IMPACT OF MICROPLASTICS ON SOIL HEALTH: SOIL-WATER RETENTION, SHRINKAGE, AND HOLDING PROPERTIES

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Impact of Microplastics on Soil Health: Soil-water Retention, Shrinkage, and Holding Properties

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While microplastics in the ocean have been heavily studied, their impact on soils have not. Current research suggests microplastics are just as prevalent in freshwater and land as in the ocean and should be considered a pollutant due to the potential hazardous effects. Once in the soil, the chemicals in the plastics, or even the plastic particles themselves, can be taken up by plants. Understanding whether or not microplastics and their additives change the soil's ability to retain water is a fundamental step towards determining the critical level of microplastics in cropland. This study reviews the effects of microplastics on the water retention and shrink/swell characteristics of soil and quantifies microplastics in the terrestrial environment. Samples were collected from the Texas A&M bioapplication field where sewage sludge has been applied, as sludge is a major vector of microplastics into soil. Samples were tested in the Pedostructure Characterization Lab for soil-water holding properties. Next, the amount of microplastics in the samples were quantified using gravimetric separation and sieving. No conclusive results about the impacts of microplastics on soil health could be drawn from this study. However, microplastics were found at a rate of 31,440 particles/kg at the bioapplication field, which is consistent with other studies quantifying microplastics in land with applied sludge.

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NOMENCLATURE

DDT	Dichlorodiphenyltrichloroethane
DEHP	Di-2-ethylhexyl phthalate
HDPE	High-density polyethylene
LDPE	Low density polyethylene
MBR	Membrane bioreactor
NOAA	National Oceanic and Atmospheric Administration
PAE	Phthalic acid esters
PC	Polycarbonate plastics
PE	Polyethylene
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
UV	Ultraviolet
WHO	World Health Organization

SECTION I

INTRODUCTION

While there is no formal definition of size, microplastics are typically defined as plastic particles smaller than 5 mm and larger than 0.3 mm. They are slightly larger than a grain of sand and slightly smaller than a grain of rice, which allows the particles to be mixed into the soil by animal and insect activity [1]. Most research concerning microplastics has focused on the ocean, where an estimated 8 million tons of plastic end up due to negligent waste management [2, 3]. Nevertheless, Hurley and Nizzetto [4] theorizes that there are more sources of microplastics for land than the ocean. In addition, Horton, et al. [2] concluded after a comprehensive literature review that current research suggests microplastics are just as prevalent in freshwater and land as the ocean, and that microplastics should be considered a pollutant due to the potential hazardous effects. Once in the soil, the chemicals in the plastics, or even the plastic particles themselves, could be taken up by plants [1]. They can also change the bulk density of the soil, which impacts water retention and soil aggregation. To further complicate matters, there are no standards for sampling methods, analytical methods, or reporting units in addition to the size discrepancies [2, 5, 6]. These inconsistencies make comparison between studies difficult.

Microplastics can be introduced to soils in a variety of ways and their effects have largely gone unstudied. Negligent waste, agriculture, sewage sludge application, and plastic mulching are all vectors of microplastics into soils [1]. One of the most prevalent introduction points of microplastics to soil is through sewage sludge application. Microplastics are numerous in urban wastewater, originating from the use of personal care products containing microplastics and washing clothing with synthetic fibers. Particles from degrading plastics also find their way into

waste water as these products are washed [7]. Approximately 98.3% of the microplastics entering wastewater treatment plants are retained in the sludge when using membrane bioreactor (MBR) filtration [7]. The solid sludge after treatment is often sold as fertilizer for agricultural fields. It is estimated that there are 440,900 tons of microplastics applied to the soil each year in the European Union via sewage sludge [2]. Furthermore, microplastics were found in fields untreated with waste fertilizer for over 15 years, indicating high retention. Microplastics are not currently required to be quantified in the US [2]. Researching the impact of microplastics on soil will help determine if the amount of microplastics applied should be monitored.

Furthermore, the additives in plastics pose a health risk as they are leached out of the microplastics into the environment. While the carbon chains of plastics themselves are inert, the chemicals added in the manufacturing process of the plastic to acquire certain properties may not be. Among other additives, BpA, phthalates, and nonylphenol are all suspected endocrine disrupters, and BpA causes an elevated risk of prostate and mammary cancer [8]. Because many of these additives are not bound to the polymer chains themselves, it is very easy for them to leach out [2]. Furthermore, microplastics absorb other chemicals as they break down [3]. The surface of the microplastic dictates what chemicals and metals are attracted to the plastics, and once saturated, the microplastic will release chemicals to the soil [8]. If chemicals are leached into the soil of croplands, there is the possibility of crops absorbing these chemicals, allowing the plastic additives with known consequences to reach the human food chain [1, 8-11].

One other study has researched the impact of microplastics; de Souza Machado, et al. [12] studied the impact of microplastics on soil water retention and aggregates and found that polyester increased water holding capacity of soil while polyacrylic and polyethylene particles had no clear trend. Their study, however, introduced microplastics to the soil. This project aims

to provide preliminary results to determine if microplastics have effects on the water retention, shrinkage, and holding properties of soil and should be further studied. This project studies soil with microplastics that have already been introduced and incorporated into the soil, which provides a more accurate picture of the long-term impact of microplastics. Understanding how and why microplastics affect crop growth can aid in production and prevention of soil degradation. Furthermore, this study serves to quantify microplastics in the terrestrial environment and help determine how prevalent microplastics are in fields with applied sewage sludge.

SECTION II

BACKGROUND

Microplastics are created in a variety of ways and they pose a health risk to both humans and animals. In addition, they have been shown to impact crop growth and their additives have been taken up by crops. Microplastics in soil are also likely to remain there for years to come. Approximately 8 trillion tons of plastics have been produced, of which only 9% is recycled [13]. With a half-life of centuries, polymers are considered by some as non-biodegradable, leading to the assumption that all plastics ever produced are still in landfills or the natural environment [2, 8, 13]. In addition, researchers have estimated that it takes 300 years to degrade a LDPE film with a thickness of 60 μm [14].

Creation of microplastics

Microplastics are subdivided into primary and secondary microplastics. Primary microplastics are intentionally manufactured for use in cosmetics, abrasives, electronics, waterborne paints, medicine, or as plastic pellets [2, 15]. Secondary microplastics break down from larger pieces of plastics exposed to UV or high temperatures, as well as mechanical weathering [2]. As of July 2018, the manufacturing of rinse-off cosmetics using microbeads, a type of primary microplastics, was banned in the US [16]. NOAA indicates that microbeads can wash directly into the ocean where they can become a hazard to marine life, as animals often consume them and can be harmed by the chemicals contained in the beads or the beads themselves [17]. The Microbead-Free Waters Act of 2015, demonstrates the public and government's action and awareness of the impact of microplastics in other areas of the environment [16].

Structure of plastics

Polymers used to create plastics are commonly developed from hydrocarbons in fossil fuels, although natural polymers include cellulose and starch, and some bacteria produce them as well [8, 13]. Polymers are then mixed with additives that compose about 7% of the plastics and are not chemically bonded to the carbon chains [6, 13]. Additives serve a variety of purposes including increasing flexibility, colorizing the plastic, provide flame retardation, and preventing degradation from UV light [6, 18, 19]. Sometimes, additives are used to make the plastic biodegradable; however, these have largely been shown to be ineffective [20]. Because they are not bound, it is possible for the additives to later leach into surroundings [6].

Mechanical and chemical breakdown

In order to degrade, the molecular structure of the polymer must change [15]. Weathering is the primary cause of breakdown of plastics. In the environment, wind and water cause dust, dirt, and sand to grate against larger plastics causing abrasion which leads to fragmentation. Plastics can also absorb some moisture, and if temperatures reach freezing, the water will freeze and expand and cause cracks in the plastics [19]. In addition to breaking apart the plastics, weathering can loosen additives from the polymer chains and release them [2].

Ultraviolet light also breaks down the polymers by increasing the molecular energy in the polymeric chains, causing oxidation. This is why UV absorber additives are necessary to preserve plastics meant for long-term use outdoors [19]. The most common polymers, polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC), have a carbon backbone that does not degrade by enzymes or hydrolysis. Instead, UV exposure is needed to break them down. When exposed to UV light or thermal energy, free radicals are created, which then bond to available oxygen. This oxidation makes the plastics more brittle and

prone to cracking [15]. UV exposure and temperature changes are greater at the soil surface level than in the ocean [2]. Thus, the negative effects of the additives seen in the ocean are likely to be exaggerated in the terrestrial environment and additives will be released more rapidly.

Mechanical breakdown plays a further role in breaking down large pieces of plastics. Agricultural activity including plowing, tillage, and driving equipment through fields further breaks down plastics. In the case of plastic mulch used for crops, introducing agrochemicals can accelerate the oxidation of polymer chains and break down plastics to remove them [9, 21]. Washing clothing with synthetic fibers is another source point of mechanically broken-down plastics which is often present in wastewater [20]. Manufacturing plants are also sources of plastics in wastewater [7].

Digestion by organisms

Due to their small size, microplastics are often unintentionally eaten by small fauna, including worms and birds, and filter feeders, which indiscriminately take in material initially. Since these animals are unable to digest the plastics, they pass through the organism, remain in the gut causing false fullness or blockages, or are absorbed into tissues [1, 2, 8]. Certain species, however, are able to consume plastics. *Galleria mellonella* moths, when in the caterpillar stage, are able to eat and digest polyethylene, which can contribute to microplastic breakdown [22]. *Aspergillus niger* degraded 26.17% of the LDPE provided, while *Streptomyces* degraded 46.7% of the LDPE. Mealworms successfully digested 47.7% of the consumed polystyrene [20]. Furthermore, on a microbial scale, UV radiation can cause plastics to form carbonyl that can be broken down by some microorganisms [9]. In the ocean, microbes have been observed to eat plastic. These microbes have evolved to allow for plastic to become a food source, although plastic must be the only available food source [3]. Certain fungi strains have also been observed

to degrade polyurethane and some bacteria have been shown to degrade PVC [23]. As these organisms eat larger plastics, they inevitably create smaller particles through waste and more fragmented larger pieces. In biodegradable plastics created by the blending of biopolymers, organisms were observed to consume just the biopolymers and leave the remaining plastics, further contributing to microplastic creation [20].

Introduction of microplastics to soil

Microplastics are primarily introduced to the soil through sewage sludge application, agricultural mulching, and contaminated air and water. Littering and mismanaged waste also serve as introduction points [6]. Figure 1 displays these sources in detail.

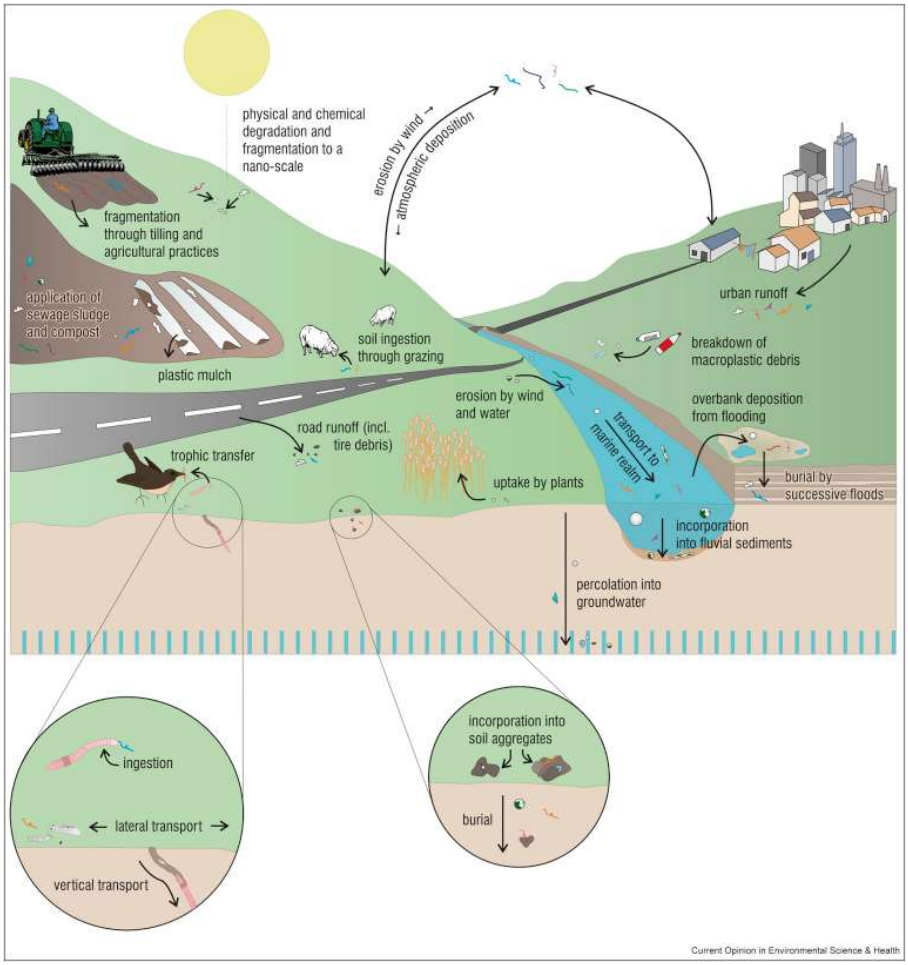

Figure 1. Introduction of microplastic to soil [4]

Contaminated sludge used as fertilizer

After treatment for microbials, solid sludge is often sold as fertilizer for agricultural fields, which acts as a common vector for microplastics. About 50% of sewage sludge is used as fertilizers in North America and Europe [4]. Approximately 98.3% of the microplastics entering wastewater treatment plants are retained in the sludge when using membrane bioreactor (MBR) filtration [7]. Microplastics occur in wastewater as byproducts of washed clothing, washed plastic items, personal care items with microplastics, and other sources discussed above [7, 20]. Considering treated wastewater can contain up to 125,000 particles per m^3, there is even more present in the sludge, leading to an estimated 44,000-300,000 tons of microplastics introduced to soils in North America annually [4, 5]. Furthermore, microplastics have been observed at a sludge application site up to 15 years after application [24].

Agricultural mulching

Mulching is commonly used in farming as it increases the heat and water retention of the soil, thereby increasing water efficiency. Particular mulches are used to prevent certain wavelengths of light from the sun from reaching the soil. This can increase the efficiency of crop growth, but it also increases the amount of additives in the plastics [9]. Mulches can also be used to prevent weed growth and prevent soil from getting on the crop [15]. Plastic mulching has been shown to give higher yields and improved crop quality; however, after 60 years of studies, the associated risks are still unknown [9]. Despite the benefits of mulching, plastic mulching is often not recycled due to the difficulty and lack of available options [21]. Since some mulches, like LDPE film, can take 300 years or more to degrade, there is high retention of the plastics in the soil and potential for the plastics to alter the chemical and physical makeup of the soil [9].

Contaminated air and water

In addition to reusing sewage sludge for fertilizer, wastewater is often used to irrigate fields. Both treated and untreated wastewater can contain microplastics; treated is estimated to contain 0 to 125,000 particles per m^3 and untreated 1,000 to 627,000 particles per m^3 [5]. If half of the irrigation requirements for cotton, a water use heavy crop, are met with treated wastewater, up to 625,000,000 particles per ha could be introduced to the soil [5]. Even if not applied to land, these microplastics are still being released into freshwater bodies via wastewater effluent, which is a major source of microplastics in freshwater [2]. As surface water often serves as a water source for crops, this can further introduce microplastics to cropland. During floods, particles from freshwater bodies could also be deposited in riparian areas and flood plains [4, 5]. Currently, microplastics are not considered pollutants in the US or EU, meaning their presence is not checked prior to discharge at wastewater treatment plants and there is no limit to what can be discharged [5]. However, in the EU, plastics are considered an indicator of water quality [3].

Despite their prevalence in surface water, it is unlikely that microplastic can contaminate groundwater due to their size and likelihood to aggregate [5]. However, Blaesing and Amelung [5] and Hurley and Nizzetto [4] suspect that nanoparticles, plastic particles smaller than 0.3 mm, could percolate. Mintenig, et al. [25] affirmed this assumption with their study of groundwater contamination. Samples were taken at the source and along various stages of drinking water treatment and transit to homes. An average of 0.7 microplastics per m^3 were observed, although researchers concluded that filtration and transport likely introduced these particles [25].

Microplastics are also prevalent in the air, especially in urban environments [2]. Dris, et al. [26] studied atmospheric fallout of microplastics in the Parisian agglomeration, which is approximately 2500 km^2 and includes both urban and suburban areas. They estimated that

between 3 and 10 tons of synthetic fibers are deposited from the atmosphere yearly over the region.

Impact of microplastics on soil and crops

Researchers have found that microplastics in soil increase the water retention, as well as change the soil structure [12]. In addition, microplastics change the microbial activity in the soil [27]. They have also been linked to increasing the amount of plastic additives in the soil [9] and negatively impacting the growth of crops [21].

Changes to water retention in soil

Recently, de Souza Machado, et al. [12] studied the effects of plastic on the structure and water retention of soils. Four types of plastics were added: polyacrylic fibers, polyamide beads, polyester fibers, and polyethylene high-density fragments. Plastic particles and fibers were added as a certain percent of soil sample weight and manually stirred in. Then the samples were buried outside with vegetation to simulate how microplastics would create changes when exposed to climate factors. Samples were analyzed after five weeks. The bulk density decreased in every soil sample, which de Souza Machado, et al. [12] hypothesizes is due to the lighter weight of plastic. Low plastic concentrations decreased water holding capacity while higher concentrations increased the water holding capacity. These results can be seen in Figure 2. Furthermore, a significant decrease in water stable aggregates was discovered in the samples containing polyacrylic fibers. Researchers concluded that polyester fibers caused the most changes in measured soil biophysical parameters and hypothesized that this was due to the flexible nature of the fibers and their ability to incorporate into the soil matrix.

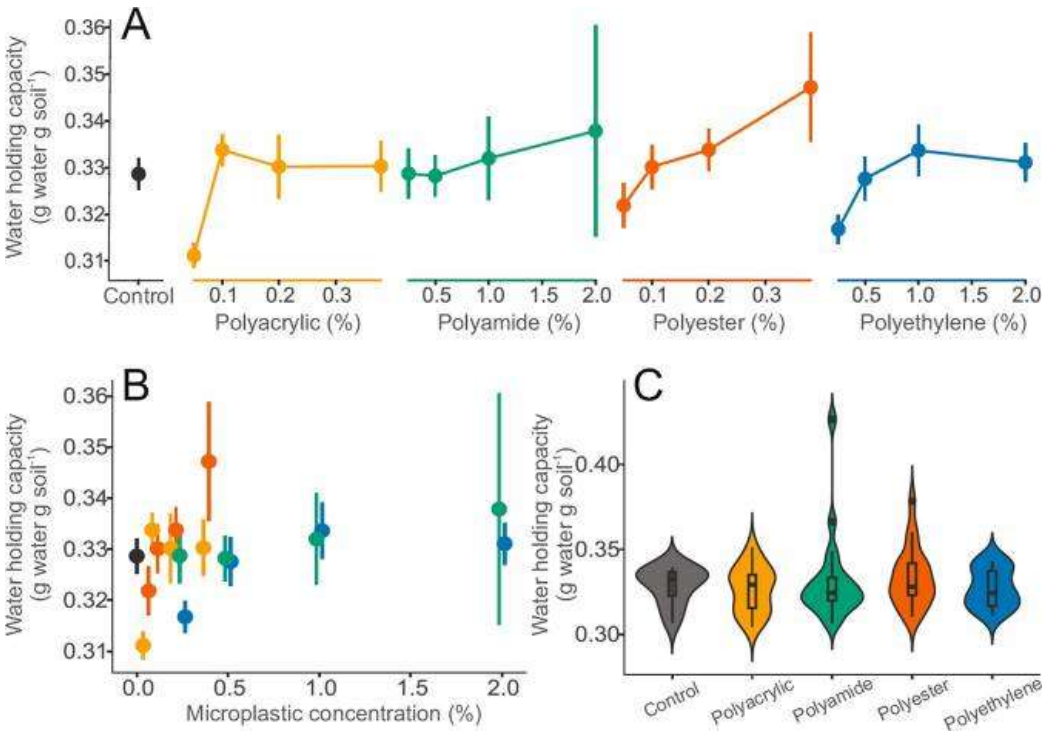

Figure 2. Response of soil water holding capacity to addition of microplastics [12]

Change in soil microbial activity

To test the impacts of plastic on microbial activity, Liu, et al. [27] added polypropylene particles smaller than 0.18 mm to soil samples, incubated the samples, then measured different markers of enzymatic microbial activity. Researchers concluded that microbial activity was likely stimulated by the accumulation of microplastics. The change in enzymatic activity can increase the amount of dissolved organic carbon, nitrogen, and phosphorus available to plants [27]. In addition, de Souza Machado, et al. [12] studied the impact of microplastics on microbial activity. They concluded that the addition polyacrylic and polyester fibers lowered microbial activity when compared to control samples or samples with nonlinear plastic particles. Particle properties mattered as much as concentration. Researchers conclude that further studies are needed to determine exactly how microplastics change soil microbe activity.

Accumulation of plastic additives and pesticides in soil

An increased level in harmful plasticizers has been found in soil that had plastic mulching. Phthalic acid esters (PAE) are the most commonly researched plastic additives in soil. They are loosely bonded within the plastic hydrocarbon chain, leading to their ease of leaching [9]. In cropland with plastic mulching in China, PAE levels have been found to be 74 to 208% higher than those without plastic mulching [9, 10]. However, the level of additives in fields with plastic mulching in Denmark and the United Kingdom are much lower than this, leading to the hypothesis that plastic film greenhouses also contribute to additive accumulation in soil [10].

In addition, microplastics have been shown to absorb pesticides into the matrix of the plastic. The plastic may also act as a pesticide collector, as researchers found that the concentration ($\mu g_{pest}/g_{material}$) of pesticides contained in microplastics was higher than in the surrounding soil [28]. Similarly, beach fragments have been observed to contain pesticides as well, including dichlorodiphenyltrichloroethane (DDTs) and HCHs [29]. Higher concentrations of DDT were found on the west coast of the United States than in other counties in 2009, despite DDT being banned since 1972 in the United States [29, 30]. Similar results could be seen for microplastics in soils and for other chemicals used in agriculture, such as herbicides. The sorption by microplastics could cause pesticides to remain in the soil longer than anticipated and change the amount that needs to be applied to fields to be effective as well.

Impact of microplastics on crops

It is possible for crops to take up additives leached by the microplastic or the microplastics themselves [8-11]. Wang, et al. [10] studied the concentrations of PAE in soil and vegetables in China; their results are shown in Figure 3. Overall, they did not find a linear correlation between the concentration in the soil and tested vegetables, but the tested vegetables

had consistently higher levels of PAEs. The European food standard for Di-2-ethylhexyl phthalate (DEHP), a common phthalate in plastic, is 1.5 mg kg^{-1} [10, 31]. About 25% of the vegetables sampled by Wang, et al. [10] exceeded this safe limit.

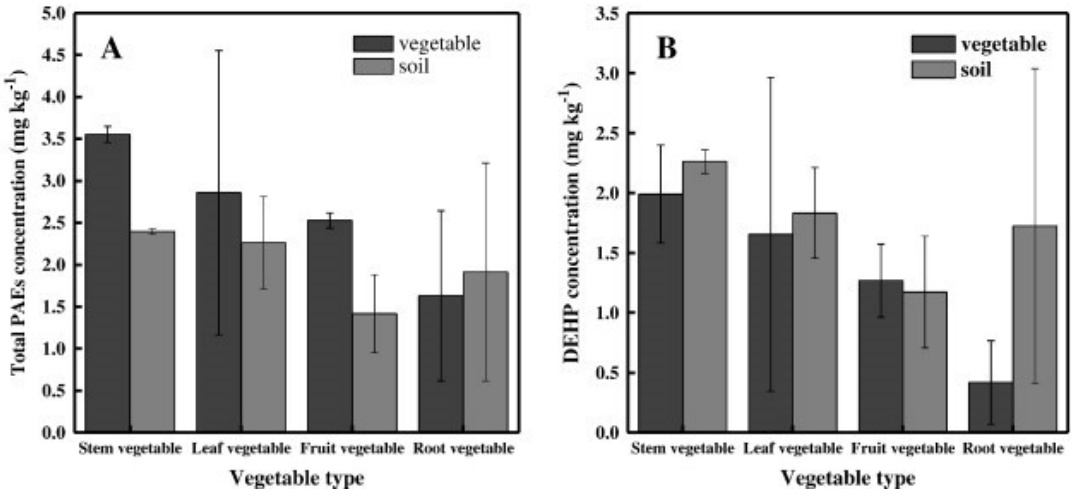

Figure 3. PAE and DEHP concentration in soil and crops [10]

Furthermore, the presence of microplastics has been shown to affect the growth of wheat. Qi, et al. [21] observed that soil containing plastic residue negatively impacted both the above ground and below ground parts of wheat. The study considered plastic mulching made of LDPE and biodegradable plastics mixed in the soil with a concentration of 1% by weight. However, Earthworms were observed to offset the negatives of the microplastics in the soil. Ultimately, Qi, et al. [21] could not determine why the wheat was impacted by the presence of microplastics. Researchers further hypothesized that microplastics altered the soil structure.

Impact of microplastics on humans

After a comprehensive literature review, WHO recently concluded that based on current data, the level of microplastics in drinking water is not a threat to human health. However, there are no studies of the impact of ingested microplastics on human health, and the data available

from studies on other organisms is limited. They also concluded that the chemical levels associated with microplastics in drinking water are very low and not currently a health risk [32]. However, there are more points of exposure for microplastics than just drinking water.

Presence in food chain

Microplastics have been found in honey, beer, salt, sugar, and fish. This is possibly due to plastic packaging, which is a likely source for fish contamination [2, 18]. Karami, et al. [33] recently quantified microplastics in canned fish. They also hypothesize that these particles occurred in canned fish due to improper gutting, translocation through tissue, and the canning process. Cox, et al. [32] reviewed 26 studies that analyzed the amount of microplastics in sugar, salt, alcohol, water, and air. They then estimated how many microplastics Americans eat based on average consumption of these goods. They concluded Americans eat an average of 74,000 to 121,000 microplastic particles per year and those that consume predominately bottled water likely consume an additional 90,000 particles. Because the studied sources only account for 15% of the average caloric intake of an American, actual figures are likely much higher.

Effects of consumed microplastics

Microplastics themselves, due to their size, can cause irritation and inflammation and even migrate through tissues of animals. More research has been done on other species than in humans. Microplastics caused intestinal blockages, inflammation, and fibrosis in earthworms [18]. Rodriguez-Seijo, et al. [34] found microplastics to have no reproductive effect on earthworms, while Qi, et al. [21] found that LDPE showed negative effects on breeding of earthworms. Furthermore, in sea bass, ninety days of exposure to PVC lead to damages that completely compromised intestinal function. In other experiments, microplastics fed to fish migrated to their liver [18]. Colin Janssen recently tested mussels for plastic particles, and found

microplastics in every single one. As the entire mussel is consumed, people who eat a substantial number of mussels are estimated to consume 11,000 plastic particles a year, of which about sixty are absorbed in the intestines [3]. Similar effects are documented in mammals. PVC was shown to migrate into the portal vein of dogs. Both rodents and humans were able to absorb some particles through the intestinal wall [18].

Effects of consumed additives

Table 1 outlines the negative side effects of some of the most common plastic additives. These chemicals have already made their way into the food chain. Researchers have found an increased concentration of phthalates in filter feeder whales and sharks and an increased amount of flame retardants in birds [35]. In sea urchins, external exposure to plastic pellets was observed to increase anomalous development in embryos. When exposed to virgin pellets, anomalous development in embryos increased by 58.1%. When exposed to particles collected on the beach, anomalous development increased by 34.6%. Researchers suspected the difference was due to desorption of additives in plastic in the environment and that the higher concentration of additives in the virgin pellets impact embryo development [36]. In addition, phthalates are one of the most common additives. They comprise about 70% of the US plasticizer market and can be found in high amounts in plastics; for example, up 80% of the weight of medical tubing can be phthalates [37]. There is some regulation to prevent these chemicals from entering the food chain. For example, PVC is banned from use in mulching in the US; however, it can be used for irrigation [9]. Nevertheless, due to negligent waste and areas without regulations, these additives can still make their way into the food chain.

Table 1. Various effects of plastic additives [8, 10, 37-41]

Additive	Common Plastic	Common Product	Effect
BpA	PC, PVC	Water bottles	• Endocrine disruptor • Increased mortality of embryos after maternal exposure • Early onset of female puberty • Hyperactivity and increased aggressiveness • Associated with mammary and prostate cancer
Nonylphenol	HDPE	Bottles for milk, detergent, and oil	• Endocrine disruptor • Increases risk of inflammatory bowel disease
Phthalates	PVC	Medical devices, food wrap, cosmetics, paints, children's toys, plastic mulching	• Suspected endocrine disruptor • Suspected to alter in-vitro development
Styrene	Polystyrene	Rubber, disposable flatware, dairy containers	• Suspected carcinogen • Eye, skin, and throat irritation • Fatigue, irritation, and forgetfulness • Mimic estrogen
Vinyl chlorides	PVC	Food wrap, medical devices	• Known carcinogen • Correlation with liver cancer

SECTION II

MATERIALS AND METHODS

Soil sampling

Standard stainless-steel soil cores with a diameter and height of 5 cm were collected from the biosolids application field where the Texas A&M Wastewater Treatment plant applies sewage sludge (Fig. 4). Six cores each were collected from sites 1, 7 and 12. Geospatial coordinates of the samples can be found in Table 2. Controls were taken from just outside the fence of the application field.

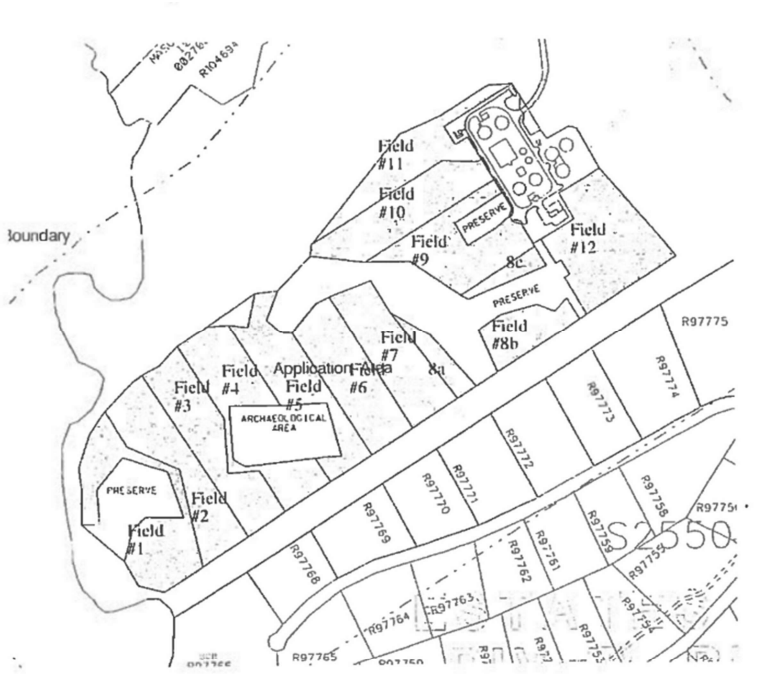

Figure 4. Map of the bioapplication fields

Table 2. Geospatial coordinates of sample sites

Site	Latitude	Longitude	Sample Number
Site 1: control	30.5594	-96.3761	96
Site 1: applied	30.59630	-96.3764	40
Site 7: applied	30.561722, 30.561738	-96.371918, -96.371948	66
Site 12: applied	30.563871	-96.369082	36

The soil map of the biosolids application field is displayed in Figure 5. Table 3 defines these soil types, gives the components, and identifies which soil type each collection site is. Half of the samples were taken on Nov. 28th, 2018 and the other half on Dec. 14th, 2018. Only cores from Nov. 28th were tested. The soil sampling site was saturated with distilled water prior to coring to ensure that the sample structure remained intact when the sample was later remoistened for removal. Due to recent heavy rains, little water was added to the samples.

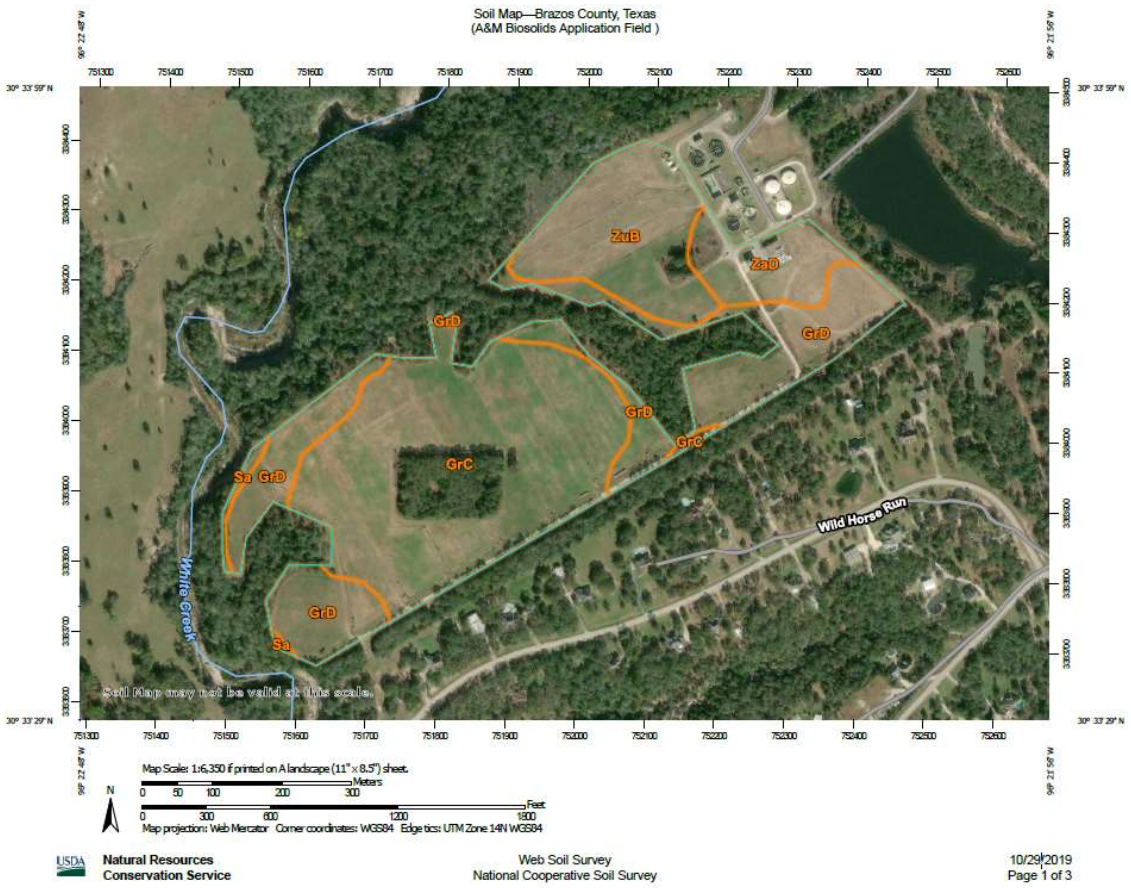

Figure 5. Soil map of the biosolids application field [42]

Table 3. Soil types of the biosolids application field [42]

Site	Sample Numbers	Map Unit Symbol	Map Unit Name	Percent Sand	Percent Clay	Percent Silt	Percent Organic Matter
1, 7	40, 66, 96	GrD	Gredge fine sandy loam, 5 to 8 percent slope	67.7	11.0	21.3	0.75
12	36	ZaD	Zack fine sandy loam, 5 to 8 percent slopes	67.7	11.0	21.3	0.65

TypoSoil® measurements

The cores were tested in the Pedostructure Characterization Lab for soil-water retention, as well as soil shrinkage and soil-water holding properties. The TypoSoil® was used for these measurements in the lab. Figures 6 and 7 show the basic components inside of the TypoSoil®. In addition, nitrile gloves were worn during handling to limit exposure to microplastics.

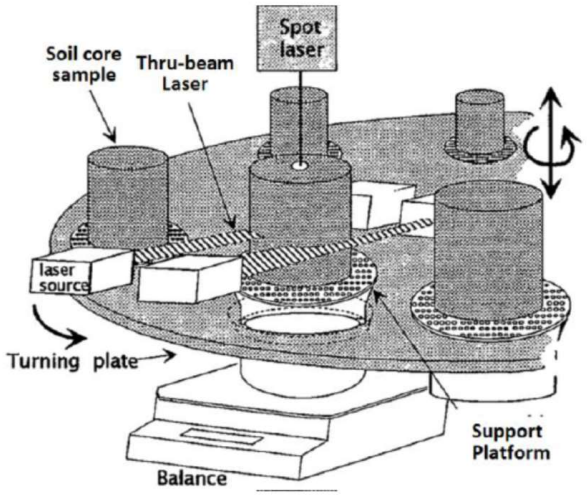

Figure 6. Illustration of the components inside of the TypoSoil® [43]

Figure 7. Components inside of the TypoSoil® with samples [43]

First, the ceramic tensiometers used to measure tension in the soil while drying were prepared. Degassed water is required for the ceramic tensiometers to correctly measure soil properties. Distilled water was boiled for approximately 10 minutes, then placed in a glass container and quickly sealed. This process degassed the water to prevent introduction of air into the ceramic needles. The needles were placed in degassed water for at least 48 hours in order to saturate them. Then the needles were thoroughly degassed using a syringe. Approximately 10 mL of degassed water was drawn into the syringe. Then the tip of the syringe was covered, and the plunger of the syringe drawn back. The tip was quickly uncovered and the gas drawn out of the water was allowed to flow out of the syringe. This process was repeated three to four times to ensure the water in the syringe was degassed. All of the air and bubbles were removed from the syringe each time. Using a small plastic tube, the saturated needle was attached to the syringe and the plunger drawn back and locked while the needle was submerged. The needle was left in this position until no more bubbles were emerging from the needle into the syringe. This process ensured that the needles were completely degassed.

Next, the soil cores were removed from the metal rings, then saturated by placing them on a ring of sand partially submerged in water (Figure 8). The cores were allowed to saturate for about 24 hours. Then the support platforms for the cores were prepared using 10 mL of degassed water. Next, the syringe was attached with a short plastic tube to the support platform. Another plastic tube was placed on the other end and was submerged in degassed water. A syringe was used to flush water through the platform with care taken not to damage the delicate membrane inside and to never draw air into the platform. After the platform had been flushed a few times, the syringe was left attached and the plastic tubing on the other side was removed. A syringe

with a hypodermic needle was used to fill water to the very edge platform tubing, then it was sealed with the small red plastic plug. Figure 9 shows a prepared soil platform.

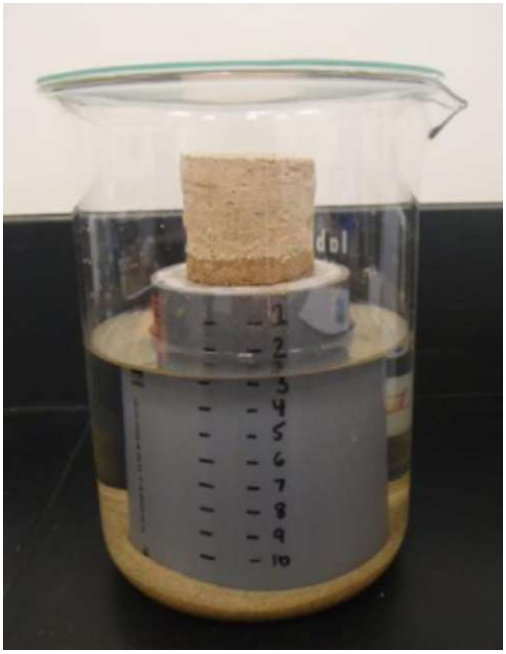

Figure 8. Sand rings used to saturated the soil cores [44]

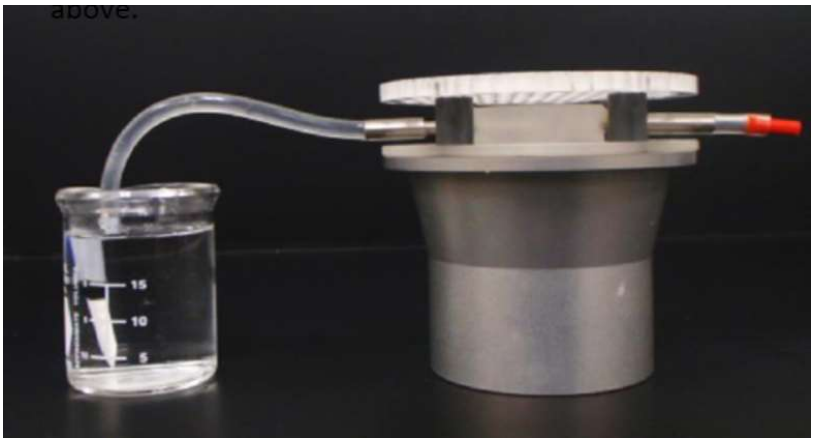

Figure 9. Prepared TypoSoil® platform [43]

Lastly, a new file was created on the TypoSoil® and the platforms were placed in the TypoSoil® to collect the tare weight. Once the platforms were weighed, they were removed and the soil cores were placed directly in the center of the of the platform. The ceramic tensiometer was inserted about two thirds of the way up the sample. The platform and sample were carefully placed back in the TypoSoil®. A small, yellow plastic square was placed on the top of the core in the center to reflect the laser measuring the height of the TypoSoil® and ensure a correct height measurement. The TypoSoil® was then started and allowed to run until the mass of the samples was no longer changing, which was approximately one week. During this time, the TypoSoil® was at 40^{o}C and completed one revolution approximately every 10-12 min. At each revolution, the TypoSoil® collected the mass, height, diameter, and pressure measured by the tensiometer of each sample. Data collected by the TypoSoil® was exported using an SD card. Further detailed procedures can be found in Assi, et al. [44], Assi, et al. [45], and Braudeau, et al. [43].

Quantifying microplastics in soil

The soil cores were analyzed to quantify the microplastics present. Most methods begin by breaking apart aggregates, then sorting by size, removing organic matter, and then removing and identifying the microplastic particles [5]. The standards set forth by NOAA for measuring the amount of microplastics in marine sediment were followed with some modifications [46]. Alternatives include using pressurized fluid extraction, Raman spectroscopy, and vis-NIR technology [5, 47, 48].

First, the soil cores were dried overnight at 110^{o}C and weighed to obtain the dry mass. Next, to disaggregate the soil, a potassium metaphosphate solution was prepared with 5.5 g per liter of water. Approximately 130 mL of a potassium metaphosphate solution was added to each

sample and the sample stirred for an hour. Next, the samples were poured through a 5 mm metal sieve placed over a 0.3 mm sieve. Material caught by the 5 mm sieve was discarded. Material retained in the 0.3 mm sieve was collected and dried for 24 hours at 90ºC, then weighed. For the density float, 100 mL of glycerol at 1.2 g/mL as added to the soil samples. The glycerol served as a substitute for NOAA's recommendation for lithium metatungstate due to its high cost. The sample was stirred and allowed to settle for three days. Particles that floated in the solution were removed and placed in the 0.3 mm sieve. The glycerol caused the larger particles to stick to the bottom of the beaker, allowing the top layer of the sample with floating particles to be poured out onto the sieve. Next, solids retained on the sieve were then collected and dried for 48 hours at 90ºC. The wet peroxide oxidation to remove organic matter was not performed on the samples because the small volume of sample allowed for visual inspection.

After baking, particles that appeared to be microplastics were removed and inspected under a microscope for verification. The samples were also handled and light pressure was applied to see if they would fracture. Particles that fracture easily under pressure are likely organic material and not plastic. Pictures were taken of the particles visual confirmed to be microplastics under a microscope with a ruler and the photos were used to measure the size of the microplastics using ImageJ. Four measurements of the diameter were taken of each particle: vertical, at a 45º angle, at a 90º, and at a 135º.

SECTION III

RESULTS

TypoSoil® results

Table 4 contains the mass and calculated volume of the soil samples. The dry mass of soil samples was obtained after baking the cores at 110°C after the TypoSoil® run. The volume was calculated using the diameter and height measurements from the TypoSoil® which uses lasers to measure the height and diameter of the soil core. The lasers have a sensitivity of 5 µm for the diameter and 10 µm for the height. To keep the results consistent, the final diameter and height measured by the TypoSoil® was used, at which time the core was at its driest in the TypoSoil®.

Table 4. Mass and volume of soil samples

Type	Sample Number	Dry Mass (g)	Dry Diameter (mm)	Dry Height (mm)	Volume (cm^3)
Control	96	149.1	50.09	56.25	110.8
Sludge Applied	66	127.9	50.94	48.62	99.03
	40	135.6	50.64	52.38	105.4
	36	119.4	51.86	46.94	99.08

Table 5 shows the soil health characteristics calculated from data collected by the TypoSoil®. Saturation (SAT) is the amount of water in the soil when all soil pores have been filled with water. At saturation, the soil is unable to absorb any more water [49]. Field capacity (FC) was originally defined by Veihmeyer and Hendrickson in 1949. Field capacity of soil is the amount of water remaining in a soil sample after it has been wetted and allowed to dry over two or three days. After which, no more water free drains from the soil and the tension applied by plant roots is at 33 kPa. The permanent wilting point (PWP) is the soil water content at which

plants are no longer able to extract water from the soil and the tension applied by plant roots is at 1500kPa [50]. Available water (AW) is the maximum amount of water in the soil that can be used by plants. The available water is calculated by subtracting the permanent wilting point from the field capacity [51]. The TypoSoil® obtains these characteristics by measuring the tension at which water flows from the soil as it dries. Results from the water retention calculations show that the addition of sludge consistently increased the saturated water content and the available water holding capacity.

Table 5. Water holding properties measured by the TypoSoil®

Type	Sample Number	SAT [kg_{water}/kg_{soil}]	FC [kg_{water}/kg_{soil}]	PWP [kg_{water}/kg_{soil}]	AW [kg_{water}/kg_{soil}]
Control	96	0.27	0.13	0.05	0.08
Sludge Applied	66	0.33	0.25	0.08	0.17
	40	0.30	0.17	0.04	0.13
	36	0.31	0.21	0.09	0.12

Quantification results

All of the samples contained a similar number of microplastics (Table 6). The control sample had one of the highest levels of microplastics. This could be due to contamination during testing. Possible exposure points include: white plastic caps on soil cores, plastic tubing used in the TypoSoil®, plastic fibers worn in the lab, and plastic vials used to transport microplastics. The average diameter of the found particles is 1.01 mm with a range of 1.3 mm. In addition, Table 7 shows the amount of microplastics per weight and volume of the soil. The weight and volume were extrapolated to kilograms and cubic meters, respectively, in order to compare them to other studies quantifying microplastics. Due to the COVID-19 pandemic, it was not possible

to weigh the microplastics to determine the exact weight of microplastics per gram of soil.

Figures of the particles and their measurements are in the appendix.

Table 6. Number and size of microplastics in each soil sample

Type	Sample Number	Number of Microplastics	Largest Diameter of Microplastics
Control	96	4	1.26 mm, 0.79 mm, 1.85 mm, 0.72 mm
Sludge Applied	66	3	0.67 mm, 0.63 mm, 0.55 mm
	40	2	0.81 mm, 1.43 mm
	36	4	1.67 mm, 0.95 mm, 1.22 mm, 0.58 mm

Table 7. Concentration of microplastics by weight and volume of soil

Type	Sample Number	Microplastics/kg of Dry Soil	Microplastics/cm^3 Dry Soil	Microplastic/m^3 of Dry Soil
Control	96	26.83	0.03610	36,100
Sludge Applied	66	23.46	0.03029	30,290
	40	14.75	0.01897	18,970
	36	33.50	0.04037	40,370
Average		24.64	0.03144	31,440

SECTION IV

DISCUSSION

The quantification results further confirm that sewage sludge is a vector for microplastics into soil. Furthermore, it is unlikely the high amount of microplastics in the control was due to contamination. None of the microplastics from the control or other samples appear to be plastics that the samples could have been exposed to. The particles found appear to be more weathered and incorporated into the soil matrix. It is possible the microplastics migrated from the applied field to the control site, which was taken a few meters outside of the applied field fence. This indicates that microplastics can be transported easily by runoff, which means applied sludge sites serve as another source of microplastics in the environment.

However, reliable conclusions on the impact of microplastics on soil health cannot be drawn from this data, because there is no correlation between the number of particles and sample location. The results from the TypoSoil® do suggest, nevertheless, that sewage sludge increases the water holding capacity of soil as the available water content was higher for each of the applied samples when compared to the control. Other studies have found applied sludge to increase the water holding capability as well [52-54]. If future studies on the impact of microplastics of soil are conducted using soil from sewage sludge application, this will need to be controlled for. In addition, because an accurate weight of the found particles is not available, comparison to other quantification studies is difficult. However, by estimating the mass of the found plastics using various assumptions, some comparisons can be made between this study and other studies done on microplastics and soil.

Estimated mass of found plastics

An estimate of the weights to compare the studies can be calculated using the following assumptions: all particles are spherical, the microplastics in terrestrial environments have similar density to those found on beaches, and the microplastics have a density of approximately 0.91 g/m^3. The particles are likely smaller than a sphere, leading to the estimated weight probably being slightly above the actual, but it is likely more accurate than assuming other shapes. The density chosen was the average density found by Morét-Ferguson, et al. [55], who quantified microplastics and plastic debris in the western North Atlantic Ocean. The density of 0.91 g/m^3 was the average density found in the beach sediments. While the waste that is found on beaches is not identical to the waste found in the terrestrial environment, especially that of applied sludge, it is more accurate than assuming a specific plastic and using that density or assuming the mean of densities in commercial plastics. When exposed to the environment and weathering, plastics often gain in density. The densities found by Morét-Ferguson, et al. [55] account for weathering and thus using their density will be a more accurate assumption than the former two. These assumptions were used to calculated the estimated mass and concentrations in Table 8.

Table 8. Estimated mass and concentration by mass of microplastics

Type	Sample Number	Estimated Mass of Microplastics (mg)	Estimated Mass of plastics (g)/Mass of Dry Soil (g)	Estimated Percent of Microplastic Mass in Dry Soil Mass
Control	96	4.679	3.139e-5	0.003139%
Sludge Applied	66	0.365	2.853e-6	0.0002853%
	40	1.488	1.097e-5	0.001097%
	36	3.828	3.206e-5	0.003206%

Comparison to studies on microplastics and soil

The particles found in this study likely behave similar to the polyethylene fragments used by de Souza Machado, et al. [12] as the other plastics used in the study were fibers and no fibers were found in the samples of this study. Polyethylene particles did impact the water holding capacity. At concentrations under 0.5% by dry weight, the microplastics decreased the water retention; over 1% by dry weight, the microplastics increased the water retention. The estimated percent by mass of microplastics from this study are significantly less than the smallest percent studied by Souza Machado, et al. [12], which was 0.5%. The smallest percent used by de Souza Machado, et al. [12] was polyacrlic at 0.05%, which did lower water holding capacity more than any other in the study. At high percentages, though, the water holding capacity with the polyacrlic was about the same as the control, leading to no clear trend on impact. Based on these observations, it is likely that microplastics at this low of concentration are decreasing the water holding potential if they are impacting it at all. It is very possible there is no impact on the water holding potential in soil at concentrations this low.

In crops, Qi, et al. [21] observed that soil containing plastic residue at 1% by weight negatively impacted both the above ground and below ground parts of wheat. The tested amount is significantly higher than the estimated found weight from the applied fields and thus reliable comparisons cannot be made between the two studies. Even so, this highlights the need for more studies on the impact of microplastics on crops at a lower percentage found in the terrestrial environment, which Qi, et al. [21] also recommended.

Comparison to quantification studies

Comparison to quantification in soil

Concentrations of microplastics in soil found in this study are similar to the concentrations found in other studies. Researchers who studied cropland and forestland in southwestern China found similar concentrations of microplastics in soil to this study. Zhang and Liu [56] found between 7,100 to 42,960 particles/kg with a mean of 18,760 particles/kg. Soils fertilized with sewage sludge and irrigated with wastewater had higher concentrations. Although the mean from this study was higher (31,440 particles/kg), all of the found concentrations in this study fit within the range found by Zhang and Liu [56]. In northwest China, researchers found microplastics in a concentration of 8.0e-6 kg/kg in agricultural soils [57]. While this is slightly less than the estimated densities found in this study, it is relatively close, especially considering the high potential for error in the estimation in the mass. The similarities between the concentrations found in this study and other found concentrations indicate that these concentrations are reliable measures of the current level of microplastics in applied sludge sights.

Comparison to quantification in the marine environment

The found microplastics per volume is 0.03144 particles/cm^3 dry soil, which is comparable to 31.44 particles/L and 31,440 particles/m^3. This concentration is much higher than that previously found in the ocean. In 2017, Horton, et al. [2] reviewed studies on the amount of particles in marine surface waters and found concentrations between 0.0005 particles/L and 16 particles/L [58, 59]. Direct comparison between microplastic counts in the terrestrial environment and the marine environment may not be reliable as soil is relatively static and plastics are likely to remain in the horizon and soil matrix while in the ocean, many microplastics eventually settle to the ocean floor [1]. Nevertheless, the higher concentration supports Horton,

et al. [2] hypothesis that microplastics are as numerous in the terrestrial environment as they are in the ocean.

SECTION V

CONCLUSION

Future studies are needed to determine how microplastics impact on soil health because no conclusive results about the impact of microplastics on soil could be made. These studies will help determine if actions to mitigate damage should be taken, such as monitoring microplastics applied in sludge or adjusting crop management techniques on impacted soil. Limitation of this study included a low budget, lack of equipment, lack of available expertise on the subject, TypoSoil® malfunctions that could not be corrected in time, no confirmation that the found microplastics were plastics via chemical analysis, and unforeseen interruptions to lab availability and lab access because of the COVID-19 pandemic.

Nevertheless, the findings of the research support that sewage sludge is a vector for microplastics into the terrestrial environment due to the high concentration of microplastics in the samples. Microplastics occur at the rate of approximately 31,440 particles/kg in the samples from the Texas A&M bioapplication field, which is consistent with other studies evaluating applied sludge sites. This concentration is also higher than found concentrations in the ocean. More studies are needed to determine what concentrations of microplastics pose threats to human and environmental health. Despite the limitations of this study, the results could prove useful for future research on quantifying microplastics in the terrestrial environment as it indicates they are as numerous as other studies indicate. Furthermore, the consistency in concentrations of microplastics between different studies provides researchers who are studying the negative impacts of microplastic approximate concentrations in the terrestrial environment to test.

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APPENDIX

Particles from sample 96

Figure 10. Sample 96, particle 1 next to ruler

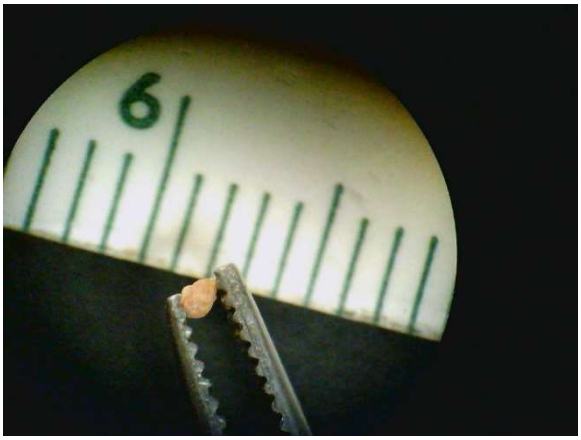

Figure 11. Sample 96, particle 1 enlarged

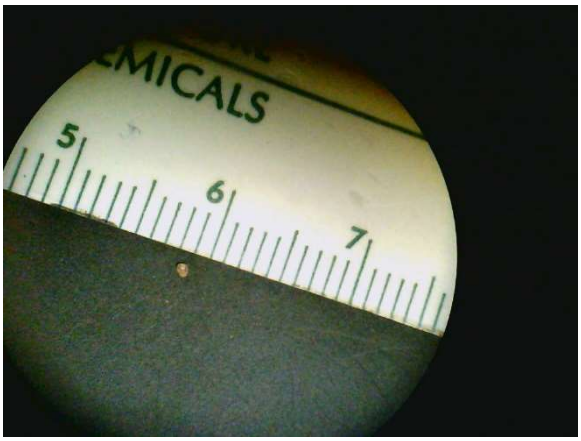

Figure 12. Sample 96, particle 2 next to ruler

Figure 13. Sample 96, particle 2 enlarged

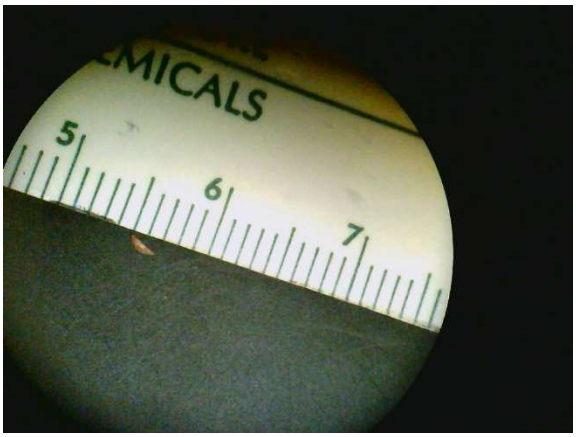

Figure 14. Sample 96, particle 3 next to ruler

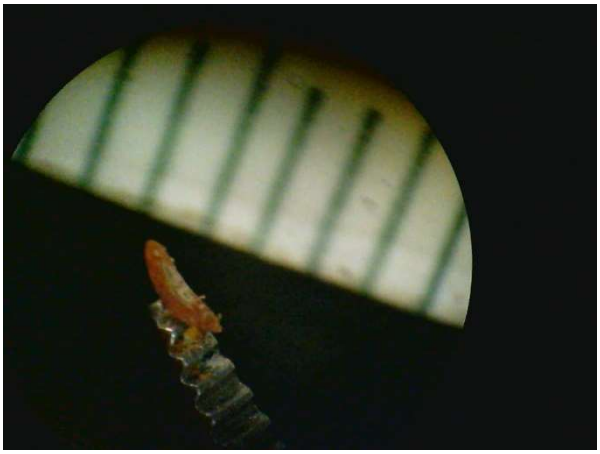

Figure 15. Sample 96, particle 3 enlarged

Figure 16. Sample 96, particle 4 next to ruler

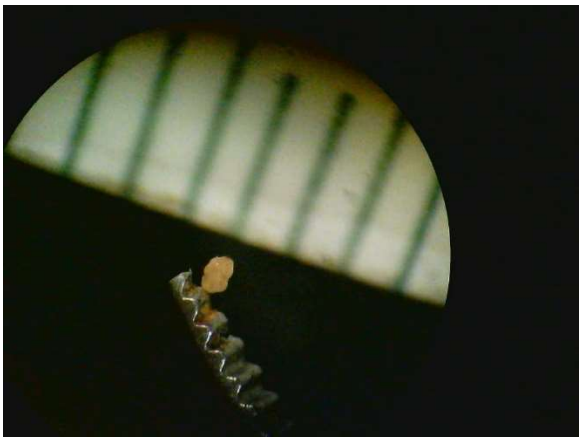

Figure 17. Sample 96, particle 4 enlarged

Particles from sample 66

Figure 18. Sample 66, particle 1 next to ruler

Figure 20. Sample 66, particle 2 next to ruler

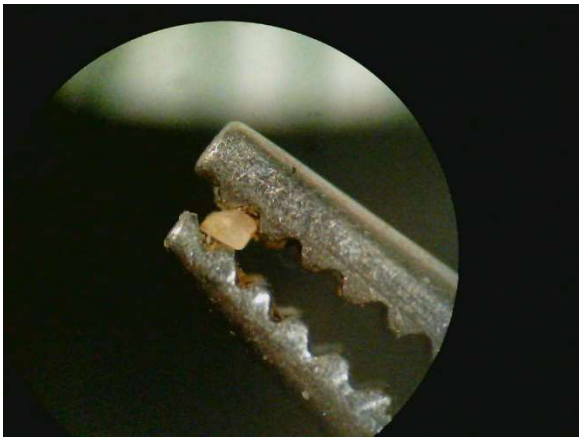

Figure 19. Sample 66, particle 1 enlarged

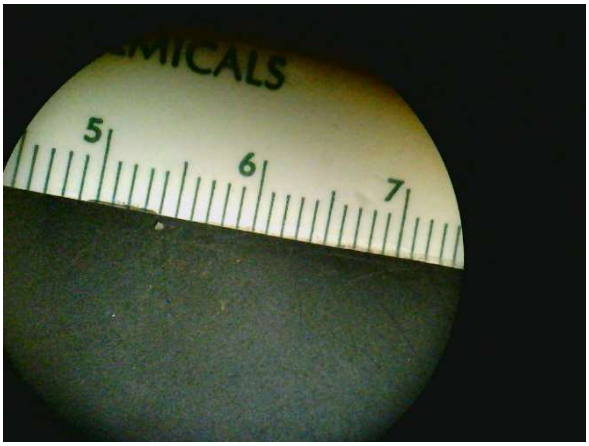

Figure 21. Sample 66, particle 3 next to ruler

Figure 22. Sample 66, particle 3 enlarged

Particles from sample 40

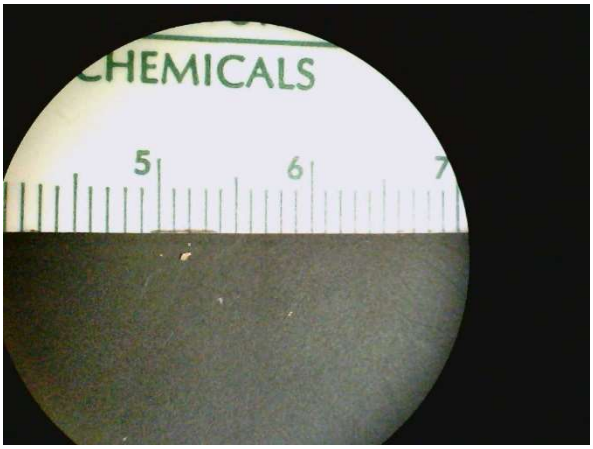

Figure 23. Sample 40, particle 1 next to ruler

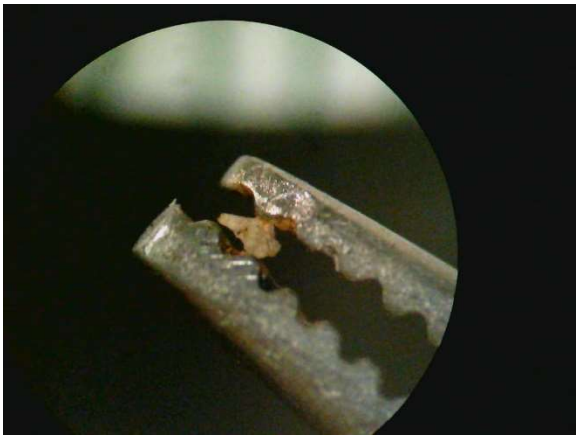

Figure 24. Sample 40, particle 1 enlarged

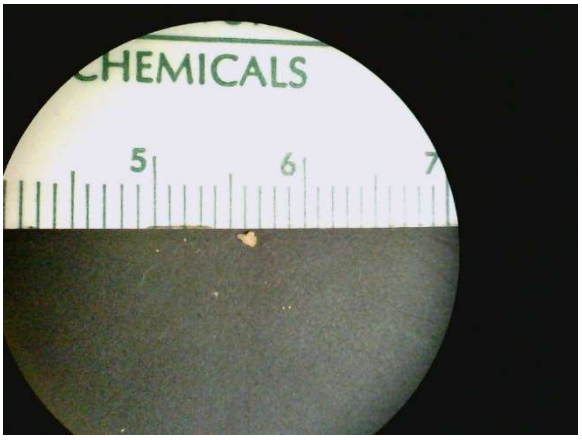

Figure 25. Sample 40, particle 2 next to ruler

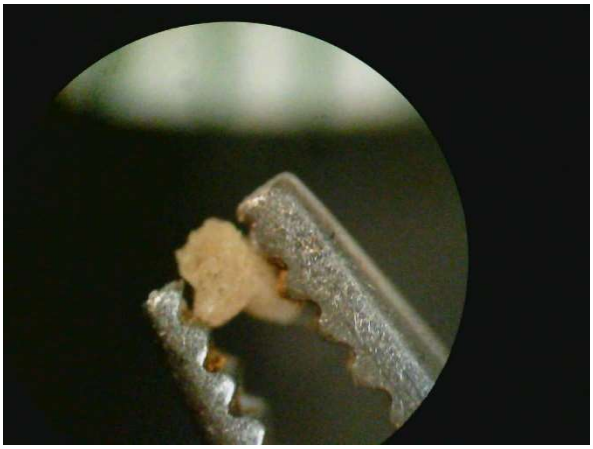

Figure 26. Sample 40, particle 2 enlarged

Particles from sample 36

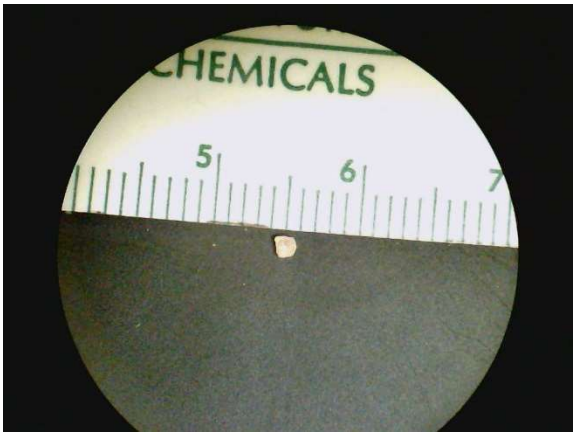

Figure 27. Sample 36, particle 1 next to ruler


Figure 28. Sample 36, particle 1 enlarged

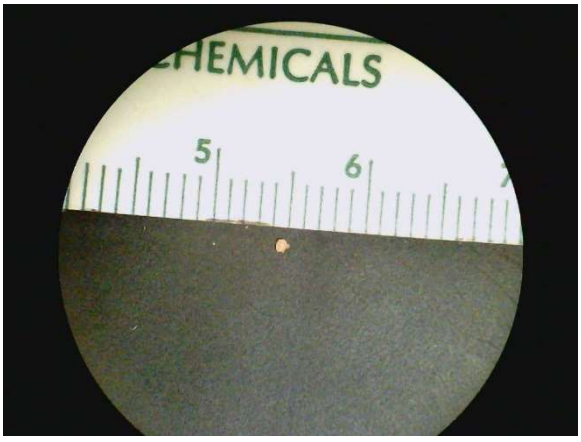

Figure 29. Sample 36, particle 2 next to ruler

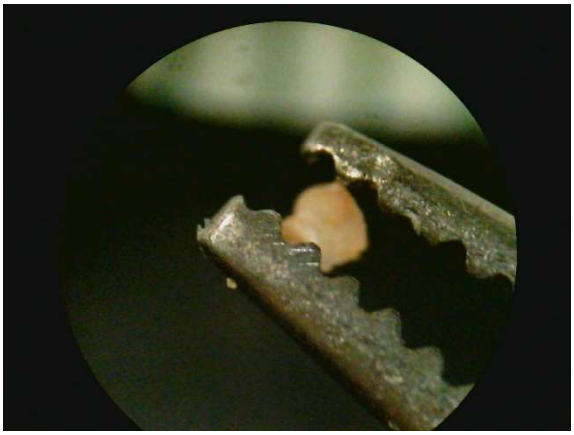
Figure 30. Sample 36, particle 2 enlarged

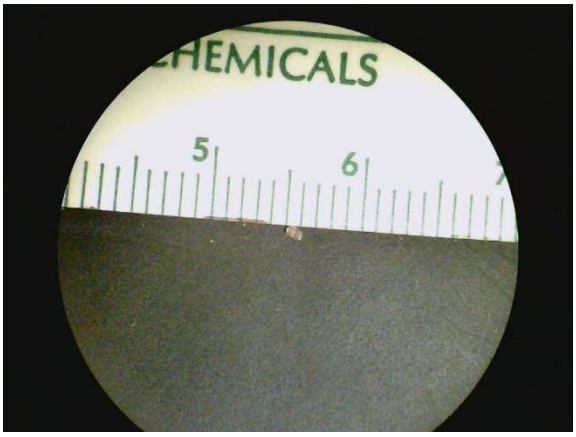

Figure 31. Sample 36, particle 3 next to ruler

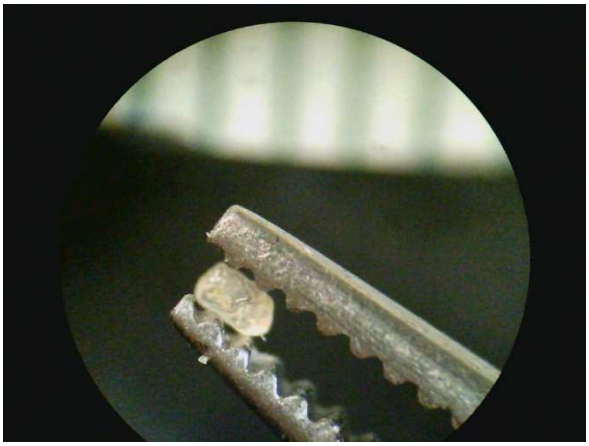
Figure 32. Sample 36, particle 3 enlarged

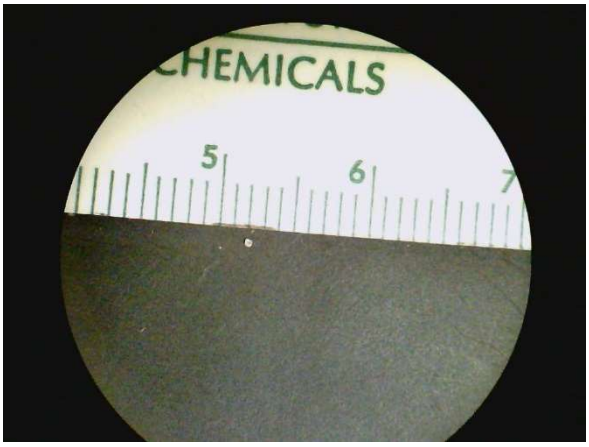

Figure 33. Sample 36, particle 4 next to ruler

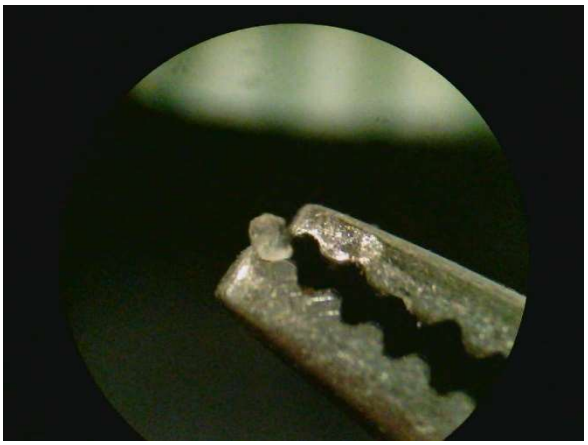
Figure 34. Sample 36, particle 4 enlarged