperature along with humidity of the room with room temperature were detailed in Appendix.

4.3.1. Plant Height

During the experiments, plant heights over time were measured and monitored individually. For sunflower plants, MP treatment -0.4% (w/w), grew fastest and reached higher length followed by the control groups. Mid-level MPs -0.8% (w/w) was relatively comparable with the control group, but the high-level MPs -1.6% (w/w) hindered the sunflower height for both sets (26 °C and 31 °C) throughout the study, and especially between 2^{nd} and 3^{rd} week after plantation (p= 0.005). As for sorghum species, similar to sunflower, low-level MP in the soil helped the plants to grow faster compared to control group, then again with the increasing level of MPs in the system decreased the growth rate. Increased biomass and plant height can be considered as a positive effect; may be related with increased aeration rate or soil permeability or increased water holding capacity as a result of MP presence in soil bodies; however, positive effect does not necessarily mean it's desirable or favorable since there is no method to recover or remove MPs from soil bodies, the accumulation will lead to higher levels of MP in the soil over time and levels will inevitably be higher than current conditions where negative impact on plant growth or other plant parameters may be more visible. Plant height data are over-time are presented in Appendix section, Table B.5 and Table B.6, respectively.

4.3.2. Shoot and Root Weight

For sunflower species, stem dry weights were comparable with each other for all sets and treatment levels, while root dry weights were increased along with MP presence (Table B.7). Statistical analysis info is available on Appendix B (Tables B.10 – 17). Significant differences were observed between the control group and lowest level of MP group (p= 0.041) at 26 ºC. Additionally, under higher temperature (31 ºC), root dry weights were significantly increased with raising MP levels in soil body, between control group and highest level of MP group (p= 0.003), low-level of MP and high-level (p= 0.004), and mid-level and high-level (p= 0.024), respectively.

For sorghum species, stem weight first decreased with low level of MPs in the soil and then increased with higher level of MP, significant difference was observed between lowest level MP and mid-level MP concentration (p=0.05). Even though, a trend was observed on root dry weight (Figure 4.12), no significant difference was observed. An interesting fact is that the effects differ depending on the plant species, especially for stem weight. Significant difference for stem dry weights were on; plant species and MP levels, further post-hoc results were detailed in the appendix section. MP presence (0.4%) first increased the stem weight of sunflower, while it decreased the weight in Sorghum species. Further increase in the concentration of MP decreased the stem weight for Sunflower but increased the weight in Sorghum species.

Significant statistical differences were observed on root dry weights for all parameters, AMF presence (detailed in section 4.3.3), plant species, and MP levels. Root dry weights showed significant difference between groups for increasing MP levels at 31 ºC, between control group and mid-level of MP group (p= 0.001), control and high-level of MP (p= 0.027), low-level and mid-level (p= 0.002), and between mid-level and high-level of MP (p=0.048) respectively.

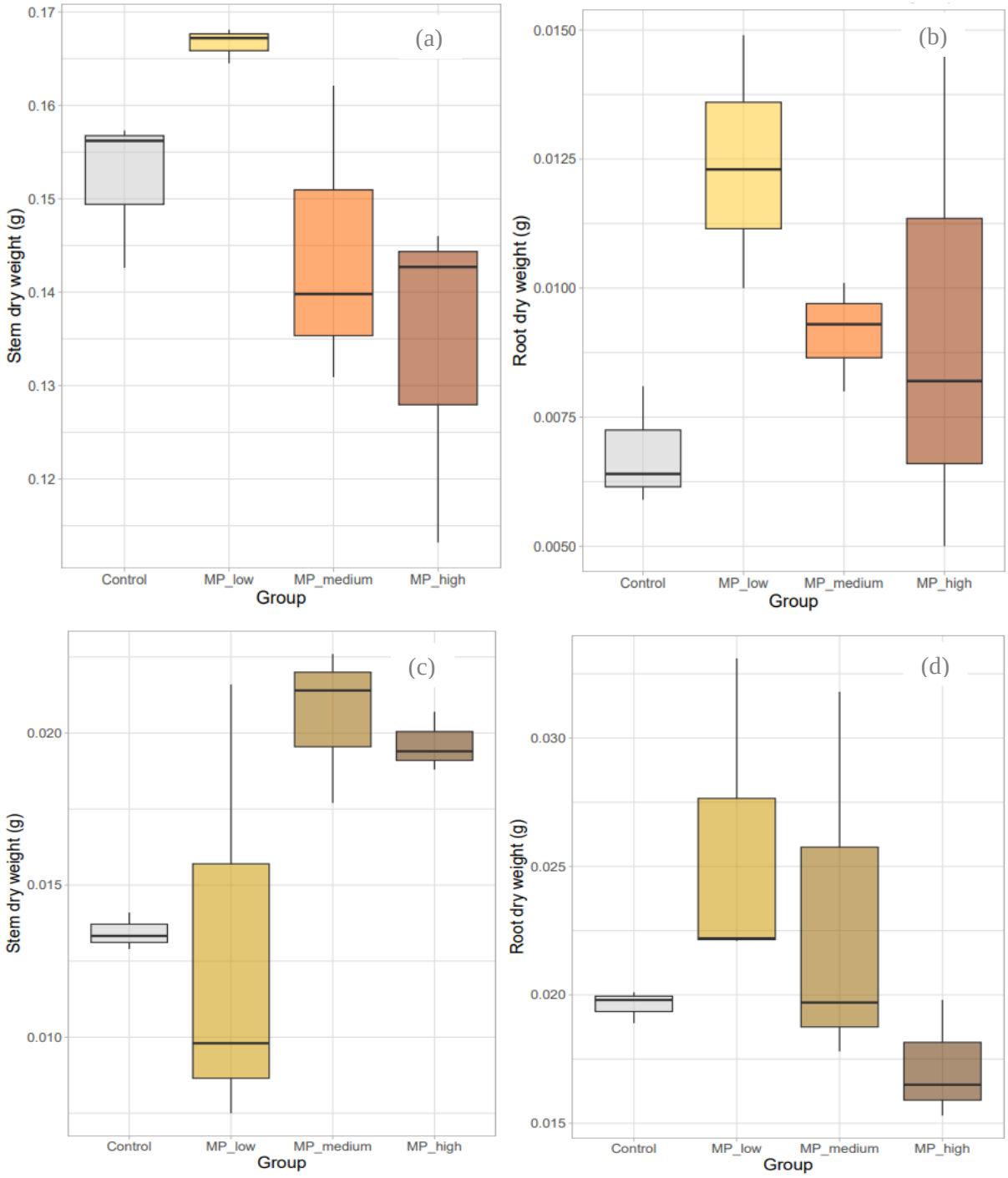

Figure 4.12. Effect of MP on sunflower (a, b) and sorghum (c, d) dry weights.

Possible effects of MPs on plant growth by other studies in the literature are relatively comparable; studies conducted with PES MP fibers on *Bidens bipinnata, Galinsoga parviflora, Plantago depressa, Medicago sativa* (Deng et al., 2022), *Lumbricus terrestris* (Prendergast-Miller et al. 2019), *Zea mais* (Ingraffia et al., 2022) have all reported that plant growth was affected in the presence of MPs. Study durations were between 30 – 35 days with MP sizes of 87-3000 µm, respectively. Deng et al (2022) presented increased plant height by 3.71 – 57.17% and increased shoot biomass by 11.67 – 72.68 % for all 4 plant species under 0.4% (w:w) PES MP fibers. de Souza

Machado et al. (2019) also found increased root biomass after inoculation with MP fibers (0.2%) into the soil media and observed ~40% increase in root biomass along with increased root length. Another research carried out by Lozano et al. (2020), utilized 0.4% MP fibers consisting of PES reached ~6% increased shoot biomass while increasing root biomass by ~90%. The results show the effect of MP at 0.4% increase plant biomass, most likely explanation is the shape and size of MP fibers acting different compared to natural components of the soil and influence soil properties via their physical characteristics such as soil bulk density and water dynamics in the system, therefore, these effects can explain the changes in the biomass allocation and plant growth. However; there are also studies which reported negative or no effect on plant species including de Souza Machado et al. (2019), Lozano et al. (2020). For instance, spherical shaped PA particles decreased the growth of *Allium fistulosum* under %0.2 MP presence (de Souza Machado et al., 2019), and PS (0.4%) type foam shaped particles had no effect on the growth of *Daucus carota* (Lozano et al. 2021). A study carried out by (Ingraffia et al., 2022) with PES MP fibers in soil (0.5% w:w) with *Zea mais* observed strong detrimental effects with ~30 decrease in plant biomass as well as total root length while reporting similarly reduced bulk density with the addition of MP fibers. Some other negative or no effect examples include but not limited to; Pignattelli et al. 2020; Qi et al. 2018, respectively. Therefore, it can be argued that the initial physical effects of MP fibers in soil can be the same; yet growth response of plants can still be different. Difference can be linked to environmental conditions, region, presence of soil organisms, MP type and concentration, plant species and other factors. A meta-analysis is needed once more data is available on the literature.

4.3.3. AMF Presence

Presence of *G. mosseae* on plant roots were demonstrated in Figure 4.14. AMF presence had positive impact on plant height, growth, and dry weights in control groups and low-level of MPs in the soil. Low level of MPs in the system increased the dry weight, between control (no inoculation) and AMF there was significant difference found on root dry weights (p= 0.0001). However, with raising level of MP plant growth was still hindered, but symbiosis with *G. mosseae* increased overall resistance and supported plant growth. Lowest growth was observed under following conditions for sunflower plant; 31°C (higher temperature), high-level of MP -1.6% (w/w) with *G. mosseae*. This effect may be related to the additional stress created by different treatments.

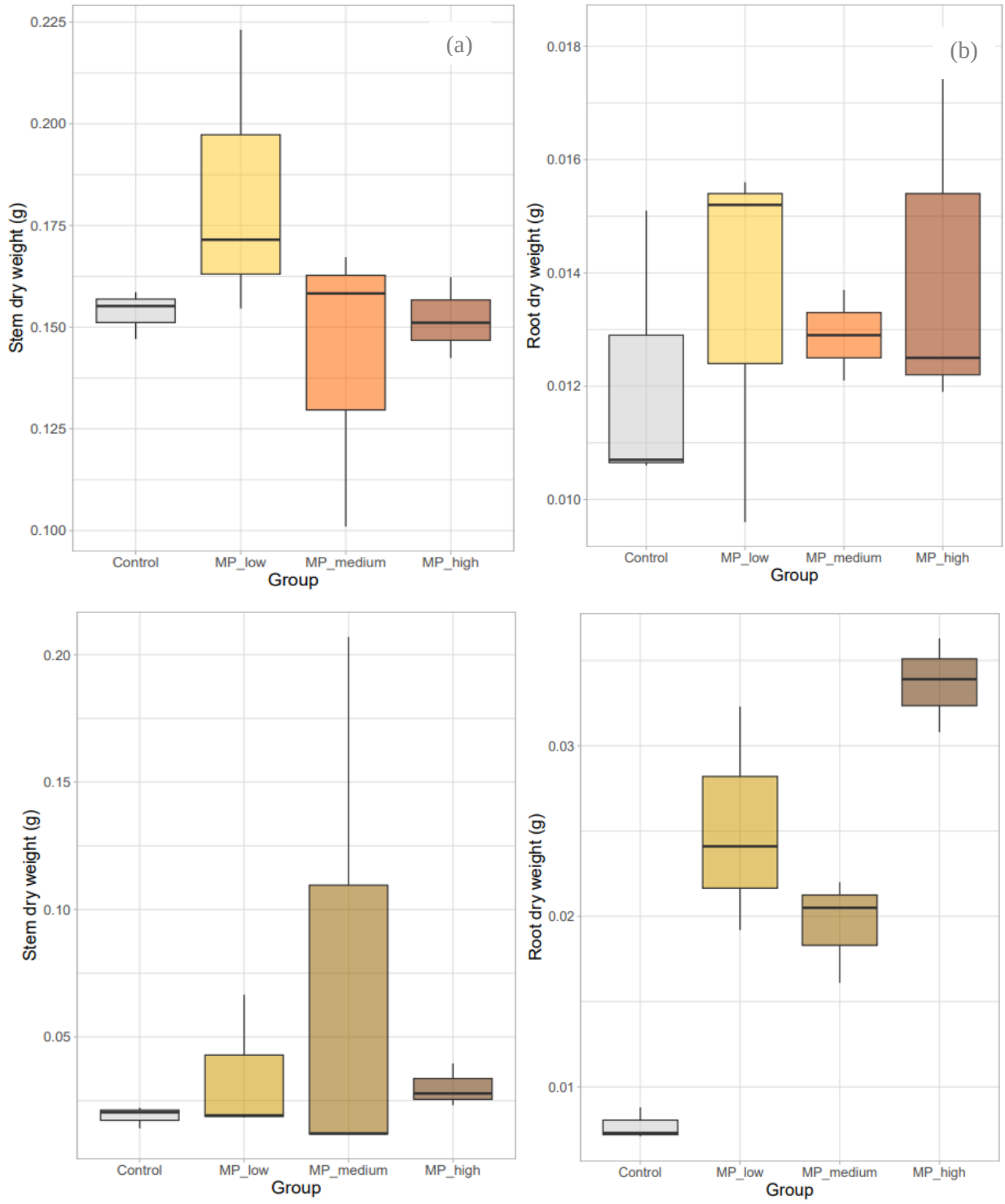

Figure 4.13. Effect of MP on sunflower (a, b) and sorghum (c, d) dry weights with AMF presence.

As for sorghum plants, similar to sunflower plants, symbiosis with *G. mosseae* promoted overall growth of sorghum plants, and root dry weights when assisted by mycorrhiza was significantly in higher levels of MP, -0.8% (w/w) and 1.6% (w/w) compared to control group (p=0.01 and p= 0.0001, respectively). Mean values of all treatment levels with AMF inoculation for both stem and root dry weights can be seen on Figure 4.13. Root growth behavior and by extension root dry weights of respective plants present more reliable comparison point compared to stem dry weights in plant

studies. Therefore, fluctuation of stem dry weights can be expected but they are still correlated with root dry weights. Root growth data in this study were consistent within groups and provided a clear outline on the effects of studied factors.

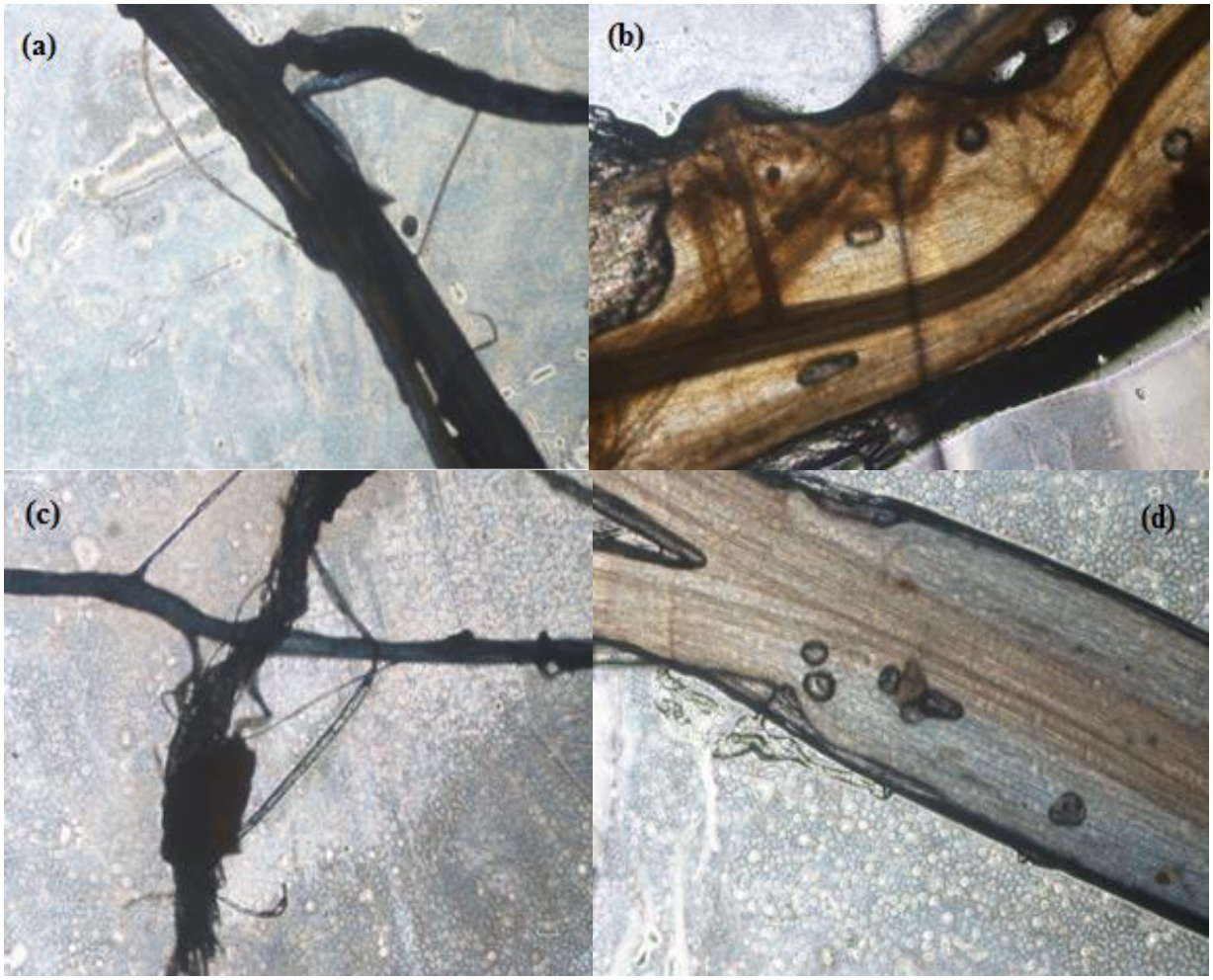

Figure 4.14. Spore, hyphal, and vesicular forms of *G. mosseae* in sunflower (a, b), Sorghum (c, d) roots.

Highest mycorrhization rate was found to be with the Sorghum plants at room temperature (26°C) compared to sunflower plants; low-level MP presence -0.4% increased the overall mycorrhization rate of control group by 10% to 76%, however mycorrhization rate was inhibited with increasing amount of MPs present in the soil. 2nd and 3rd MP levels; 0.8%, and 1.6% w/w performed worse than control by 11.45%, and by 19.3%, respectively. Mycorrhization rates of sorghum and sunflower plants can be seen in Table B.8, Figure 4.15, and Figure 4.16, respectively.

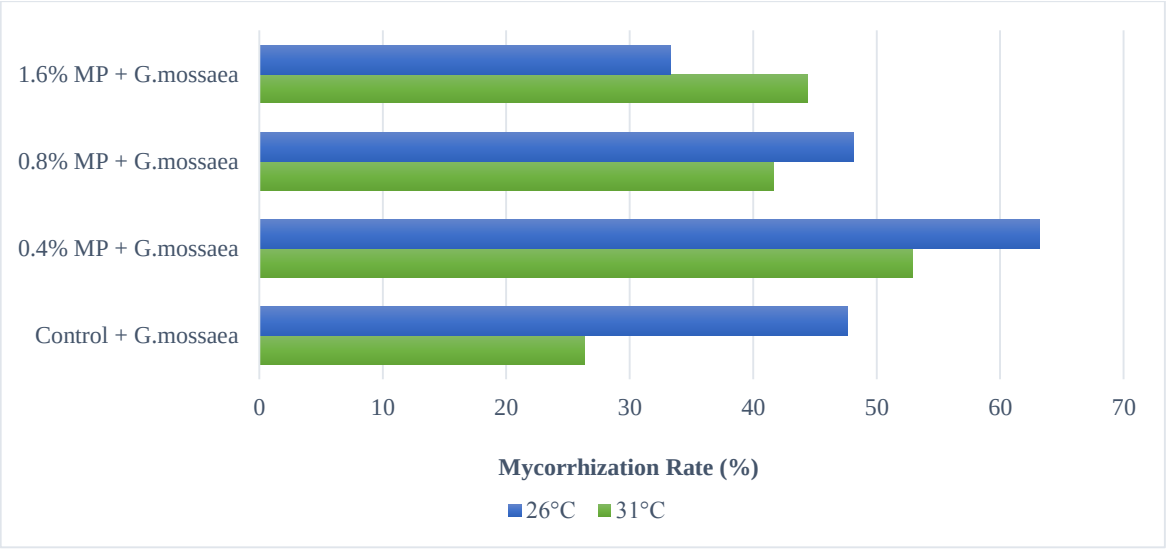

Figure 4.15. Comparison of mycorrhization rate of sorghum roots.

Sunflower symbioses with *G. mosseae* species had similar colonization rate to sorghum plants when exposed to various levels of MP. At lower temperature of 26°C; control group of sunflowers had the mycorrhization rate of 47.62%, significantly better than high temperature group of 31°C with 37.5% root colonization. Relatively lower level of MP presence (0.4%) increased the rate to 63.16%; while, increasing amount of MPs levels of 0.8%, and 1.6% performed worse with colonization with 48.15%, and by 33.33%, respectively. Root mycorrhizal symbiosis for plants showed significant difference in terms of colonization behavior with different levels of MP present in the soil body.

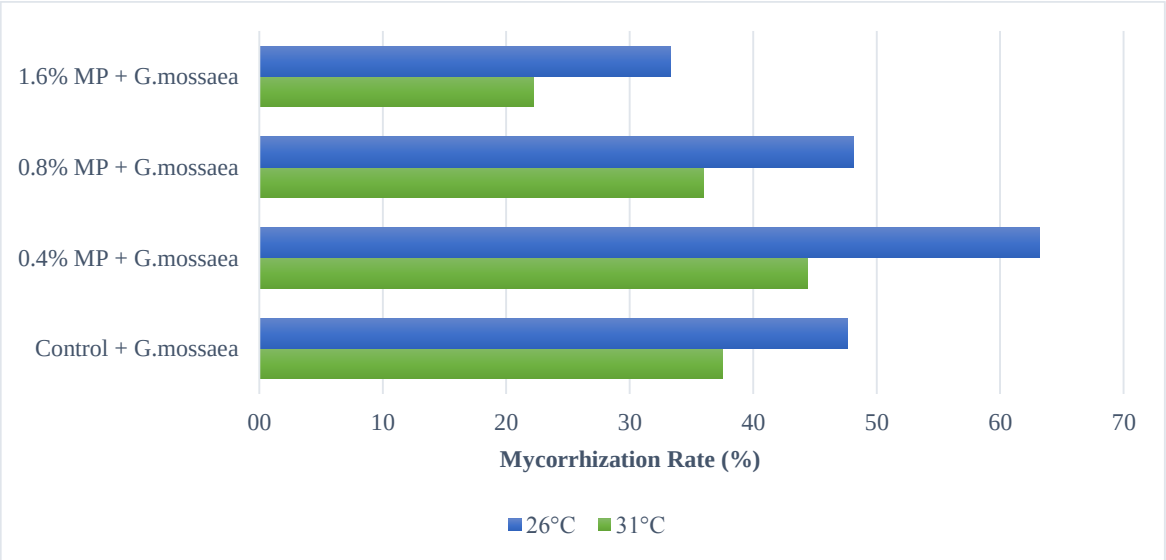

Figure 4.16. Comparison of mycorrhization rate of sunflower roots.

Both plants had the ability to form symbiosis with *G. mosseae* while low amount of MPs helped forming stronger and wider colonization; while after a breaking point colonization slowed down and weakened by increasing amount of MP. This phenomenon can be explained by physical properties of MPs and their ability to incorporate into soil and between soil aggregates, combined with the increased surface tension and water holding capacity it gives plant roots available room spreading and easier growth. However, increased levels also interfere with the root development and mycorrhizal colonization by causing possible blockage of water transport systems within root structure, and by being stuck on the surface of the root area to decrease the growth ability of the respective plant.

In the literature, it is also reported that root symbiosis is affected by MPs present in the system. 0.2% (w:w) PES MP fibers increased the root colonization of AMF ~ 8-fold while with the presence of PP MPs observed increase on root colonization was ~1.4 fold. On the other hand, PET type MPs in the system have decreased the root colonization by ~ 50% (de Souza Machado et al., 2019). Another study carried out by Lehmann et al. (2020), utilized 0.4% PES MP fibers in soil with 3 different AMF species *Rhizoglomus irregulare, Funneliformis caledonium, Funneliformis mosseae* and worked with *Allium cepa*. The authors reported highest colonization of mycorrhiza was observed with MP fibers and under well-watered conditions, however, they also reported that the plant biomass did not increase under the presence of AMF specie. Which is in accordance with the findings of our study. It can be speculated that the mycelial network of mycorrhizal activity associated with plant roots supporting the water-nutrient transportation is also correlated with the root cortex area and root colonization by AMF in turn, promoting root growth of the host plant. Another aspect is the effect of MPs on AMF species. Leifheit et al. (2021) reported that the release of the additives of MPs will be released, which may affect AMF directly, together with possible indirect effects mainly related to alteration of soil properties by MPs.

4.3.4. Increased Temperature

Increased temperature utilized as a mean to incorporate global climate change as a factor in this study. 5 ºC difference in temperature levels were set between difference to observe the temperature effect clearly and to maintain a reliable base point for comparison between sets, in accordance with the prediction for global climate change and related studies (IPCC, 2014, Rillig et al., 2019). Higher temperature (31ºC) hindered the growth of sunflower in terms of produced biomass and height compared to lower temperature (26ºC). Higher average temperature assisted the growth of sorghum plants as opposed to sunflower species. Additionally, symbiosis with *G. mosseae* promoted overall

growth of sorghum plants. Similar trend was observed for plant growth in response to MP levels in the soil under different temperatures. In terms of plant height best performance of Sorghum plants were observed under following conditions; 31°C (higher temperature), high-level of MP -1.6% (w/w) with *G. mosseae.* This effect may explain the survivability instinct of sorghum plants by adapting the conditions faster and building up the symbiosis with mycorrhizal fungi under different sources of stress. On the other hand, least growth of sorghum plants was observed in; 26°C (lower temperature), moderate - high level of MP -0.8% (w/w) and 1.6% (w/w) and without the presence of *G. mosseae.* Comparison of means of stem dry weight and root dry weight of sunflower and sorghum plants on different temperature levels can be seen in Figure 4.17 and Figure 4.18.

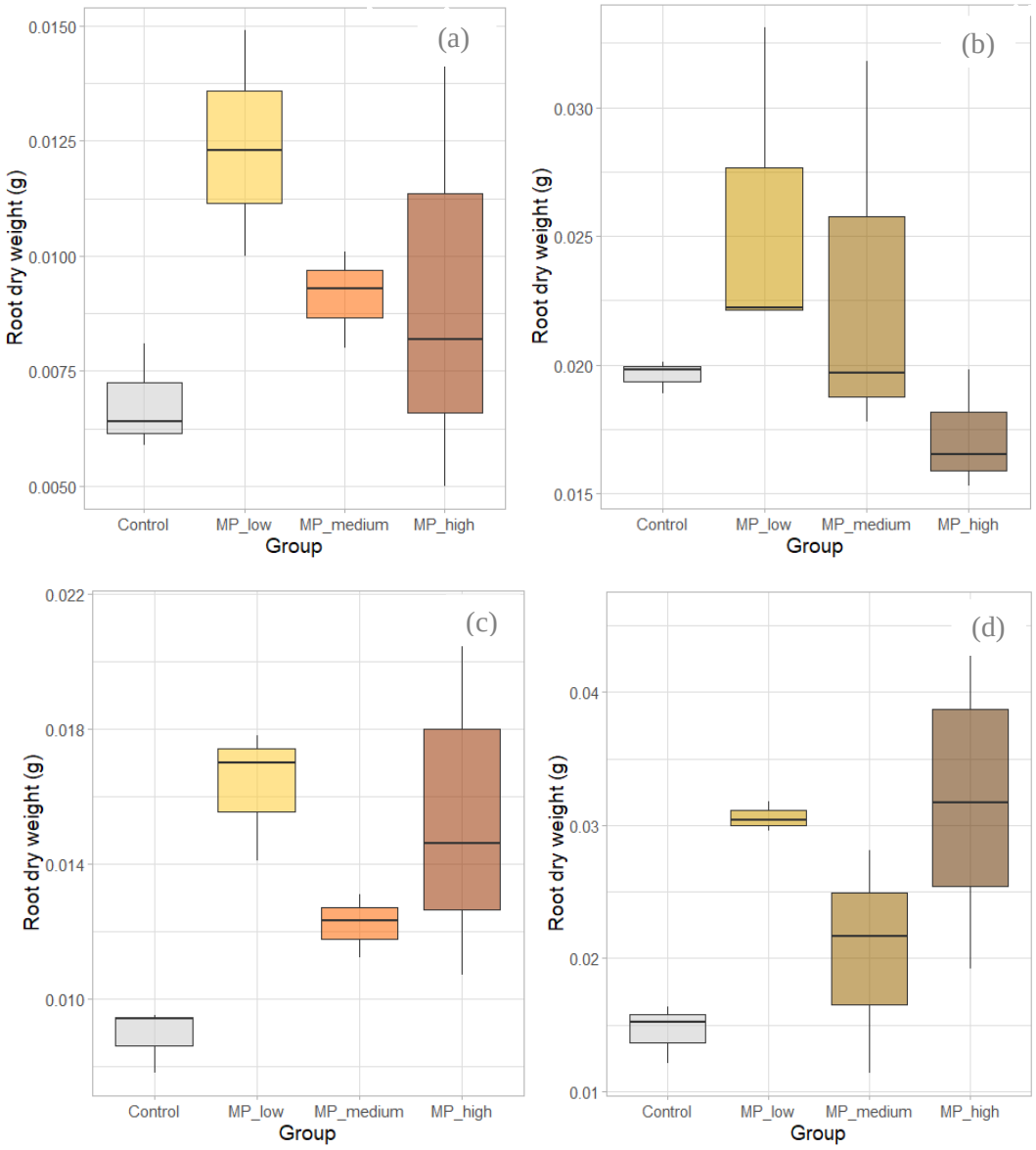

Figure 4.17. Root dry weights of sunflower (a, b) and sorghum plants (c ,d).

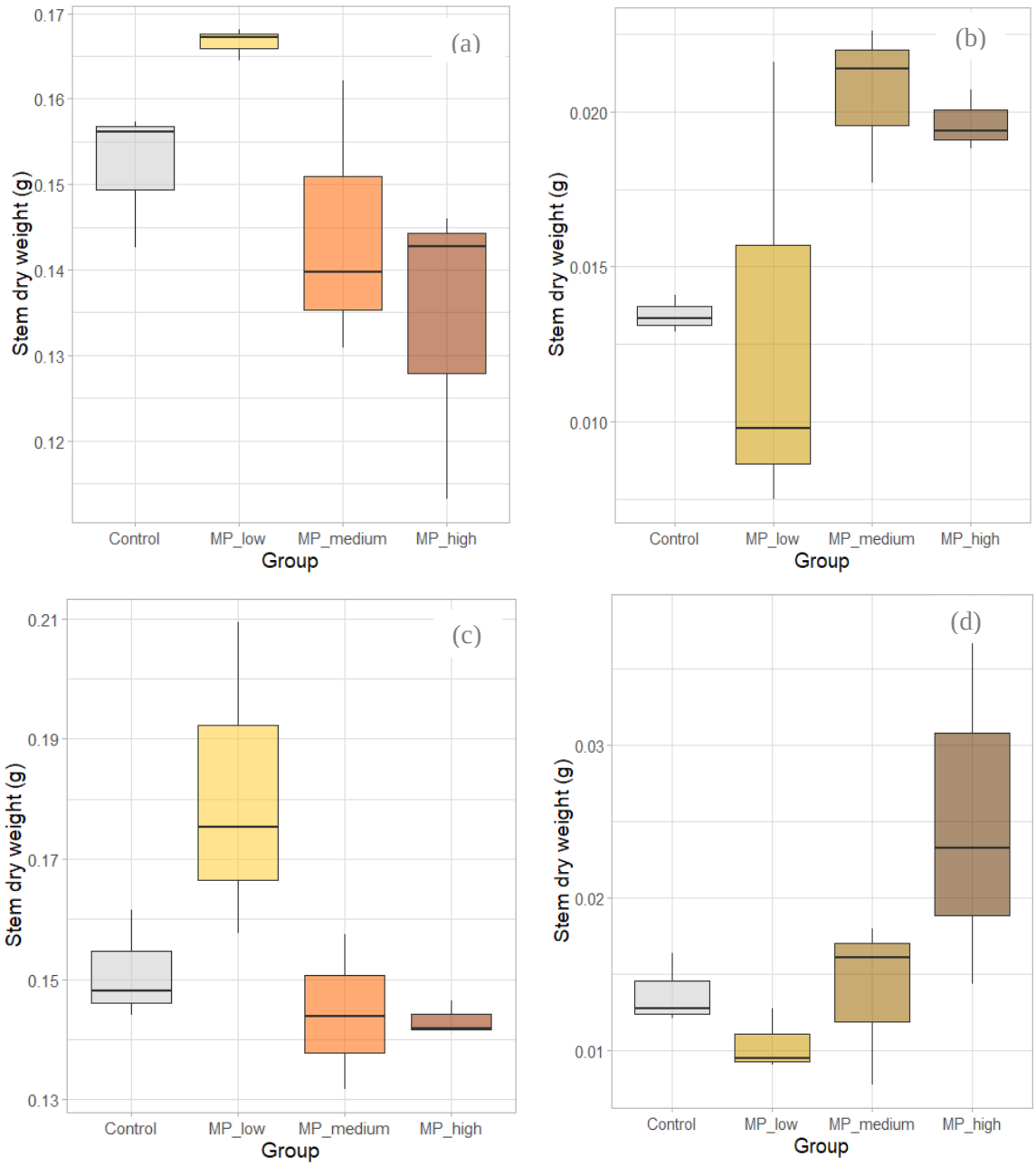

Figure 4.18. Stem dry weights of sunflower (a, b) and sorghum plants (c ,d).

4.3.5. Glomalin Concentration in the Soil

Glomalin; a glycoprotein, is produced as a result of AMF activity under critical conditions. Studies have demonstrated that under suboptimal conditions for AMF activity and hyphal growth, glomalin contents were increased (Rillig et al., 2002). There are various stress factors or sources for the increased production of glomalin and in this study there were two main factors; one being different temperature levels and other is the levels of MP fibers present in the soil. GRSP levels in soil samples gathered from rhizosphere area of sunflower and sorghum plants.

Glomalin levels of sunflower and sorghum planted soils were escalated with lower amount of MPs 0.4% w/w in the soil body. Produced glomalin levels by *Glomus mosseae* and sunflower symbiosis, which had root colonization rate of 22.22% - 63.16%, in lower temperature level (26°C) produced higher amount of GRSP with 6.91 mg/kg while higher temperature (31°C) yielded 5.75 mg/kg at 0.4% w/w MPs; while control group with AMF on each temperature had 2.89 and 1.01 mg/kg of glomalin related soil protein; respectively (Figure 4.19).

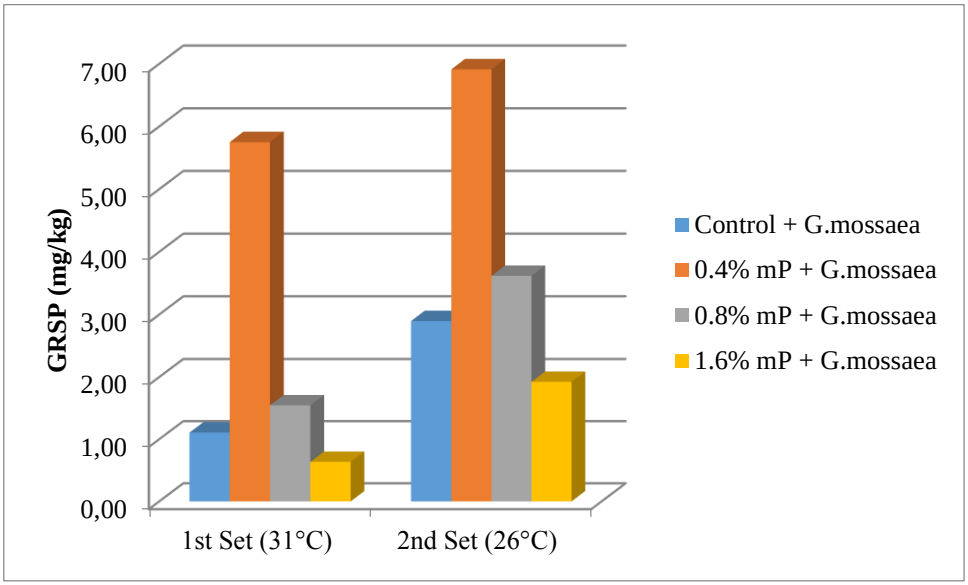

Figure 4.19. GRSP concentrations of sunflower planted soils

Soils glomalin content by plantation of sorghum with symbiosis of *Glomus mosseae*, had mycorrhization rate between 26.32% - 76%. Similar to sunflower planted soils; lower temperature level (26°C) produced higher amount of GRSP at 0.4% w/w; with 7.26 mg/kg and higher temperature (31°C) resulted in 6.25 mg/kg. Control groups with AMF had 3.77 and 1.2 mg/kg of glomalin related soil protein, respectively (Figure 4.20). Details of GRSP breakdown is presented on Table B.9.

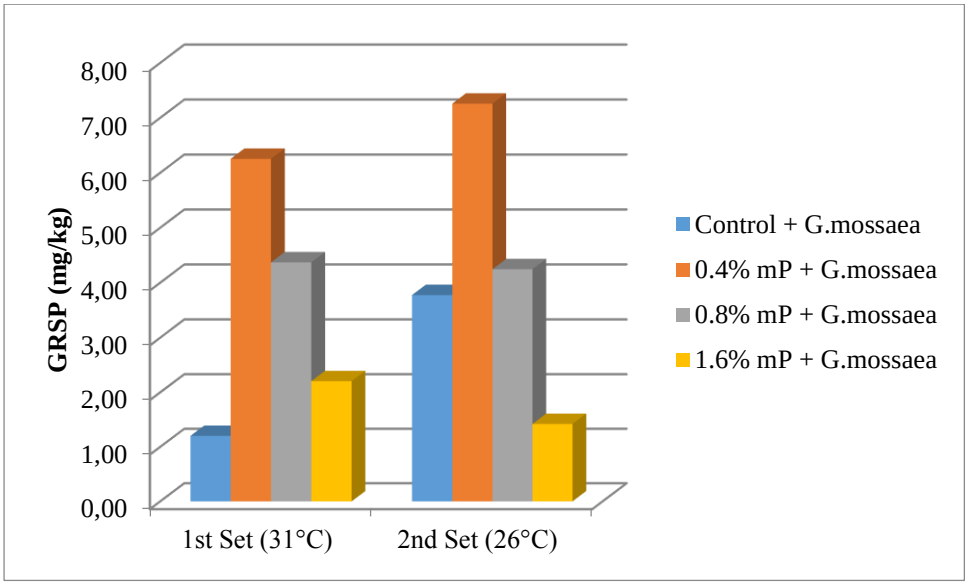

Figure 4.20. GRSP concentrations of sorghum planted soils.

Humic substances' extraction with Bradford assay may interfere with glomalin content of the soil; yet correlation between glomalin related soil protein is proportional. Hence, indicating a comparison point for soils gathered from different type of applications (Jorge-Arauho et al., 2015). Enhanced glomalin yield was observed in soils planted with sunflower and sorghum with various MP levels. The correlation between glomalin content in rhizosphere area with MP presence could be the result that can be linked to escalated mycorrhization rate and increased activity of AMF and it can be the mechanism to improve the fungal habitat in soil bodies (Rillig et al., 2007). Comparison between mycorrhization and glomalin content were detailed in Table 4.4.

Table 4.4. Comparison of average mycorrhization and GRSP levels.

Plant - Sunflower	Average Mycorrhization (%)		Glomalin (mg/kg)	
	1st Set (31°C)	2nd Set (26°C)	1st Set (31°C)	2nd Set (26°C)
Control + *G.mossaea*	37.50	47.62	1.11	2.90
0.4% MP + *G.mossaea*	44.44	63.16	5.75	6.91
0.8% MP + *G.mossaea*	36.00	48.15	1.55	3.62
1.6% MP + *G.mossaea*	22.22	33.33	0.64	1.92
Plant - Sorghum	Average Mycorrhization (%)		Glomalin (mg/kg)	
	1st Set (31°C)	Set (26°C)	1st Set (31°C)	2nd Set (26°C)
Control + *G.mossaea*	26.32	66.67	1.20	3.77
0.4% MP + *G.mossaea*	52.94	76.00	6.25	7.26
0.8% MP + *G.mossaea*	41.67	54.55	4.37	4.24
1.6% MP + *G.mossaea*	44.44	47.37	2.20	1.42

5. INSIGHTS AND PERSPECTIVE

Regarding the investigation of MPs in soils, there are uncertainties contributing to the outcome of the evaluation of MPs in soil systems. Research on the abundance of MPs in soils are being studied around the world, conceptually they are comparable; however, there is no common method to quantify MPs in soils. Due to the use of different digestion and density separation agents, as well as the use of different pore-sized filters, it is not possible to make comparisons on solid basis. Another point regarding the size while the upper limit of the size range is clear in most of the studies, the lowest limit depends on the filter pore size. Therefore, a standardized method is urgently needed to make the results comparable, as well as to use the results of the studies for possible meta-analysis studies.

When it comes to the effects of the MPs on plants, PES fibers which is readily available was used in this study. Due to the complexity of MPs in terms of chemical composition and shape, the effects may differ, thus it is not possible to generalize that MPs effect plant growth. More studies should be conducted with different polymer types, and shapes as well as with different species to see the full picture. Another important point is that most of the ecotoxicity studies used virgin MPs, which are not weathered or aged to the time of this thesis. However, the effect of aged or weathered MPs may be different than those are virgins. Other ingredients of plastic particles such as dyes and additives may also have different effects than the MP alone. Additionally, the interaction of MPs with other soil contaminants such as pesticides, heavy metals, and organic pollutants, and the combined effect on plant growth remains as a virgin topic in the literature to date.

Recommendations for future researches/studies; first and foremost, is to understand the uncertainties and fluctuations of MPs present in the system and conceptualizing the research according to the study area and/or experimental design considering the gaps mentioned in previous paragraphs. To improve our understanding on the effects of MPs, current levels in terrestrial systems should be investigated in detail using a standard method for detection and quantification of in soils can be investigated and established to ensure one of the most crucial steps of our understanding is universal. Number of studies on quantification of MPs in soils system should increase over time in terms of their region/area, land use, and future outlook should be included in those studies in order to provide a comparison point for future research. As for establishing the effects of MPs on soil organisms and plants; an interdisciplinary approach should be considered to better understand the plausible chemical pathway of deterioration of plastic materials, ecotoxicological/biological impact of respective elements as well as their interaction with other soil contaminants through both

experimental and modeling systems with respect to the environmental conditions of the region. Also, other global change factors should be considered together with MP pollution to model the experimental studies more similar to the real environmental scenarios.

6. CONCLUSION

Determination of current MP content of soils from areas which had different land use revealed that MP particles or fibers were present in all of the samples. Soil samples from Belgrad Forest had the highest amount of MP among all locations, and other two recreational areas (Yıldız Park and university campus) having higher MP content compared to industrial and residential areas further support the accumulation scenario of MP in soil. Lowest average MP content was found in recreational areas and results have been consistent for that aspect. It can also be argued that shape of MPs can be different due to the location of contamination and various other factors. For recreational areas including forest area, microfibers were the most common type with an average of 36.92%. While industrial areas had more share on round/spherical MP particles with 32.85% and residential areas with 34.26%, respectively.

As for MP effects on seed germination and plant growth; this study supports that MP presence inhibits the seed germination rate for plants noticeably, however beneficial soil organisms such as AMF can relatively help with germination of these seedlings and can potentially support their growth depending on MP levels in soil, species involved, and increased temperature. The effect of MPs on plant growth varies depending on the present levels in the soil. General trend for both plant species was the increased plant and root growth in low MP levels (0.4%) compared to the control groups, and with increasing levels; mid (0.8%) and high (1.6%) MP overall plant growth was decreased proportionally based on their height and dry biomass (stem and roots). Similar responses to increasing MP levels were observed under lower average temperatures (26 ºC) on plant growth of both plant species; while higher average temperature (31ºC) hindered the growth of sunflower it assisted the growth of sorghum plants.

Introduction of AMF species (*G. mosseae*) have had positive impact on plant height, growth, and biomass in control groups and low level of MP in soil. Even though symbiosis with *G. mosseae* increased overall plant resistance the effect on plant growth was similar with increasing level of MP in soils. Mycorrhization rates were also affected by MP presence in the soil; whereas, low (0.4% w:w) levels of MP had the best colonization rate and it started to decrease by mid (0.8%) levels of MP, mid-level MP still had higher mycorrhization when compared to control group without inoculation; however, high-level (1.6%) MP inhibited the colonization ratios including control groups for both plants. So, it can be argued that the ability to form symbiosis with *G. mosseae* while low amount of MPs helped forming stronger and wider colonization; after a certain breaking point colonization slowed down and weakened by proportional to MP levels present in the soil. In relation with AMF

activity the Glomalin content of the rhizosphere were escalated with lower amount of MPs -0.4%; where 6.91mg/kg and 5.75 mg/kg GRSP was produced in lower (26°C) and higher temperature (31°C) while control group with AMF on each temperature had 2.89 and 1.01 mg/kg of glomalin related soil protein; respectively.

REFERENCES

Almond, J., Sugumaar, P., Wenzel, M., Hill, G. and Wallis, C. (2020) Determination of the carbonyl index of polyethylene and polypropylene using specified area under band methodology with ATR-FTIR spectroscopy. e-Polymers, Vol. 20 (Issue 1), pp. 369-381. https://doi.org/10.1515/epoly-2020-0041.

Alauddin, M., Choudkury, I. A., Baradie, M. A., Hashmi, M. S. J.1995. Plastics and their machining: a review.Materials ProcessingTechnology.54: 40–46.

Avella, M., Bonadies, E., Martuscelli, E., and Rimedio, R. 2001.European current standardization for plastic packaging recoverablethrough composting and biodegradation.Polymer Testing.20: 517–521. https://doi.org/10.1016/S0142-9418(00)00068-4.

Bl¨asing, M., Amelung, W., 2018. Plastics in soil: analytical methods and possible sources. Sci. Total Environ. 612, 422–435. https://doi.org/10.1016/j.scitotenv.2017.08.086.

Bolgar M., Hubball J., Groeger J., Meronek S., 2008. Handbook for the Chemical Analysis of Plastic and Polymer Additives CRC Press, - 498 p. https://doi.org/10.1201/9781420044881.

Bosker, T., Bouwman, L.J., Brun, N.R., Beherns, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. Chemosphere 226, 774–781. https:// doi.org/10.1016/j.chemosphere.2019.03.163.

Brundrett, M.C., 2002. Co-evoloution of roots of mycorrhizas of land plants, New Phytologist, 154, 275-304.

Campanale, C., Galafassi, S., Savino, I., Massarelli, C., Ancona, V., Volta, P., Uricchio, V. F., 2022. Microplastics pollution in the terrestrial environments: Poorly known diffuse sources and implications for plants. Sci. Total Environ. 805, 150431 https:// doi.org/10.1016/j.scitotenv.2021.150431.

Cao, D., Wang, X., Luo, X., Liu, G., Zheng, H., 2018. Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil. IOP Conf Ser. Earth Environ. Sci. 61, 012148. https://doi.org/10.1088/1755-1315/61/1/012148.

Chen, Y., Leng, Y., Liu, X., Wang, J., 2020. Microplastic pollution in vegetable farmlands of suburb Wuhan, central China. Environ. Pollut. https://doi.org/10.1016/j.envpol.2019.113449.

Choi, Y.R., Kim, Y.N., Yoon, J.H., Dickinson, N., Kim, K.H., (2020). Plastic contamination of forest, urban, and agricultural soils: a case study of Yeoju City in the Republic of Korea. Journal of Soils and Sediments, https://doi.org/10.1007/s11368-020-02759-0.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Corradini, F., Meza, P., Eguiluz, R., Casado, F., Huerta-Lwanga, E., Geissen, V., 2019. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. Sci. Total Environ. 671, 411–4.

Çullu, A.F., Sönmez, V.Z., Sivri, N., 2021. Microplastic contamination in surface waters of the Küçükçekmece Lagoon, Marmara Sea (Turkey): Sources and areal distribution, Environmental Pollution, Volume 268, Part B, 115801, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2020.115801.

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Chang. Biol. https://doi.org/10.1111/gcb.14020.

de Souza Machado, A.A., Lau, C.W., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Görlich, A.S., Rillig, M.C., 2019. Microplastics Can Change Soil Properties and Affect Plant Performance, Environ. Sci. Technol. *53* (10), 6044-6052. https://doi.org/10.1021/acs.est.9b01339.

Deng, J., Zhou, L., Zhou, W., Wang, Q., Yu, D., 2022. Effect of microfibers combined with UV-B and drought on plant community, Chemosphere, https://doi.org/10.1016/j.chemosphere.2021.132413.

Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y., Guo, X., 2020. The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.137525.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: A case study in Greater Paris. Environ. Chem. https://doi.org/10.1071/EN14167.

Fakour, H., Lo S-L., Yoashi, N.T., Massao, A.M., Lema, N.N., Mkhontfo, F.B., Jomalema, P.C., Jumanne, N.S., Mbuya, B.H., Mtweve, J.T., Imani, M., 2021. Quantification and Analysis of Microplastics in Farmland Soils: Characterization, Sources, and Pathways. *Agriculture*. https://doi.org/10.3390/agriculture11040330.

Fortin, J.A., Bécard, G., Declerck, S., Dalpé, Y., St-Arnaud, M., Coughan, A.P., Piché, Y., 2002. Arbuscular Mycorrhiza on root-organ cultures, Canadian Journal of Botany, 80, 1-20.

Fuller, S.G., Gautam, A., 2016. A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. Environmental Science & Technology, https://doi.org/10.1021/acs.est.6b00816.

Gerstenbacher, C.M., Finzi, A.C., Rotjan, R.D., Novak, A.B., 2022. A review of microplastic impacts on seagrasses, epiphytes, and associated sediment communities, Environmental Pollution, Volume 303, 119108, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2022.119108.

Haward, M., 2020. Science-based solutions to plastic pollution. One Earth 2 (1), 5–7 ISSN, 2590-3322. https://doi.org/10.1016/j.oneear.2020.01.004.

Hildebrant, L., Nack, F.L., Zimmerman, T., Pröfock, D., 2021. Microplasticsas a Trojan Horse for Trace Metals, JHM Letters 2- 100035. https://doi.org/10.1016/j.hazl.2021.100035.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J., Lahive, E., 2017. Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2016.09.004.

Huang Y., Liu, Q., Jia, W., Yan, C., Wang, J., 2020. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environ. Pollut. 260, 114096 https://doi.org/10.1016/j.envpol.2020.114096.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., Van Der Ploeg, M., Besseling, E., Koelmans, A.A., Geissen, V., 2016. Microplastics in the Terrestrial Ecosystem: Implications for Lumbricus terrestris (Oligochaeta, Lumbricidae). Environ. Sci. Technol. https://doi.org/10.1021/acs.est.5b05478.

Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.8b01517.

Jansen, J., 2015. Plastic Failure Through Molecular Degradation, Plastics Engineering, Available at: http://read.nxtbook.com/wiley/plasticsengineering/january2015/consultantscorner.html. Accessed 20 Oct 2022.

Ingraffia, R., Amato, G., Bagarello, V., Carollo, F. G., Giambalvo, D., Iovino, M., Lehmann, A., Rillig, M. C., and Frenda, A. S, 2022. Polyester microplastic fibers affect soil physical properties and erosion as a function of soil type, SOIL, 8, 421–435, https://doi.org/10.5194/soil-8-421-2022.

Ingraffia, R., Amato, G., Bagarello, V., Iovıno, M., Rillig, M.C., Giambalvo, D., Iovino, M., Frenda, A. S, 2022. Polyester microplastic fibers in soil increase nitrogen loss via leaching and decrease plant biomass production and N uptake, Environ. Res. Lett. 17 054012, https://doi.org/ 10.1088/1748-9326/ac652d.

Intergovernmental Panel on Climate Change (IPCC), "Climate change 2014: Synthesis report. A contribution of working groups I, II, and III to the fifth assessment report of the Intergovernmental Panel on Climate Change," Core Writing Team, R. K. Pachauri, L. A. Meyer, Eds. (IPCC, Geneva, Switzerland, 2014).

Jiang, X.J., Liu, W., Wang, E., Zhou, T., Xin, P., 2017. Residual plastic mulch fragments effects on soil physical properties and water flow behavior in the Minqin Oasis, northwestern China. Soil Tillage Res. https://doi.org/10.1016/j.still.2016.10.011.

Judy, J.D., Williams, M., Gregg, A., Oliver, D., Kumar, A., Kookana, R., Kirby, J.K., 2019. Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota, Envir Poll. https://doi.org/10.1016/j.envpol.2019.05.027.

Jorge-Arauho, P., Quiquampoix, H., Matumato-Pintro, T., Staunton, S., 2015. Glomalin-related soil protein in French temperate forest soils: interference in the bradford assay caused by co-extracted humic substances, European Journal of Soil Science, 66, 311–319.

Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean,

Marine Pollution Bulletin, Volume 115, Issues 1–2, Pages 307-314, ISSN 0025-326X, https://doi.org/10.1016/j.marpolbul.2016.12.025.

Kasirajan, S., Ngouajio, M., 2012. Polyethylene and biodegradable mulches for agricultural applications: A review. Agron. Sustain. Dev. https://doi.org/10.1007/s13593-011-0068-3.

Khalvati, M.A., Hu, Y., Mozafar, A., Schmidhalter, U., 2005. Quantification of water uptake by mycorrhizal hyphae and its significance for leaf growth, water relations and gas exchange of barley subjected to drought stress, Plant Biology, 7, 706-712.

Khan, A.G., 2006. Mycorrhizoremediation—an enhanced form of phytoremediation", Journal of Zhejiang University Science B, 7(7), 503–514.

Kim, S.W., Waldman, W.R., Kim, T., Rillig, M.C., 2020. Effects of Different Microplastics on Nematodes in the Soil Environment: Tracking the Extractable Additives Using an Ecotoxicological Approach Environ. Sci. Technol. 54 (21), https://doi.org/10.1021/acs.est.0c04641.

Lahive, E., Walton, A., Horton, A.A., Spurgeon, D.J., Svendsen, C., 2019. Microplastic particles reduce reproduction in the terrestrial worm Enchytraeus crypticus in a soil exposure. Environ. Pollut. https://doi.org/10.1016/j.envpol.2019.113174.

Lehmann, A., Leifheit, E.F., Feng, L., Bergmann, J., Rillig, M.C., 2020. Microplastic fiber and drought effects on plants and soil are only slightly modified by arbuscular mycorrhizal fungi. Soil Ecol. Lett. https://doi.org/10.1007/s42832-020-0060-4.

Lehmann, A., Leifheit, E. F., Feng, L., Bergmann, J., Wulf, A., & Rillig, M. C. (2022). Microplastic fiber and drought effects on plants and soil are only slightly modified by arbuscular mycorrhizal fungi. Soil Ecology Letters, 4(1), 32–44. https://doi.org/10.1007/s42832-020-0060-4.

Leifheit, E. F., Lehmann, A., & Rillig, M. C. (2021). Potential Effects of Microplastic on Arbuscular Mycorrhizal Fungi. Frontiers in Plant Science, 12(February). https://doi.org/10.3389/fpls.2021.626709

Liang Y, Lehmann A, Yang G, Leifheit EF and Rillig MC (2021) Effects of Microplastic Fibers on Soil Aggregation and Enzyme Activities Are Organic Matter Dependent. Front. Environ. Sci. 9:650155. https://doi.org/10.3389/fenvs.2021.650155

Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size, Environmental Pollution, Volume 265, Part A, 114721, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2020.114721.

Lozano Y.M., Rillig, M.C., 2020. Effects of Microplastic Fibers and Drought on Plant Communities, Environmental Science & Technology 54 (10), 6166- https://doi.org/10.1021/acs.est.0c01051.

Lozano, Y.M., Aguilar-Trigueros, C.A., Onandia, G., Maaß, S., Zhao, T., Rillig, M.C., 2021. Effects of microplastics and drought on soil ecosystem functions and multifunctionality, Journal of Applied Ecology, https://doi.org/10.1111/1365-2664.13839.

Lusher, A., Hollman, P., Mendoza-Hill, J., 2017. Microplastics in fisheries and aquaculture: Status of knowledge on their occurrence and implications for aquatic organisms and food safety, FAO fisheries and aquaculture technical paper. ARRAY(0x9fa9fac), Rome, Italy.

Mehdinia, A., Dehbandi, R., Hamzehpour, A., Rahnama, R., 2020. Identification of microplastics in the sediments of southern coasts of the Caspian Sea, north of Iran. Environ. Pollut. https://doi.org/10.1016/j.envpol.2019.113738

Munno, K., Helm, P.A., Jackson, D.A., Rochman, C., Sims, A., 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.3935

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environ. Sci. Process. Impacts 18, 1050–1059. https://doi.org/10.1039/C6EM00206D

O'Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W.M., Hou, D., 2019. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles, Environmental Pollution, Volume 249, Pages 527-534, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2019.03.092.

Pagès, P., 2015. Characterization of polymer materials using FT-IR and DSC techniques, Thermal Analysis. Fundamentals and Applications to Material Characterization, 2005: 121-140. ISBN: 84-9749-100-9.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic on vesicular-Arbuscular Mycorrhizal Fungi for rapid assessment of infection, Transactions of British Mycological Society, 55, 157-160.

Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C., Laforsch, C., 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. Sci. Rep. https://doi.org/10.1038/s41598-018-36172-y

Pignattelli S, Broccoli A, Renzi M., 2020. Physiological responses of garden cress (L. sativum) to different types of microplastics, Sci Total Environ 727:138609 https://doi.org/10.1016/j.scitotenv.2020.138609.

Prendergast-Miller, M.T., Katsiamides, A., Abbass, M., Sturzenbaum, S.R., Thorpe, K.L., Hodson, M.E., 2019. Polyester-derived microfibre impacts on the soil-dwelling earthworm Lumbricus terrestris, Environmental Pollution, https://doi.org/10.1016/j.envpol.2019.05.037.

Qi, R., Jones, D.L., Li, Z., Liu, Q., Yan, C., 2020. Behavior of microplastics and plastic film residues in the soil environment: A critical review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134722

Qi, Y., Yang, X., Mejia Pelaez, A., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P., Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (Triticum aestivum) growth. Sci. Total Environ. 645, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229

Rafique, A., Irfan, M., Mumtaz, M., Qadir, A., 2020. Spatial distribution of microplastics in soil with context to human activities: a case study from the urban center. Environmental Monitoring and Assessment, 192(11), 671 https://doi.org/10.1007/s10661-020-08641-3

Rillig, M., 2004. Arbuscular Mycorrhizae and terrestrial ecosystem processes, Ecology Letters, 7 (8), 740-754.

Rillig, M.C., Caldwell, B.A., Wösten, H.A.B., Sollins, P., 2007. Role of proteins in soil carbon and nitrogen storage: controls on persistence, Biogeochemistry, 85, 25-44.

Rillig, M.C., Lehmann, A., 2020. Microplastic in terrestrial ecosystems, Science 368, 1430–1431. https://doi.org/10.1126/science.abb5979.

Rillig, M.C., Wright, *S.F.*, Eviner, V., 2002. The role of Arbuscular Mycorrhizal Fungi and glomalin in soil aggregation: comparing effects of five plant species, Plant and Soil, 238, 325–333.

Rillig, M.C., Ryo, M., Lehmann, A., Aguilar-Trigueros, C., Buchert, S., Wulf, A., Iwasaki, A., Roy, J., Yang, G., 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity, Science 336, 886–890. https://doi.org/10.1126/science.aay2832.

Rillig, M.C., Ziersch, L. & Hempel, S., 2017. Microplastic transport in soil by earthworms. Sci Rep 7, 1362. https://doi.org/10.1038/s41598-017-01594-7.

Rillig, MC., 2012. Microplastic in terrestrial ecosystems and the soil? Environmental Science & Technology 46: 6453–6454. https://doi.org/10.1021/es302011r.

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss Floodplain Soils. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.7b06003.

Selonen, S., Dolar, A., Kokalj, A.J., Skalar, T., Dolcet, L.P., Hurley, R., van Gestel, C.A.M., 2020. Exploring the impacts of plastics in soil – The effects of polyester textile fibers on soil invertebrates, Sci. Total Environ 700, 134451 https://doi.org/10.1016/j.scitotenv.2019.134451.

Şener, M., Doğruyol, P., Balkaya N., 2019. Microplastic pollution in the Black Sea Coast of the Anatolian side of Istanbul, Turkey, Desalination and Water Treatment, 172, https://doi.org/10.5004/dwt.2019.25111.

Sobhani, Z., Fang, C., Naidu, R., Megharaj, M., 2021. Microplastics as a vector of toxic chemicals in soil: Enhanced uptake of perfluorooctane sulfonate and perfluorooctanoic acid by earthworms through sorption and reproductive toxicity, Environmental Technology & Innovation, Volume 22, 101476, https://doi.org/10.1016/j.eti.2021.101476.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., Schaumann, G.E., 2016. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.01.153.

Thompson R C, Olsen Y, Mitchell R P, Davis A, Rowland S J, John A W, McGonigle D and Russell A E 2004 Lost at sea: where is all the plastic? Science 304 838. https://doi.org/10.1126/science.1094559.

Tunali, M., Adam, V. Nowack, B., 2023, Probabilistic environmental risk assessment of microplastics in soils, Geoderma, https://doi.org/10.1016/j.geoderma.2022.116315.

Tunali, M., Uzeofuna, E.N., Tunali, M.M., Yenigun, O, 2020. Effect of microplastics and microplastic-metal combinations on growth and chlorophyll a concentration of Chlorella vulgaris, Sci Total Environ, 743, 140479, DOI 10.1016/j.scitotenv.2020.140479.

Tunali, M.M., 2015. Plant-Microbe interactions in the Phytoremediation of Heavy Metal Contaminated Soils, M.S. Thesis, Boğaziçi University.

Tunali, M.M., Myronyuk, O., Tunali, M. et al., 2022. Microplastic Abundance in Human-Influenced Soils in Recreational, Residential, and Industrial Areas. Water Air Soil Pollut 233, 433. https://doi.org/10.1007/s11270-022-05901-5.

Wang, J., Li, J., Liu, S., Li, H., Chen, X., Peng, C., Zhang, P., Liu, X., 2021. Distinct microplastic distributions in soils of different land-use types: A case study of Chinese farmlands. Environ. Pollut, https://doı.org 10.1016/j.envpol.2020.116199.

Wang, J., Lv, S., Zhang, M., Chen, G., Zhu, T., Zhang, S., Teng, Y., Christie, P., Luo, Y., 2016. Effects of plastic film residues on occurrence of phthalates and microbial activity in soils. Chemosphere. https://doi.org/10.1016/j.chemosphere.2016.02.076.

Wessel, C.C., Lockridge, G.R., Battiste, D., Cebrian, J., 2016. Abundance and characteristics of microplastics in beach sediments: Insights into microplastic accumulation in northern Gulf of Mexico estuaries, Marine Pollution Bulletin, Volume 109, Issue 1, Pages 178-183, ISSN 0025-326X, https://doi.org/10.1016/j.marpolbul.2016.06.002.

Yang, X., Bento, C.P.M., Chen, H., Zhang, H., Xue, S., Lwanga, E.H., Zomer, P., Ritsema, C.J., Geissen, V., 2018. Influence of microplastic addition on glyphosate decay and soil microbial activities in Chinese loess soil. Environ. Pollut. https://doi.org/10.1016/j.envpol.2018.07.006.

Yang, W., Cheng, P., Adams, C.A., Zhang, S., Sun, Y., Yu, H., Wang, F., 2021. Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with ZnO nanoparticles, Soil Biology and Biochemistry, Volume 155, https://doi.org/10.1016/j.soilbio.2021.108179.

Yuan, W., Liu, X., Wang, W., Di, M., Wang, J., 2019. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China, Ecotoxicology and

Environmental Safety, Volume 170, Pages 180-187, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.126.

Zbyszewski, M., Corcoran, P.L., 2011. Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. Water. Air. Soil Pollut. https://doi.org/10.1007/s11270-011-0760-6.

Zeng, L.S., Zhou, Z.F., Shi, Y.X., 2013. Environmental problems and control ways of plastic film in agricultural production, in: Applied Mechanics and Materials. https://doi.org/10.4028/www.scientific.net/AMM.295-298.2187.

Zhanbei, L., 2008. Soil microbial and Arbuscular Mycorrhizal communuties in afforsted ecosystems of the Nebraska National Forest, *ETD collection for University of Nebraska - Lincoln* Paper AAI3308324, University of Nebraska.

Zhang, G.S., Liu, Y.F., 2018. The distribution of microplastics in soil aggregate fractions in southwestern China. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.06.004.

Zhang, L., Xie, Y., Liu, J., Zhong, S., Qian, Y., Gao, P., 2020. An Overlooked Entry Pathway of Microplastics into Agricultural Soils from Application of Sludge-Based Fertilizers. Environ. Sci. Technol. https://doi.org/10.1021/acs.est.9b07905.

Zhang, Y., Liang, J., Zeng, G., Tang, W., Lu, Y., Luo, Y., Xing, W., Tang, N., Ye, S., Li, X., Huang, W., 2020. How climate change and eutrophication interact with microplastic pollution and sediment resuspension in shallow lakes: A review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.135979.

Zhou, Q., Zhang, H., Fu, C., Zhou, Y., Dai, Z., Li, Y., Tu, C., Luo, Y., 2018. The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea, Geoderma, Volume 322, Pages 201-208, ISSN 0016-7061, https://doi.org/10.1016/j.geoderma.2018.02.015.

Zhou, Y., Liu, X., Wang, J.,2020, Ecotoxicological effects of microplastics and cadmium on the earthworm Eisenia foetida, Journal of Hazardous Materials, https://doi.org/10.1016/j.jhazmat.2020.122273.

Zubris, K.A., Richards, B.K., 2005. Synthetic fibers as an indicator of land application of sludge. Environ Pollut 138, 201–211. https://doi.org/10.1016/j.envpol.2005.04.013.

APPENDIX A: SUPPLEMENTARY FILES FOR MP ABUNDANCE

Table A.1. R-Statistical data comparison of microplastic/area.

$Landuse

diff	lwr	upr	p adj
Recreational-Industrial	1755.5556	563.1603	2947.9508 0.0032813
Residential-Industrial	-644.4444	-1836.8397	547.9508 0.3824980
Residential-Recreational	-2400.0000	-3592.3952	-1207.6048 0.0001116

Linear Hypotheses:

	Estimate Std.	Error	t value	Pr(>|t|)	
Campus - Ataturk == 0	2266.7	684.9	3.309	0.07278	.
Darussafaka - Ataturk == 0	-133.3	684.9	-0.195	1.00000	
Dilovasi - Ataturk == 0	1400.0	684.9	2.044	0.53522	
Forest - Ataturk == 0	3600.0	684.9	5.256	0.00131	**
Ikitelli - Ataturk == 0	2133.3	684.9	3.115	0.10438	
Levent - Ataturk == 0	666.7	684.9	0.973	0.98386	
Park - Ataturk == 0	2933.3	684.9	4.283	0.01023	*
Zekeriyakoy - Ataturk == 0	1066.7	684.9	1.557	0.81466	
Darussafaka - Campus == 0	-2400.0	684.9	-3.504	0.05010	.
Dilovasi - Campus == 0	-866.7	684.9	-1.265	0.92906	
Forest - Campus == 0	1333.3	684.9	1.947	0.59421	
Ikitelli - Campus == 0	-133.3	684.9	-0.195	1.00000	
Levent - Campus == 0	-1600.0	684.9	-2.336	0.37140	
Park - Campus == 0	666.7	684.9	0.973	0.98387	
Zekeriyakoy - Campus == 0	-1200.0	684.9	-1.752	0.71007	
Dilovasi - Darussafaka == 0	1533.3	684.9	2.239	0.42298	
Forest - Darussafaka == 0	3733.3	684.9	5.451	< 0.001	***
Ikitelli - Darussafaka == 0	2266.7	684.9	3.309	0.07254	.
Levent - Darussafaka == 0	800.0	684.9	1.168	0.95362	
Park - Darussafaka == 0	3066.7	684.9	4.477	0.00677	**
Zekeriyakoy - Darussafaka == 0	1200.0	684.9	1.752	0.71023	
Forest - Dilovasi == 0	2200.0	684.9	3.212	0.08730	.
Ikitelli - Dilovasi == 0	733.3	684.9	1.071	0.97157	
Levent - Dilovasi == 0	-733.3	684.9	-1.071	0.97157	
Park - Dilovasi == 0	1533.3	684.9	2.239	0.42352	
Zekeriyakoy - Dilovasi == 0	-333.3	684.9	-0.487	0.99987	
Ikitelli - Forest == 0	-1466.7	684.9	-2.141	0.47793	
Levent - Forest == 0	-2933.3	684.9	-4.283	0.01040	*
Park - Forest == 0	-666.7	684.9	-0.973	0.98388	
Zekeriyakoy - Forest == 0	-2533.3	684.9	-3.699	0.03398	*
Levent - Ikitelli == 0	-1466.7	684.9	-2.141	0.47824	
Park - Ikitelli == 0	800.0	684.9	1.168	0.95357	
Zekeriyakoy - Ikitelli == 0	-1066.7	684.9	-1.557	0.81462	
Park - Levent == 0	2266.7	684.9	3.309	0.07262	.
Zekeriyakoy - Levent == 0	400.0	684.9	0.584	0.99949	
Zekeriyakoy - Park == 0	-1866.7	684.9	-2.725	0.20590	

Signif. codes: 0 ‘***’ 0.00 1 ‘**’ 0.01 ‘*’ 0.05 ‘ ’ 0.1 ‘ ’ 1

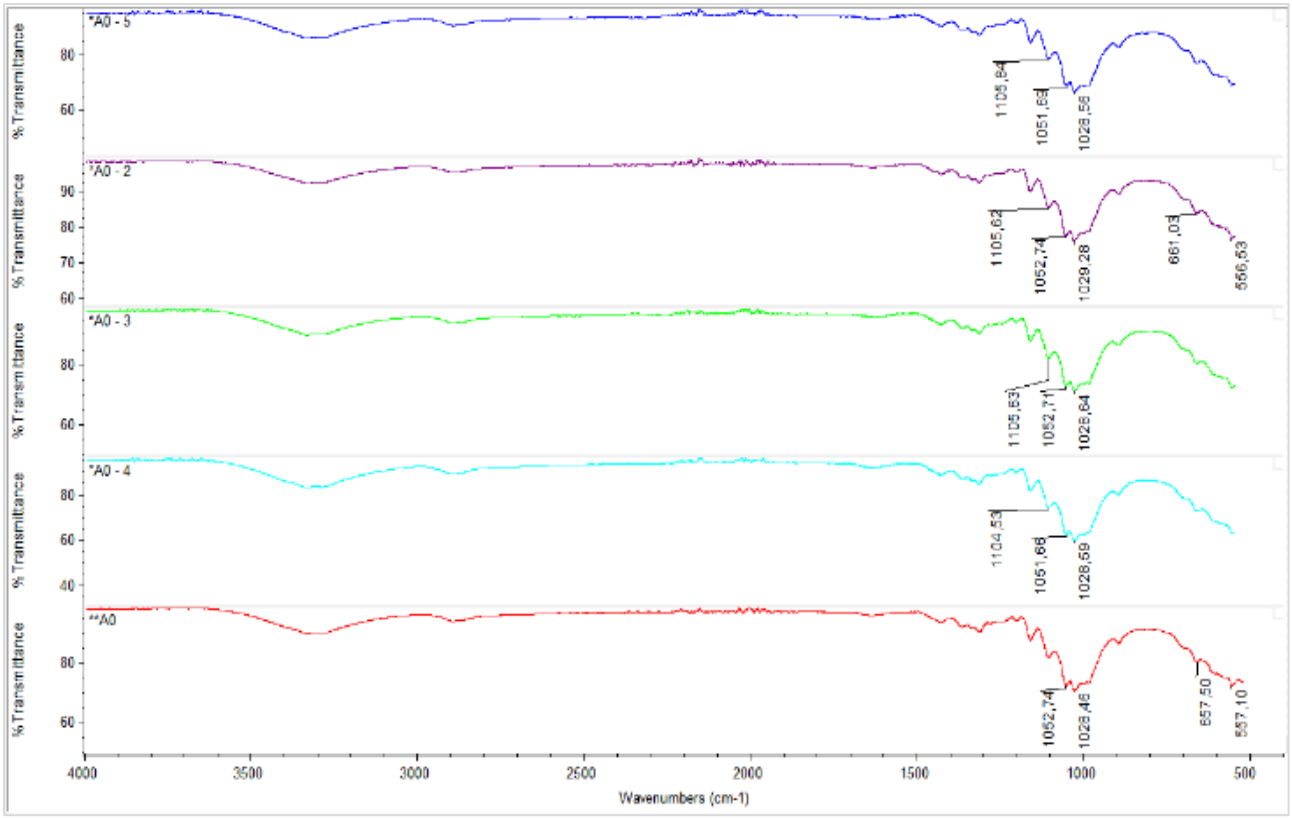

Figure A.1. FT-IR results of control group.

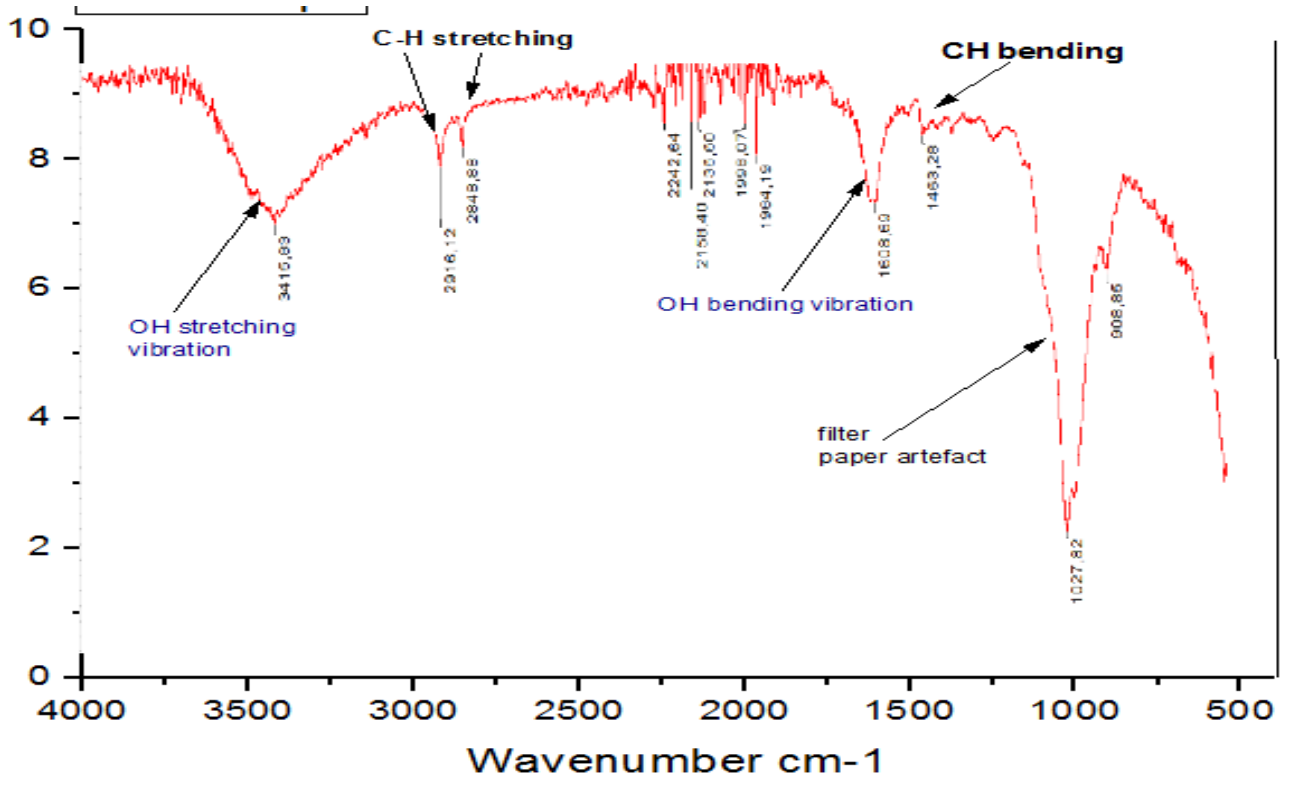

Figure A.2. FT-IR spectra of the residential area 1.

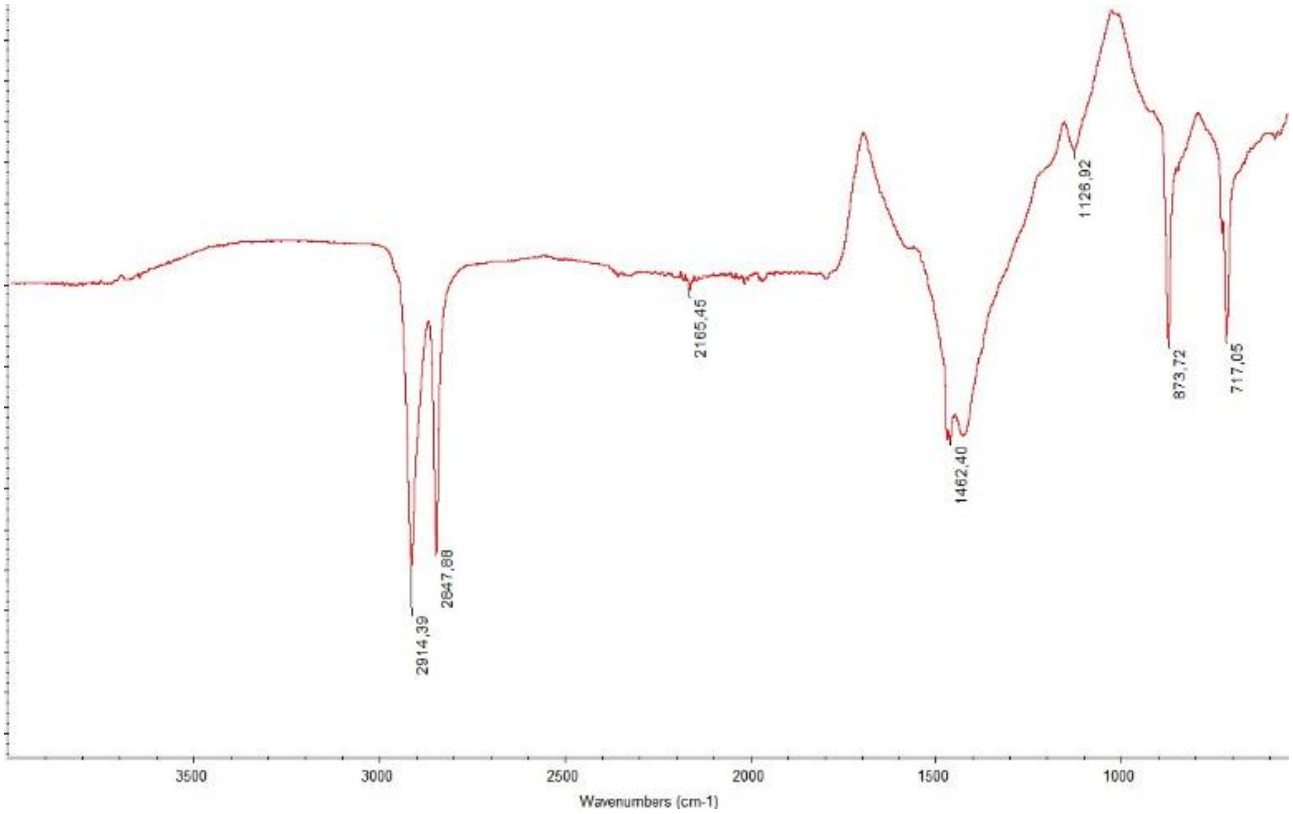

Figure A.3. FT-IR spectra of the industrial area 2.

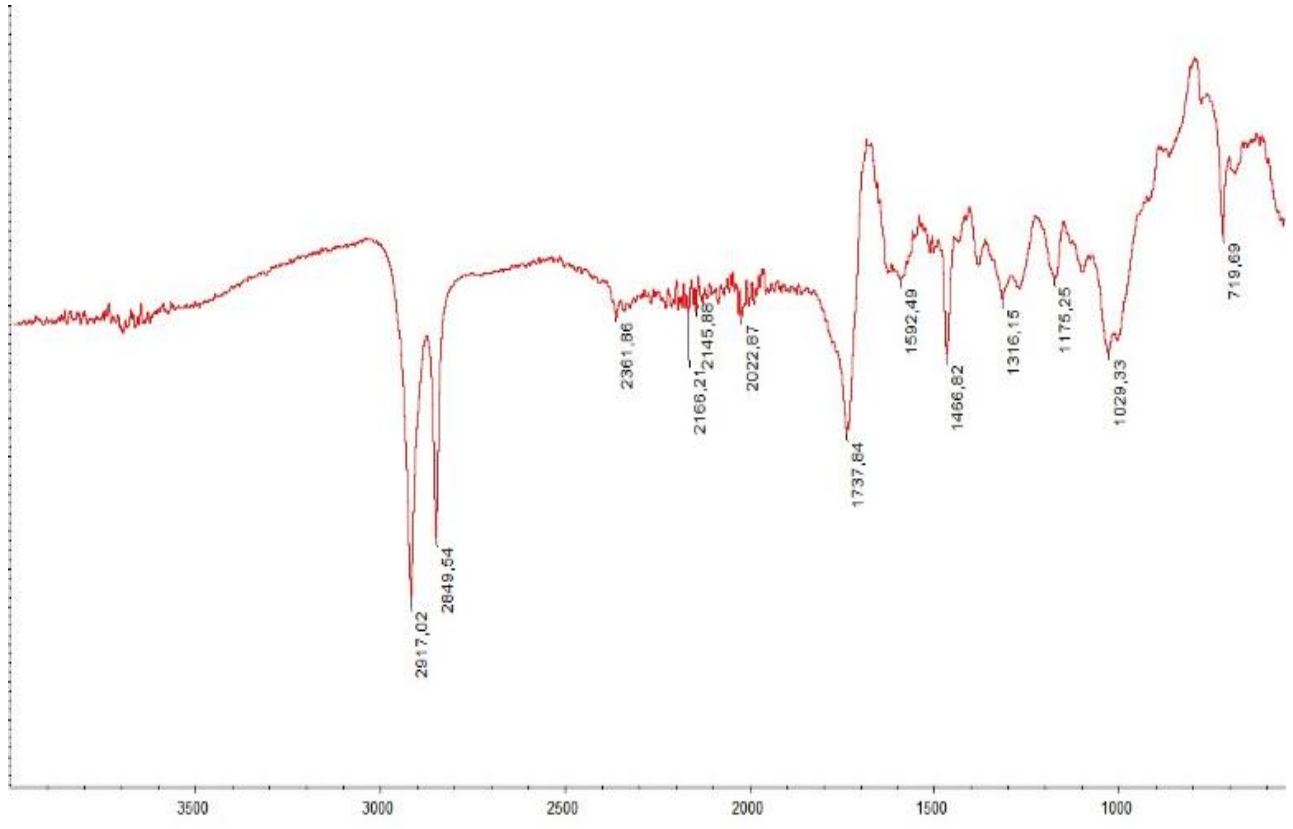

Figure A.4. FT-IR spectra of the recreational area 1.

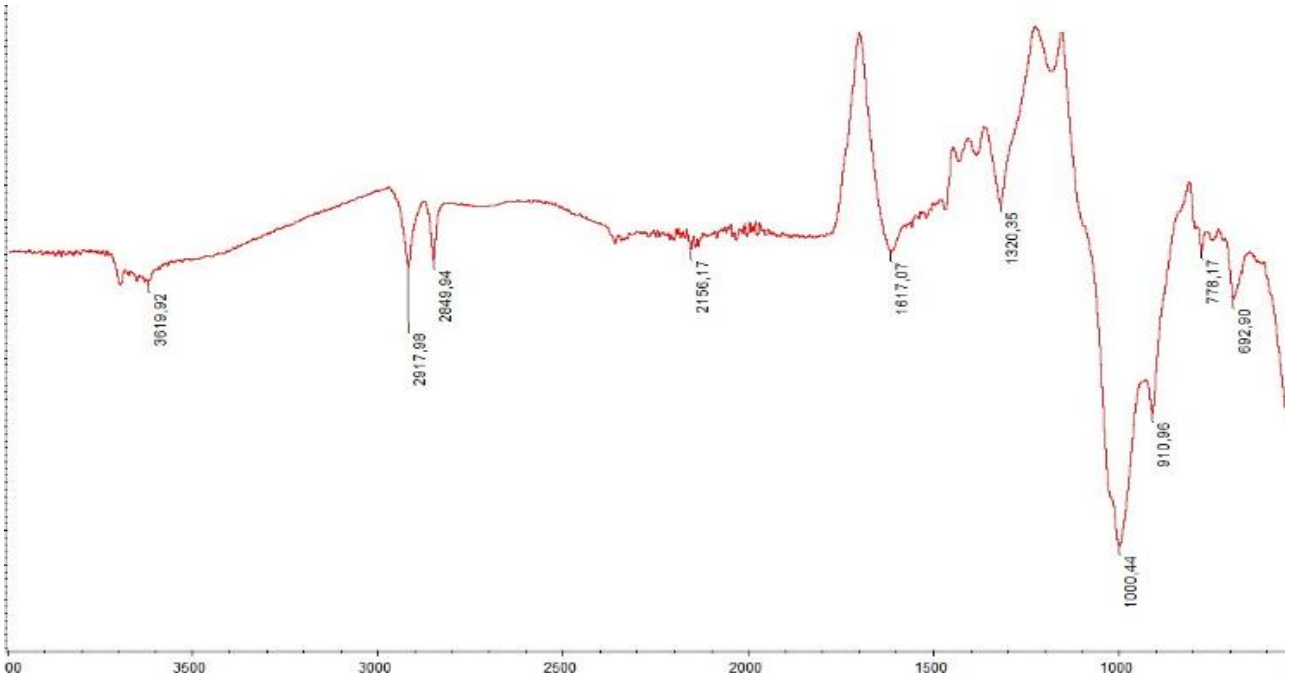

Figure A.5. FT-IR spectra of the recreational area 2.

APPENDIX B: OPERATIONAL PARAMETERS AND SUPPLEMENTARY INFORMATION ON THE EFFECT OF MPs ON PLANT GROTH

Table B.1. Operating soil pH conditions of plant growth.

pH (Sunflower)									
1st Set				1st Avg	2nd Set				2nd Avg
6.76	6.84	6.88	6.89	6.84	6.82	6.93	6.84	6.87	6.87
pH (Sorghum)									
1st Set				1st Avg	2nd Set				2nd Avg
7.1	7.2	7.15	6.8	7.06	7.3	7.35	7.29	7.25	7.30

Table B.2. Humidity and room temperature during plant growth.

Time	Humidity (%)	Room Temp	Time	Humidity (%)	Room Temp
Day 1	60	24.1	Day 20	69	26.9
Day 2	62	28.2	Day 21	56	28.4
Day 3	48	29	Day 22	57	27.7
Day 4	49	29.1	Day 23	45	26.5
Day 5	52	29.1	Day 24	50	26.8
Day 6	58	27.6	Day 25	52	26.7
Day 7	62	29.2	Day 26	51	26.8
Day 8	60	28	Day 27	52	27.1
Day 9	54	28.3	Day 28	40	28.1
Day 10	53	27.9	Day 29	47	25.8
Day 11	55	28.1	Day 30	54	25.9
Day 12	58	28.3	Day 31	52	26
Day 13	58	28.8	Day 32	48	26.1
Day 14	59	28.9	Day 33	50	25.9
Day 15	64	28.4	Day 34	54	25.9
Day 16	63	28.9	Day 35	56	25.8
Day 17	62	28.5	Day 36	55	26.2
Day 18	67	28.3	Day 37	46	26.1
Day 19	68	27.8			

- 1st set represent 31ºC and 2nd set represents 26ºC in all tables

Table B.3. Operating soil temperatures for plant growth (1st Set).

Time	Soil Temperature (°C) - 1st Set				Avg
	Sunflower		Sorghum		
Day 1	28.35	28.25	27.83	27.97	28.10
Day 2	32.19	30.81	31	31.5	31.38
Day 3	32.94	31.44	32.88	31.62	32.22
Day 4	33	31.62	32.38	31.69	32.17
Day 5	32.19	31.56	32	31.44	31.80
Day 6	32.44	33	32.88	31.87	32.55
Day 7	32.56	32.81	32.69	32.13	32.55
Day 8	31.19	31.5	31.25	31.35	31.32
Day 9	31.62	30.94	30.62	30.44	30.91
Day 10	31.31	30.62	30.46	30.5	30.72
Day 11	31.37	30.94	30.75	30.88	30.99
Day 12	31.56	31.25	31	31.3	31.28
Day 13	31.5	30.62	31.33	31	31.11
Day 14	32.25	32.35	31.31	31.19	31.78
Day 15	31.42	31.25	31.75	31.34	31.44
Day 16	31.56	31.62	31.94	31.13	31.56
Day 17	31.62	31.87	31.56	31.62	31.67
Day 18	31.31	31.02	31.19	31.74	31.32
Day 19	31.19	31.25	31.44	31.56	31.36
Day 20	31.44	31.69	31.25	31.36	31.44
Day 21	31.15	31.84	31.12	31.42	31.38
Day 22	30.94	30.81	31.38	30.62	30.94
Day 23	29.88	29.96	30.15	30.19	30.05
Day 24	30.56	30.54	30.25	30.15	30.38
Day 25	30.33	30.22	30.74	30.57	30.47
Day 26	30.68	30.96	30.45	30.52	30.65
Day 27	30.89	30.23	30.5	30.12	30.44
Day 28	29.44	27.87	29.31	28.25	28.72
Day 29	30.42	29.94	29.78	31.15	30.32
Day 30	28.94	29.32	29.25	29.92	29.36
Day 31	29.22	30.33	29.15	30.67	29.84
Day 32	28.37	30.06	28.25	30.78	29.37
Day 33	30.42	29.78	29.31	31.25	30.19
Day 34	30.15	30.78	31.02	30.66	30.65
Day 35	29.88	30.52	31.05	29.45	30.23
Day 36	30.31	30.35	30.24	30.77	30.42
Day 37	30.62	30.14	30.46	30.32	30.39

Table B.4. Operating soil temperatures for plant growth (2nd Set).

Time	Soil Temperature (°C) - 2nd Set				Avg
	Sunflower		Sorghum		
Day 1	23.6	23.67	23.6	23.82	23.6725
Day 2	27	26.96	27.12	27.1	27.045
Day 3	27.1	27.02	26.9	26.84	26.965
Day 4	27	26.91	26.9	27.12	26.9825
Day 5	27.1	26.8	26.72	26.96	26.895
Day 6	27.6	27.42	27.35	27.4	27.4425
Day 7	27.8	27.65	26.96	27.66	27.5175
Day 8	27.2	27.15	26.92	27.12	27.0975
Day 9	26.5	26.12	26.84	26.64	26.525
Day 10	26.2	26.82	26.36	26.3	26.42
Day 11	26.4	26.25	26.72	26.34	26.4275
Day 12	26.8	26.75	26.33	26.54	26.605
Day 13	27.4	27.64	27.25	27.33	27.405
Day 14	27.2	27.15	27.88	27.44	27.4175
Day 15	26.2	26.56	26.12	26.19	26.2675
Day 16	26.8	26.77	26.15	26.45	26.5425
Day 17	26.2	26.88	26.34	26.14	26.39
Day 18	26.1	26.15	26.58	26.51	26.335
Day 19	25.9	26.65	26.19	26.25	26.2475
Day 20	26	26.35	26.54	26.33	26.305
Day 21	26.4	26.96	26.5	23.24	25.775
Day 22	25.8	25.44	25.73	25.63	25.65
Day 23	25.6	25.68	25.13	26.25	25.665
Day 24	25.8	25.35	25.78	25.56	25.6225
Day 25	25.5	25.54	25.22	25.67	25.4825
Day 26	25.4	26.15	25.33	25.58	25.615
Day 27	25.9	26.15	25.74	25.53	25.83
Day 28	26.8	26.44	26.15	26.72	26.5275
Day 29	25.4	25.88	25.23	25.5	25.5025
Day 30	25.6	26.12	25.78	25.45	25.7375
Day 31	25.3	25.88	25.15	25.25	25.395
Day 32	25.5	25.44	25.02	25.72	25.42
Day 33	25.7	25.32	25.45	25.68	25.5375
Day 34	25.5	25.47	25.3	25.62	25.4725
Day 35	25.8	25.75	25.42	25.63	25.65
Day 36	26.1	26.15	26.43	26.02	26.175
Day 37	26.1	26.33	26.38	26.22	26.2575

Table B.5. Average plant height data of Sunflower over-time.

1st Set								
Plant - Sunflower Avg.	Day 4	Day 5	Day 6	Day 9	Day 16	Day 21	Day 28	Day 34
Control	1.91	3.61	5.41	10.86	14.76	16.48	18.37	21.59
0.4% MP	2.56	5.34	8.11	11.59	13.97	16.60	18.81	22.01
0.8% MP	4.72	8.22	9.67	10.92	12.83	14.86	18.07	20.61
1.6% MP	2.86	6.11	7.77	9.84	12.81	14.76	17.81	19.98
Control + *G.mossaea*	2.96	4.79	5.88	12.57	14.42	16.30	18.82	23.58
0.4% MP + *G.mossaea*	3.30	7.61	8.56	12.66	15.97	18.30	21.56	22.09
0.8% MP + *G.mossaea*	1.24	5.54	6.71	11.88	14.86	16.23	17.49	20.88
1.6% MP + *G.mossaea*	1.23	6.52	7.52	12.12	14.20	15.78	16.88	18.82
2nd Set								
Plant - Sunflower Avg.	Day 4	Day 5	Day 6	Day 9	Day 16	Day 21	Day 28	Day 34
Control	1.89	3.02	5.43	9.92	13.24	15.09	17.37	20.88
0.4% MP	5.23	7.10	8.29	11.84	14.56	17.47	19.47	24.82
0.8% MP	4.34	6.71	7.40	10.37	13.87	15.60	16.88	23.43
1.6% MP	3.78	7.37	8.01	9.67	12.78	16.02	17.24	21.58
Control + *G.mossaea*	3.54	5.66	7.18	8.84	11.89	14.03	17.07	27.46
0.4% MP + *G.mossaea*	3.88	4.91	5.63	8.23	12.13	15.47	16.78	22.49
0.8% MP + *G.mossaea*	3.31	4.41	4.99	8.36	11.21	15.01	16.34	22.06
1.6% MP + *G.mossaea*	7.33	8.27	8.73	10.97	12.03	14.56	16.52	20.37

Table B.6. Plant height data of Sorghum over-time.

1st Set								
Plant - Sorghum Avg.	Day 4	Day 5	Day 6	Day 9	Day 16	Day 21	Day 28	Day 34
Control	2.60	3.50	4.10	11.60	12.80	14.70	18.40	24.20
0.4% MP	3.69	7.06	9.12	10.79	12.66	14.56	16.76	20.47
0.8% MP	6.30	7.94	8.44	9.02	9.71	11.57	13.00	19.97
1.6% MP	6.02	8.70	9.31	9.53	9.74	12.11	14.51	18.90
Control + *G.mossaea*	6.64	8.09	8.63	9.86	12.20	13.67	15.40	23.00
0.4% MP + *G.mossaea*	3.71	7.77	9.11	10.43	12.17	13.89	15.56	17.61
0.8% MP + *G.mossaea*	5.82	8.29	9.79	11.04	13.33	14.78	16.29	18.71
1.6% MP + *G.mossaea*	6.27	7.63	8.63	10.58	13.21	14.99	17.08	22.92
2nd Set								
Plant - Sorghum Avg.	Day 4	Day 5	Day 6	Day 9	Day 16	Day 21	Day 28	Day 34
Control	2.01	3.43	4.87	7.73	9.76	11.93	13.14	17.70
0.4% MP	4.69	6.18	7.17	10.27	12.60	14.64	15.62	19.18
0.8% MP	6.01	7.43	8.12	10.03	12.22	14.09	15.11	16.50
1.6% MP	6.31	7.62	7.87	9.61	11.39	13.24	16.82	17.32
Control + *G.mossaea*	1.96	3.22	4.41	6.93	10.01	13.54	15.63	19.47
0.4% MP + *G.mossaea*	4.41	6.81	7.69	10.03	13.78	17.68	20.41	21.86
0.8% MP + *G.mossaea*	6.96	7.47	7.82	9.78	11.99	14.15	16.06	20.06
1.6% MP + *G.mossaea*	7.99	8.43	8.68	10.73	12.76	17.41	18.86	19.87

Table B.7. Plant dry weight data (stem and roots), mean values of 9 plants (Avg).

	1st Set		2nd Set	
	Dry Weight (Avg.)	Root Weight (Avg.)	Dry Weight (Avg.)	Root Weight (Avg.)
Plant - Sunflower				
Control	0.1431	0.0054	0.1520	0.0068
0.4% MP	0.1547	0.0061	0.1666	0.0124
0.8% MP	0.1437	0.0102	0.1443	0.0091
1.6% MP	0.1564	0.0190	0.1340	0.0092
Plant - Sunflower + AMF				
Control +	0.1512	0.0089	0.1536	0.0121
0.4% MP+	0.1808	0.0163	0.1831	0.0135
0.8% MP+	0.1443	0.0122	0.1422	0.0129
1.6% MP+	0.1433	0.0156	0.1519	0.0142
Plant - Sorghum				
Control	0.0139	0.0125	0.0134	0.0196
0.4% MP	0.0176	0.0138	0.0130	0.0258
0.8% MP	0.0157	0.0240	0.0206	0.0231
1.6% MP	0.0152	0.0186	0.0196	0.0172
Plant - Sorghum + AMF				
Control +	0.0138	0.0146	0.0189	0.0077
0.4% MP+	0.0105	0.0306	0.0347	0.0252
0.8% MP+	0.0140	0.0204	0.0770	0.0195
1.6% MP+	0.0253	0.0322	0.0302	0.0337

Table B.8. Mycorrhization data of each plant, MP and AMF combination.

Plant - Sunflower	Mycorrhization Rate (%)	
	31 ºC	26 ºC
Control	12.50	14.29
0.4% MP	14.29	11.11
0.8% MP	5.88	5.00
1.6% MP	5.00	6.25
Plant - Sunflower + AMF		
Control + *G.mossaea*	37.50	47.62
0.4% MP + *G.mossaea*	44.44	63.16
0.8% MP + *G.mossaea*	36.00	48.15
1.6% MP + *G.mossaea*	22.22	33.33
Plant - Sorghum		
Control	11.11	13.64
0.4% MP	10.00	11.11
0.8% MP	5.56	5.56
1.6% MP	10.00	6.25
Plant - Sorghum + AMF		
Control + *G.mossaea*	26.32	66.67
0.4% MP + *G.mossaea*	52.94	76.00
0.8% MP + *G.mossaea*	41.67	54.55
1.6% MP + *G.mossaea*	44.44	47.37

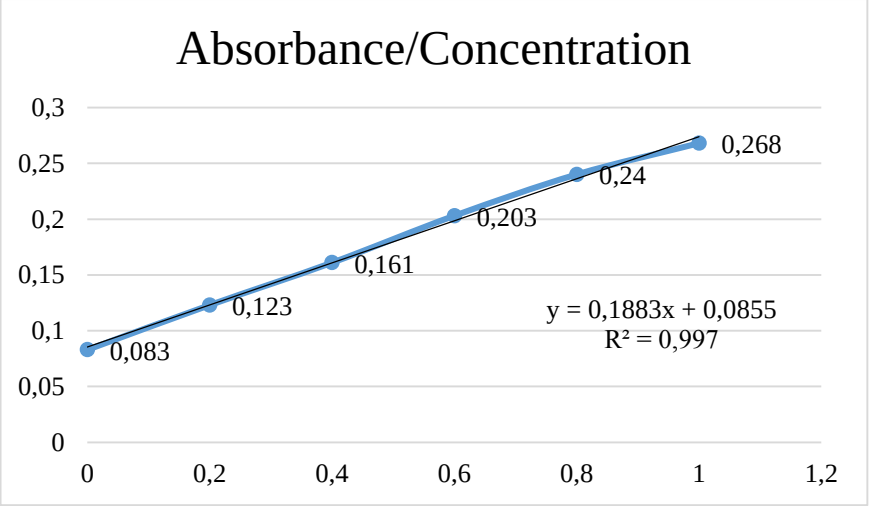

Figure B.1. Calibration curve prepared with protein standard for GRSP analysis.

Table B.9. Glomalin levels in soil after harvest.

Plant - Sunflower + AMF	1st Set GRSP(mg/kg)				2nd Set GRSP(mg/kg)			
	1st Parallel	2nd Parallel	3rd Parallel	Avg.	1st Parallel	2nd Parallel	3rd Parallel	Avg.
Control + *G.mossaea*	1.5773	0.7299	1.0124	1.1065	2.1422	3.8369	2.7071	2.8954
0.4% MP + *G.mossaea*	7.979	4.967	4.308	5.751	6.944	6.944	6.850	6.912
0.8% MP + *G.mossaea*	2.0480	0.8241	1.7656	1.5459	2.3305	6.0023	2.5188	3.6172
1.6% MP + *G.mossaea*	0.353	0.824	0.730	0.636	1.295	2.613	1.860	1.922
Plant - Sorghum + AMF	1st Set GRSP(mg/kg)				2nd Set GRSP(mg/kg)			
	1st Parallel	2nd Parallel	3rd Parallel	Avg.	1st Parallel	2nd Parallel	3rd Parallel	Avg.
Control + *G.mossaea*	0.7299	1.7656	1.1065	1.2007	2.3305	5.9082	3.0837	3.7741
0.4% MP + *G.mossaea*	3.649	8.639	6.473	6.253	8.544	5.437	7.791	7.258
0.8% MP + *G.mossaea*	1.8597	6.9438	4.3076	4.3704	3.8369	4.3076	4.5901	4.2448
1.6% MP + *G.mossaea*	1.766	2.142	2.707	2.205	1.012	1.671	1.577	1.420

Table B.10. Descriptive stats for dry weight data (stem and roots) for statistical analysis.

		Value Label	N
G. mossaea	,00	No AMF	48
	1,00	*G. mossaea*	48
Temperature	1,00	1st Set	48
	2,00	2nd Set	48
Plant Species	1,00	Sunflower	48
	2,00	Sorghum	48
MP abundance	,00	Control	24
	1,00	MP - lvl 1 (0.4g/100g)	24
	2,00	MP - lvl 2 (0.8g/100g)	24
	3,00	MP - lvl 3 (1.6 g/100g)	24

Table B.11. Tests of Between-Subjects Effects (dry weight data).

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Dry Plant Weight	,430[a]	31	,014	23,637	,000
	Dry Root Weight	,005[b]	31	,000	8,965	,000
Intercept	Dry Plant Weight	,734	1	,734	1250,532	,000
	Dry Root Weight	,026	1	,026	1343,995	,000
AMF	Dry Plant Weight	,002	1	,002	3,639	,061
	Dry Root Weight	,000	1	,000	15,885	,000
Set	Dry Plant Weight	,001	1	,001	1,988	,163
	Dry Root Weight	3,038E-7	1	3,038E-7	,016	,900
Plant	Dry Plant Weight	,410	1	,410	698,712	,000
	Dry Root Weight	,002	1	,002	117,679	,000
MPlevel	Dry Plant Weight	,002	3	,001	1,255	,297
	Dry Root Weight	,001	3	,000	18,854	,000
AMF * Plant	Dry Plant Weight	,000	1	,000	,252	,618
	Dry Root Weight	3,267E-7	1	3,267E-7	,017	,896
AMF * MPlevel	Dry Plant Weight	,000	3	,000	,272	,846
	Dry Root Weight	,000	3	,000	6,232	,001
Set * Plant	Dry Plant Weight	,001	1	,001	1,333	,253
	Dry Root Weight	6,720E-6	1	6,720E-6	,353	,555
Set * MPlevel	Dry Plant Weight	,001	3	,000	,573	,635
	Dry Root Weight	9,438E-5	3	3,146E-5	1,652	,186
Plant * MPlevel	Dry Plant Weight	,005	3	,002	3,037	,035
	Dry Root Weight	,000	3	5,256E-5	2,760	,049
AMF * Set * Plant	Dry Plant Weight	,001	1	,001	1,043	,311
	Dry Root Weight	9,204E-5	1	9,204E-5	4,834	,032
AMF * Set * MPlevel	Dry Plant Weight	,001	3	,000	,355	,786
	Dry Root Weight	,000	3	,000	5,359	,002
AMF * Plant * MPlevel	Dry Plant Weight	,001	3	,000	,809	,493
	Dry Root Weight	,001	3	,000	8,853	,000
Error	Dry Plant Weight	,038	64	,001		
	Dry Root Weight	,001	64	1,904E-5		
Total	Dry Plant Weight	1,202	96			
	Dry Root Weight	,032	96			
Corrected Total	Dry Plant Weight	,468	95			
	Dry Root Weight	,007	95			

a. R Squared = ,920 (Adjusted R Squared = ,881)

b. R Squared = ,813 (Adjusted R Squared = ,722)

Table B.12. Multiple Comparisons- Post-hoc analysis (dry weight data).

Dependent Variable		(I) MP abundance	(J) MP abundance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Dry Plant Weight	LSD	Control	MP - lvl 1 (0.4g/100g)	-,0126	,00699	,076	-,0266	,0014
			MP - lvl 2 (0.8g/100g)	-,0052	,00699	,459	-,0192	,0088
			MP - lvl 3 (1.6 g/100g)	-,0020	,00699	,777	-,0160	,0120
Dry Root Weight	LSD	Control	MP - lvl 1 (0.4g/100g)	-,0070*	,00126	,000	-,0095	-,0045
			MP - lvl 2 (0.8g/100g)	-,0055*	,00126	,000	-,0080	-,0030
			MP - lvl 3 (1.6 g/100g)	-,0090*	,00126	,000	-,0115	-,0065

Table B.13. Post-hoc analysis between MP Groups of Sunflower plants' heights.

Dependent Variable		(I) MP Levels	(J) MP Levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Day 4	LSD	Control	MP Low	-1,1667*	,47967	,021	-2,1437	-,1896
			MP Medium	-,8306	,47967	,093	-1,8076	,1465
			MP High	-1,2250*	,47967	,016	-2,2021	-,2479
		MP Low	Control	1,1667*	,47967	,021	,1896	2,1437
			MP Medium	,3361	,47967	,489	-,6409	1,3132
			MP High	-,0583	,47967	,904	-1,0354	,9187
		MP Medium	Control	,8306	,47967	,093	-,1465	1,8076
			MP Low	-,3361	,47967	,489	-1,3132	,6409
			MP High	-,3944	,47967	,417	-1,3715	,5826
		MP High	Control	1,2250*	,47967	,016	,2479	2,2021
			MP Low	,0583	,47967	,904	-,9187	1,0354
			MP Medium	,3944	,47967	,417	-,5826	1,3715

Based on observed means.

The error term is Mean Square(Error) = 9,91E-006.

Table B.14. Post-hoc analysis between MP groups of Sorghum plants' heights.

Dependent Variable		(I) MP Levels	(J) MP Levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Day 4	LSD	Control	MP Low	-,5528	,61519	,376	-1,8059	,7003
			MP Medium	-2,9611*	,61519	,000	-4,2142	-1,7080
			MP High	-3,4056*	,61519	,000	-4,6587	-2,1525
		MP Low	Control	,5528	,61519	,376	-,7003	1,8059
			MP Medium	-2,4083*	,61519	,000	-3,6614	-1,1552
			MP High	-2,8528*	,61519	,000	-4,1059	-1,5997
		MP Medium	Control	2,9611*	,61519	,000	1,7080	4,2142
			MP Low	2,4083*	,61519	,000	1,1552	3,6614
			MP High	-,4444	,61519	,475	-1,6975	,8087
		MP High	Control	3,4056*	,61519	,000	2,1525	4,6587
			MP Low	2,8528*	,61519	,000	1,5997	4,1059
			MP Medium	,4444	,61519	,475	-,8087	1,6975
Day 5	LSD	Control	MP Low	-1,4472*	,42473	,002	-2,3124	-,5821
			MP Medium	-3,0694*	,42473	,000	-3,9346	-2,2043
			MP High	-3,3833*	,42473	,000	-4,2485	-2,5182
		MP Low	Control	1,4472*	,42473	,002	,5821	2,3124
			MP Medium	-1,6222*	,42473	,001	-2,4874	-,7571
			MP High	-1,9361*	,42473	,000	-2,8013	-1,0710
		MP Medium	Control	3,0694*	,42473	,000	2,2043	3,9346
			MP Low	1,6222*	,42473	,001	,7571	2,4874
			MP High	-,3139	,42473	,465	-1,1790	,5513
		MP High	Control	3,3833*	,42473	,000	2,5182	4,2485
			MP Low	1,9361*	,42473	,000	1,0710	2,8013
			MP Medium	,3139	,42473	,465	-,5513	1,1790

Based on observed means.

The error term is Mean Square(Error) = 2,82E-005.

*. The mean difference is significant at the ,05 level.

Table B.15. Post-hoc analysis of dry weight data (stem and roots) – 1st Set

LSD

Dependent Variable	(I) MP Levels	(J) MP Levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Stem Dry Weight	Control	MP Low	-.01457	.01045	.201	-.0387	.0095
		MP Medium	.00777	.01045	.479	-.0163	.0319
		MP High	.01807	.01045	.122	-.0060	.0422
	MP Low	Control	.01457	.01045	.201	-.0095	.0387
		MP Medium	.02233	.01045	.065	-.0018	.0464
		MP High	.03263*	.01045	.014	.0085	.0567
	MP Medium	Control	-.00777	.01045	.479	-.0319	.0163
		MP Low	-.02233	.01045	.065	-.0464	.0018
		MP High	.01030	.01045	.353	-.0138	.0344
	MP High	Control	-.01807	.01045	.122	-.0422	.0060
		MP Low	-.03263*	.01045	.014	-.0567	-.0085
		MP Medium	-.01030	.01045	.353	-.0344	.0138
Root Dry Weight	Control	MP Low	-.00560*	.00230	.041	-.0109	-.0003
		MP Medium	-.00233	.00230	.341	-.0076	.0030
		MP High	-.00243	.00230	.322	-.0077	.0029
	MP Low	Control	.00560*	.00230	.041	.0003	.0109
		MP Medium	.00327	.00230	.194	-.0020	.0086
		MP High	.00317	.00230	.206	-.0021	.0085
	MP Medium	Control	.00233	.00230	.341	-.0030	.0076
		MP Low	-.00327	.00230	.194	-.0086	.0020
		MP High	-.00010	.00230	.966	-.0054	.0052
	MP High	Control	.00243	.00230	.322	-.0029	.0077
		MP Low	-.00317	.00230	.206	-.0085	.0021
		MP Medium	.00010	.00230	.966	-.0052	.0054

*. The mean difference is significant at the 0.05 level.

Table B.16. Post-hoc analysis of dry weight data (stem and roots) – 1st Set

Dependent Variable		(I) MP Levels	(J) MP Levels	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Root Dry Weight	LSD	Control	MP Low	-.0059*	.00237	.024	-.0109	-.0009
			MP Medium	-.0029	.00237	.237	-.0079	.0021
			MP High	.0000	.00237	.994	-.0050	.0050
		MP Low	Control	.0059*	.00237	.024	.0009	.0109
			MP Medium	.0030	.00237	.227	-.0020	.0080
			MP High	.0059*	.00237	.025	.0009	.0109
		MP Medium	Control	.0029	.00237	.237	-.0021	.0079
			MP Low	-.0030	.00237	.227	-.0080	.0020
			MP High	.0029	.00237	.240	-.0021	.0079
		MP High	Control	.0000	.00237	.994	-.0050	.0050
			MP Low	-.0059*	.00237	.025	-.0109	-.0009
			MP Medium	-.0029	.00237	.240	-.0079	.0021
	Bonferroni	Control	MP Low	-.0059	.00237	.146	-.0130	.0012
			MP Medium	-.0029	.00237	1.000	-.0101	.0042
			MP High	.0000	.00237	1.000	-.0072	.0071
		MP Low	Control	.0059	.00237	.146	-.0012	.0130
			MP Medium	.0030	.00237	1.000	-.0042	.0101
			MP High	.0059	.00237	.148	-.0013	.0130
		MP Medium	Control	.0029	.00237	1.000	-.0042	.0101
			MP Low	-.0030	.00237	1.000	-.0101	.0042
			MP High	.0029	.00237	1.000	-.0042	.0100
		MP High	Control	.0000	.00237	1.000	-.0071	.0072
			MP Low	-.0059	.00237	.148	-.0130	.0013
			MP Medium	-.0029	.00237	1.000	-.0100	.0042
Stem Dry Weight	LSD	Control	MP Low	-.0070	.00548	.217	-.0187	.0046
			MP Medium	.0003	.00548	.954	-.0113	.0119
			MP High	.0059	.00548	.294	-.0057	.0176
		MP Low	Control	.0070	.00548	.217	-.0046	.0187
			MP Medium	.0074	.00548	.197	-.0042	.0190
			MP High	.0130*	.00548	.031	.0014	.0246
		MP Medium	Control	-.0003	.00548	.954	-.0119	.0113
			MP Low	-.0074	.00548	.197	-.0190	.0042

			MP High	.0056	.00548	.320	-.0060	.0172
		MP High	Control	-.0059	.00548	.294	-.0176	.0057
			MP Low	-.0130*	.00548	.031	-.0246	-.0014
			MP Medium	-.0056	.00548	.320	-.0172	.0060
	Bonferroni	Control	MP Low	-.0070	.00548	1.000	-.0235	.0094
			MP Medium	.0003	.00548	1.000	-.0162	.0168
			MP High	.0059	.00548	1.000	-.0105	.0224
		MP Low	Control	.0070	.00548	1.000	-.0094	.0235
			MP Medium	.0074	.00548	1.000	-.0091	.0238
			MP High	.0130	.00548	.184	-.0035	.0295
		MP Medium	Control	-.0003	.00548	1.000	-.0168	.0162
			MP Low	-.0074	.00548	1.000	-.0238	.0091
			MP High	.0056	.00548	1.000	-.0109	.0221
		MP High	Control	-.0059	.00548	1.000	-.0224	.0105
			MP Low	-.0130	.00548	.184	-.0295	.0035
			MP Medium	-.0056	.00548	1.000	-.0221	.0109

Based on observed means.

The error term is Mean Square(Error) = 9.00E-005.

*. The mean difference is significant at the .05 level.

Table B.17. Multiple Comparisons (dry weight data).

Dependent Variable		(I) MP abundance	(J) MP abundance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Dry Plant Weight	LSD	Control	MP - lvl 1 (0.4g/100g)	-,0126	,00699	,076	-,0266	,0014
			MP - lvl 2 (0.8g/100g)	-,0052	,00699	,459	-,0192	,0088
			MP - lvl 3 (1.6 g/100g)	-,0020	,00699	,777	-,0160	,0120
Dry Root Weight	LSD	Control	MP - lvl 1 (0.4g/100g)	-,0070*	,00126	,000	-,0095	-,0045
			MP - lvl 2 (0.8g/100g)	-,0055*	,00126	,000	-,0080	-,0030
			MP - lvl 3 (1.6 g/100g)	-,0090*	,00126	,000	-,0115	-,0065