THE FLORIDA AGRICULTURAL AND MECHANICAL UNIVERSITY

COLLEGE OF AGRICULTURE AND FOOD SCIENCES

CHARACTERIZING THE OCCURRENCE AND DISTRIBUTION OF MICROPLASTICS IN AN URBAN/RURAL REGIONAL HYDROLOGICAL SYSTEM: CASE STUDY TALLAHASSEE, FLORIDA.

By:

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A thesis submitted to the

Department of Soil and Water Science

In partial fulfillment of the

requirements for the degree of

Master's

Tallahassee, Florida

Spring, 2024

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DEDICATION

Dedicated to God Almighty, my beloved family, mentors, and cherished friends, your unwavering support has been my guiding light throughout this journey. Thank you for standing by me every step of the way

ACKNOWLEDGMENT

I extend my heartfelt gratitude to my supervisory committee members, colleagues, staff, and family for their invaluable contributions to the success of my thesis.

I am deeply thankful to my major advisor, Dr. Katherine Milla, for her exceptional guidance, unwavering support, and patience throughout my master's program. Her mentorship has been instrumental, and I am grateful for the nurturing research environment she provided. Dr. Milla's support transcended academic boundaries, and I am truly fortunate to have her as both an advisor and a friend.

I am indebted to Dr. Jane Amita for her assistance and guidance during sampling and laboratory analysis. Her meticulous feedback and dedication to improving my thesis were invaluable. I also express my appreciation to Dr. Maia McGuire for her invaluable contributions and willingness to participate in my thesis, despite her busy schedule. Her expertise provided a solid foundation for the methodology used in this research.

A special acknowledgment goes to Dr. Odemari Mbuya for his fatherly support, constant care, and invaluable advice since the inception of my program. I am also grateful to Dr. Muchovej for his unwavering support and commitment to ensuring my understanding of research writing.

I extend my sincere appreciation to Dr. Lucy Ngatia, Mrs. Constance Bernard, and Mrs. Agnes Coppin for their unwavering support and valuable advice. Special thanks to Dr. Edwin Duke, Alacia Murphy, and Mrs. Linda Sapp for their assistance during field sampling and ongoing support.

I am immensely grateful to my loving husband, Simeon Chancha, and my children, Benicia Chancha and Janelle Chancha, for their unconditional love, prayers, and encouragement. I am also

thankful to my parents and siblings. I am thankful to Prof. Hassan Makun, the Late Dr. Mercy Bankole, Prof. Akim Osunde, Prof. Fagbenro, Dr. Eze P. C. Eniola Olaoye, Cassey Stark, Saheed Bolarinwa, and fellow graduate students of CAFS for their selfless assistance throughout my journey.

I acknowledge the support received from the National Institute of Food and Agriculture (NIFA) Capacity Building Grant, which made this research project possible.

Finally, I offer my deepest gratitude to God Almighty for His guidance, strength, and blessings, which made this thesis a reality.

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ABSTRACT

Nearly all plastic pollution originates from terrestrial sources, with urban areas acting as an essential contributor to microplastics in aquatic environments. The ubiquitous nature of microplastics in the environment has raised concern about their presence in surface water and potential contamination of connecting groundwater. This study was conducted in the Tallahassee, Florida, urban and downgradient region. Dye tracing and cave diving studies have confirmed hydrologic connectivity between surface waters in Tallahassee and the downgradient springs in the region. This study conducted a comprehensive sampling of urban stormwater and groundwater wells, to gain a better understanding of the behavior of microplastics in a hydrologic system with surface water-groundwater connectivity. Complications encountered using filtration methods developed for marine samples necessitated developing a new filtering method for separating the microplastics. 1000 ml water sample was collected from different freshwater sources (lakes, ponds, springs, wells, estuarine), treated with 0.75% aqueous solution of NaOCl, and allowed to sit for 48 hrs to reduce through oxidation the organic matter and algae that clog the filters and also reduce the number of natural fibers, hence reducing filtration time and the time spent in counting the microplastics trapped by the filter. Samples were filtered using a Rocker 400 vacuum pump, and hydrophilic filter papers of 47 mm diameter and 0.45µm pore size were loaded into 300ml filtration funnels and inserted into the manifold chambers. Filter papers was then observed under a microscope, and the number of microplastics was counted and documented. Results demonstrated generally high numbers of microplastics in surface waters and shallow monitoring wells at the Tallahassee wastewater reuse facility, where treated wastewater is used to irrigate crops. High numbers of plastic fibers were sampled from Wakulla Springs, which directly connects to Tallahassee stormwater inputs. In contrast, groundwater sampled from the City of Tallahassee's public supply wells did not exceed background level concentrations of plastic fibers. The City

wells draw from the deep, confined Upper Floridan Aquifer. These findings contribute new understanding of the hydrodynamics of microplastic transport and demonstrate that environmentally ubiquitous microplastics can invade interconnected groundwater.

CHAPTER 1
INTRODUCTION AND RATIONALE

There is an increasing interest in microplastics as a major world concern due to their accumulation, persistence, and harm to biological systems (Du et al., 2020; Chenye et al., 2020). Most societies widely use plastic (Mendoza & Balcer., 2019). There has been a rapid increase in global plastic production due to the industrial revolution, population growth, urbanization, and development of new technologies (Waad et al., 2021) since commercial production first began in the 1950s (Gayer et al., 2017; Nastaran et al., 2022), with packing materials having the highest market (Jambeck et al., 2015).

Microplastics are plastic particles with a diameter of less than 5 mm (Arthur et al., 2009). There are two categories of microplastics. Primary microplastics are those manufactured with an original size of less than 5 mm. They are intentionally produced and found in products like virgin pellets, microbeads from cosmetic products, abrasives, powders for injection molding, medicine, or ink for 3D printers. (Cole et al., 2011; Mendoza & Balcer, 2019). Secondary microplastics are those derived from the breaking down of plastics when exposed to harsh environmental conditions such as high temperatures, chemicals (Cole et al., 2011), photodegradation, and physical and biological interaction (Thompson et al., 2009). The significant components of microplastics are polyamide (PA), polypropylene (PP), polyethylene (PE), polyester (PET), polybutylene adipate-co-terephthalate (PBAT), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polylactide (PLA) etc. (Ivleva, 2021; Yanyan, 2021). Microplastics can be classified as fragments, films, fibers, granules, and foams based on their shape.

Microplastics are fast-growing in our environment and find their way into the food chain (Wagner et al., 2014). Microplastics are diversely dispersed and available to organisms at different trophic levels (Cole et al., 2011; De Sá et al., 2018). Organisms confuse these brightly colored

plastics for food and can be ingested by fish, mussels, turtles, and birds. Microplastic fibers have the potential to entangle with appendages, gill filaments and the gastrointestinal system of organisms causing harm to the organism both directly and physiologically (Rebelein et al., 2021). Microplastics may be carriers of pathogens, support dense biofilm colonies and antibiotic resistant genes (ARGs) (Kaur et al., 2022). These pathogens are toxic and are spread in the ecological environment. Biofilm formation facilitates horizontal transfer of ARGs to organisms, including humans, that feed on them, posing a major threat to human health and health care (Kaur et al., 2022; Vandermeersch et al., 2015; Prinz & Korez, 2020). The role of biofilms on microplastic retention is still unknown. However, much attention has been drawn to the bio-coating growing on plastic surfaces in the environment. Certain microbes have a particular affinity for plastic surfaces. The ecosystem formed by plastics and microbes is called plastisphere (Zettler et al., 2013; Guasch et al., 2022). Fluvial biofilm may be composed of microorganisms such as viruses, archaea, bacteria, algae, cyanobacteria, and fungi. These organisms produce a matrix of extracellular polymeric substances that may become sticky and thickened under high light and nutrient availability, trapping suspended sediments, including microplastics (Romani, 2010; Fleming & Wingender, 2010) and transporting them through the hydrological cycle.

Nearly all plastic pollutants are derived from terrestrial sources (Bakir et al., 2012). Many studies have focused on ocean plastic pollution, but more research needs to be done on freshwater and terrestrial plastic pollution (Lambert & Wagner, 2018; Yanyan, 2021). 25.3 million metric tons of mismanaged microplastics were released into the world's oceans from 1961 to 2017 (Isobe & Iwasaki, 2022). Ocean plastics accounted for 4.7% of the (mismanaged plastics) that are littered or inadequately disposed of, while terrestrial plastics accounted for 95.3% (Isobe & Iwasaki, 2022).

Microplastics are ubiquitous and are distributed through the hydrological cycle. They are suspended and transported in the atmosphere and can be found in precipitation. This is especially true for fibers that are very small in size, have a low density compared with soil and dust particles, have high drag, and have low settling velocity (Abbasi & Turner, 2021). The soil is a significant sink for microplastic (Boots et al., 2019). Soil plays a vital role in the downward movement of microplastics through soil pores via leaching (Zhefal et al., 2021). The soil acts as a sink and a potential source of microplastics, and is influenced by agricultural activities, precipitation, runoff, and flooding (Meng et al., 2020).

Plastic particles smaller than soil pore spaces and cracks can move through the soil profile as water washes down microplastics, conveying them to shallow groundwater (Zhefal et al., 2021). Zhou et al., 2021 observed that subsoil (10-15cm) contained more microplastics than topsoil (0-5cm). There is evidence of microplastics in groundwater and, most notably, in drinking water taken from different levels of purification (Mintenig et al., 2019). As runoff moves, it carries these particles and deposits them into water bodies such as ponds, lakes, and streams until they end up in the ocean.

Factors such as population density, land use, land cover type, presence of impervious surfaces, and types of watershed management influence the amount and dispersal of microplastics into the aquatic environment (Yonkos et al., 2014; Baldwin et al., 2016; McCormick et al., 2014). Other studies have shown a correlation between microplastic accumulation, population density, and land use (Baldwin et al., 2016).

Microplastics research has focused mainly on their abundance and distribution in the environment, especially the marine environment, while their pathways have been understudied (Mendoza & Balcer, 2019). Their sources, transportation, and sinks have also received less

attention (Wagner & Lambert, 2018). The goal of this study is to assess the occurrence and distribution of microplastics in interconnected terrestrial surface and ground waters through comprehensive sampling of an urban region.

1.1 Study Site

This study focuses on the regional area of Tallahassee, Florida. Tallahassee is the largest city in the Northwest Florida region. For this study, samples were collected from Leon, Wakulla and Jefferson Counties in the Tallahassee region (Fig. 1). Leon County is home to Florida's capital, Tallahassee. This county has a total area of 702 square miles (1,820 km^2), of which 667 square miles (1,730 km2) are land, and 35 square miles (91 km2) (5.0%) are water (https://en.wikipedia.org/wiki/Tallahassee,_Florida; U.S Census Bureau, 2023). Wakulla County is in the Big Bend region in the northern portion of the US state of Florida. It has a population of about 33,764. This county has a total area of 736 square miles (1910 km^2), of which 606 square miles (1,570 km2) are land, and 129 square miles (330 km^2) 17.6% are water (https://en.wikipedia.org/wiki/Wakulla_County,_Florida; U.S Census Bureau, 2023).

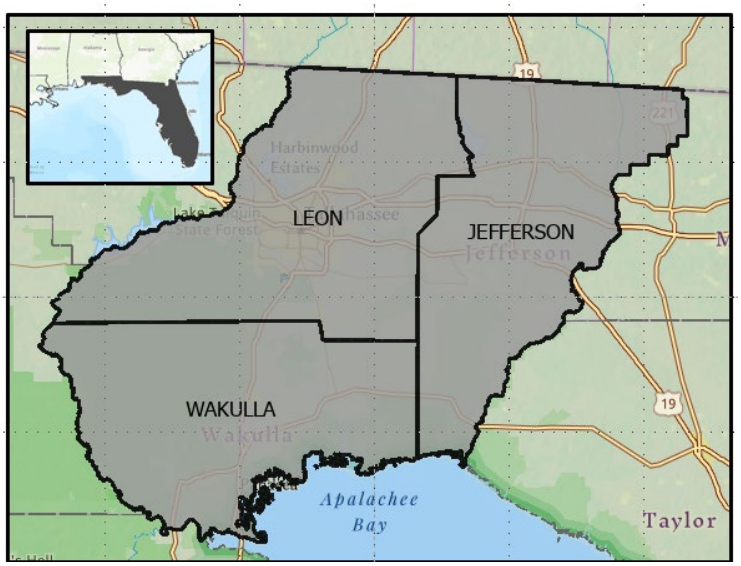

Figure 1. Map showing the various Counties that were sampled in this study.

Jefferson County is also located in the Big Bend region in the Florida's rolling hills. It is the only county that extends from Georgia in the north to the Gulf of Mexico in the south. As of the 2020 census, Jefferson County has a population of 14,510. Covering a total area of 637 square miles (1,650 km^2), the county comprises 598 square miles (1,550 km^2) of land and 38 square miles (98 km^2) of water (U.S Census Bureau, 2023; http://www.jeffersoncountyfl.gov/p/about-jefferson).

1.1.1 Tallahassee Stormwater

In an undisturbed landscape, precipitation interacts with the region's natural features. Rain falling on surfaces can undergo a variety of processes. Trees and vegetation intercept rainfall and return it to the atmosphere via evapotranspiration. Rainfall infiltrating the soil can be returned to surface water through subsurface flow to waterbodies, streams, wetlands, and springs. Surface runoff moves in response to the region's natural topography, flowing downslope into waterbodies and streams. The area drained by a specific waterbody or stream is referred to as a watershed.

Figure 2 shows delineated storm watersheds in a portion of Tallahassee. Each watershed drains into a waterbody within the watershed. Many waterbodies are drained by streams, ditches, and underground conduits that pass directly from one watershed into an adjacent watershed.

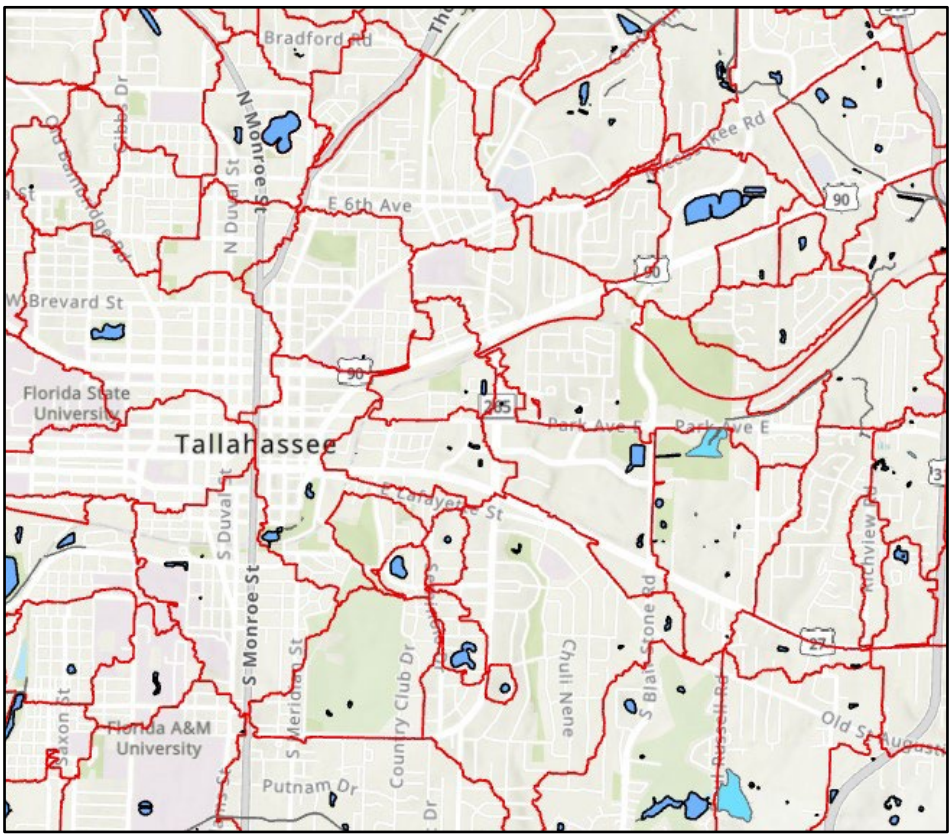

Figure 2. Delineated storm watersheds in a portion of Tallahassee. (GIS data from Tallahassee-Leon County GIS department).

In mature urban settings, the natural landscape has been highly disturbed by construction, paving of surfaces, channelizing, rerouting of natural drainage ways, burying natural streams and creeks in underground conduits, excavation, and infilling of depressions and channels. The original drainage network of the landscape is modified to accommodate changing hydrologic conditions, which include increased runoff, flooding, and erosion due to expansion of impervious surfaces; reduced infiltration of rainfall due to changes in land cover; and alterations in the flood pulsing cycles of wetlands. Natural waterbodies, wetlands, and streams are appropriated to manage stormwater. New waterbodies are constructed to detain, retain, and route stormwater.

The Tallahassee urban area encompasses various land uses, land covers, and population densities (Fig. 3), including residential, industrial/commercial, agricultural, recreational, transportation, and open lands. Although land uses do not imply specific land covers, land use and land cover are intimately linked. For example, transportation and commercial land uses require paved surfaces to accommodate vehicle traffic. Residential uses incorporate paved surfaces, vegetated spaces (e.g., lawns, trees, gardens), and waterbodies (e.g., streams, creeks, ponds).

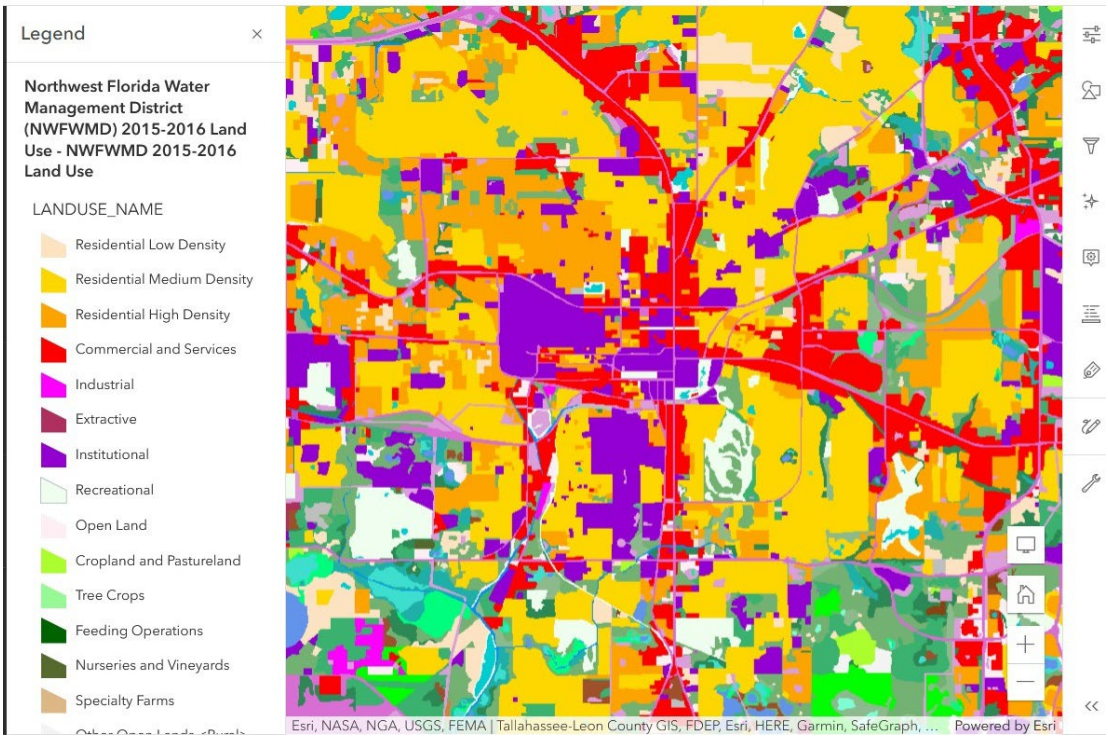

Figure 3. Land uses in Tallahassee urban area. (Data from the Northwest Florida Water Management District)

When varied land uses and hydrologic alterations are superimposed upon the original landscape, a complex system of surface water interactions between natural and human systems develops. A major concern is the chemical contamination of surface waters that cycle through an

urban environment and are then returned to downstream natural waterbodies and ultimately to the oceans. There is a growing awareness that physical contamination of surface waters has been delivering vast amounts of plastics to the world's oceans, where they are accumulating and disrupting marine environments. In this project, a comprehensive analysis of stormwaters in Tallahassee will be conducted to understand how microplastic fibers are distributed and travel through an urban environment.

1.1.2 Hydrogeologic Setting and Surface Water/Groundwater Connectivity

The underlying regional hydrogeologic framework controls stormwater flow in the study area. The city of Tallahassee sits at the upland edge of an east-west trending topographic transition known as the Cody Scarp, which was first identified by Puri & Vernon (1964). North of the Cody Scarp, the terrain is characterized by lakes surrounded by rolling hills of red clay and sand that comprise the Miocene Hawthorn Group and Pliocene Miccosukee Formation geologic units. South of the Cody Scarp, the terrain is flat, has a lower elevation, and is characterized by limestone of the Miocene St. Marks Formation near the surface with numerous sinkholes and other karst dissolution features (Fig. 4) (Pratt et al., 1996; Bostick et al., 2018; Upchurch et al., 2019).

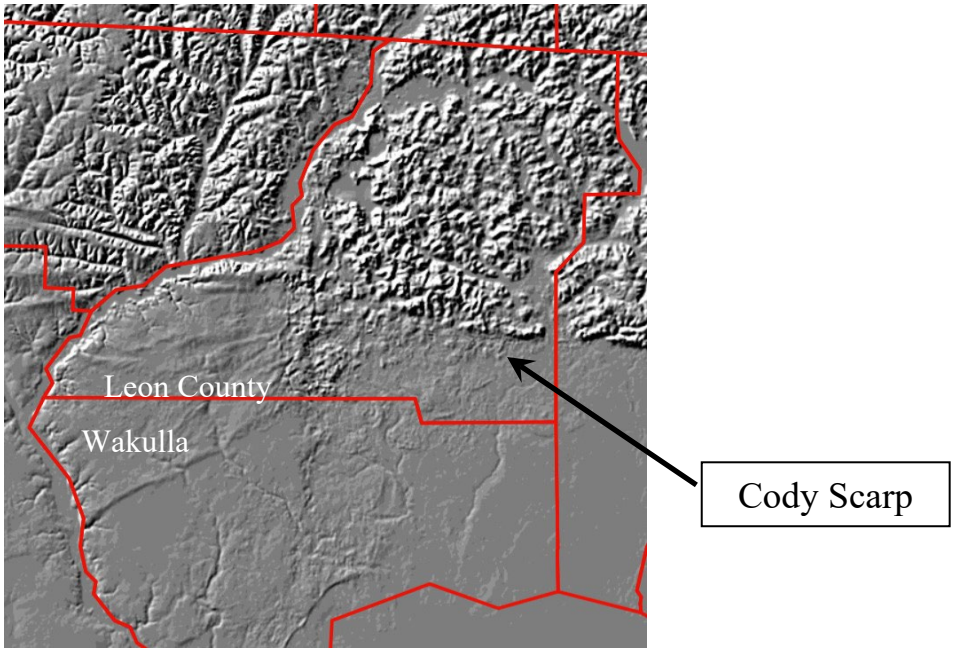

Figure 4. Leon County topographic hillshade image showing drainage patterns and the Cody Scarp. Data from Environmental Systems Research Institute.

The Cody Scarp (Fig. 4) is a topographic expression of the erosional processes resulting from a Pleistocene high sea level. Wave action of the advancing-retreating shoreline and fluvial erosion scoured away the Miocene/Pliocene sands and clays and exposed the underlying Miocene limestone. Percolation of acidic surface waters has resulted in the numerous karsts features south of the Cody Scarp (e.g., sinkholes, springs, disappearing streams) that typify a near-surface, easily dissolved limestone lithology (Upchurch et al., 2019). As a result, the region south of the Cody Scarp to the Gulf Coast shoreline is referred to as the Woodville Karst Plain (WKP), named for the city of Woodville, located in the region.

The Miocene limestone underlying the WKP is part of the Upper Floridan Aquifer (UFA), which provides the regional public water supply in Leon and Wakulla Counties (Fig. 5). North of

the Cody Scarp, the UFA is overlain by low permeability clays and sands (Fig. 5), which protect groundwater in the aquifer from surface water contamination. In the WKP, the UFA is mantled by a thin permeable layer of sand and sediments. This results in extensive connectivity between surface and groundwater south of the Cody scarp, resulting in high vulnerability of the UFA to contamination from surface water inputs.

In addition to precipitation and runoff that infiltrates directly to groundwater, a significant amount of surface stormwater and treated municipal wastewater from the Tallahassee urban area are delivered to the aquifer in the WKP region through 1) downgradient runoff across the Cody scarp and 2) land application of treated wastewater at the northern edge of the WKP. Each of these sources of surface water is discussed below.

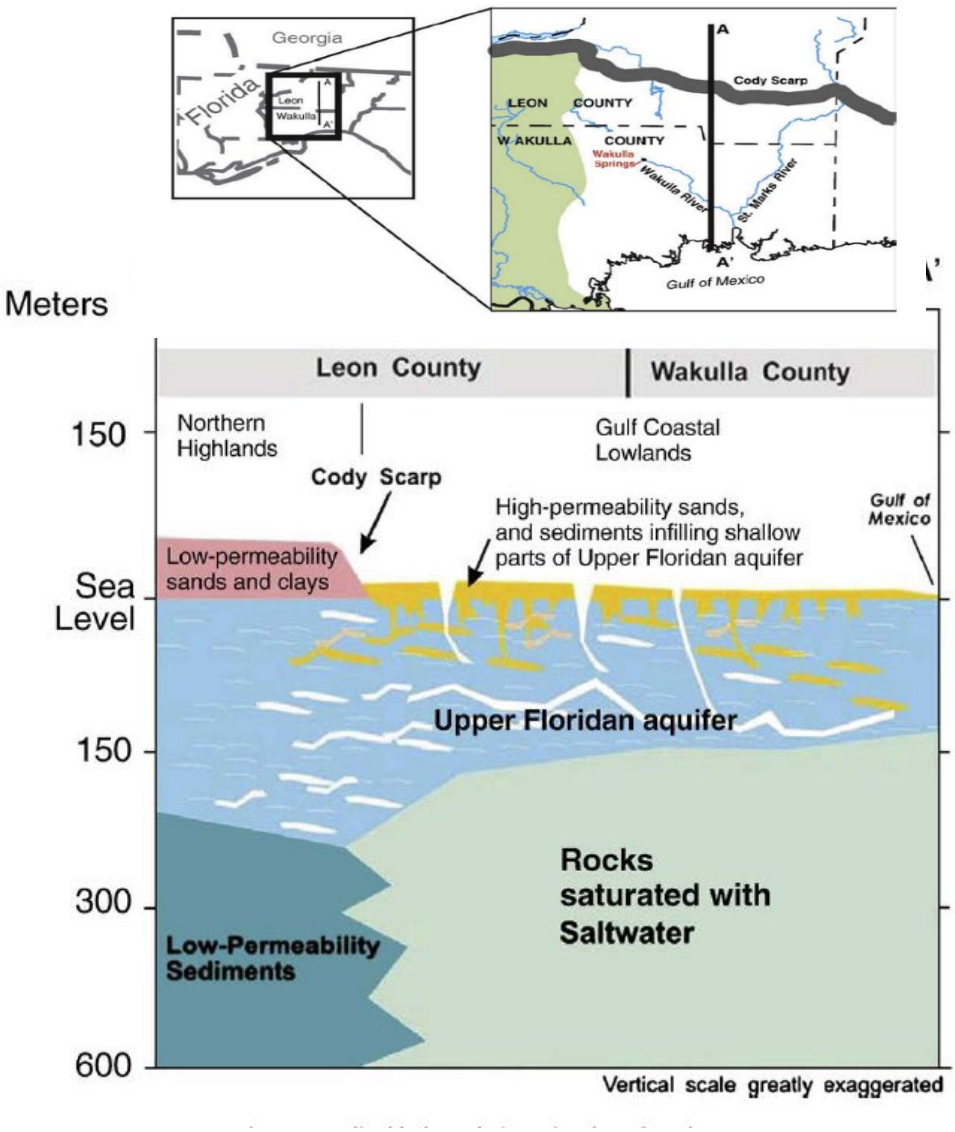

Figure 5. North-south hydrogeologic cross section through Leon and Wakulla Counties showing the Upper Floridan Aquifer that provides public water supply to the region (from Katz et al., 2009).

1.1.3 Runoff

Runoff refers to excess precipitation that flows over the land surface into water bodies. In terrains overlain by impermeable or semi-impermeable materials, rainfall produces more runoff than in terrains with more permeable materials. In the Tallahassee urban region, the presence of clay-rich soils and impervious surfaces such as roads, buildings, and parking lots results in significant runoff that flows into surface water bodies. A large portion of Tallahassee is drained by Lake Munson in south Tallahassee (Fig. 6a). Water from Lake Munson drains southward and eventually disappears into a series of swallets (sinkholes into which a stream flows underground) in the WKP (Fig. 6b).

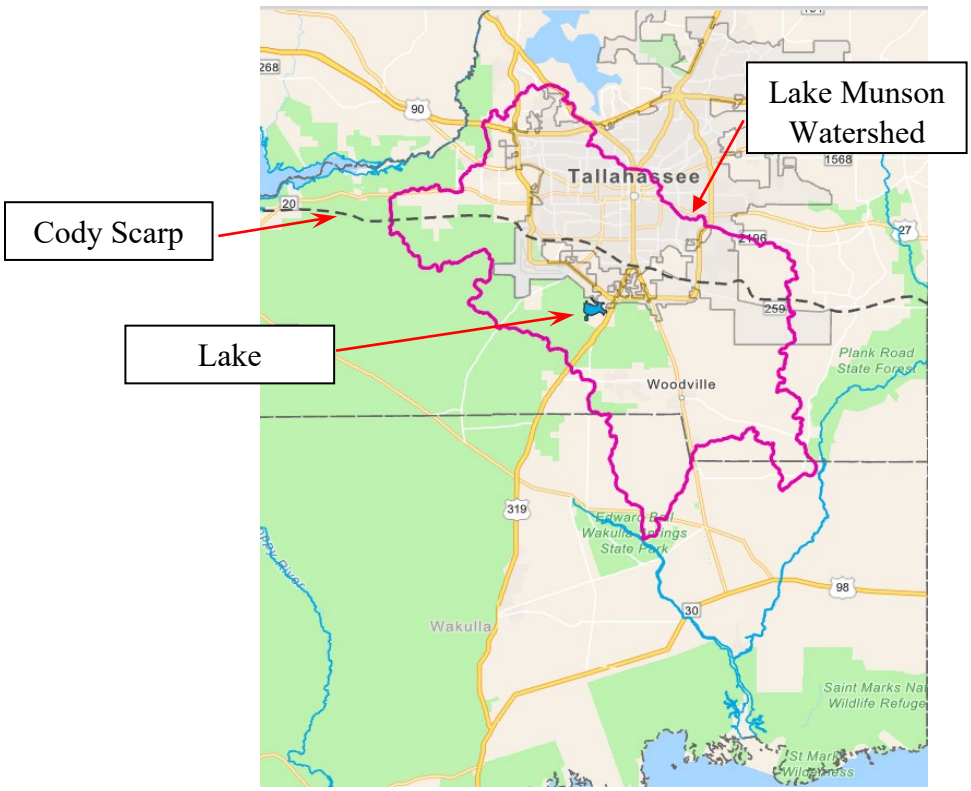

Figure 6:a) Map showing the Lake Munson watershed in Tallahassee. Runoff from the southern and western urban area flows to Lake Munson. Cartography by Katherine Milla.

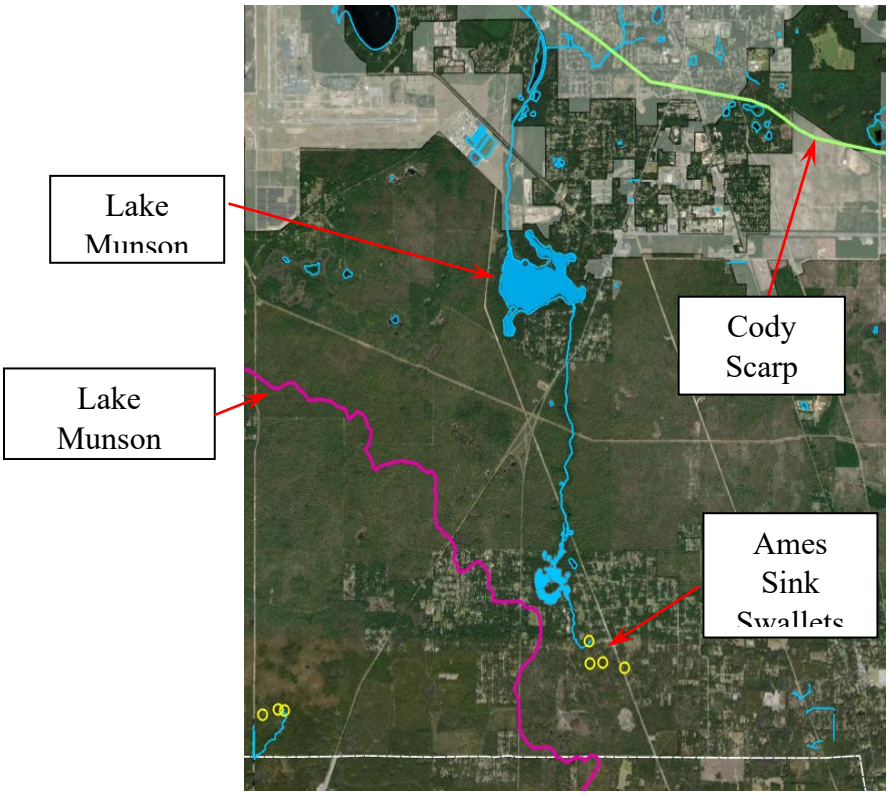

Figure 6b) Map showing the flow of runoff from Lake Munson to swallets in the Woodville Karst Plain.

1.1.4 Wastewater

Tallahassee's wastewater is treated at the Thomas P. Smith (TPS) Water Reclamation Facility, located in southwest Tallahassee. The facility processes an average of 17 million gallons per day (MPD). The reclaimed water is pumped to the City of Tallahassee water reuse facility, where the vast majority is used to irrigate feed and fodder crops at the City's Southeast Farm Spray field (Figure 7). Note in Fig. 7 that both Lake Munson and the spray field are south of the Cody scarp and thus discharge runoff and reclaimed water into the permeable WKP, where they infiltrate the thin sandy soils and recharge groundwater in the UFA and move southward with the hydrologic gradient.

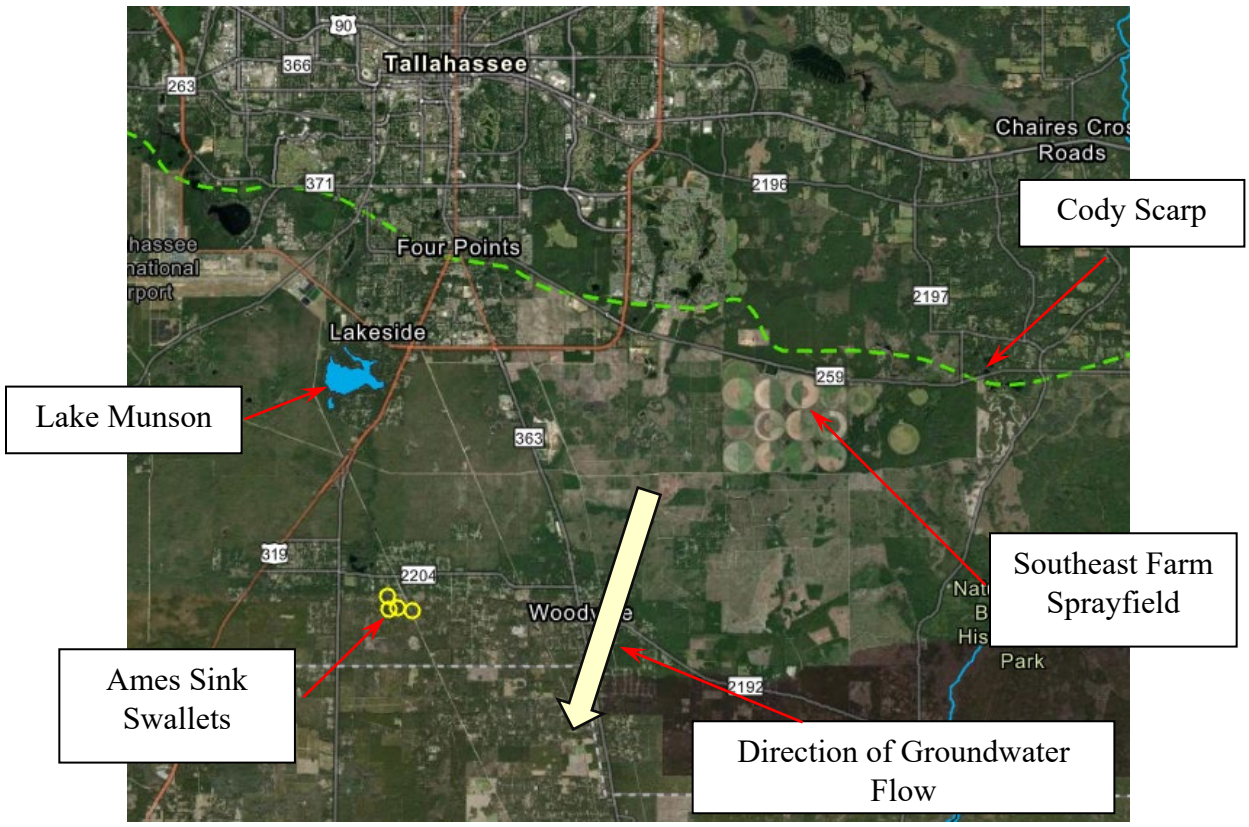

Figure 7. Direction of groundwater flow from spray field to Wakulla Spring. Cartography by Katherine Milla.

Concerns about groundwater contamination in the WKP from the spray field led to several studies of the region's hydrology. Initial concerns focused on nitrate concentrations in Wakulla Springs, located in northern Wakulla County (Figure 8). Wakulla Springs is one of the world's largest and deepest freshwater springs. It is a major tourist attraction and has a significant positive economic impact on the region. Hydrologic modeling, dye tracing studies, and underwater cave diving in and around the spring have demonstrated direct connections from the spray field and Lake Munson swallets to Wakulla Springs (Fig. 8) (FDEP, 2012; Ahmed et al., 2021).

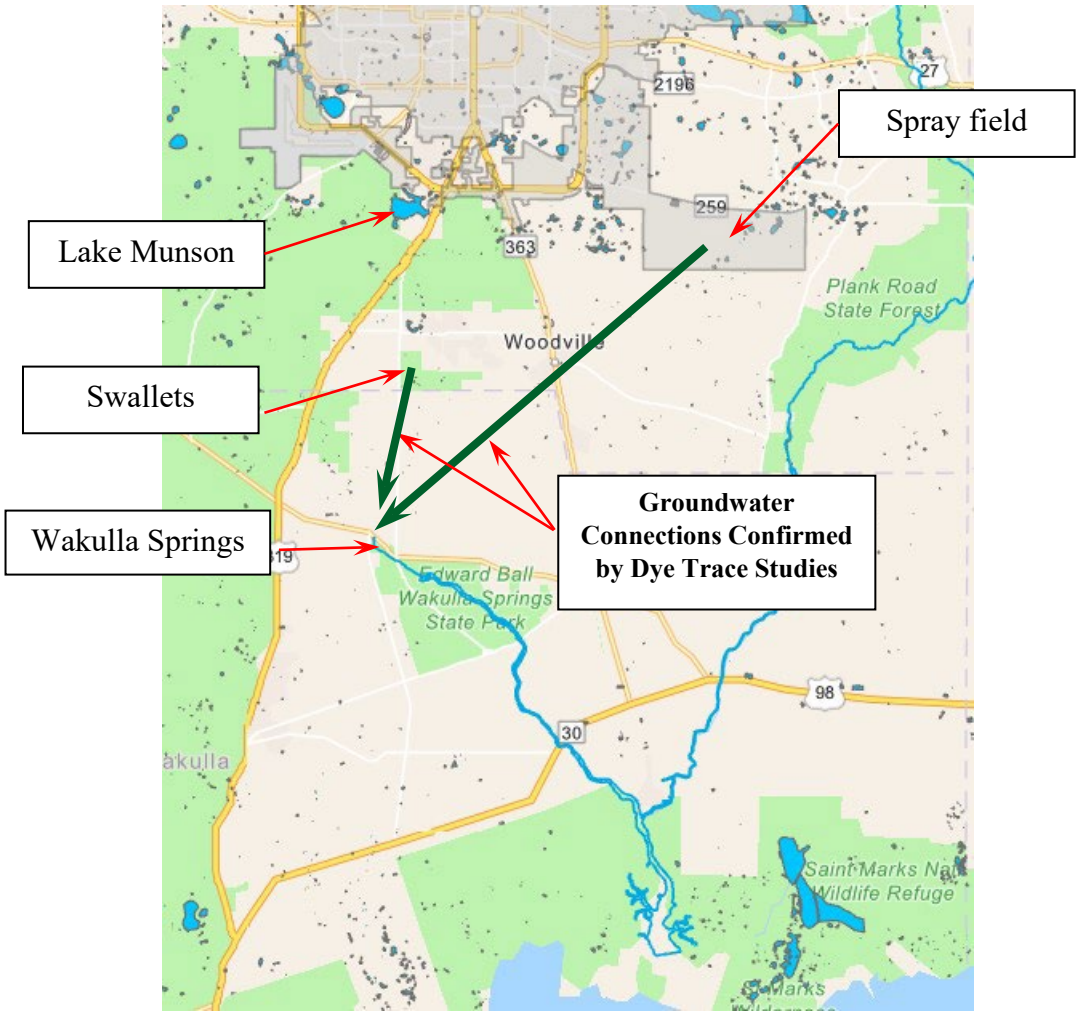

Figure 8. Surface water-ground water connections between Lake Munson and the Southeast Farm Spray field and Wakulla Springs demonstrated by dye tracing studies. Cartography by Katherine Milla.

Awareness of the connectivity between urban storm- and wastewater sources and Wakulla Springs has resulted in a greater understanding of the influence of "upstream" urban hydrodynamics on the quality of downstream groundwater systems. Most hydrologic studies in the Leon-Wakulla Counties region have focused on chemical contaminants (including pollutants such as nutrients and contaminants of emerging concern (CEC)) and natural and anthropogenic chemical components as tracers (such as isotopes and caffeine). In light of recent awareness of the

ubiquity of plastic pollution in the hydrologic cycle, a significant outcome sought for this study is to increase the body of scientific knowledge about the occurrence, distribution, and abundance of microplastic fibers in an urban/rural regional hydrologic system. Due to surface and groundwater interaction complexities, the Tallahassee regional area provides a unique study site for this investigation.

1.2 Goals and Objectives

The goal of this study is to assess the occurrence and distribution of microplastics in interconnected terrestrial surface and ground waters through comprehensive sampling of an urban region. This goal will be accomplished by conducting a comprehensive analysis of the distribution and abundance of microplastic fibers in surface water and groundwater in Tallahassee urban region and in downstream groundwater hydrologically connected to surface water inputs.

CHAPTER 2
LITERATURE REVIEW

2.1 Brief history and definition of microplastics

Plastic has become a widely used material since its creation in 1869 by John Wesley Hyatt as an alternative to ivory. Attempts made in the 1800s to develop synthetic polymers such as polystyrene (PS) and polyvinyl chloride (PVC) for commercial use failed as these materials were too brittle, would not retain their shape, and hence were not commercially viable (Lambert & Wagner, 2018). In 1907, Leo Baekeland developed the first synthetic polymer, Bakelite, produced in mass (Lambert & Wagner, 2018).

Modern forms of polyethylene terephthalate (PET), polyurethane (PUR), polyvinyl chloride (PVC), high-density polyethylene (HDPE) and polypropylene (PP) were developed later in the 1900s (Lambert, 2015; Brandsch, & Piringer 2000). Different types of polymers and plastic formulations have been introduced, and their low density, low resistance to corrosion, low thermal and electric conductivity, and low price contribute to their massive manufacture and use (Shashoua, 2012).

The widespread use of plastics in food packaging, medical equipment (e.g., syringes, surgical gloves, insulin pens, catheters, IV tubes, and other medical materials intended for one-time use) and technological application has led to large quantities of plastic waste in marine and freshwater ecosystems (Gutow et al., 2015). Plastics are long-chain polymeric materials that can be molded and shaped, usually by applying heat and pressure. (Scott, 1999). Plastic wastes resist many environmental influences, and only about 9% of plastics have been recycled (Geyer et al., 2017). The remaining are weathered or broken down into smaller fragments by extreme environmental conditions such as high temperature, intense sunlight, and wave actions in the

ocean. These resulting plastic pieces less than 5 mm in diameter are called microplastics (Arthur et al., 2009).

Microplastics are defined as particles less than 5mm in diameter with no specific lower limit (Zhang et al., 2019; Li et al., 2018; Gago et al., 2016; Cole et al., 2011; Arthur et al., 2009). This definition was modified by Cole et al. (2011) to distinguish microplastics according to their different sources or origin. According to the source, microplastic is grouped into two categories, primary microplastic (those manufactured initially to have a size of less than 5mm in diameter) and secondary microplastic (those that are derived from the breaking down of large plastic materials due to exposure to extreme environmental conditions).

There is still no clear consensus on an all-encompassing definition of microplastics (Barboza et al., 2019; Wagner & Lambert, 2018; Zeng, 2018). Several authors have adopted different definitions and methodologies in the study of microplastic pollution, making comparison of results obtained difficult. Some of the critical aspects in the definition of microplastics are the types and shapes (Frias et al., 2018), upper and lower size limit (Barboza et al., 2018), physiochemical properties, and polymetric matrix (Verschoor, 2015).

There is still disagreement on microplastics' upper and lower size limits. Most studies use the definition by (Arthur et al. 2009) of "microplastics as plastic particles smaller than 5mm in diameter with no specific lower size limit" This definition does not exclude visible components of small plastic spectrum. The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) further endorsed the definition worldwide as plastic particles less than 5 mm in diameter, and this includes the nano-size range (1 nm) as there is no lower size limit (GESAMP, 2016). Other authors refer to the upper size limits ranging from 500 μm to 5mm, while the lower size limits may range from 1 to 20 μm (Ryan, 2015; DeWitte et al.,

2014). Technological limitations exist on the current potential size range limit (20 and 100 μm) identifiable using micro-Fourier Transformed Infrared Spectroscopy (Frias et al., 2018). Hence the need for advanced technologies that could lower this range to 1 μm.

The common shapes and composition of microplastics are pellets, fragments, fibers, films, ropes, filaments, sponges, foams, microbeads, and rubber. However, the most frequently reported in studies are pellets, fragments, and fibers. Color is not a crucial factor in the definition of microplastics but plays a vital role in studying organisms that ingest microplastics, as many visual feeding organisms exhibit color preference (Wright et al., 2013).

Verschoor (2015) proposed an all-inclusive definition of microplastics. "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which is insoluble in water".

This study defines microplastics as plastic particles or fibers less than 5 mm in diameter/length with a lower size limit of 0.45 μm. This lower size limit is a result of the pore size of the filter used while conducting this experiment. In practicality, the lower limit is the resolving power of the stereomicroscope used in this study to detect and identify the fibers, which has a maximum magnification of 40X.

2.1.1 Hydrological Factors in the Florida Upper Aquifer

In Tallahassee and Leon County, a large geologic formation called the Floridan Aquifer provides a natural abundance of water supply. In the central and northern parts of Leon County, thick sands and clay on top of the aquifer provide an impermeable protective layer through which surface water percolates and filters into the aquifer. The southern part of Leon County and the

eastern part of Wakulla County have a thin layer of sand over the limestone formation, resulting in rapid surface water movement directly into the aquifer (Kincaid et al., 2008).

Tallahassee drinking water is pumped from 27 deep wells throughout the city, ranging in depth from 199 to 483 feet. The city's drinking water receives standard chlorination treatment against bacteria and fluorination to prevent tooth decay. The city of Tallahassee public supply wells (COT wells) are used majorly for the withdrawal of drinking water. They are deep into the aquifer and are located in the central and northern part of Leon County, where the presence of sand and clay provides impermeable protection of the aquifer from contaminants in surface water. In contrast, the spray field and Wakulla spring wells are monitoring wells. They are shallow wells located south of the Upper Florida Aquifer.

2.2 Microplastic in Urban Stormwater

Urban areas are significant contributors of microplastics into the environment, with runoff acting as a primary vector in the movement of microplastics from the source to the sink (Auta et al., 2017). The characteristics of urban runoff are complex due to its diverse sources. Runoff is either discharged into nearby water bodies directly or through stormwater systems. Microplastics in stormwater have increased with the increase in the urban population’s production and use of plastics (Campanale et al., 2022).

Common sources of microplastic in stormwater include atmospheric deposition and urban litter, such as single-use plastics derived from tire wear particles formed from friction between vehicle tires and road surfaces (Campanale et al., 2022). Other sources include landfill leachates, laundry of synthetic fabrics, paint fragments, plastic rainwater facilities, and industrial activities (Grbic et al., 2020; Zhang et al., 2022).

Atmospheric deposition of microplastic is widely recognized as a source in urban environments (Müller et al., 2020; Ross et al., 2023). Due to low weight and size, a microplastic particle can be transported a great distance or may participate in local air-ground exchange (Zhang et al., 2020). Microplastics in the atmosphere can undergo wet deposition (resulting from raindrops or snowflakes); dry deposition, which is the settling and finally, bulk deposition, a combination of wet and dry atmospheric deposition. Quantifying the contribution of atmospheric deposition to stormwater microplastic pollution is challenging, with dry deposition most difficult to assess (Smyth et al., 2021).

Wastewater treatment plants (WWTPs) represent a notable source of microplastic contamination in aquatic ecosystems. Microplastics in WWTPs primarily stem from various consumer products such as facial cleansers, exfoliating scrubs, cosmetics, sunscreen, nail polish, hair dye, eye shadow, shower gels, toothpaste, and synthetic textiles, which continuously shed fibers during washing processes (Suaria et al., 2020). Among these, laundry fibers constitute a significant portion of microplastics found in WWTPs. These fibers are released throughout the lifecycle of textiles, including during manufacturing, usage, disposal, and most notably, during laundering. Several factors, including fabric type, mechanical agitation, detergent composition, water temperature, and wash cycle duration, influence the release of microfibers from textiles.

Microbeads, synthetic polymers employed as abrasives and exfoliants in consumer and personal care items, pose a challenge due to their non-biodegradable nature (Suaria et al., 2020). While high temperatures can break them down, wastewater treatment plants (WWTPs) typically lack the means to apply such heat during treatment, allowing these plastic particles to pass through and ultimately enter water bodies. The Microbead-Free Waters Act of 2015 in the United States prohibits the use of microbeads in consumer cosmetic products (McDevitt et al., 2017),

representing a progressive measure against microplastic pollution. However, the effectiveness of this ban has been limited. Microplastic persistence and accumulation in wastewater has a toxic impact on organisms through ingestion, (Wang et al., 2019).

Urban littering is inevitable due to the widespread use of plastics. These litters degrade into microplastics. Litter is grouped into three categories based on its sources. The first is personal products or activities, including clothes, textiles, cigarette filters, personal protection equipment, single-use food containers, and shopping bags (Kole et al., 2017; Huang et al., 2022).

The second source of microplastic litter is landscaping and construction materials/activities, including building insulation, wraps, geotextiles, and ropes. The third includes vehicle parts. Tire and road wear particles formed by continuous attrition of tire treads and road surfaces significantly contribute to microplastics in urban stormwater. Both tire rubber and road pavement contain polymers (Kole et al., 2017).

Microplastics from urban environments end up in various sinks. They can be transferred to nearby water bodies, including streams, lakes, rivers, reservoirs, estuaries, and oceans. Some are deposited in water body sediments or trapped in soil or stormwater retention structures (Shruti et al., 2021).

2.3 Sources of Microplastics in Freshwater Systems

Microplastics enter freshwater environments through various routes. An essential route in one geographical region may not be necessary in another region (Lambert & Wagner, 2018). Three significant pathways of microplastic pollution in freshwater systems include terrestrial inputs, direct release of plastics into surface water, and atmospheric transportation (Isobe & Iwasaki, 2022).

Terrestrial sources of microplastic account for a large amount of microplastics in freshwater. Plastics produced and disposed of on or into soils after use are exposed to extreme temperatures, abrasion, and UV rays that break them into smaller fragments called secondary microplastics (Ng et al., 2018). The resulting microplastics are washed into surface water or leached down the soil profile.

Land-based microplastics include diverse sources (1) Laundering: according to a report by the International Union for the Conservation of Nature, clothes laundering contributes about 35% of the ocean microplastic fibers (Boucher & Friot, 2017); (2) Paints and coatings. Polymers are used as binding agents in paints, which are applied to many different types of surfaces, including roadways, structures, and vessels. Paint particles are often smaller in size due to their brittleness, (3) Vehicle tire wear: a study by Kole et al. (2017) estimated an average per person contribution of 4.7 kg/year of tire wear microplastics in the US, (4) Plastic burning: The burning of plastic trash and electronic waste (e-waste) releases microplastics and additives into the environment, (5) Wastewater treatment plants (WWTPs): Wastewater treatment plants are sources of microplastics in aquatic environments. Wastewaters contain microplastics and polymer additives. Although some wastewater treatment plants have improved their cleanup process, microplastic's fate during wastewater treatment is influenced by its particle densities.

Microplastics can also be released directly into freshwater through fishing, aquaculture, debris, and vessels. Discarded ropes, nets, lines, floats, and traps could be potential sources of microplastics in surface water (Yuan et al., 2019). This fishing equipment is made of plastics and synthetic fiber and may degrade after a long time when exposed to harsh environmental conditions (Yuan et al., 2019).

Atmospheric transportation is another important source of microplastics into freshwater that has received little attention. Microplastics are lightweight and high drag and may be transported over thousands of kilometers before they are deposited (Allen et al., 2019). Their deposition is facilitated by rainfall and snow.

2.3.1 Methods for extracting microplastics from freshwater samples

Various sample treatment methods for microplastics separation are documented in the literature. Examples include density separation with salt solutions (Browne et al., 2011) and use of various reagents, including sodium polytungstate solution (Corcoran et al., 2009); calcium chloride (Stole et al., 2015); sodium iodide solution (Nuelle et al., 2014), zinc chloride (Imhof et al., 2012), and hydrogen peroxide solution with mixed mineral acid to remove the interfering organic matter (Zattler et al., 2013). Most of these methods are either time consuming, expensive, hazardous, and/or require complex laboratory procedures and instrumentation. Additionally, some chemicals change the microplastic morphology, affecting the determination of size distribution and identification of smaller plastics. Some oxidizing acids can cause damage to microplastics that are less tolerant to low pH (Browne et al., 2011; Claessens et al., 2011; Hanvey et al., 2017; Jingyi et al., 2018; Monteiro et al., 2022).

2.4 Uptake and Impact of Microplastics on Freshwater Species

Regardless of trophic level, all aquatic and terrestrial species encounter microplastics due to their ubiquitous nature, and exposure is a prerequisite to manifesting toxic effects (Hammer et al., 2012). In freshwater ecosystems, microplastics could cause toxicity by directly harming the organism. Particularly, filter-feeders like mussels and zooplankton, may ingest microplastics mistaking them for food. This can lead to physical blockages in the digestive tract, reduced feeding efficiency, and impaired growth and reproduction. Microplastics can absorb and concentrate toxic

chemicals from the surrounding environment. When organisms ingest these microplastics, they may also ingest these harmful chemicals, leading to toxicity and potential health issues (Tamis et al., 2021).

The direct hazardous effects of microplastics on biota are majorly due to the size, shape, and texture of plastic ingested by these organisms, leading to physical and histophysiological damage (Lei et al., 2018). Microplastic alters the structure of freshwater ecosystems affecting both producers (bacteria and algae) and consumers, causing physical damages such as breaking cell walls and cell membranes, altering feeding, locomotion, reproduction, nutrition, growth, and survival of freshwater organisms (Du et al., 2021).

Histophysiological damages caused by microplastics depend on the exposure pathways, either waterborne or diet borne. These exposures may affect freshwater organisms' eyes, gills, liver, intestine, and brain (Ding et al., 2018; Jabeen et al., 2018; Lei et al., 2018).

Microplastics combine with organic compounds and metal ions derived from microplastic leachate or the surrounding aquatic environment. Microplastics can act as carriers or sorbents for metal ions present in the environment, and these ions can be released into surrounding water or soil through a process known as leaching. However, the sorption of pollutants may be influenced by competitive interaction with other chemicals (Bakir et al., 2012). Research shows that microplastics and sorbed pollutants accumulate in organisms after ingestion (Scopetani et al., 2018; Hermsen et al., 2018).

2.5 Effects of Leaching Chemicals from Microplastics

Plastic materials are made up of residual starting substances, monomers, oligomers, catalysts, solvents, additives, plasticizers, antioxidants, heat stabilizers, pigments, and non-intentionally added substances (impurities, polymerization by-products, products from its breakdown) (Groh et

al., 2019). Plastic packaging is associated with over 4000 chemicals (Groh et al., 2019). Assessing the composition and concentration of leachable compounds in microplastics is difficult as this depends on the environment's physical, chemical, and biological conditions (Lithner et al., 2012). Also, the total number of plastic chemicals and their mixture toxicity is unknown (Zimmermann et al., 2020).

Chemicals can leach from packages into packaged food since they are not covalently bonded to the polymer matrix. This is relevant, especially regarding food contact materials, as humans are exposed to chemicals that migrate into their foods. Wildlife is also exposed to chemicals leaching from plastic litter (Wooten et al., 2021).

2.6 Microplastics as Vector for Other Contaminants

Microplastics are potential vectors of contaminants as they may adsorb chemical contaminants and get attached to a biological agent. This interaction between the absorbed/attached chemical or biological agents modifies the mobility, aggregation, bioavailability, toxicity and interaction of microplastics with organisms in the environment and increases their risk (Mei et al., 2020).

The chemical composition, surface characteristics, carbon chains, crystallinity, and functional groups of plastic materials (the nature of carbon bonds) play a significant role in their interaction with contaminants and the type of bonding between them. The chemical interactions could be hydrophobic, electrostatic interaction-repulsion forces, van der Waals, or hydrogen bonding (Luo et al., 2022). Chemical contaminants associated with microplastics in a freshwater environment include persistent organic pollutants (POPs), organochlorine pesticides (OCP), polybrominated diphenyl ethers (PBDEs), dichloro-diphenyl-trichloroethane (DDT), polychlorinated biphenyls (PCBs), pharmaceuticals, metals and microbes forming biofilms

(Torres et al., 2021; Yang et al., 2020; Eder et al., 2021). When there is a high concentration and density of contaminants in microplastics, they are considered vectors of contaminants.

2.7 Degradation of Microplastics

Microplastic originates from the degradation of macroplastics, while nano plastics are derived from the degradation of microplastics. The size difference of plastic material in the environments shows that plastics degrade. Four broad degradation mechanisms include photodegradation, chemical degradation, thermal degradation, and biodegradation (Liu et al., 2022).

2.7.1 Thermal degradation

Thermal degradation and pyrolysis are not the same. Pyrolysis is the conversion of long-chain microplastics into substances with small molecular weight by thermal degradation in inert gas at a temperature of about 300ºC to 900ºC. On the other hand, thermal degradation is the breakage of the polymeric backbone, deterioration of the polymer tensile strength, crystallinity, durability, cracks, and alteration of polymer color (Lambert et al., 2014). It can be used as a pretreatment of microplastics to enhance polymer biodegradability (Liu et al., 2021). Thermal degradation occurs in nature through exposure to Ultraviolet (UV) radiation from the sun.

2.7.2 Chemical degradation

Chemical degradation is the depolymerization of polymer to monomers. There are two chemical degradation processes, hydrolytic degradation, and oxidative degradation. Hydrolytic degradation is the decomposition of plastic in the water, and it is influenced by the presence of hydrolysable covalent bonds in plastics. Examples include ester, anhydride, amide, ether, ester amide groups, and carbamide. Hydrolysis efficiency is also affected by factors such as temperature, pH, water activities, and time (Lucas et al., 2008; Liu et al., 2022). Oxidative degradation involves forming hydroxyl and carbon monoxide functional groups by adding oxygen

to polymers (Lambert et al., 2014). This process is induced by light or heat and serves as a biodegradation precursor. Oxygen is a vital degradation factor and it can attack covalent bonds in plastics, thereby generating free radicals.

2.7.3 Photodegradation/photocatalytic degradation

Photodegradation is the degradation of the polymer and cross-linked reactions by UV radiation. Photodegradation in nature is a necessary process that involves chain breaking (Al-Salem, 2009). It begins the degradation of plastic in nature. UV radiation comes in varying wavelengths, the specific wavelengths of UV radiation that can affect the mechanical properties of plastics typically fall within the UVB (280-315 nm) and UVA (315-400 nm) ranges and have different destructive capacities influenced by the nature of the polymer material and the chemical bond. UV radiation also affects the mechanical properties of plastics. Photocatalytic degradation is an environmentally friendly process that utilizes semiconductor materials to degrade microplastics. This process takes advantage of the energy provided by photons (light particles) to initiate chemical reactions on the surface of the semiconductor material (Nakata & Fujishima, 2012).

2.7.4 Biodegradation

Biodegradation is a process where microorganisms mineralize plastics by colonizing and forming biofilms on plastic surfaces. These organisms secrete enzymes that act on the main skeletal structure and chains and depolarize the polymers producing oligomers, dimers, monomers, and other by-products such as carbon dioxide and water. It is environmentally friendly and widely documented. (Zhang et al., 2020; Polman et al., 2020). Biodegradation occurs both in aerobic and anaerobic environment. In an aerobic condition, the product is carbon dioxide and water, while in an anaerobic condition, the product is methane and carbon dioxide (Giacomucci et al., 2020).

2.7.5 Bacterial degradation

Many studies on the degradation of plastics and microplastics by bacteria have been carried out. It was found that Actinobacteria and different *Bacillus* strains could degrade microplastics. Most bacteria that degrade plastics were isolated from landfills, including Proteobacteria, Firmicutes, and Actinobacteria (Matjai et al., 2020). For example, the *Pseudomonas aeruginosa* DSM 50071 isolated from super worm gut *Zophobas morio*, effectively degraded polystyrene in an experimental condition (Kim et al., 2020). In Peninsular Malaysia, a *Bacillus* strain was isolated from the mangrove ecosystem (Auta et al., 2018). One major limitation of bacterial degradation is that it occurs slowly. It takes about 30 years to produce a weight loss of about 1-10 % (Roager & Sonnenschein, 2019).

2.7.6 Fungal degradation

Fungi also utilize microplastics as carbon and energy sources. Fungi can effectively utilize substrates such as PP, PVC, PET, Pa, polyurethane (PPU), polyester, and polystyrene sulfonate (Hu et al., 2021). Intracellular processes such as detoxification, adaptation abilities, and enzymatic processes that release hydrolase influence fungi degradation of plastics. The marine fungus *Zalerion maritimum* utilized polyethylene for growth, reducing the size and mass of polyethylene particles (Paca et al., 2017). When compared to bacteria, fungi have more advantages. They showed greater PE degradation (Muhonja et al., 2018) and can attach to hydrophobic substrates by synthesizing hydrophobin. Fungi adapt to various environmental conditions (Raghukumar, 2017). Fungi are relatively understudied in terms of microplastic degradation, so there is a need for more research.

2.7.7 Enzymatic biodegradation

Almost all polymer degradation that occurs in nature requires enzymatic degradation. It is one of the major principles involved in biodegradation. (Zurier & Goddard, 2020). Enzymatic degradation relies on well-defined catalytic reactions and enzyme species and requires a long incubation period. It is still an essential tool in plastic degradation. Both bacterial and fungi degradation of microplastics are mainly associated with microbial enzymes. However, the major limitation of these processes is that the purity level of naturally occurring enzymes is low. It takes a long time for naturally occurring enzymes to degrade plastics. However, the introduction of biotechnology, which has led to the genetic engineering of these enzymes, has increased its efficiency, making this method of degradation superior (Liu et al., 2022).

2.7.8 Algae degradation

Algae can degrade plastic materials and synthesize biodegradable materials with better water resistance and mechanical properties than traditional bio-based degradable plastics (Wen et al., 2020). Algal degradation is a potential technique as it is easy to grow algae and there is a short harvest period, which is time and land-efficient (Tang et al., 2020). When algae colonize plastic surfaces, they produce extracellular polysaccharides and lignin on plastic surfaces that help degrade them (Sarmah & Rout, 2018). Some algae that degrade plastics include the green alga *Scenedesmus dimorphus*, the diatom *Navicula pupula*, and the blue-green alga *Anabaena spiroides* (Ramachandran et al., 2017). Some bacteria and fungi capable of plastic degradation must be better adapted to the marine environment where most plastic waste is deposited. Algae overcome this drawback. Algae can also produce PETase (Liu et al., 2022).

2.7.9 Degradation by other organisms

In recent studies, several insect species can eat and degrade plastics, particularly the larvae of wax moths, darkling beetles, meal moths, and meal worms. For example, the Indian meal moth *Plodia interpunctella* can ingest and degrade polystyrene, larvae of yellow meal worms *Tenebrio molitor*, greater and lesser wax moths *Galleria mellonella* and *Achroia grisella* degrade polyethylene and polystyrene, respectively (Yang et al., 2014; Kundungal et al., 2019).

2.8 Plastisphere and microplastic degradation

Specific groups of fungi, algae, bacteria, and viruses play a significant role in biofilm formation that helps in degrading microplastics. Plastispheres are biospheres formed on plastic surfaces. Their hydrophobic nature encourages the development of microorganisms on plastic surfaces (Gosh et al., 2019). Microorganisms colonize microplastic by attaching and forming a matrix. These microbes alter the physiochemical characteristics of the substrate. (Guasch et al., 2021; Lobelle, & Cunliffe, 2011). Plastisphere is a complex community of microorganisms from autotrophs to heterotrophs (algae and cyanobacteria) and (fungi, protozoa, and bacteria), respectively.

Microorganisms are a potential tool for plastic waste clearing in surface water by breaking down its polymeric structures into monomers utilized by these organisms as energy sources. Microorganisms have a high adaptive ability to survive in harsh environmental conditions and break down complex polymers such as ethylene, pectin, lignin, keratin, and chitin (Gosh et al., 2019).

There is a need for a good understanding of the ecology, efficiency, and mechanisms of microbial communities attracted to plastic surfaces and environmental factors like high temperature, intense wave action, and UV radiation that break down plastics. Factors such as pH,

moisture, temperature, and the nature of the polymer influence the thickness and composition of biofilm developing on the surface (Roger & Sonnenschein, 2019). The secretion of enzymes and polysaccharides initiates the chemical degradation of microplastic. While physically degrading polymers into monomers that can serve as energy sources. Finally, microbes convert these monomers into a secondary metabolite that is released back into the environment as waste (da Luz et al., 2014).

Microplastics can be removed from surface water through processes like biofouling and biodegrading. The number of microbial communities identified and explored for the degradation of microplastic is limited. It is because of the problem associated with isolation, identification of microbes, and laboratory culturing, which can only account for about 1% of the entire microbial community (Kumar et al., 2021).

Correctly understanding the molecular mechanism of microbial communities degrading microplastic is required. The metagenomic approach would be a great tool, and most importantly, bioinformatics and sequencing techniques will help better analyze many environmental samples (Shilpa et al., 2022).

2.9 The role of metagenomics in microplastic remediation

Scientific studies have identified and explored microbial communities that can degrade plastics (Kumar et al., 2021). Very few microbes have been identified due to the limitations associated with the laboratory culturing of microorganisms. There are limited numbers of microorganisms that can degrade microplastics. However, with a metagenomics approach, a better understanding of the microbial community's molecular mechanism could help overcome the challenges associated with culturing.

With metagenomics, DNA can be isolated directly from the environment without isolating its pure isolates, and many microbes can be identified simultaneously without trying to identify them individually. Community genomes' function, structure, adaptability, survival, and fitness could be well understood. Hence, using sequencing and various omics tools to engineer microorganisms and their metabolisms could feasibly increase plastic degradation processes (Zhu et al., 2022).

Microbial profiling 16s ribosomal RNA based on sequencing of conserved and hypervariable regions is a potential approach for identifying plastic-degrading microbes. The microorganism can upcycle microbial degradation waste into numerous valuable products, such as *Pseudomonas stutzeri* isolated from PET waste, to upcycle the resulting monomers (TPA) from PET degradation into PHB (Liu et al., 2021).

CHAPTER 3
MATERIALS AND METHODS

3.1 Sample Collection and Laboratory Analysis

3.1.1 Sample Collection

Freshwater samples were collected from different components of the hydrologic cycle in Tallahassee and downstream locations, including stormwater ponds, lakes, springs, drainage ditches, streams, groundwater monitoring wells, the Tallahassee Wastewater Reclamation Facility, Tallahassee municipal drinking water wells. Sample locations and sampling dates are shown in Appendix 1. Figure 9 shows a map of sample locations. Figures 10 and 11 show an example of field location and sampling method.

Samples were collected in clean 1000 ml plastic bottles rinsed with filtered water (see filtration method below). In the field sample bottles were rinsed three times with the water to be collected before collecting a 1000 ml water sample. Duplicate samples were collected at a subset of locations to assess sampling variability. Sample blanks were prepared in the laboratory by filling rinsed 1000 ml plastic bottles with filtered tap water. In the field sample blank bottles were opened at the sampling site, allowed to stand open during sample collection and recapped. Blanks were processed using the same techniques as field samples. These blanks were used to assess combined microplastic contamination from laboratory prep procedures, atmospheric contamination in the field, and contamination during sample processing.

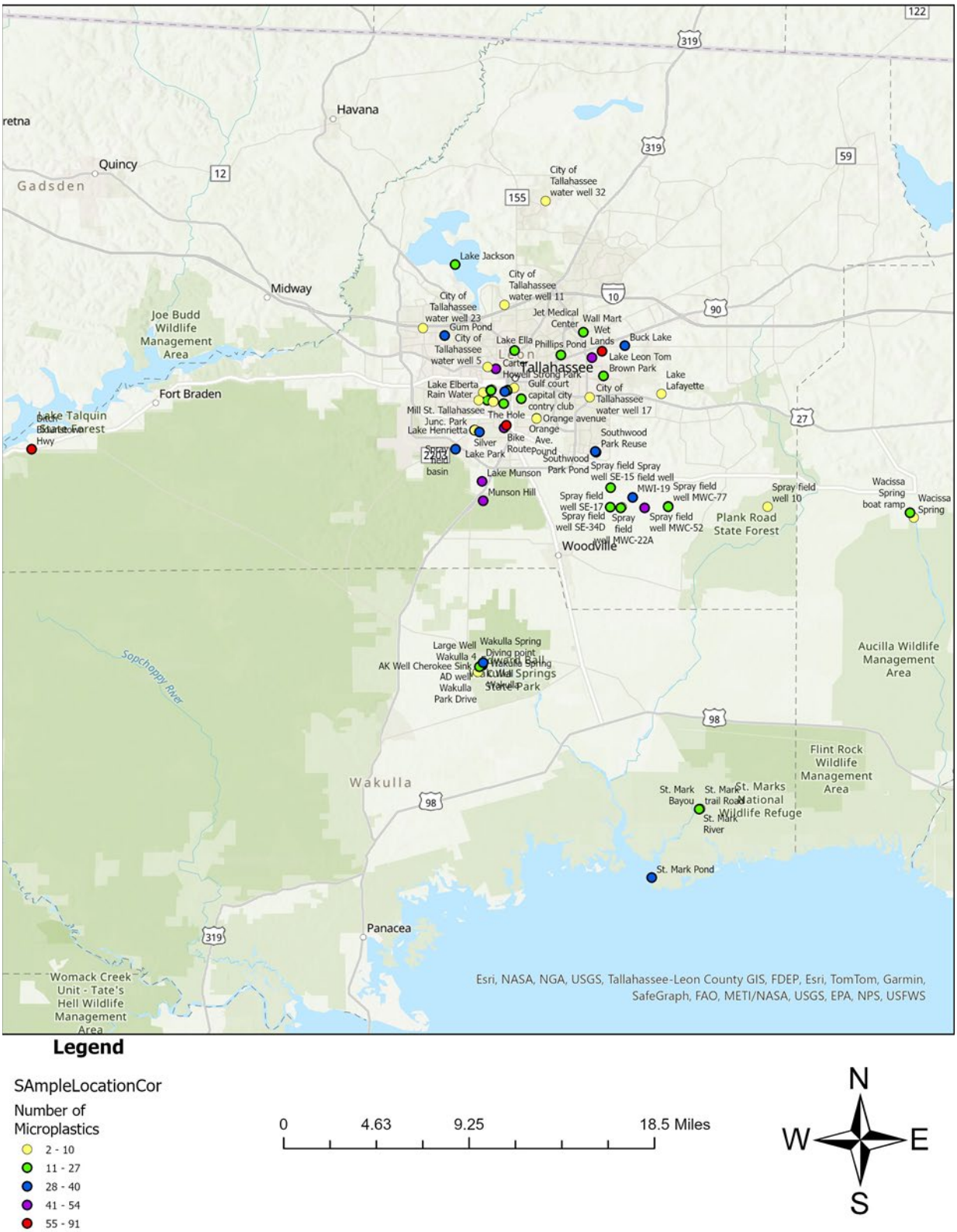

Figure 9. Map showing sample locations.

Figure 10. Photograph of plastic debris in Lake Henrietta

Figure 11. Photograph showing collection of water sample from a drainage ditch.

3.1.2 Sample Preparation and Extraction

Samples were initially prepared using the method from Florida Microplastic Awareness Project (https://flseagrant.ifas.ufl.edu/microplastics/) Volunteer Manual (McGuire, 2017). This method involves filtration of 1L water samples with a hand vacuum pump through a 0.45 μm gridded filter. Samples with sediment and debris are allowed to sit in a separatory funnel until suspended solids have settled and the solids are tapped off and removed. Using this method, vacuum filtration of most untreated freshwater samples collected for this study required exceedingly prolonged (several hours) filtration times, a phenomenon that is apparently not as common with marine samples. This was not a plausible method for this study as it involves lots of samples, hence the need for a simple and effective method for separating microplastics from freshwater samples. After researching the literature on different sample treatment methods for separating microplastics, a new treatment method was developed for this project to reduce filtration times.

3.1.2.1 Testing the optimum extraction method

Two commonly available oxidizing agents, sodium hypochlorite (NaOCl, the oxidizing agent in household chlorine bleach) and hydrogen peroxide (H_2O_2, readily available as a wound disinfectant) were chosen to test their effectiveness in reducing sample filtration times. Although the specific causes for long filtration times are not obvious, it is assumed to be related to organic matter such as algae clogging the filter.

Freshwater samples from different stormwater ponds in the Tallahassee urban area were used to conduct the experiments. During informal preliminary trials, samples were treated with solutions of either chlorine bleach or hydrogen peroxide in different concentrations and allowed to sit for different lengths of time before filtering. Household bleach (CLOROX Performance

Bleach) with a concentration of 8.3 % NaOCl and hydrogen peroxide with a concentration of 10 % H_2O_2 were used as treatment reagents. Different volumes of the reagents were added to samples to attain different concentrations of sodium hypochlorite and hydrogen peroxide and different waiting times were tested for their ability to improve the extraction time of microplastic from freshwater samples. Results from these initial trials clearly indicated that bleach solutions were more effective in reducing filtration times than the hydrogen peroxide solutions. Addition of up to 300 ml hydrogen peroxide to 1000 ml sample volume with a waiting time of 24 hours still resulted in filtration times exceeding one hour, whereas bleach additions reduced filtration times to as little as five minutes.

Following the result obtained from the trials, a more detailed study on NaOCl was conducted to determine the optimum combination of NaOCl concentration and waiting time for the freshwater samples. Different volumes of bleach were added to each 1000 ml sample. Thus, 1000 ml of freshwater samples from each site were treated with 20 ml, 50 ml, 100 ml, and 300 ml of 8.3% NaOCl. For each concentration, samples were allowed to sit for 5 hrs. or 24 hrs. to determine the better holding time for the oxidation process to complete. Samples were then filtered, and filtration times were recorded. The final concentrations of NaOCl in each treatment solution (shown in Table 1) were calculated using the formula below:

$$\text{NaOCl concentration} = \frac{\text{Volume of bleach X Concentration of NaOCl}}{\text{Total volume (volume of bleach + volume of water)}}$$

Table 1. Table showing NaOCl concentrations for each treatment

Bleach volume (ml) added to 1000 ml sample	NaOCl concentration in treated sample
20	0.16 %
50	0.39 %
100	0.75 %
300	1.92 %

3.1.2.2 Testing the effect of sodium hypochlorite solution on microplastics

When developing a new analytical procedure, it is essential to determine if and how the procedure affects the analyte. Although it has been established that plastics persist in the environment, they can still experience degradation when exposed to specific chemicals (Chamas et al., 2020). The effect of sodium hypochlorite treatment on plastic fibers was evaluated by placing various natural and synthetic fibers in a Petri dish, covering the fibers with a 9 % bleach solution (0.75 % NaOCl), and allowing the samples to sit overnight. The samples were then dried on a hot plate and examined under a microscope.

3.1.2.4 Sample extraction, filtration, and identification

Microplastics were extracted by treating 1000 ml water samples with 0.75 % sodium hypochlorite solution as discussed above. Samples were treated based on the level of clarity, organic matter content, particles, and sediments contained in the samples as determined by physical examination. Water samples with sediments and particles were placed in a separatory funnel where they were allowed to sit for at least 24 hrs for easy removal of sediments and particles from the samples, after which 0.75 % concentration solution of NaOCl was added and allowed to

sit for 48 hrs. Clear samples, with no visible particles and sediments, were treated directly with 0.75 % NaOCl and allowed to sit for 48 hrs.

Treated samples were filtered using a three-chamber filtration manifold attached to a Rocker 400 vacuum pump. 47 mm diameter, 0.45 µm pore size filter were loaded into 300 ml filtration funnels and inserted into the manifold chambers. The funnel was covered with a cap to prevent contamination of the samples from air-borne fibers. The pump filtered the samples by creating a vacuum that drives water through the filter. Fig. 12. shows the filtration setup.

Identification and counting of microplastics: after the filtration process were completed, filter papers were transferred into a clean petri-dish, allowed to dry, and observed under a 10x-40x zoom stereo microscope. The number of plastic pieces was counted and documented.

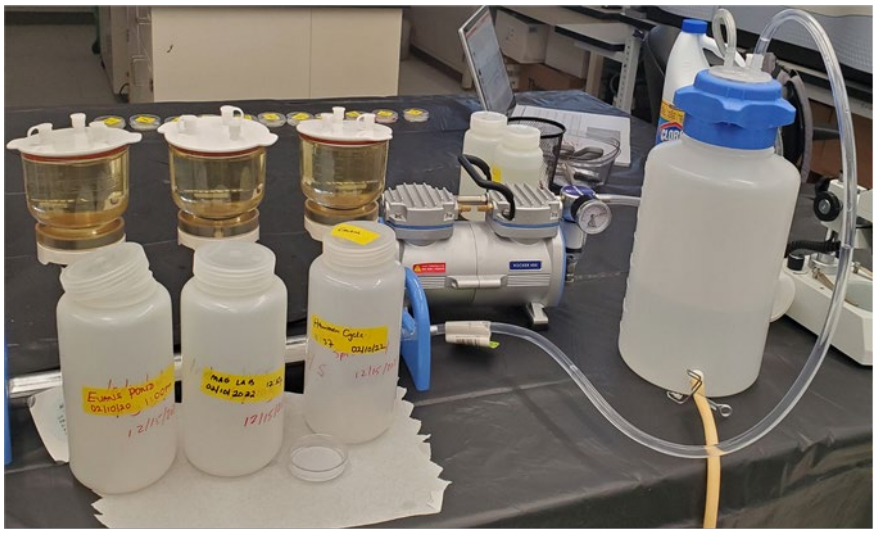

Figure 12.A filtration setup consisting of a pump, filtration funnels, and a filtrate collection unit

3.1.2.5 Differentiating plastics from natural fibers.

Microplastic fibers were differentiated from natural fibers using methods developed by the Florida Microplastics Awareness Project (McGuire, 2017). The method involves using

tweezers to pick up the fiber from the Petri dish while viewing it under the microscope, placing the fiber approximately one cm above a candle flame for about one second, returning it to the Petri dish, and observing it. Fibers that burn into ash that can be crushed are natural, while microplastic fibers melt, coil up or produce ash that cannot be crushed when pricked with tweezers (De Witte et al., 2014) (Fig. 13). Fibers can also be viewed under a compound microscope to check for the presence or absence of cellular structures. Microplastic fibers do not have cellular structures, but natural fibers do (Hidalgo-Ruz et al., 2014). Microplastic fibers should be equally thick throughout their length but are not always homogenous in color.

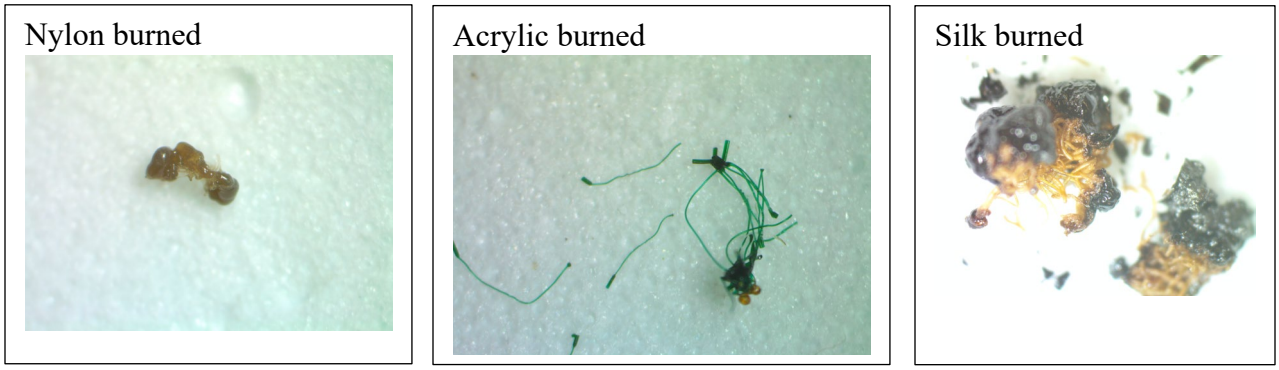

Figure 13.Photographs showing examples of burned plastic and natural fibers.

3.1.2.6 Quality assurance and Control Procedure

Laboratory equipment (filtration funnels, filtration chambers, sample bottles, treatment plates, and Petri dishes) was washed and rinsed with filtered tap water three times. The rinse water was filtered using the above filtration method. During sample treatments and filtration, samples were covered with plastic caps to prevent contamination. During the identification and counting of microplastics, plastics were tested using the hot tweezers method and by burning over a candle to ensure that natural fibers were not counted as plastics.

3.2 Data Analysis

Samples collected for this study were separated into groups to examine the possibility that microplastics can be used as an indicator of surface and groundwater interactions. Stormwater reservoirs were sampled to test the assumption that urban surface waters are contaminated with microplastics by comparing them to sample blanks. Treated wastewater from the SFRF and associated monitoring wells was sampled to test the assumption that wastewater is contaminated with microplastics (presumably primarily from laundering cloth made from synthetic fabrics). The municipal public supply wells were sampled to test the assumption that deep aquifer groundwater (not subject to mixing with surface water) is not contaminated with microplastics.

The overall approach to statistical analysis was designed as follows. Statistical tests include ANOVA, If ANOVA indicates a difference, then the Levene's test was performed to test for variance homogeneity. Since the variance was not homogenous the Dunnett T3 test was applied to evaluate mean differences among the individual groups (Fig.14). Graphical representations were constructed using basic box plots in IBM SPSS grad pack version, with charts also created in Microsoft Excel. Results were interpreted and recommendations for further study were made.

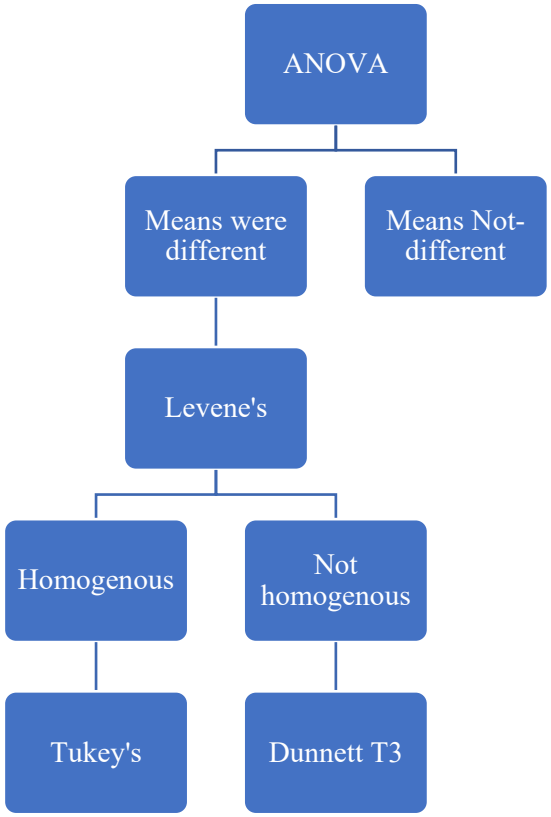

Figure 14.Sequential steps in statistical analysis.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Extraction of Microplastics from Freshwater Samples

Trial results indicated that the 0.16 % NaOCl treatment had minimal effect on filtration times and that 1.92 % NaOCl treatments demonstrated no significant advantage over the 0.75% treatment. More extensive testing, as described above, was done on the 0.39 % and the 0.75 % NaOCl concentrations. Ranges of filtration times for samples from each pond site treated with 0.39 % and 0.75 % and subjected to 5-hr or 24-hr waiting times are shown in Table 2. The result showed that 0.75% NaOCl concentration and a waiting time of 24 hours was generally the best combination for most of the samples in this study. For a few of the samples this combination was still not enough to reduce filtration times to a reasonable length. For these samples a longer waiting time of 48 sufficiently reduced filtration times. NaOCl reduce through oxidation the organic matter, algae and other materials (leaves and wood particles) that clog the filters. This treatment also reduces the number of natural fibers, hence reducing filtration time and the time spent in counting the microplastics trapped by the filter.

Table 2. Average filtration times of samples treated with different NaOCl concentrations waiting times

	NaOCl Concentration	
	0.39 %	0.75 %
Filtration time range for samples with 5-hour treatment waiting times	40-60 mins	18-30 mins
Filtration time range for samples with 24-hour treatment waiting times	15-25 mins	5-10 mins

4.2 Effect of Sodium Hypochlorite Solution on Microplastics

Photographs of the fibers were taken before and after treatment application for comparison (Figures 15 and 16). The synthetic fibers included fabrics made of nylon, acrylic, polyester, acetate, satin, rayon, spandex, and polyvinyl chloride (PVC). Natural fibers included cotton, cat hair, silk, and wool fabrics.

The natural fibers (silk, cat hair, wool, and cotton) were oxidized by NaOCl as shown in Fig. 15. The cat hair was completely oxidized with nothing observable left in the Petri dish. The silk, wool and cotton were almost completely oxidized with some particles left in the Petri dish. The plastic fibers were not affected by the treatment (Fig. 16). Acrylic, rayon, polyesters, spandex, and PVC fabrics displayed no observable changes either in color or in size. The nylon, and acetate satin displayed some color changes, presumably due to oxidation of the fabric dyes, but the fibers remained intact.

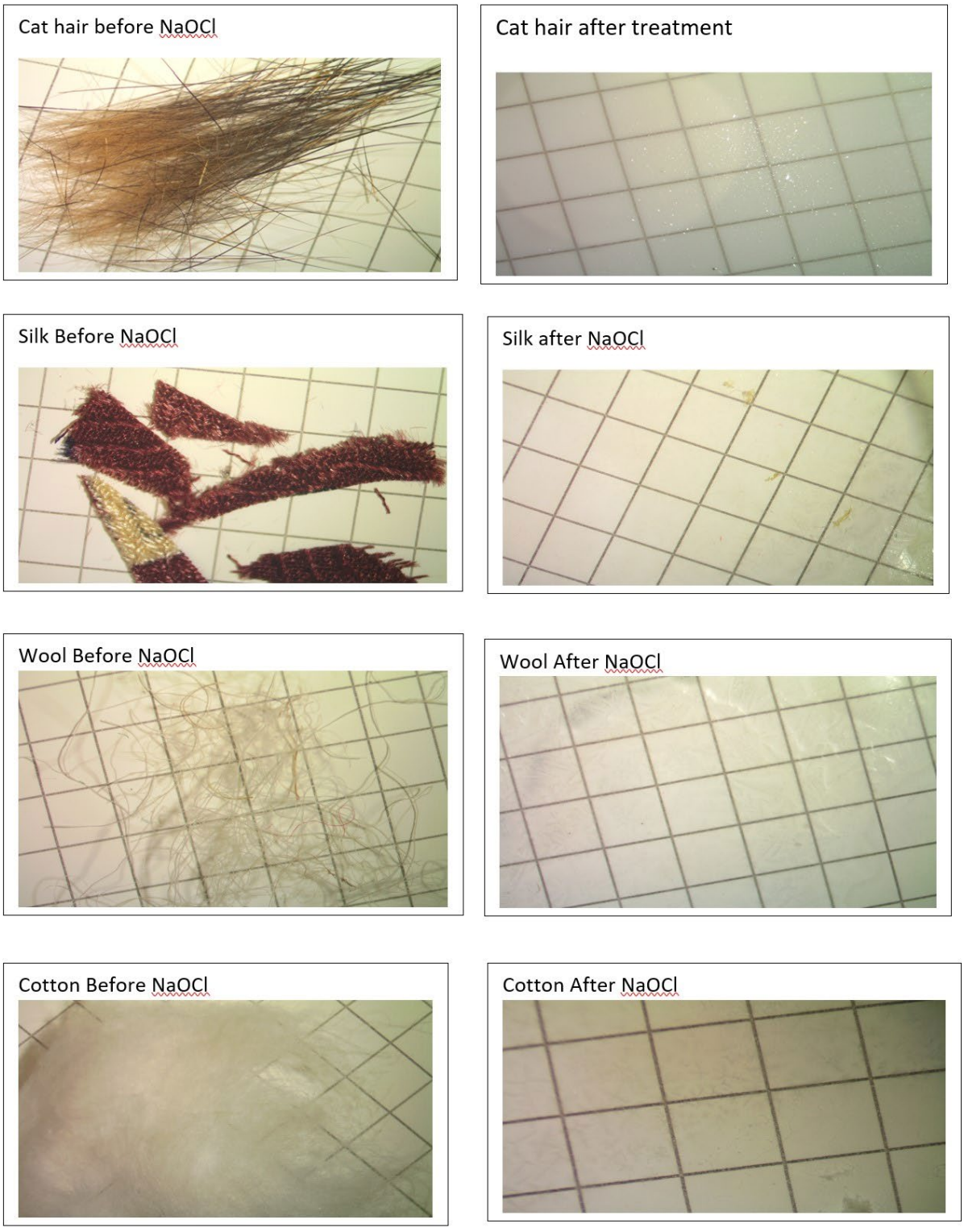

Figure 15. Photographs showing examples of natural fibers before and after treatment with 0.75% NaOCl solution with a waiting time of 24 hours.

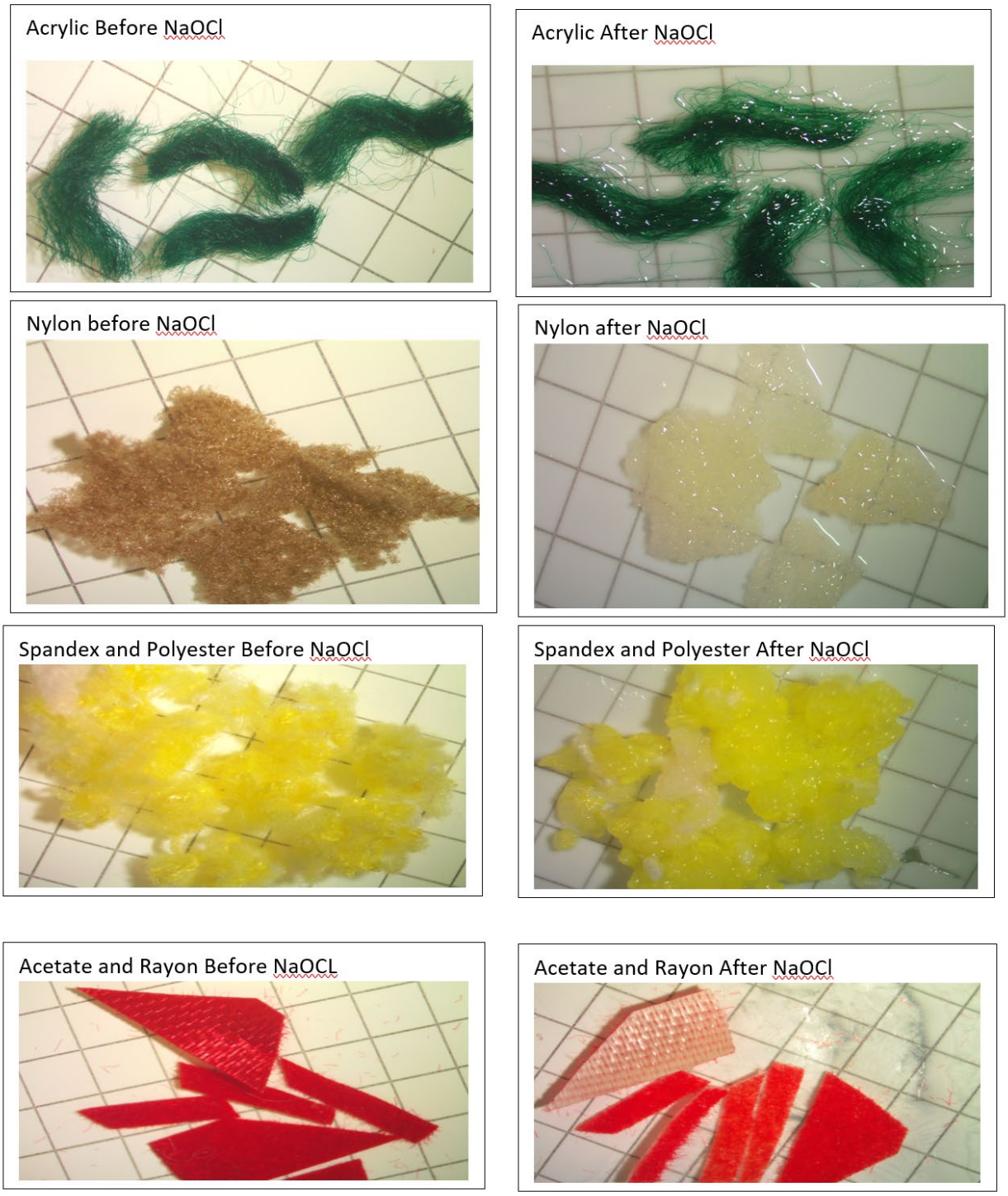

Figure 16.Figure 16. showing plastic fibers before and after treatment with 0.75 % NaOCl and a waiting time of 24hrs

4.3 Comparison of Microplastic Counts Across Different Freshwater Groups

Most microplastics identified in this study were in the form of fibers. Other forms include microbeads and plastic fragments. Microplastic counts for each sample, including replicate samples and blanks, are shown in Appendix I, II, and III. Results of Levene's test for equality of variances are found in Table 2 of Appendix IV. The result showed the variances were not equal at a p value of < 0.001. ANOVA showed that means were significantly different. Result of mean separation (Dunnett T3 test) are found in Table 5 of Appendix IV.

Water samples from the city of Tallahassee public water supply wells (referred to as COT wells) had the lowest microplastic count (Figure 17). Statistical analysis revealed that the microplastic counts in the COT wells were significantly lower compared to those in the Wacissa Springs, Figure 18. Urban surface water and treated wastewater from the Spray-field are found to be contaminated with microplastics. Stormwater, Spray-field well water, and Reuse water exhibit significantly higher microplastic levels compared to the COT wells. Urban areas serve as prominent sources of microplastic pollution, with runoff acting as a principal vector. Microplastics originating from urban surface water and wastewater are conveyed to connecting springs and groundwater. Highest microplastic counts were observed in stormwater and Wakulla Spring, Figure 18.

Comparing two springs located in rural areas, Wacissa Springs, which does not receive urban inputs, exhibited significantly lower microplastic levels ($M = 34.75$, $SD = 9.953$) compared to Wakulla Spring, which receives water from the spray field and Lake Munson ($M = 15.38$, $SD = 10.155$), $t(14) = 3.854$, $p = <0.001$, Cohen's $d = 1.011$ (Table 3). This finding is consistent with dye tracing studies, indicating the transport of fibers from the Spray-field and Lake Munson into Wakulla spring.

A broad spectrum of microplastic counts was observed across stormwater samples, ranging from negligible levels in certain ponds to exceeding 90 counts per liter in others. Analysis through boxplot visualization revealed a bimodal distribution within the stormwater dataset. The highest microplastic counts were observed in water samples collected during or shortly after rainfall events, possibly due to the deposition of suspended microplastics from the atmosphere.

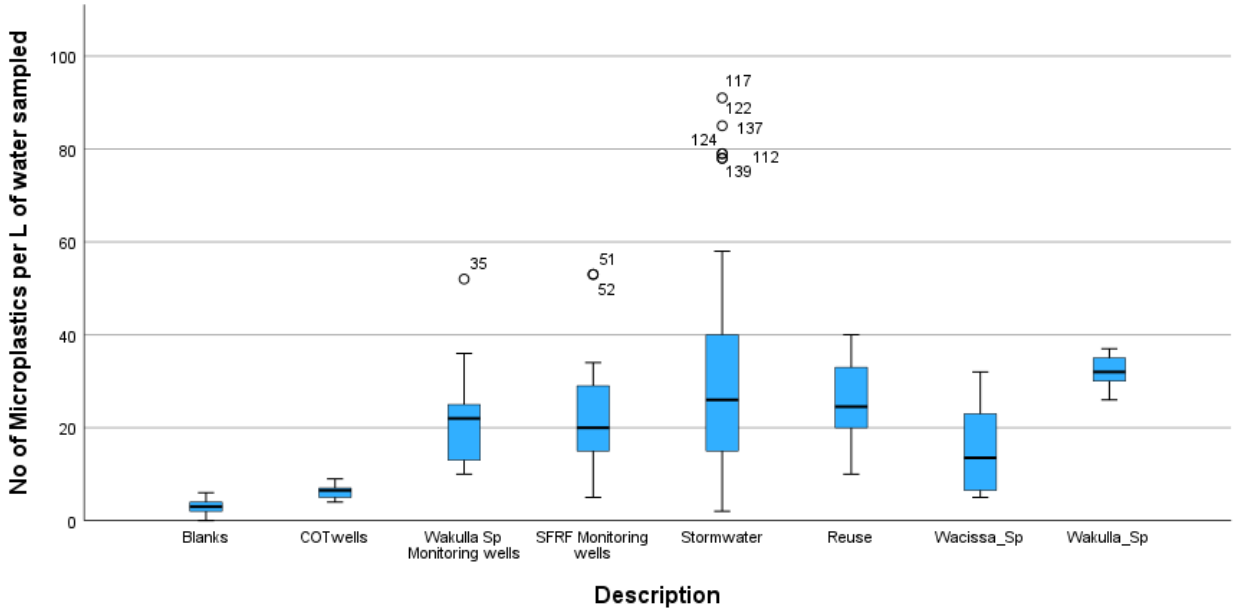

Figure 17. Comparison of microplastic counts across various water groups. The Blanks are sample blanks prepared in the laboratory as described in Chapter 3, COT wells refer to the City of Tallahassee public supply wells, and Reuse water refers to the Spray-field irrigation water.

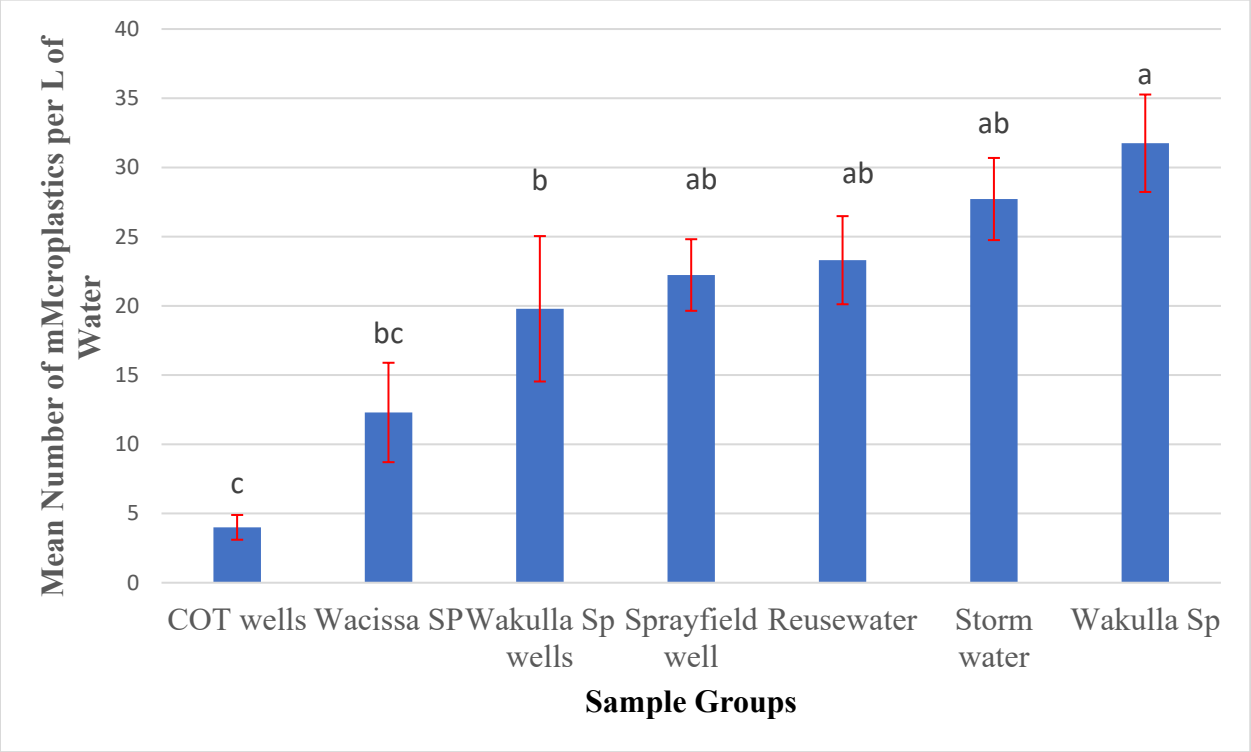

Figure 18. The occurrence and distribution of microplastic across various water groups, means with different letters are significantly different at an alpha level of < 0.05 using Dunnett T3 test for significance.

Table 3. Independent t-test comparing means of Wakulla sp. and Wacissa sp.

Groups	**Number of Samples**	**Means**	**SD**	**t-value**	**DF**	**P value**	**Cohen's d**
Wakulla Sp.	8	34.75	9.953	3.854**	14	<0.001	1.011
Wacissa Sp.	8	15.38	10.155				

4.4 Distribution of Microplastic Fibers in Groundwater (Upper Florida Aquifer)

An analysis of wells across the study area was conducted to determine whether microplastic fibers in urban stormwater and spray field water is transported through the soil into the Upper Florida Aquifer (UFA). Microplastic fibers were seen in every 1000 ml of groundwater sampled. Fig.17 shows that the COT wells are significantly lower in fibers than the Spray-field monitoring wells and Wakulla spring monitoring wells.

The low number of microplastic fibers in COT wells could be attributed to the depth into the aquifer and the presence confining layer (layers of clays and sand) with low permeability that protects the aquifer from contaminants. While the high amount of microplastic fibers in Spray-field monitoring wells and Wakulla spring monitoring wells indicates the contribution of urban stormwater ponds and WWTP to groundwater pollution. The absence of natural filtering clay allows these fibers to infiltrate through the thin layers of sand into the aquifer. No significant difference existed between the Spray-field monitoring wells and Wakulla spring monitoring wells (Fig. 18).

CHAPTER 5
CONCLUSIONS

The prevalence of microplastics in the environment has raised concerns about their presence in surface water and potential contamination of connected groundwater. This study conducted a thorough sampling of urban stormwater and groundwater wells to better understand how microplastics behave in a hydrologic system with surface water-groundwater connectivity. Challenges encountered during the filtration of freshwater samples led to the development of a new filtration method specifically designed for separating microplastics from freshwater samples, addressing complications observed with existing filtration methods developed for marine samples.

Based on quantitative analysis, it was found that microplastics are widespread, with microplastic presence detected in every 1000 ml of surface and groundwater sampled. Urban runoff and wastewater effluent were identified as significant contributors to the transport and distribution of microplastics in water bodies, with the wastewater treatment process found to be ineffective at removing microplastics from water. Microplastics were also found in significant amounts in the setting pond at the wastewater treatment plant and in the reuse water collected at Southwood.

Microplastics were found to be present in Tallahassee surface water, with stormwater showing high levels of contamination. Treatment of ponds such as Cascade Park and Lake Ella with alum was found to reduce microplastic levels, making them appear less contaminated. However, deep percolation of surface water, particularly reuse water, did not entirely prevent microplastics from entering groundwater, as significant amounts of microplastics were detected in shallow monitoring wells and deep wells. The comparative analysis between Wakulla Spring and Wacissa Spring reveals that urban stormwater, laden with microplastics, is infiltrating Wakulla

Spring. This influx suggests a potential pathway for the introduction of microplastics from urban environments into natural aquatic systems.

In addition to chemical contaminants, microplastics pose a threat to the Florida aquifer, which is a significant source of the city's drinking water supply. However, the generalizability of these results is limited by the low number of samples carried out, especially across groundwater. Detailed sampling of wells such as COT wells, Spray-field monitoring wells, and Wakulla spring monitoring wells is needed for a more thorough analysis of the extent of microplastic pollution in the aquifer.

Overall, these findings contribute to a better understanding of the hydrodynamics of microplastic transport and highlight the potential for environmentally ubiquitous microplastics to contaminate interconnected groundwater. Urgent action is needed to conserve and protect the aquifer from microplastic contamination.

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APPENDIX

Table A1. Sample locations and microplastic counts per liter.

Name	Lat	Long	Date	Number of Microplastics
Bike Route	30.404923	-84.286372	7/21/2022	44
Lake Anita Favors	30.43216	-84.28372	7/21/2022	36
Orange Ave. Pound	30.41174	-84.26295	7/8/2022	14
Mill St. Tallahassee Junc. Park	30.42473	-84.29858	7/21/2022	21
Skate Park Pond	30.43249	-84.29537	7/21/2022	2
Lake Henrietta	30.40318	-84.30768	8/3/2022	26
Speed Spencer Stephens	30.42408	-84.29428	7/21/2022	48
Speed Spencer Stephens I	30.42378	-84.2942	7/21/2022	6
Carter Howell Strong Park	30.4475	-84.2924	7/17/2023	50
Southwood Park Reuse	30.38737	-84.22016	7/8/2022	31
Spray field basin	30.38943	-84.3216	6/30/2022	39
Lake Munson	30.36636	-84.30227	7/21/2022	52
Lake Ella	30.46064	-84.27881	7/17/2023	18
Lake Elberta	30.43033	-84.30163	6/16/2022	5
Cascades Park	30.43378	-84.27903	6/9/2022	4
Lake Jackson	30.5227606	-84.3217039	8/12/2022	26
Gum Pond	30.4715436	-84.3292541	8/12/2022	40
Phillips Pond	30.457475	-84.2455931	8/12/2022	26
Lake Lafayette	30.4294184	-84.1728066	8/12/2022	14
Buck Lake	30.4641984	-84.1993507	8/12/2022	32
Jet Medical Center	30.4738935	-84.229161	8/12/2022	22
Skate Park Ditch cloudy	30.431946	-84.296068	7/21/2022	78
Lake Anita Favors	30.43185	-84.2843	7/21/2022	26
FAMU-Way Dith Clear	30.43191	-84.29591	7/21/2022	20
AK Well Cherokee Sink	30.22882	-84.30545	1/9/2023	13
C Well Wakulla	30.23366	-84.30219	1/9/2023	36
The Hole	30.40673	-84.28483	8/3/2022	91
Weems Road Pond	30.4556391	-84.223017	9/1/2022	50
Lake Leon Tom Brown Park	30.44241	-84.2147	9/1/2022	26
Large Well Wakulla 4	30.23419	-84.30225	12/14/2022	52
Wall Mart Wetlands	30.45997	-84.21567	9/1/2022	85
D well Wakulla Park Drive	30.23263	-84.30435	1/19/2023	23
K Well Cherokee Sink	30.22882	-84.30545	1/19/2023	30
B Well Wakulla	30.23366	-84.30219	1/19/2023	21

AD well Wakulla Park Drive	30.23263	-84.30435	1/19/2023	19
Spray field well MWC-22A	30.3476	-84.20161	3/20/2023	8
City of Tallahassee water well 5	30.44879	-84.29844	5/10/2023	6
City of Tallahassee water well 32	30.5686	-84.25641	5/10/2023	14
City of Tallahassee water well 11	30.49352	-84.28617	5/10/2023	6
City of Tallahassee water well 23	30.47681	-84.34494	5/10/2023	9
City of Tallahassee water well 17	30.42696	-84.22462	5/10/2023	7
Spray field well SE-15	30.36168	-84.20949	3/20/2023	18
Spray field well SE-17	30.34777	-84.20983	3/20/2023	29
Spray field well SE-34D	30.34777	-84.20983	3/20/2023	21
Spray field well 10	30.34786	-84.0963	3/20/2023	5
Spray field well MWC-2	30.34723	-84.20234	3/20/2023	20
Spray field well MWC-77	30.3483333	-84.16805	3/27/2023	73
Spray field well MWC-52	30.34722	-84.185	3/27/2023	53
Spray field well MWC-78	30.34805	-84.16805	3/27/2023	22
Spray field well MWI-19	30.35472	-84.1938	3/27/2023	34
Wacissa Spring	30.34023	-83.99106	2/18/2023	10
Wacissa Spring boat ramp	30.34375	-83.99357	2/18/2023	6
Wakulla Spring	30.2356359	-84.3014502	6/29/2022	32
Orange avenue	30.4116681	-84.2629248	7/8/2022	7
Southwood Park Pond	30.387788	-84.2207251	7/8/2022	40
Ditch Blountstown Hwy	30.38934	-84.62728	7/21/2022	79
Munson Hill	30.35238	-84.30158	7/21/2022	50
FAMU retention Pond Entry	30.4311	-84.28586	9/1/2022	58
FAMU retention Pond Exit	30.4311	-84.28586	9/1/2022	33
Gulf course Capital City Country Club	30.425968	-84.273999	9/1/2022	20
Wakulla Spring Diving point	30.2356359	-84.3014502	10/6/2022	37
FAMU pond USDA Tele Center	30.42228	-84.28678	6/23/2023	20
Silver Lake Park	30.40174	-84.30411	6/23/2023	30
Rain Water	30.4248437	-84.3048256	7/17/2023	12

Table A2. Replicate samples.

SAMPLE NAME	No. of Fibers per sample				DATE
B Well Wakulla	21	12			1/19/2023
City of Tallahassee water well 11	6	7			5/10/2023
Wacissa Spring boat ramp	6	5			2/18/2023
Wakulla Spring	32	32	28		10/13/2022
FAMU pond USDA Tele center	20	15			6/23/2023
Silver Lake Park	30	15			6/23/2023
Rain Water	12	18			7/17/2023
Southwood Park Reuse	31	23	23	20	7/8/2022
St. Mark Pond	23	20			10/6/2022
Jet Medical Center	21	22			8/12/2022
Phillips Pond	26	22			8/12/2022
City of Tallahassee water well 5	6	5			5/10/2023
St. Mark River	27	27			10/6/2022
Large Well Wakulla 4	23	25	23		12/14/2022
Lake Munson	52	40			7/21/22

Replicate samples were collected at subset of sample locations. Due to the small sample size, duplicates were included in the box plot and were collected to get an idea of sample variability.

Table A3. Microplastic counts in field blanks.

Field Blanks	No of MPs	Date
St. Mark pond	4	10/6/2022
Large well Wakulla	3	12/14/2022
AD well Wakulla	2	1/19/2023
AK well Wakulla	3	1/19/2023
BC well Wakulla	4	1/19/2023
Spray field wells	4	3/20/2023
City well 5	1	5/10/2023
city well 11	4	5/10/2023
city well 32	2	5/10/2023
Lake Munson	3	6/23/2023
Lake Munson	0	6/23/2023
Silver Lake Park	2	6/23/2023
Lake Henrietta	2	6/23/2023
FAMU pond USDA Tele center	1	6/23/2023
Carter Howell Strong Park	6	7/17/2023
Carter Howell Strong Park	3	7/17/2023
Lake Ella	3	7/17/2023
Wacissa Spring	2	7/17/2023
Wacissa Spring Boat rentals	4	7/17/2023
Rain Water	1	7/17/2023

Experiment field blanks prepared in the laboratory and exposed to field conditions to determine errors from laboratory preparation and atmospheric contamination during sampling.

Table A4. Levene test of homogeneity of variance, ANOVA, and multiple mean comparisons by Dunnett T3.

Table A4a. Descriptive statistics.

No of Microplastics								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
COTwells	10	4.00	2.828	0.894	1.98	6.02	1	11
Wacissa Sp	8	12.38	10.155	3.590	3.89	20.86	2	29
Sprayfield wells	13	22.23	18.939	5.253	10.79	33.68	2	70
StormWater	67	27.72	21.167	2.586	22.55	32.88	0	88
Reuse	10	23.30	10.067	3.183	16.10	30.50	7	37
Wakulla Sp wells	14	19.79	11.102	2.967	13.38	26.20	7	49
Wakulla Sp	8	31.75	9.953	3.519	23.43	40.07	23	55

The descriptive statistics give a summary of the measures of the central tendency and variability of the data within each group.

Table A4b. Leven's test of homogeneity of variance.

Tests of Homogeneity of Variances					
		Levene Statistic	df1	df2	Sig.
No. of Microplastics	Based on Mean	4.178	6	123	0.001
	Based on Median	3.132	6	123	0.007
	Based on Median and with adjusted df	3.132	6	93.030	0.008
	Based on trimmed mean	3.840	6	123	0.002

Table A4c. ANOVA results for number of microplastics.

ANOVA Analysis					
No of Microplastics					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6742.471	6	1123.745	3.649	0.002
Within Groups	37875.75	123	307.933		
Total	44618.22	129			

Table A4d. ANOVA effect size

ANOVA Effect Size					
		Point Estimate	95% Confidence Interval		
			Lower	Upper	
No of Microplastics	Eta-squared	0.151	0.023	0.232	
	Epsilon-squared	0.11	-0.024	0.194	
	Omega-squared Fixed-effect	0.109	-0.024	0.193	
	Omega-squared Random-effect	0.02	-0.004	0.038	

Effect size indicates the proportion of total variance in the dependent variable that can be explained by the independent variable(s) in the model.

Table A4e. Dunnett test results.

Multiple Comparisons							
Dependent Variable: No of Microplastics							
Dunnett T3							
(I) Description	(J) Description	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
					Lower Bound	Upper Bound	
COT wells	Wacissa Spring	-8.375	3.7	0.494	-23.54	6.79	
	Spray-field wells	-18.231	5.328	0.076	-37.74	1.27	
	Storm Water	-23.716*	2.736	<.001	-32.28	-15.15	
	Reuse	-19.300*	3.307	0.002	-31.91	-6.69	
	Wakulla spring wells	-15.786*	3.099	0.002	-26.78	-4.79	
	Wakulla Spring	-27.750*	3.631	0.001	-42.61	-12.89	
Wacissa Spring	COT wells	8.375	3.7	0.494	-6.79	23.54	
	Spray-field wells	-9.856	6.363	0.903	-31.78	12.06	
	Storm Water	-15.341	4.425	0.057	-30.97	0.29	
	Reuse	-10.925	4.798	0.452	-27.98	6.13	
	Wakulla spring wells	-7.411	4.658	0.88	-23.84	9.02	
	Wakulla Spring	-19.375*	5.027	0.031	-37.46	-1.29	
Spray-field wells	COT wells	18.231	5.328	0.076	-1.27	37.74	
	Wacissa Spring	9.856	6.363	0.903	-12.06	31.78	
	Storm Water	-5.486	5.855	0.999	-25.72	14.75	

	Reuse	-1.069	6.142	1	-22.2	20.06	
	Wakulla spring wells	2.445	6.033	1	-18.3	23.19	
	Wakulla Spring	-9.519	6.323	0.92	-31.31	12.27	
Storm Water	COT wells	23.716*	2.736	<.001	15.15	32.28	
	Wacissa Spring	15.341	4.425	0.057	-0.29	30.97	
	Spray-field wells	5.486	5.855	0.999	-14.75	25.72	
	Reuse	4.416	4.101	0.997	-9.36	18.2	
	Wakulla spring wells	7.931	3.936	0.617	-4.82	20.69	
	Wakulla Spring	-4.034	4.367	0.999	-19.4	11.33	
Reuse	COT wells	19.300*	3.307	0.002	6.69	31.91	
	Wacissa Spring	10.925	4.798	0.452	-6.13	27.98	
	Spray-field wells	1.069	6.142	1	-20.06	22.2	
	Storm Water	-4.416	4.101	0.997	-18.2	9.36	
	Wakulla spring wells	3.514	4.352	1	-11.31	18.34	
	Wakulla Spring	-8.45	4.745	0.776	-25.29	8.39	
Wakulla spring wells	COT wells	15.786*	3.099	0.002	4.79	26.78	
	Wacissa Spring	7.411	4.658	0.88	-9.02	23.84	
	Spray-field wells	-2.445	6.033	1	-23.19	18.3	
	Storm Water	-7.931	3.936	0.617	-20.69	4.82	
	Reuse	-3.514	4.352	1	-18.34	11.31	
	Wakulla Spring	-11.964	4.603	0.275	-28.16	4.23	
Wakulla Spring	COT wells	27.750*	3.631	0.001	12.89	42.61	
	Wacissa Spring	19.375*	5.027	0.031	1.29	37.46	
	Spray-field wells	9.519	6.323	0.92	-12.27	31.31	
	Storm Water	4.034	4.367	0.999	-11.33	19.4	
	Reuse	8.45	4.745	0.776	-8.39	25.29	
	Wakulla spring wells	11.964	4.603	0.275	-4.23	28.16	
* The mean difference is significant at the 0.05 level.							

Dunnett T3 test serves as a multiple comparison technique, particularly suitable for situations involving small sample sizes and when there's evidence of heterogeneous variances among groups. It's utilized to determine significant differences among means of compared groups. A p-value <0.05 indicate statistical significance between groups.