The Fate of Microplastics in Tres Rios, a Constructed Treatment Wetland

by

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ABSTRACT

Plastics are an emerging issue in aquatic ecosystems due to their slow degradation and ability to fragment into smaller more mobile parts. Concluding this process, plastics <5mm are categorized as Microplastics, MPs. Currently, the majority of MP studies bring attention to marine pollution and the impacts that follow. However, it remains a high priority to understand how MPs move through urban aquatic environments, and the impacts this may have for surrounding urban ecosystems. Little is known about how MPs move through tertiary treated wastewater plants, such as constructed wetlands, and how much, if any, remain trapped in abiotic and biotic material such as soil or plant life, respectively. An analysis of MP distribution using Tres Rios, a tertiary wastewater treatment wetland, as the study site may help to shed light on the source-occurrences of MPs. Microplastics extraction was performed on soil, plant, and water samples that were collected along major access points within the system with emphasis on inflow and outflow. The inflow of the wetland receives between 246-398 MPs/L vs the outflow of 90-199 MPs/L. Tres Rios soil concentrations ranged between 1,017-10,100 per kg and 133-700 MPs per kg in sampled vegetation throughout the wetland. The distribution of soil and vegetation samples differed throughout Tres Rios, as soil sampled exhibited higher quantities towards inflow site and vegetation MP occurrences were increased throughout the middle of the system. Additionally, this study aimed to determine if seasonality impacted the concentration of plastics seen throughout the system. There was no evidence that suggested seasonal variations were occurring in any sample type. Atmospheric deposition fluxes of microplastics were considered as a potential additional influx but even at the measured 1510 MP m^{-2} day^{-1} they were small compared to the water influx. Overall, the results

suggest that the Tres Rios wetland removed 55% of the microplastics it receives and hence performs a substantial ecosystem service.

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1. INTRODUCTION

Microplastics, plastic particles <5 mm in size, are formed through continuous environmental exposure, such as abrasion, chemical oxidation, or photodegradation from UV rays causing larger plastics to break down into smaller particles (Zhang et al., 2021). As an emergent field of study, the long-term impacts of microplastics (MPs) are not yet well understood. Given their widespread distribution, it is essential to understand their environmental occurrence and their fluxes within ecosystems. While little is known about the potential ecological and health impacts, MP research has had a relatively lengthy history focused almost exclusively on marine sea life. As a result, 87% of plastic pollution studies focus on marine environments (Blettler et al., 2018). Although early research focused on marine environments, (Browne et. al, 2011) MP presence has now been detected in a wide array of environmental compartments and their inhabitants, including freshwater, soil, air, and terrestrial/marine organisms. Equally important, MPs are not spatially limited to clearly anthropogenically impacted areas such as urbanized settings, but they have been detected in any environment on every continent, including Antarctica (Bessa et al., 2019). Acting on the concept of their ubiquitous presence, a more detailed understanding of how MPs spread was the motivation for this study. This research aims to investigate the occurrence and transport of MPs within an engineered wetland, Tres Rios in Phoenix, Arizona. Furthermore, this controlled environment can be utilized to understand the potential for MP retention.

1.1 Origins and Distribution

MPs can be classified as either primary or secondary, depending on their origin. Primary MPs are microplastics manufactured as such for commercial use (i.e., microbeads in facewash). Secondary MPs result from breakdown of larger plastics as they degrade through environmental exposure (Kasmuri et al., 2022). Both types originate from a variety of human activities, from household plastic use, to synthetic fiber clothing to processes such as tire wear. These plastics and MPs then spread through means such as road run-off, wastewater, and wind (Boucher and Friot, 2017). All plastics are manufactured on land, and their spread can be attributed to inadequate waste management practices, illegal dumping, or accidental and improper discharges into stormwater. The mobility, robustness and persistence of the MPs ensure their widespread distribution. As the demand for plastic products persists, the waste associated with plastic products not only fills up landfills, but also results in plastic and microplastic discharge into waterways, as well as terrestrial and aquatic ecosystems through numerous pathways (Tang and Hadibarata, 2021).

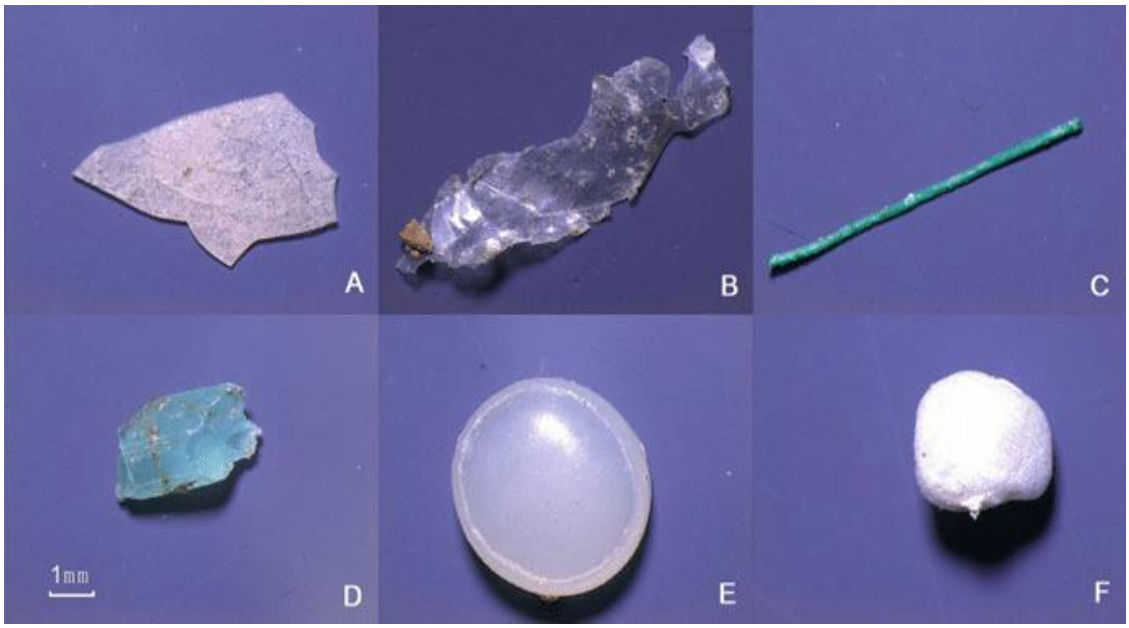

Figure 1. Shapes of common microplastics A. Sheet, B. Film, C. Fiber, D. Fragment, E. Pellet, F Foam. Microplastics are produced with a multitude of polymers and various additives. Microplastics can be any size < 5 mm. Microplastics are described with at least 7 morphologies. Source: Microplastic pollution in inland waters focusing on Asia

1.2 Microplastic Characterization and Environmental Health Effects

The rich literature on microplastics in the environment has used a range of analytical approaches to detect and characterize MPs. However, there are still no established standardized methods, and as a result, studies differ in methodologies, which are nearly as varied as the microplastics they study. Traditionally, the common aspect of most methods aimed at microplastics is that MPs are isolated through filtration from water directly or after density separation (flotation) from soil suspensions or digested solids. The isolated microplastics are analyzed by microscopy for counts, size, and shape (Ye et al., 2022). Visual identification with the naked eye or a microscope is a common practice, however when not combined with other methodologies, such as spectroscopy, misidentification can occur (Fahrenfeld et al., 2019). In some cases, a primary objective of

the research is to chemically characterize the plastics and techniques such as micro-Raman or micro-IR spectroscopy are used to identify the chemical nature of the polymer (Xu et al., 2020). In recent years, alternative approaches have emerged to quantify not only microplastics but also smaller nanoplastics (<1 µm) using instrumental analytical techniques such as gas chromatography-mass spectrometry (GC/MS). When the samples are thermally treated, they release degradation products (often their monomers) that can be identified and quantified using GC/MS (Cai et al., 2021). Furthermore, changes in density separation by floatation methodology can be used to select MPs for characterization (i.e., shape or polymer).

Although not deliberated in this literature review, microplastics exhibit a wide range of morphological characteristics (*see Figure 1*) including color, shape, and size. The size of microplastics varies and ranges from 5 mm to the detection limit of the analytical method, e.g., 5 µm, which is typical for optical microscopes (Masura et al., 2015). Morphology is an important consideration as certain shapes often shed from a specific source (Rochman et al., 2019). The heterogeneity of shapes found in MPs is a prevailing topic in the literature, as microplastics of different shapes interact with ecosystem components in variable manners. For example, MP-biota interactions vary with MP shape. In particular, in aquatic environments MP beads have the highest ingestion rates, though it is not clear as to why (Scherer et al., 2018). In addition to shape, the acquisition of MPs in aquatic animals can be directly linked to particle size, as this is related to the typical size range of their feeding habits (Fueser et al., 2019). MPs are often mistaken for food and ingested by aquatic animals. While much of the literature focuses on the negative impacts of MP presence, some studies suggest that certain MPs, such as fibers, found in the soil,

can have positive effects by retaining water for extended periods of time, making them favorable for increased growth of plant biomass (Lozano et al., 2021). Due to their complex morphological characteristics, there are innumerable interactions, positive and negative, that MPs can contribute to in ecosystems.

There are varying levels of health impacts that can be linked to MP presence. As a general perspective on this topic, associated risks can be categorically addressed into direct and indirect effects. There can be direct effects from the particles themselves. For example, in terrestrial environments the incorporation of microplastics into soil may increase the hydraulic conductivity (Yu et al., 2023). In addition to physically disrupting their surroundings, the toxic byproducts that MPs leach, increase as they age, making them of dual concern (Luo et al., 2022). Additives are regularly used in the production of plastics to enhance their properties (e.g., phthalates are plasticizers, and they are added to make plastics more malleable), and their leaching can enhance the general toxicity. Substances such as polybrominated esters (flame retardants), phthalates (plasticizers), and bisphenols (plasticizers) are known disruptors of endocrine function in humans and are common additives in plastic manufacturing (Sixto et al., 2021). Apart from ingestion, the effects of a multitude of other interactions are predominately unknown. Several studies (Raju et al., 2022, Courtene-Jones et al., 2022) have explored the presence of plastic material in aquatic animals, internally or externally, by means of attaching to the outside, and the effects this type of pollution can have on overall fitness. As an example, studies have explored the effects of fish ingesting plastics and the resulting effects include neurological and oxidative stress (de Sá et al., 2018). Indicator species, such as bivalves, are more vulnerable as filter feeders, as studies have shown co-occurring interactions of multiple MP additives,

resulting in DNA modification at the transcription level (Rios-Fuster et al., 2022). Although the potential for MP presence within aquatic organisms is probable, the aspect of animal interactions is not considered within the confines of this thesis (*see Figure 2*). There can also be 'Trojan Horse' effects, were MPs act as vectors for other pollutants. Heavy metals and microplastics are believed to fall into this co-transportation category, and with the evident impacts of heavy metals on freshwater system this could lead to exacerbation of toxicity to aquatic organism, food web dynamics and human health (Zhao et al., 2023). In aquatic environments, the diverse pathways of adverse effects are extensive, as each plastic is enhanced with its own combination of additives that homogenize in the water column (Beiras et al., 2021). As a result, they individually impact aquatic environments and their inhabitants in their unique trajectories.

1.3 Microplastics in Urban waterways

Plastics and microplastics can end up in the ocean through their release into freshwater and subsequent flow towards estuaries. It is worth noting that North American rivers contribute about 4.5% of global ocean bound plastics, while Asia's riverways contribute over 80% (Meijer et al., 2021). This should be of no surprise, as Asia is the most populous continent and bears the burden of much of the world's industrial productivity. Urban centers are likely sources for the release of plastics and microplastics into the environment through stormwater and wastewater pollution. Household products such as face washes and toothpastes are known to contain numerous microbeads while washing of synthetic fabric clothing releases microfibers. For reference, one wash with an exfoliant can release between 4,500–94,500 microbeads (Napper et al., 2015). Consequently, a sizable number of MPs make their way to wastewater treatment plants (WWTPs), daily.

Authors reported that in a WWTP on Plum Island, South Carolina, MP loads at inflow ranged from 8,000 to 20,000 million MPs a day (Conley et al., 2019). Stormwater can also contribute to the spread of MPs in urban areas by carrying plastics from mismanaged waste, tire wear, and other sources. While some stormwater is treated at WWTPs, untreated stormwater can also end up directly in the environment, carrying microplastics with it (Mangistu, 2023).

Although WWTPs typically achieve significant removal rates of MPs after primary and secondary treatment, occurrence of microplastics has been reported downstream (Kay et al., 2018). There are some inconsistencies within the literature regarding removal rates, as some studies suggest that there is almost a complete removal (99.9%, Gies et al., 2018) while others are more conservative and report above 88% retention (Sun et al., 2019). However, there is a consensus within the literature that the removal capacity increases from primary to secondary treatment. Studies which include a tertiary treatment reported almost a complete removal at 88-94% (Iyare et al., 2020). The high removal rate of MPs in the primary phase of water treatment is the result of the removal of biosolids (Lofty et al., 2022). The byproduct of solid removal in WWTPs is sewage sludge, which is separated, dried, and then transported to be applied for agricultural use. Despite the potential for sludge to contain a multitude of chemicals, the MPs are redistributed into a terrestrial setting (Rillig, 2012) where they can be reintroduced to the aquatic environment through runoff or transfer to groundwater. This poses a concern as MPs cycle from urban disposal in WWTPs to agricultural settings and back into the water system. Recent research shows, that, while once thought to be a site for transportation, rivers have the capability of retaining MPs, through a process called hyporheic exchange in which surface water mixes with water

in the riverbed to trap lightweight microplastics that otherwise might be expected to float (Drummond et al., 2022). Overall, the presence of microplastics in urban water represents a concern for aquatic and marine ecosystems, if these waterways eventually drain into the ocean. However, a substantial fraction of the plastics might be retained in the urban or terrestrial ecosystems where they can accumulate overtime locally (Wang et al., 2020).

Constructed wetlands are a notable urban environment to study the fate of MPs, as one can quantify the number of MPs entering, exiting, and the distribution of MPs between water, soil and biomass. Wetlands in small landlocked states, such as Arizona, are less likely to have substantial impacts on ocean bound MPs but rather offer unique opportunities to study the fate and transport of MPs. As urban populations continue to expand, sustainable methods to treat water for excess nutrient contamination and, in this work, potential MP pollution are necessary. Constructed treatment wetlands serve as an ecofriendly approach to utilizing nature as a regenerative design tactic for urban water (Childers, 2020). Due to the complex nature of MP pollution, identifying sources and potential sinks, may help facilitate management strategies to effect change. Constructed wetlands simultaneously carry out several objectives: reducing excess nutrients loads, promoting biodiversity, and aiding in flood control in some cases, (Dumax et al., 2021). With the many advantages linked to urban treatment wetlands, they can be a source of effective, natural tertiary wastewater treatment solution. However, there are few studies on the distribution occurrences of MPs in these settings, which is a concern as MPs are often discovered downstream from WWTP. This study investigates the Tres Rios Constructed Wetland in Phoenix AZ USA, to better understand the occurrence, the fate and transport of microplastics in a wetland ecosystem.

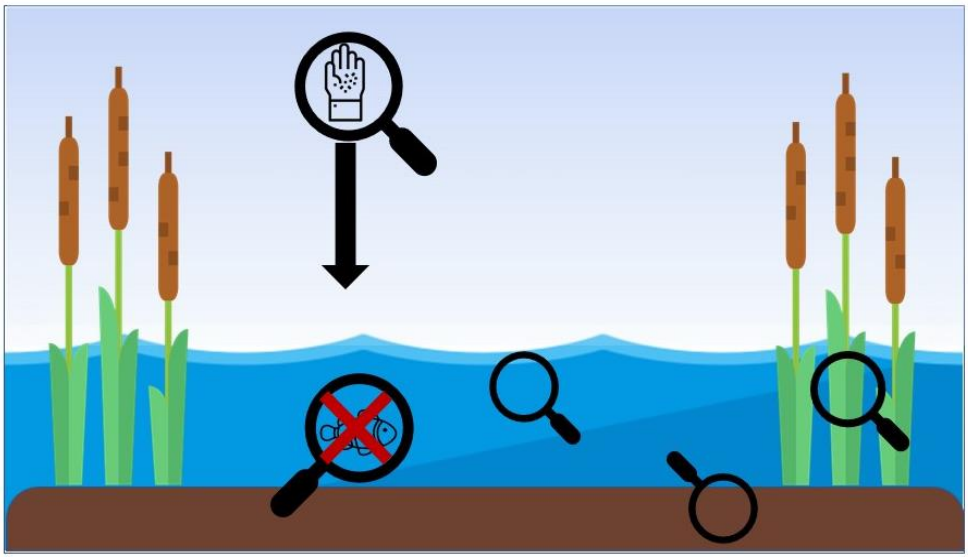

Figure 2. Graphical abstract representative of microplastics that were examined as a potential source site within Tres Rios. These include atmospheric pollution, vegetation, soil, and surface water samples. Another likely occurrence may reside among aquatic life; however, this aspect was not tested.

The previous sections summarized the current state of knowledge on environmental microplastics, highlighting the limited understanding of their occurrence and partition in the environment.

Here we present a study on Tres Rios, a constructed wetland, to: (1) investigate the distribution and fluxes of MPs within a constructed wetland in regards to its abiotic and biotic components- soil, water, and plant matter (2) assess the efficacy of the wetland as tertiary treatment process by monitoring the concentrations by which MPs are entering and exiting the system. (3) determine the retention rate of the wetland.

Tres Rios constructed wetland was selected as a site to investigate these possible processes. Indirectly, this study anticipates delivering potential solutions for mitigation of pollution control. Two hypotheses were developed to explore the extent, if any, of MP distribution within Tres Rios and its change over time (1) the wetland components (soil

and plant material) will retain significant amounts of MPs, acting as a sink, indicated by a reduced number leaving the system via surface water transportation than being received and (2) microplastic contamination varies with seasonal changes resulting in an increase of MPs in wetland components during summer months.

2. METHODS

2.1. Study site

Figure 3. Tres Rios Wetland study site. Red arrows represent inflow (north) and outflow (south). Yellow arrows indicate collection sites for vegetation and soil samples.
Source: Google Earth Pro

Samples were collected from the Tres Ríos wetland, a constructed wetland in Phoenix, Arizona. In 2010, the City of Phoenix began to utilize the constructed wetland for tertiary treatment of wastewater as a result of increased population demands, in association with the 91st Avenue Wastewater Treatment Plant, the largest municipal treatment plant in the Phoenix metropolitan area (https://www.phoenix.gov/waterservices/tresrios/planning-

your-trip). The adoption of more natural methods to address anthropogenic problems, like excess nutrients discharged into riverways, is termed Urban Ecological Infrastructure (UEI) and functions as an ecological approach to provide ecosystem services for urban settings (Childers et al., 2019). Tres Rios can be categorized as one of the many UEIs that have been implemented into cities with a successful outcome. In 2011, CAP-LTER, the Central Arizona–Phoenix Long-Term Ecological Research program (https://sustainability-innovation.asu.edu/caplter/) began studying the wetland, to improve the understanding of water, nutrient, and treatment dynamics of constructed treatment wetlands in arid, urban environments. Today, Tres Rios serves as a long-term site for collecting data regarding nutrient concerns in conjunction with the city of Phoenix which operates the site. Tres Rios was completed to address effluent polishing needs in a cost effective and environmentally friendly manner, (i.e., Urban Ecological Infrastructure). In addition to providing a sustainable means of water purification, the wetland also serves as a research basin for CAP LTER to study various ecosystem services (Elkins, 2011).

Approximately 100 ha in size, Tres Rios is composed of three wetland treatment cells with marshland and two flow regulating basins. One of the three wetland basins is used for research (such as this study) and is approximately 42 ha in size. Open water areas span roughly half of the total area, and the remaining 21 ha are vegetative marshland comprised with densely packed rooted macrophytes. The wetland vegetation consists primarily of Cattail species: *Typha latifolia* and *Typha domingensis*, in a slightly reduced capacity, Bulrush species: *Schoenoplectus californicus, Schoenoplectus tabernaemontani* and *Schoenoplectus acutus* are present. Prior to the construction of the Roosevelt Dam, this area originally served as a riparian area (Brown et al., 2011). To pay homage to its original

landscape, Mesquite, Cottonwoods and desert shrubs, endemic Sonoran Desert plants that were once prevalent to the area, were planted in the surrounding area of the wetland. Due to the size and ecosystem resource availability, Tres Rios attracts a wide variety of animals– raccoons, coyotes, and beavers. Furthermore, the riparian habitat doubles as a hotspot for bird communities, allowing many migratory and residential birds to use the wetland as a temporary home along their travels, or a permanent residence.

Varying seasonally, the wetland can receive up to 400,000 m^3 day^{-1} of wastewater effluent from the treatment facility (Childers et al., 2020). The WWTP effluent is treated partially to the tertiary stage, where the effluent water then becomes Tres Rios's influent. Treatment of the water in the wetland occurs as vegetation for pulling nutrients from the water, resulting in a more polished effluent. The total water residence time (length of time for inflow water to leave the system) is water is four days. In this short time, the vegetation pulls an average of 22% of nitrate and 48% of ammonium from the water (Sanchez et al., 2016). This is crucial as the water is discharged into the Salt River corridor where it is later utilized by local farmers for non-edible food crops.

2.2 Sample collection

All samples were collected over a two-year period, 2020 – 2022, on a quarterly basis during the months of March, July, September, and December.

Water

Water samples were collected from inflow and outflow to determine the changes in water entering and leaving the system (*Figure 3. Red arrows*). All three cells have

hydraulic control stations that release the outflow water to the Salt River on the south end. Therefore, each cell should receive similar water distributions and should not vary in MP occurrence. Water was sampled in one-liter amber glass bottles. Prior to collection, bottles were prepared in the lab by washing with laboratory grade detergent and baking them at 450 ° C to ensure complete removal of organic material. Bottles remained sealed with lids until in use, then were processed with a triplicate rinse before taking each initial water sample. Water sampling occurred directly at the inflow and outflow pipe. Water samples were then stored on ice until taken back to the lab where they were kept in the refrigerator, at 4°C, until they were analyzed. The same procedure was followed for both influent and effluent water samples.

Soil and Vegetation

Soil and vegetation samples were collected from three sampling locations commonly used as water quality monitoring sites throughout the wetland (*Figure 3.* yellow arrows). Soil and vegetation samples were collected only from the inner workings of the cell to determine where MP partitioning occurred. The monitoring sites were located in one of the three cells that is used for research purposes only and is not open to the public. The sites chosen were evenly distributed from one another and selected to evaluate soil and vegetation near the entrance (Q1), middle (Q2) and the exit of the wetland (Q3). Soil and vegetation samples were collected in wide-mouth glass bottles. These bottles were rinsed with DI water, washed with lab-grade detergent, and baked at 450°C overnight. Once at sampling site (Q1-Q3), both soil and belowground biomass were collected using a 5in diameter metal coring pipe. For soil, core was submerged until hitting the clay layer at

every site (roughly ½ a meter). A cap was then placed over the top of the core to provide enough suction to remove the core from the water. A smaller solid pipe was then placed at the end of the sample core to push the soil sample out of the opposite end while removing the excess water. The same was done for belowground biomass, expect core was placed over the top of the individual Typha, the core was pushed into the sediment until root mass was covered by core, then extracted in the same manner. Above ground biomass for individual Typha species was collected by clipping the entire plant directly above the point of submersion. Above ground biomass was folded and stored into large jars. Samples were kept on ice until taken back to the lab where they were stored in the freezer while awaiting processing.

Atmospheric Deposition

In addition to water, soil and vegetation samples, deposition samples were collected in the winter of 2022 to estimate atmospheric deposition. Deposition samples were collected over a week's time period in late December of 2022. Amber deposition canisters with attached funnels were placed in crates and positioned near the inflow and outflow. Prior to placing them, the 4L glass canisters were prepared by baking to remove other particulates and stored with aluminum foil over funnel until use. Funnels were covered back up with aluminum while being transported back to the lab to ensure accuracy of MP deposition counts. After being brought back to the lab, atmospheric samples were analyzed immediately.

2.3 Sample Processing

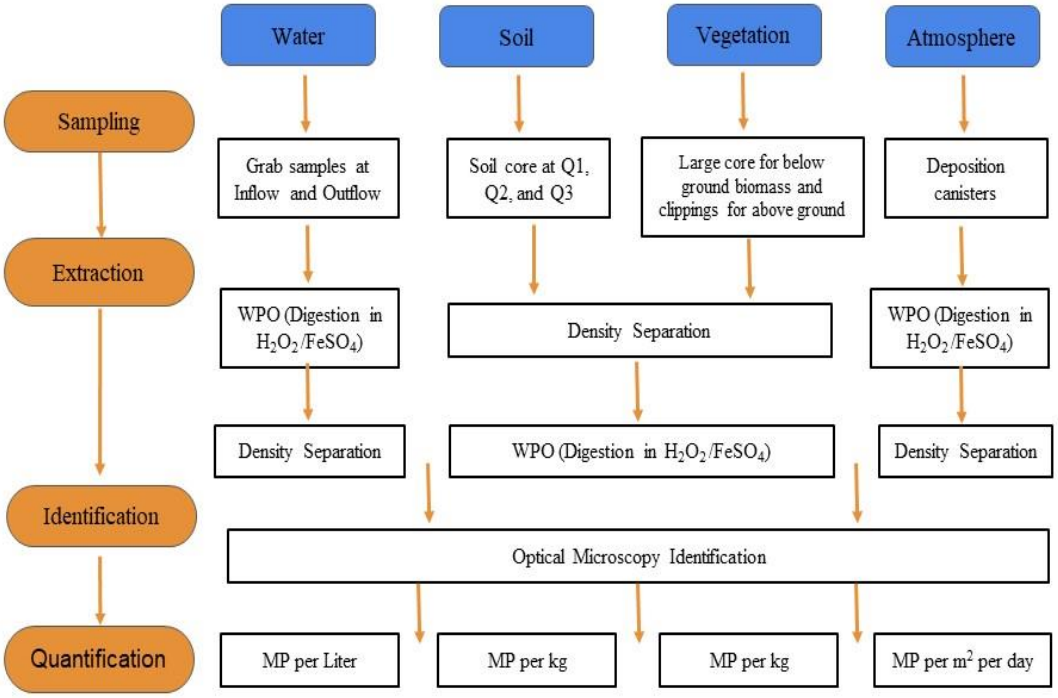

Figure 4. Flow chart of analytical methods followed for the different sample types.

Once taken back to the lab, all samples were processed using a method patterned after the NOAA Marine Debris Program method for sediments (Masura et al., 2015). Figure 4 describes the process for each sample type.

Water and atmospheric deposition samples were first treated with the Wet Peroxide Oxidation, WPO, method and then processed by density separation, leading directly into filtration. Water samples were transferred to new vials, lightly rinsing collecting bottles with ultrapure water (Purelab Flex, IL, USA) to ensure all plastics were removed. Water samples from inflow and outflow were collected at a volume of one liter. Once atmospheric deposition containers were brought back to the lab, each funnel and bottle were rinsed three times totaling to 500 ml of ultrapure water and then transferred to individual beakers.

Bottles were given one final rinse with ultrapure water to ensure no plastics were left behind.

Contrary to water and atmospheric deposition samples, soil and vegetation samples utilized only the decanted portion of the water (portion that floated plastics). This indicated the need for density separation prior to WPO so only the plastics and water proceed to the next steps. Samples were weighed first wet, as received, then dried in an oven at 60°C for 12 hours in a Muffle furnace (Vulcan 3-1759, CA, USA). Soil and vegetation samples varied between 24-48 hours. After drying, soil samples were measured to 60g per sampling site (Q1, Q2, Q3) and placed into individual vials for further analysis. Vegetation samples were weighed up to 30g with a combination of above and belowground biomass, then transferred to a mortar to be ground down with a pestle into fine particles in order to process. They were then placed into individual vials based on sampling site (Q1, Q2, Q3) for further processing.

2.4 Density separation

During the density separation phase, a highly saturated salt mixture was added to each sample. This solution acted as a means to which the lighter substance within each solution would float to the surface. 150g of NaCl, sodium chloride, was dissolved in 500ml of DI water. Each sample type was allotted a 24-hour period to settle before proceeding to the remaining steps.

2.5 Wet peroxide oxidation

Each sample was subjected to wet peroxide oxidation (WPO). During this time, an aqueous solution of 10 ml (varying based on sample type reference supplementary material in Appendix B) was prepared by adding 0.05 Fe (II) as $FeSO_4$ to each sample that had settled overnight succeeding the density separation. Following the addition of $FeSO_4$, 10-20 ml of 30% H_2O_2 (Sigma Aldrich, MO, USA) was added to each sample and allowed to settle for 5 minutes uncovered, under the fume hood. The time spent cooling varied based on sample type. Soil and vegetation samples had an initial 10 ml of H_2O_2 and were allowed 10 minutes to cool prior to the mixture being heated on a hot plate, while being stirred (*See Figure 5 for reaction*). These samples were exposed to a temperature of 75°C with continuous monitoring until the reaction ceased. Additional H_2O_2 was added during this process until a reaction was no longer present. This was indicated by a cease in bubbling. No samples needed additional H_2O_2 over 6 hours. Water samples were not exposed to heat. These samples came to room temperature naturally after having an initial 20 ml H_2O_2. Due to the abundance of small vegetative particles all samples were then run through a 5 mm sieve; these sieves were then rinsed with DI water.

Figure 5. Wet Peroxide Oxidation of soil sample from Q3. Reaction is currently taking place, indicated by presence of bubbles.

2.6 Filtration and Microscope examination

Following the sieving, each individual sample was vacuum filtered through a glass microfiber filter (Whatman GF/A, Sigma Aldrich, MO, USA). Loosely covered in aluminum foil, individual filters were then dried at 60°C for 5-6 hours in a Muffle furnace (Vulcan 3-1759, CA, USA) or until reaching complete dryness. After completion, filters examined under an optical microscope with the 40X lens (Leica DM6B-Z, Germany) equipped with the Leica DFC7000 T camera and Leica Application Suite software (LAS X). The size range of interest was 5mm to 5µm. During examination various morphologies

were observed, but not noted—the purpose of examination was purely on MP number. All material was visually identified (See *Figure 6.*) and all particles that fell apart when pressure was applied with a pin were excluded from total. All samples were stored covered in aluminum and placed in a freezer until use.

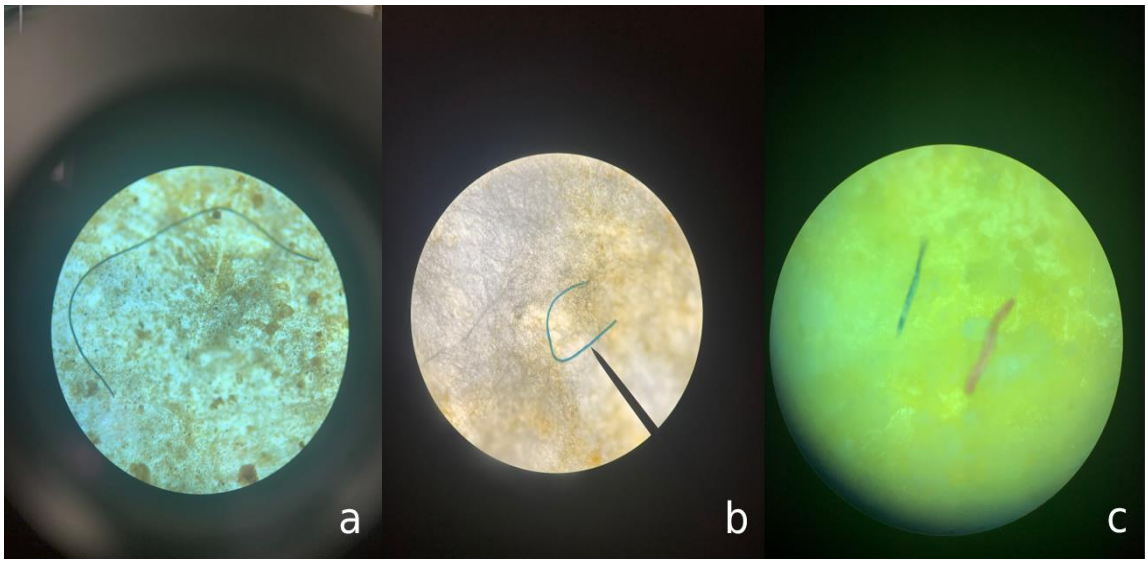

Figure 6. Examples of microplastic fibers found in soil (c) and vegetation samples (a and b).

2.6 Statistics

Following microscopy examination, all statistical analyses were performed using RStudio (2020). Following the completion of sample collection and processing, statistical analysis was performed as a t-Test, ANOVA, and Chi squared to determine if a significant difference was observed between sample groups.

3. RESULTS AND DISCUSSION

3.1 Water

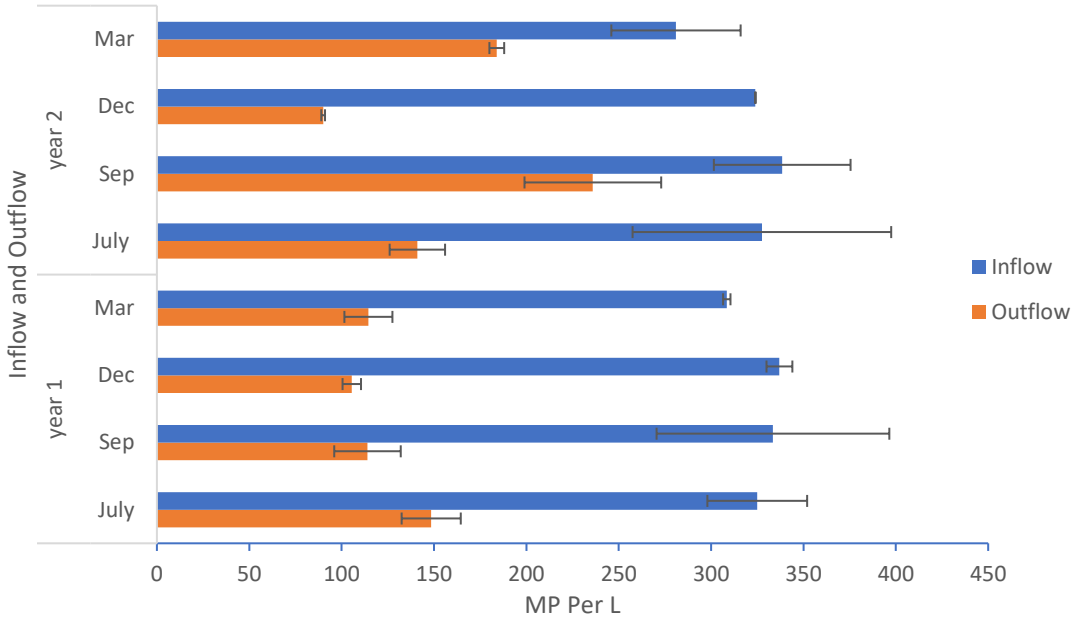

Figure 7. Distribution of MPs in inflow and outflow across the study period. The bars indicate min and max concentration while the bar indicates the median.

The Tres Rios wetland receives about 320 MPs/L on average (246-398), based on our inflow concentration measurements. These concentrations are substantially higher than those seen in natural wetlands. Lalu, a natural wetland in Tibet, China saw a range of 0.06 MPs/L to 3.05 MPs/L in surface water samples (Lui et al., 2022). When compared to other Arizona waterways such as The Santa Cruz River (Eppehimer et al., 2021), researchers found a slightly higher concentration of 19.5 MPs/L in water column samples. However, as Tres Rios is a treatment wetland, the inflow concentrations should be compared to WWTP effluent concentrations after secondary or partial tertiary treatment as these are the kind of waters that Tres Rios receives. Here the concentrations are higher than most WWTPs in the literature that have effluent concentrations <100 MPs/L. For example, a

WWTP in China sees an influent concentration of 126 MPs/L and an effluent of 32 MPs/L (Jiang et al., 2020).

This increase in MP presence may be indicative of the volume of water the 91st Ave WWTP treats, as it is the largest in Arizona. Another possible explanation for the variation in MP presence may be attributed to size. Within the scope of this study the size distribution of plastics identified ranged from 5 mm- 5µm, though other studies may choose to include only certain morphologies of MPs or MPs that are below this size or even consider nanoplastics.

Figure 7 shows the distribution of MP concentrations measured in inflow and outflow samples across all seasons. On average the number of MPs found in inflow, determined by the mean, were 322 MPs L^{-1} vs 145 MPs L^{-1} in the outflow. A t-test resulted in a p value <0.01 (7.56E-11), confirming significant difference between inflow and outflow, suggesting that the Tres Rios wetland removes microplastics. The average removal rate based on inflow and outflow concentrations of MPs was 55%, indicating that roughly half of the microplastics are being removed. As nutrient removal is a primary objective of Tres Rios, a comparison of nitrogen removal may provide a broader context as to how other particulates move through the system. In the case of ammonium, a 48% removal occurs in months of high transpiration (i.e., summer months) (Sanchez et al., 2016). This removal rate is lower than studies in laboratory constructed wetlands which saw only % and 0.003% of the total microbeads and fibers, respectively, introduced into the system in the effluent, indicating a removal efficiency of 99.9% (Rozman et al., 2023). However, Lula, the natural wetland mentioned above, had an average MP removal rate of 55%, which aligns with our observations in Tres Rios. In another study (Bydalek et al.,

2023) final effluent of the constructed wetland had a MP concentration of 0.30 MP/L. When comparing removal rates, (Long et al., 2022) found a removal rate of 89.3–97.4% in Tianjin, China. This was the relative average of WWTPs constructed treatment wetlands in China. Additionally, much of the other literature states removal rates in relation to the entire treatment process, WWTP influent, through primary, ending at secondary treatment. Some include tertiary treatment if this is a part of the WWTP process. This makes comparison difficult as the numbers for influent and primary treatment of the 91st Ave WWTP associated with Tres Rios are unknown. Tres Rios receives secondary treatment effluent from the WWTP, we know a portion of the MP concentrations, but not the whole picture. Future research may choose to quantify this.

Seasonality

The highest MP concentration of 398 MPs L^{-1} was found in an inflow sample from July in 2021, while the lowest concentrations was detected in an outflow concentration from December 2022 with 90 MPs L^{-1}. In the inflow data there is no seasonality apparent (*see Figure 7*) in terms of concentrations. The outflow concentration data is more variable but again no clear seasonality is apparent and only 1 statistically significant difference was observed in pairwise tests which was between December 2021 and March of 2022. We have hypothesized that summer months would showcase increased MP occurrences, this single difference lacked overall relevant correlation. Overall, the lack of variability in microplastic concentration is in stark contrast to other pollutants such as phthalates observed at this site. In a study conducted concurrently with ours, they found a clear

distinction between seasons, with significantly higher phthalates detected in summer months (Storey, 2023).

Total plastic load and ecosystem function

Table. 1 MP concentrations in comparison with daily influent loads

Date	Inflow (MGD)	Liters per Day	MP per Liter	Billions of MP per day
Mar 2021	76 MGD	287,691,296 L/D	309 MP/L	88 B MP/D
July 2021	91 MGD	347,398,597 L/D	325 MP/ L	112 B MP/D
Sep 2021	90 MGD	343,461,768 L/D	334 MP/ L	114 B MP/D
Dec 2021	82 MGD	314,071,831 L/D	337 MP/ L	105 B MP/D
Mar 2022	74 MGD	283,481,918 L/D	281 MP / L	79 B MP/D
July 2022	58 MGD	220,155,764 L/D	328 MP / L	72 B MP/D
Sep 2022	89 MGD	340,009, 473 L/D	344 MP / L	116 B MP/D
Dec 2022	109 MGD	415,036,335 L/D	324 MP/ L	134 B MP/D

We have only considered plastic concentrations up to this point, but it is well-known that both the nature of urban waste as well as the discharge flows from wastewater effluents change over time (Forrest et al., 2022). Consequently, we looked at the discharge rates into the wetland on the days we sampled and calculated the loads of microplastics received. The average is about 100 billion pieces (*Table 1)* of microplastics per day, ranging from 72 to 134, suggesting no large variation in terms of total load, which is not inconsistent with the concentrations observed. As can been seen in *Table 1,* MP concentrations were not directly linked to daily loads, as one would expect the

concentrations on MPs to increase with increased load size, which is not evident in this data set. Considering the total loads, one can assess the magnitude of the ecosystem function that the Tres Rios wetland performs. At 100 billion microplastics per day received, it removes 55 billion MPs each day that would otherwise end up in downstream waterways.

Given that the wetland removes such a large amount of MPs, it is important to understand where the plastics end up. In the next sections we will investigate MP concentrations in soils and biomass.

3.2 Soil

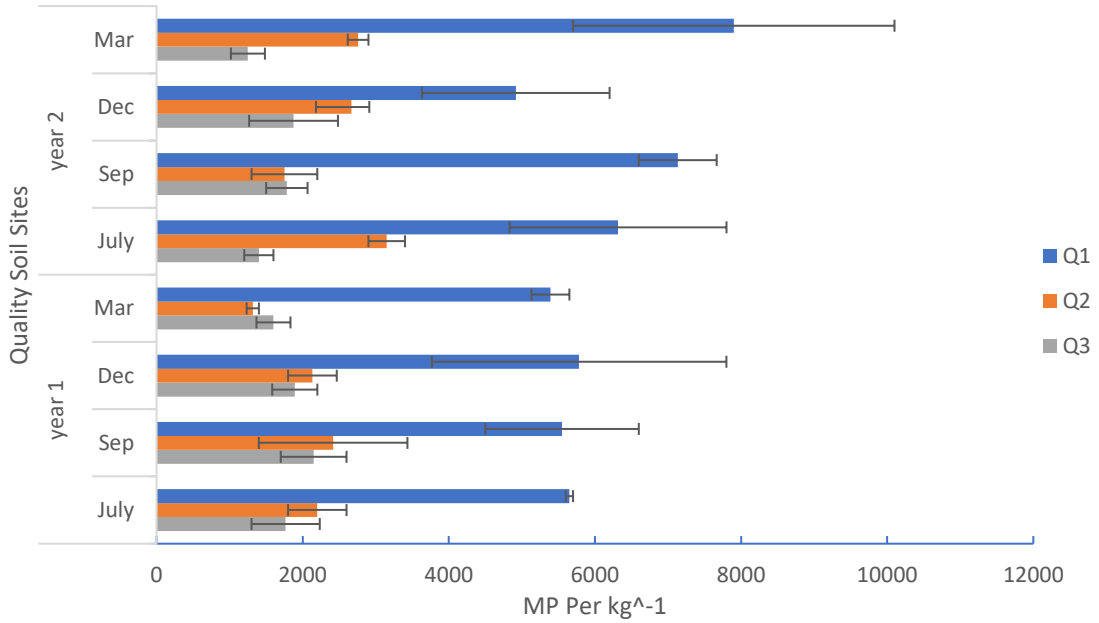

Figure 8. Distribution of MP concentrations in soil samples at the three testing sites (Q1, Q2, Q3) across the whole sampling period. The bars represent the minimum and maximum concentrations while the box represents the median.

The concentration of microplastics seen within the wetland range from 1,017-10,100 MPs/kg based on each sample site. These concentrations are rather large, but in contrast to literature, these concentrations vary considerably. When compared to other wetlands, Townsend and coworkers (Townsend et al., 2019) found a range of 2 MPs/kg

dry sediment to 147 MPs/kg within 20 wetlands. Tres Rios exhibited several magnitudes higher concentrations than these values, however these wetlands occurred all across Australia and were natural wetlands, not treatment wetlands. Other studies have shown MP concentrations at a much higher level with ranges between 334–3,068 MPs/kg (Heloski et al., 2020). The wetland in this latter study is a restored-natural wetland but is situated in the middle of the highly populated city, Washington, DC. also, fits more within the range of observations within the Tres Rios wetland, still, location, size of plastic, and type of wetland are all variables to consider when comparing different studies, also for soils.

Seasonality

Seasonality was tested by merging the sampling site data into season (March, July, September, and December) categories by combing into "Year 1" and "Year 2". September showcased the highest abundance of MPs, (reference *Table 2*) statistical analysis when comparing to other months displays no evidence of seasonality ((Supplementary data tables found in Appendix A). Overall, the highest values of plastics were identified in soil samples, in comparison to other sample types (i.e., atmospheric deposition and vegetation samples), signifying that the majority of sequestration within the system occurs at the soil level.

Table 2. Soil data transformed into year sets across all quality sites.

Soil Samples Across Year Periods in MP/ kg of all seasons			
	Year 1 MP kg $^{-1}$	Year 2 MP kg $^{-1}$	Total MP kg^{-1}
July	19,233	21,733	**40,967**
Sep	20,233	21,333	**41,567**
Dec	19,617	18,917	38,533
Mar	16,617	23,817	40,433

Spatial distribution

As sampling sites were selected to assess the wetland as a whole (beginning, middle, and end), the spatial distribution of microplastics along the flow path is worth exploring. Sampling sites 1, 2, and 3 respectively show an average of 6,080 MPs kg^{-1}, 2,299 MP kg^{-1}, and 1,715 MPs kg^{-1}. MP concentration was lowest in the month of March in Q2 2021 and also the highest in Q1 of 2022. The relative decrease in soil concentrations from Q1 to Q2 was 62% and between Q2 and Q3 the percentage decrease of MPs was 25%. We see a clear reduction from each sampling site to the next (*see Figure 8*). In a study comparing 5 constructed wetlands, sediment abundance of MPs was generally higher at the inlet (736 -3,480 MP/kg) and decrease towards the outlet (19-1060 MP/kg) (Lu et al., 2022). Considering that the abundance in soil samples were relatively high in comparison to other studies, biomass may likely follow suit. This will be investigated in the following section.

Influent daily loads showed (*Table 1*) the wetland retains an average of 50 billion MPs per day. Through further calculations, the partitioning of MPs presence was made clear. An estimation of ~ 190 billion MPs are retained in the soil. This approximation was calculated by using the area of the wetland (42 ha), the typical depth of soil (10 cm), and the average occurrence of MPs per kg of soil. This demonstrates a distinct overall presence, and represents soil is the main sink component of the wetland, however additional research may need to be conducted to further refine these values.

3.3. Vegetation

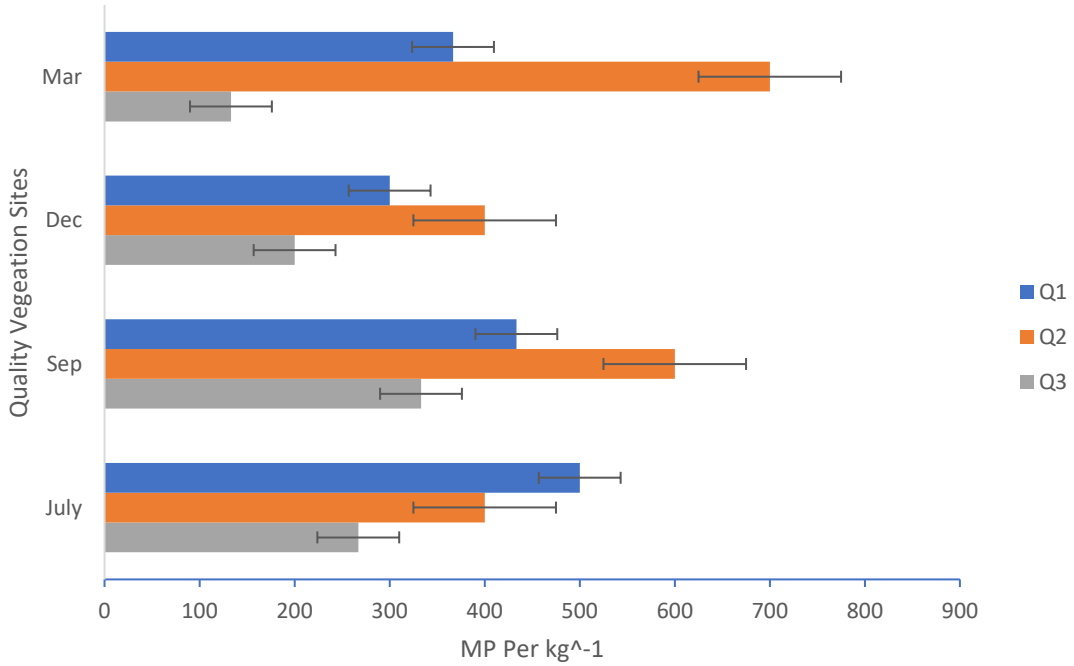

Figure 9. Distribution MP concentrations in vegetation samples in three testing sites (Q1, Q2, Q3) across the whole sampling period. The bars represent the minimum and maximum concentrations while the box represents the median.

The wetland ecosystem sees an average of 386 microplastics ranging from 133-700 MPs kg^{-1}. Q1 and Q2 showed similar averages of 400 MPs kg^{-1} and 525 MPs kg^{-1},

respectively. Q3 showed a reduction in MP occurrence with an average of 233 MPs kg^{-1}. Looking at this from a system perspective, peak aboveground biomass ranges from 1,586 to 2,666 gdw m^{-2} (grams of dry weight), of which Typh represents >60% (Weller et al, 2016). Based on these values, the wetland would contain between 12,240,000 MPs – 20,580,000 MPs in the entire aboveground biomass. Looking at this from a system perspective, the wetland retains ~50 billion MPs, while vegetation is contributing to the overall retention, it is not the main sink. When comparing vegetation to that of soil, we can see the majority of plastics are removed by means of soil.

Typha samples showed a smaller abundance of MPs relative to soil samples, as 1 kg of dry biomass of vegetation ranges from 133-700 MPs and 1 kg of dry sediment ranges from 1017-10100 MP. One explanation for this is the relative age gap between individual Typha and the duration of the soil's existence in that area. Typha are periodically thatched to promote fresh growth, in this we see new Typha biomass. Soil, however, remains constant and can therefore continue to act as a sink for plastics over longer periods of time (i.e., accumulating more MPs than Typha). Additionally, when vegetation is thatched, and there are plastics affixed to the blade and stems, they would be lost to machinery workings or possibly fall back into the water column or soil below. Both reasons could attribute to the reduced amount of MPs found on/in vegetation compared to soils.

Seasonality

Determining seasonal variation was done in an analogous manner to soil samples. A t-Test was used to interpret the difference between the highest plant MP concentration month and the lowest. No seasonal variation occurred statistically between vegetation samples.

The second indirect measurement of trends within seasons was to test for biological tide in relation to MPs. Biological tide occurs as a result of vegetation *pulling* water from the open surface water to the outskirts because the evaporative loss is so great in summer months that more extreme water quantities are required (Bois et al., 2017). This water loss results in a force that drives a lateral flow of surface water from open water areas into the vegetated marsh. Based on this phenomenon, soil and vegetation samples in the month of July were thought to show an increase in MPs. This, however, was not indicative of the data for soil that had the highest number occurring overall in September. Likewise, vegetation's highest averages MP occurrence in September. Initially thought to influence results, the plant-mediated "biological tide" showed no influence over the soil and vegetative samples in July.

Spatial distribution

The spatial distribution of MPs in vegetation samples along the flow path varied significantly. The sampling sites displayed statistically significant differences from one another, with the largest difference (56% reduction) observed between Q2 and Q3. The majority of MPs were found in Q2 with an average of 525 MPs kg^{-1} (see *Figure 9*). Contrary to soil samples the MP occurrence in vegetation was highest in the middle (Q2) section on the wetland while for soil, the highest concentration occurred at the inlet section of the wetland (Q1). In both sample types we see the lowest quantities of MPs in the section nearest the exit (Q3). This aligns with what was hypothesized, that a significant amount of MPs will be retained within the system, which can be indicated by a reduction in plastics in the outflow samples.

3.4 Atmosphere

Microplastics were detected and quantified at both atmospheric deposition sampling sites, thereby confirming also an ubiquitous atmospheric input into the study site. Over the span of 7 days, Site 1 saw an atmospheric deposition flux of 1364 MPs m^{-2} day^{-1} while site 2 showed a flux of 1656 MPs m^{-2} day^{-1} (*Table 3*). These concentrations are quite high, though no known testing for atmospheric MPs have been performed regarding the Tolleson, AZ area (city in the nearest vicinity of Tres Rios), just East of this in Tempe, AZ MPs were found at an average deposition flux of 178 MPs m^{-2} day^{-1} (Chandrakanthan et al., 2023). Future work conducted in this area should look into reasons for the locally high atmospheric deposition fluxes.

Table 3. Microplastic abundance in Atmospheric Deposition samples of 500 ml^{-1} and fluxes.

Location	Number of MP Counted	Deposition Fluxes
Site 1	168	1364 MPs m^{-2} day^{-1}
Site 2	204	1656 MPs m^{-2} day^{-1}

An estimate of the impact of atmospheric deposition fluxes of MPs in the Tres Rios wetland revealed a negligible contribution compared to the MP already present in the water, in fact it would yield 0.5 MPs/L or <1% of even the outflow concentrations. Atmospheric deposition samples show a clear presence of plastics in the surrounding area of Tres Rios. However, the overall atmospheric fallout was not significant enough to impact the system. Comparing this study's average atmospheric flux, 1510 MP m^{-2} day^{-1}, a more pronounced number is found. This demonstrates some inconsistency for the locality

of the area, which is likely, given that only two atmospheric samples were tested at Tres Rios. Additionally, the week of deployment for deposition samples had consistent rainfall events, which may influence this variability.

4. CONCLUSION

This study has shown various analysis to establish microplastic concentrations throughout a treatment wetland. Inflow and outflow water samples were used to determine if the system provided a retention of MPs—indicated by a reduced number leaving through outflow. Soil and vegetation samples were measured for their ability to serve as a MP sink. Microplastic concentrations in water samples show a retention rate of 55%, signifying MPs are being held within the wetland. Based on the p value of inflow and outflow data (p value <0.01), the null hypothesis was rejected, meaning the alternative was accepted, indicating a retention of MPs. The soil and vegetation samples retained a range of MPs between 1,017- 10,100 MPs kg^{-1} and 133-700 MPs kg^{-1}, respectively, indicating a removal from the surrounding surface water. While Atmospheric deposition samples were higher than other studies from surrounding cities reported, they had no clear influence over additional MPs added to the system. Although originally anticipated seasonal variation would occur within the system, there was no evidence to suggest this was occurring over our timescale.

Future work may choose to focus on the elevated atmospheric deposition samples in the area as these fluxes may be indicative of a unique source to the area. Additionally, it is understood that Tres Rios receives variable loads of influence from the 91st Ave WWTP, over a larger temporal scale, one might see changes in MP concentrations if studied more in depth. Lastly, being that plastics are a common occurrence in every wetland component, it is likely they exist in the existing animal community. Addressing if this remains true would further the ecological perspective of this study.

Though there is evidence for constructed wetlands to act as a retention barrier for MPs from tributary sources of urban water, the effects of plastics on existing biotic

communities within the initial system is problematic. Given the importance of this habitat for not only its primary role, water treatment, but its ecosystem service as a hotspot for wildlife communities, it is essential to understand the adverse effects of utilizing wetlands as a sink. The distribution of MPs found in Tres Rios indicates a higher presence in soil and water samples, which may shape future research regarding how adjacent ecosystem inhabitants are impacted. With the lack of incentives to properly dispose, reduce consumption, and change overall behavior surrounding plastic use, issues will continue to arise as a result of mismanagement of plastic waste.

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APPENDIX A

SAMPLE'S RAW DATA

Table A1.

MP Abundance In Soil in kg^-1

	Q1	Q2	Q3
July	5600	1800	1300
July	5700	2600	2233
Sep	6600	1400	2600
Sep	4500	3433	1700
Dec	3767	1800	2200
Dec	7800	2467	1583
Mar	5133	1233	1367
Mar	5650	1400	1833
July	7800	3400	1600
July	4833	2900	1200
Sep	7667	2200	2067
Sep	6600	1300	1500
Dec	3633	2183	2483
Dec	6200	3150	1267
Mar	5700	2900	1483
Mar	10100	2617	1017

Table A2.

MP Abundance in Vegetation in kg^-1

	Q1	Q2	Q3
July	500	400	267
Sep	433	600	333
Dec	300	400	200
Mar	367	700	133

Table A3.

MP Abundance in Water Per Liter

	Inflow	Outflow
July	352	132
July	298	165
Sep	396	96
Sep	271	132
Dec	330	100
Dec	344	111
Mar	306	128
Mar	311	101
July	398	126
July	257	156
Sep	376	273
Sep	301	199
Dec	324	90
Mar	316	180
Mar	246	188

APPENDIX B

METHODOLOGY VARIATIONS FOR SAMPLE TYPE

Table B1 Methods followed for individual sample types regarding collection and sampling process

Water	Samples were collected at the hydrologic station directly from the pipe dispersal. Each water sample was gathered in duplicate, Each sampling bottle was rinsed twice prior to collecting the final sample. This was completed at both the inflow and outflow.
Soil	Samples were collected at each quality site. A random number was generated to select the distance along a 50 m transect that would indicate where to source a soil sample from. Soil samples were collected using a soil coring device.
Vegetation	Samples were collected at each quality site. A random number was generated to select the distance along a 50 m transect that would indicate where to source a vegetation sample from. A single *Typha* sample was sourced by clipping the exposed section and coring the root portion.
Atmospheric	Deposition samples were collected twice during the remaining month of the project. Deposition samplers were set up along the outskirts of the wetland and were collected after two weeks.

Table B2. Methods followed for individual sample types regarding Density Separation

Water	300g NaCl was directly poured into the water samples that were measured individually of 1000ml. Samples were stirred vigorously for 10 mins. *note Wet Peroxide Oxidation step proceeds the density separation phase in water samples because no decanting was necessary. After NaCl was added to water, preceded by WPO, water was allotted 24 hours to align with other samples methodology.
Soil	Salt solution was poured over the previously measured 60g soil vials until reaching the top and stirred vigorously for 10 mins. They were allowed to settle for 24 hours
Vegetation	Ground samples were added to vials and mixed with salt solution until reaching top of the vial. Samples were mixed vigorously for 10 mins and a small beaker was added to the top of vegetation to keep fully submerged while allowing for plastics to rise. This mixture was allowed to settle for 24 hours.
Atmospheric	A salt solution (150g NaCl) was added to 500ml samples from the previous step, mixed vigorously and allowed to settle for 24 hours. The same procedure was followed for deposition samples as was done for water samples (WPO done first).

Table B3. Methods followed for individual sample types regarding WPO

Water	10 ml of $FeSO_4$ was added to individual vials then the addition of H_2O_2 at a volume of 10 ml. These samples went on to the Density separation phase next.
Soil	After settling overnight the top layer was decanted into a new vial leaving behind soil residue. Then 10 ml of $FeSO_4$ was added to individual vials then the addition of H_2O_2 at a volume of 20 ml. (See fig. 5). After the initial reaction took place, contents were allowed to cool, then placed on a hotplate with a stir bar. Peroxide was added in 10 ml increments until no reaction took place.
Vegetation	After settling overnight the top layer was decanted into a new vial leaving behind previous soil residue. All plant material was rinsed with DI water to ensure MP removal. Then 10 ml of $FeSO_4$ was added to individual vials then the addition of H_2O_2 at a volume of 20 ml. (See fig. 5). After the initial reaction took place, contents were allowed to cool, then placed on a hotplate with a stir bar. Peroxide was added in 10 ml increments until no reaction took place.
Atmospheric	10 ml of $FeSO_4$ was added to individual vials then the addition of H_2O_2 at a volume of 10 ml. These samples went on to the Density separation phase next.

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