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First investigation of micro-plastics pollution in the River Nile in Khartoum, Sudan: using Nile Tilapia (Oreochromis niloticus) as a bio-indicator

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First investigation of micro-plastics pollution in the River Nile in Khartoum, Sudan: using Nile Tilapia (*Oreochromis niloticus*) as a bio-indicator

By

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Science

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Co-supervisor**: Dr. Heidi Richards**

October 2025

DECLARATION

I declare that this dissertation is my own work, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other university.

........

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ABSTRACT

Microplastics (MPs) have become the bane of the environment for decades, and their impact on freshwater systems is still understudied. In addition to the inevitable and potentially irreversible imbalances in ecosystems caused by particulate matter pollution, environmental standards and coordinated actions are limited by the lack of knowledge about their abundance, distribution, and fate in the ecosystem. This project aimed to generate new data on the occurrence and characteristics of MPs in the River Nile in Khartoum, Sudan, using Nile Tilapia *(Oreochromis niloticus)* as a bio-indicator. The study also aimed to assess the contribution of anthropogenic activities to MP pollution in the River Nile.

To achieve this, thirty Nile Tilapia fish samples (freshly caught) were bought from Al-Mawrada fish market. Samples were frozen and transferred to the MPs laboratory at the University of the Witwatersrand, Johannesburg, South Africa. The digestive tracts were removed and individually digested with 10% potassium hydroxide (KOH) to degrade biogenic and natural organic matter that could interfere with MP identification. MP particles were separated according to their density using Sodium iodide (NaI) as a density separator. Nikon light microscope (stereomicroscope) was used to detect and identify MPs based on size, shape, and colour. The surface morphology of MP particles was examined by scanning electron microscope (SEM), and Raman spectroscopy was used to identify the polymer types.

The weight of the fish ranged from 0.11 to 1.39 kg with an average of 0.48 ± 0.38 kg. Two-thirds of the fish samples (twenty fish) were identified as males, leaving ten female samples. The values of condition factor (K), except for one sample, were greater than 1, implying that the fish were in good health. A total of 567 particles were recovered from the digestive tracts of the Nile Tilapia, ranging from 5 to 45 MPs/individual fish with an average intensity of 18.90 ± 9.17 MPs/individual. The average abundance of MPs in the fish samples was 72.07 ± 62.06 particles/kg.

The examination of the physical characteristics of MPs revealed a predominance of fibers (85.19%), small-sized particles of less than 0.5 mm (30.51%), and coloured MPs (83.60%). However, MPs of other shapes and sizes were also present in considerable percentages, for instance, 25.93% of MPs were detected in the size range of 0.5 - 1 mm, and 27.69% were in the range of 1 - 2 mm. In terms of shape, fragments accounted for 9.52% of the total MPs, while other shapes were present in very small quantities.

The chemical characterization of MPs in this study showed two types of polymers, polyethylene (PE) and polypropylene (PP).

The high prevalence of fibers and fragments indicates that MP contamination in the River Nile is from secondary sources. These shapes are formed by the fragmentation of macro-plastics. Their potential sources include domestic and industrial effluent, recreational activities, and surface run-off from urban centres. This study is the first investigation to assess MPs in the River Nile in Sudan. It provides the first data on their presence and bioavailability, thus providing a benchmark for guiding future monitoring studies and identifying research gaps.

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Conference Presentations

Saad D. and Alamin H., 2024, Saad D., Nile Tilapia reveal microplastic contamination in the River Nile in Khartoum, Sudan, ACS Spring 2025, March 23 - 27, 2025, San Diego, US.

Popular Articles

1. Nile Tilapia Reveal the Hidden Plastic Threat in Africa's Lifeline, Science featured series, Mar 2024. https://sciencefeatured.com/2024/03/14/nile-tilapia-reveal-the-hidden-plastic-threat-in-africas-lifeline/#

2. Microplastics found in Nile River's tilapia fish: new study, The Conversation, Feb 2024. https://theconversation.com/microplastics-found-in-nile-rivers-tilapia-fish-new-study-221655

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List of abbreviations and acronyms

Attenuated total reflection	ATR
Bioaccumulative toxins	PBT
Dichlorodiphenyltrichloroethanes	DDT
Differential scanning calorimetry	DSC
Dimethyl sulfide	DMS
Focal plane array	FPA
Fourier-transform infrared spectroscopy	FTIR
Gillnets, trawls, hand lines, and long lines are important elements of Abandoned, Lost or Discarded Fishing Gear	ALDFG
Hexachlorocyclohexane	HCH
High-density polyethylene	HDPE
Low-density polyethylene	LDPE
Microplastics	MPs
Near-infrared spectroscopy	NIR
Nuclear Magnetic Resonance	NMR
National Oceanic and Atmospheric Administration	NOAA
Polyamide	PA
Polychlorinated biphenyls	PCB
polycyclic aromatic hydrocarbons	PAH
Polyethylene	PE
Polyethylene terephthalate	PET

Polypropylene	PP
Polystyrene	PS
Polyurethane	PUR
Polyvinyl Chloride	PVC
Potassium hydroxide	KOH
Pyrolysis-Gas Chromatography-Mass Spectrometry	Pyr-GC/MS
Ultra Violate	UV
Scanning electron microscopy	SEM
Sodium Iodide	NaI
Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry	TED-GC/MS
Wastewater treatment plants	WWTPs

Chapter 1

INTRODUCTION

This chapter presents a general background about plastic and their uses, manufacturing, and types; followed by information about microplastics "MPs"; Nile Tilapia (Oreochromis niloticus); and the research problem statement and motivation, and the aim and objectives of the research.

1.1 General background

The use of plastic products has become an important part of everyday life, with applications ranging from clothes, furniture, and electronics to highly specified products such as prosthetics and engineered nano-plastics for drug delivery (Crawford and Quinn 2017; Heller et al. 2020). The term "plastic" is derived from the Latin "plasticus" which means capable of being shaped or moulded. These include multi-element materials such as carbon, oxygen, hydrogen, nitrogen, sulfur, and chlorine (Brydson 1999; Hammer, Kraak, and Parsons 2012; Beltrán Armenta et al. 2024). Structurally, plastics are formed by the composition of many repeating chains of monomers, combined with chemical additives, and can be moulded and cast into various shapes. They are polymers with long molecular chains formed by combining short repeating subunits in a chemical process called polymerization (Kesy et al. 2016).

Plastics are made from both natural and synthetic materials, often derived from oil, natural gases, or coal (Orem et al. 2014). Most plastics are based on carbon atoms except for silicones, which are based on the silicon atom. Carbon atoms are linked to oxygen, hydrogen, nitrogen, chlorine, or sulfur (Brydson 2014). Other polymers are inorganic with silicon or phosphorus backbones. When such connections form long one-dimensional chains, the polymer is called thermoplastic. Thermoplastics have the same repeating units and are known to melt and deform when heated. These include commonly used plastics such as Polyethylene (PE), Polypropylene (PP), Polyvinyl Chloride (PVC), Polyamide (PA), and Polystyrene (PS) (Maldonado et al. 2025). On the other hand, thermoset plastics have two- or three-dimensional networks; they cannot be melted or reshaped. Therefore, thermoset plastics are not recyclable, however, they are more durable than thermoplastics. Polyurethane (PUR), silicone, acrylic, and epoxy resins are examples of thermoset plastics. Thermoplastics are used more frequently than thermoset plastics (Wu et al. 2024; Busto et al. 2025).

The first mass-produced stable thermoplastic polymer was Bakelite (a phenol formaldehyde resin), which was discovered in 1907 by Leo Baekeland when he mixed phenol and formaldehyde at high temperature and pressure (Baekeland 1909). More synthetic polymers were developed in the late 19^{th} and early 20^{th} centuries, some of them were accidentally discovered (Harper 2000). For example, in 1933, a leak in an experimental tank led to the development of PE, one of the most commonly used plastics today, when trace amounts of oxygen reacted with ethylene under high pressure. However, the researchers were initially unable to replicate the product. As scientists

learned more about the synthesis process, new materials were developed in the early 20th century, including PVC, PET, PUR, PS, and Low-density polyethylene (LDPE). PP and High-density polyethylene (HDPE) were first developed in the early 1950s (Hammer et al. 2012).

Although it varies between types, all plastics can withstand certain conditions, such as extreme heat and the leaching of harmful substances (Liu et al. 2022). However, three types of polymers, including HDPE, PET, and PP, are considered safer due to their thermal and chemical stability (Zimmermann et al. 2019). Despite ongoing efforts to establish sustainable methods and strategies for recycling, among all types of plastics, HDPE and PET remain the most commonly recycled types.

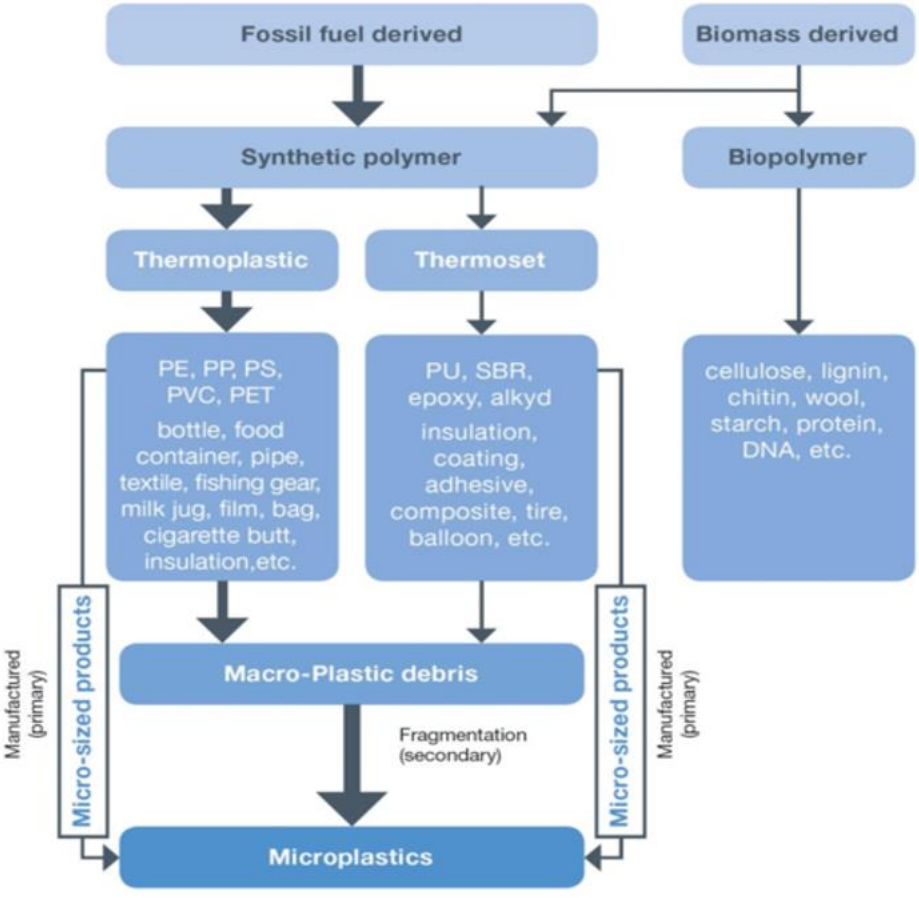


Fig 1.1: A generalized schematic of plastics production (Thompson 2016)

The physical and chemical properties of plastics, namely, the long, flexible, and interlinked chains of molecules, result in outstanding properties, such as resistance to corrosion, thermal and electric insulation, strength, durability, etc. As a result, many everyday products incorporate plastics and are used in numerous applications, including textiles, construction, packaging, and components in electronics and vehicles, to name a few (Maity et al. 2023).

Various additives are added to polymers during production processes to change some characteristics and/or to improve desired properties (Kantaros et al. 2024). This allows plastics to take many forms, with different appearances, durability, and performance. Common additives include plasticisers, thermal stabilizers, dyes and pigments, ultraviolet blockers, and flame retardants (Harding, 2016). Some of these additives are toxic even at low concentrations, and when they leach out of plastics, they can be harmful to humans and the environment. Plastics undergo biological, physical, and chemical degradation processes that convert them into smaller fragments called microplastics "MPs".

MPs are plastic particles less than 5 mm in length. Due to their persistence and lightweight they have been reported in almost all aquatic compartments, including water columns, sediment, and biota, in seas, oceans, rivers, and lakes worldwide (Ramaremisa et al. 2024). Due to their wide distribution, MPs are bioavailable to organisms living both in benthic and pelagic environments (Carbery et al. 2018).

MP research has primarily focused on marine systems, with limited freshwater studies. There is still a significant lack of knowledge about their existence, abundance, pathways, transport, and fate in freshwater environments. Geographically, MP research is mainly concentrated in Europe and North America, with Africa, Asia, and South America being underrepresented (Jambeck et al. 2018). Recently, there has been a growing interest in studying MPs in freshwater systems where vast numbers of people depend on rivers for essential activities and services. Rivers are the main transport routes for large amounts of plastic waste and MPs from land-based sources into the oceans (Carbery et al. 2018).

In recent years, MPs have been found in the water, soil, and biota of some of the world's major rivers, including the Thames (UK), the Rhine (Germany), and the Seine (France) in Europe, the Yangzte (China), and the Amazon (South America) (Dris et al. 2015; Mani et al. 2015; Lechner and Ramler 2015; Schmid et al. 2018; Zhao et al. 2014). A notable river not on this list is probably the most famous one, the River Nile.

The Nile is the longest river in Africa and the second longest in the world; it is the only permanent river that crosses the Sahara Desert. The White Nile and Blue Nile are the two main tributaries of the Nile (Zeidan 2024). The White Nile is considered a source river and flows in a wide straight channel that begins at Lake Victoria and flows through Uganda and South Sudan. Although narrow with very pronounced meandered, the Blue Nile originates in Lake Tana in Ethiopia, accounting for 80% of the Nile water and lower in the Nile mud (Bangs 2024). The White Nile joins the Blue Nile in Khartoum, the capital of Sudan, and flows towards Egypt, where it empties into the Mediterranean Sea.

Khartoum is one of Africa's largest cities. It is a growing metropolis city situated at the confluence of the Blue Nile and the White Nile (Zerboni et al. 2021). The River Nile covers an area of 3.18 million km^2 which is about 10% of the land area of Africa and is shared by 11 countries, namely, Burundi, Democratic Republic of the Congo, Egypt, Eritrea, Ethiopia, Kenya, Rwanda, Sudan, South Sudan, Tanzania and Uganda (Mohamed 2016). It is home to over 300 million people and supports various economic and social activities including agriculture, fisheries, transport, and tourism. Not only does it provide a permanent source of water and fertile soil for agriculture, but it has been a key to the rise of ancient civilizations (e.g., Sudanese and Egyptian civilizations). However, the adverse effects of plastic and particulate matter pollution are a common threat that has yet to be addressed (Dumont 2009). This project aimed to investigate the presence and extent of MPs in the River Nile using Nile Tilapia as a bio-indicator.

Nile Tilapia fish *(Oreochromisniloticus)* is often called the aquatic chicken and is considered the second most farmed fish in the world, after carp (El-Sayed and Fitzsimmons 2023a). It is a freshwater cichlid native to the River Nile basin, the northern half of Africa, the southwestern Middle East, the Niger, Volta, Senegal, Benue, Albert, Edward, Kivu, and Tanganyika rivers (Stauffer Jr et al. 2022). It has been introduced to over fifty countries on every continent except Antarctica and is now virtually ubiquitous in the tropics (Bonham 2022). Nile Tilapia can grow up to 60 cm (24 in) in length and weigh up to 5 kg (11 lb); males reach a larger size and grow faster than females. It is found in different freshwater environments, including lakes, rivers, streams, ponds, and canals, and at elevations ranging from sea level to 1,830 meters (6,000 ft) (Degsera 2019). They also live in brackish water but cannot survive in saline water for long periods. Nile Tilapia live at water temperatures between 8 and 42°C (46 and 108°F). They are among the most common freshwater fish due to their fast growth, resistance to low oxygen levels, and simple

farming (El-Leithy et al. 2019). Known as Bulti in the Sudanese Arabic dialect, Nile Tilapia is an economically and ecologically dominant species in Sudan and is widely consumed. Therefore, it is a suitable target organism to bio-monitor MPs in the region.

1.2 Problem statement and motivation

Plastics have been an integral part of our ecosystem since their introduction in the early 1960s. Most plastics are not biodegradable and pose significant environmental challenges. In the past decade, MPs have gained significant attention as emerging pollutants, becoming a key area of global environmental research. Concerns related to MPs arise from potential risks to the environment and human health due to properties such as (i) bio-accessibility: due to their small size, they are readily available for ingestion and potentially susceptible to transmission through the food chain; (ii) non-biodegradability: resistance to environment biodegradation involves long environmental residence time; (iii) prone to further fragmentation: due to natural degradation processes, MPs are expected to gradually decrease in size over time (Leslie and Depledge, 2020; Saad D. 2023).

As a developing continent, Africa has a high demand for plastic products due to their low cost and availability. Although Africa has lower plastic production and consumption rates than China, India, Western Europe, and North America, it has the highest plastic emissions in the world due to poor waste mismanagement (Environment et al. 2018). Therefore, African freshwater ecosystems are at higher risk of MP pollution (Gómez et al. 2020).

Research on MP pollution in African freshwater systems has started late compared to other continents. Until recently, MP research on the continent was limited to marine and coastal areas, and this study is the first assessment of MP occurrence in the River Nile in Khartoum. Due to their correlation with water and sediments, aquatic biota are recommended “as suitable bio-indicators” to study the level of MP contamination in water bodies. Nile Tilapia inhabit shallow waters and are omnivorous, feeding on plankton, insects, smaller fish, and other plant materials. Nile Tilapia is a dominant economic and ecological species in the River Nile in Sudan, and it is widely distributed and frequently consumed. Upon it, the presence of MPs in the Nile Tilapia could provide valuable information to better understand the fate of MPs in the River Nile (Khan et al. 2020). The River Nile basin has supported human life since the earliest days of human civilization. It covers about 10% of the African landmass and hosts 20% of the African population who depend

on livestock and agriculture for their livelihoods. The River Nile also plays a key role in energy generation and industry, as well as its social and tourism importance (Batisha 2023). To date, there is no single study reporting the presence of MPs in aquatic compartments in the River Nile in Sudan. The slow economic growth influences the prioritization and application of capital to stimulate development. Therefore, such environmental issues receive little attention. Sudan in particular suffers from poor waste management due to a lack of institutional organization, limited funds, poor waste collection equipment, and lack of urban planning and legislation. Much solid waste, including plastics, ends up in landfills or is dumped illegally. Khartoum is a densely populated city and urban center, as well as the largest industrial centre in Sudan (Ille et al. 2020). Based on this, and given the size of the River Nile and the populations and activities it supports, the likelihood of MP pollution in the River Nile is significant.

1.3 Aim of the research

This study aimed to address the knowledge gap in MP research in the African context and the lack of data on MP pollution in African freshwater systems. Using Nile Tilapia as a target organism to bio-monitor MPs, this study aimed at generating new data on the occurrence, characteristics, and potential sources of MPs in the River Nile in Khartoum.

1.3.1 Specific objectives

- To investigate the existence, intensity, and abundance of MPs in the digestive tract of Nile Tilapia collected from the River Nile in the city of Khartoum.
- To characterize the MPs found in the samples in terms of size, shape, surface morphology, and colour.
- To identify polymer types to predict sources, pathways, and potential anthropogenic inputs around the River Nile.
- To assess the biotic factors that influenced the consumption of MPs by Nile Tilapia (feeding habits, sex, and size/age).

1.3.2 Research questions

The study was designed to answer the following questions:

- Do Nile Tilapia fish ingest MPs?

- What types of MPs are ingested (size, colour, shape, and polymer composition)?
- Is there a correlation between the shape and colour of MPs ingested and the preferential feeding habits of Nile Tilapia?
- Is there a correlation between the size, length, and sex of fish and the number of plastic particles they ingest?
- What are the possible sources of MP pollution in the Nile River in Khartoum?

1.3.3 Hypotheses

- The River Nile, as an urban river that flows through some of the most densely populated cities in Africa, will most likely be contaminated by MPs.
- Nile Tilapia ingest MPs as long as they are present in the environment.
- Ingestion of MPs by the fish may be influenced by both biotic and abiotic elements.
- According to their feeding habits, Nile Tilapia fish may preferably consume certain shapes, colours, or sizes.
- Different anthropogenic activities may contribute to the MP pollution in the River Nile.

Chapter 2

LITERATURE REVIEW

This chapter provides an overview of the literature on plastics manufacturing, the global microplastic pollution situation, and the risks they pose to water resources, human life, and aquatic life worldwide; their common sources; their physical and chemical properties; and the analytical techniques commonly used to characterize them

2.1 Plastics production

Plastics are composed of high molecular weight synthetic polymers (derived from fossil fuels) and natural organic polymers (derived from biomass). They are formed under a certain pressure and temperature and have different properties depending on the requirements of the end product (Desidery and Lanotte 2022). Plastics have improved the quality and ease of our lives due to their beneficial properties such as low costs, strength, durability, corrosion resistance, and thermal and electrical insulation. They are used in virtually every aspect of society, such as agriculture, transportation, sports, recreation, architecture and construction, design, manufacturing, electronics, and currency (Beltrán Armenta et al. 2024). About 80% of the world's total demand is covered by polyolefin polyethylene (PE) and polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyurethane (PUR), which are divided into two groups: (i) polymers with C-C backbone and (ii) polymers with C-O backbone as shown in Fig 2.1. PE, PP, PVC, and PS are C-C backbone polymers accounting for 77% of the total market share, while PET and PU are heteroatomic polymers accounting for about 18% of the total market share (Gewert et al. 2015). The C-C backbone polymers are resistant to biodegradation and hydrolysis and sensitive to thermal oxidation, in contrast to polymers with heteroatoms, which can be treated by hydrolysis, biodegradation, and photo-oxidation (Krueger et al. 2015).

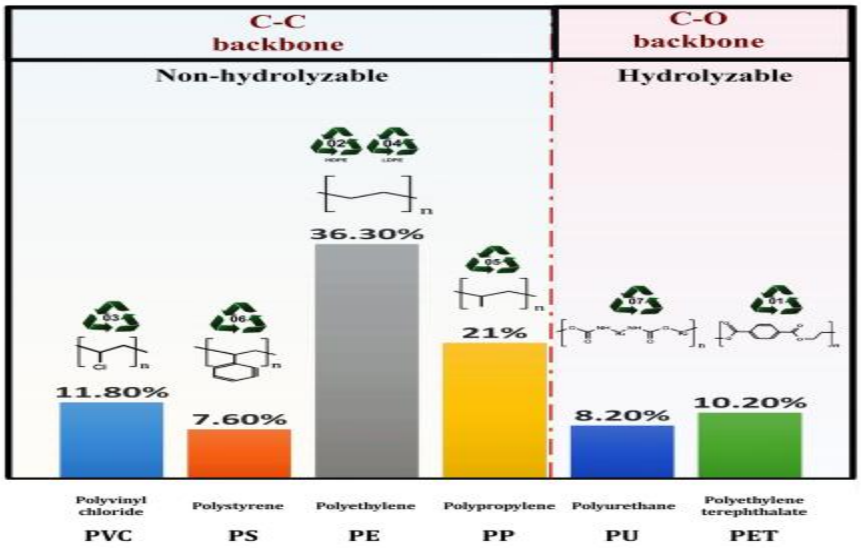


Fig 2.1: The most prevalent plastics, and their classification according to chemical backbone structure (Ali et al. 2021)

The chemical composition of a polymer affects its properties, including physical, thermal properties, degree of crystallinity, and density (Beltrán Armenta et al. 2024). For example, polymers are made into fibers, films, and foams, which affect their size, shape, and other properties, including density. For example, foams are plastics with a light cell structure as they contain a porous or foaming component that creates open, interconnected, or closed cavities (Ghosh et al. 2023). Plastics in the environment are as diverse as the products they come from, with shapes, sizes, densities, and chemical compositions ranging from small to large (Duis and Coors 2016) as shown in Table 2.1. The structure, composition, and associated physical and chemical properties of plastics directly affect their behavior in the environment, including routes of transport and fate in aquatic systems.

Table 2.1: Popular polymer types, their densities and related products (Zhao et al. 2018)

Type of polymer	**Typical use**	**Density (g/cm^{-3})**
Natural rubber	Vehicle tyres	0.29
Polyethylene	Supermarket bags, plastic bottles	0.91–0.96
Polyethylene* - low density	all-purpose containers, shower curtains, packaging,, and floor tiles	0.91–0.93
Polyethylene* - high density	Bottles, pipes	0.94–0.98
Polypropylene	Packaging, bottle caps, ropes, carpets, laboratory equipment, drinking straws	0.85–0.92
Polystyrene (expanded)	Cool boxes, floats, cups	0.016 - 0.36

Type of polymer	Typical use	Density (g/cm^{-3})
Polystyrene	Packaging foam, disposable cups, food containers, CDs, building materials	0.96 - 1.05
Polystyrene (high impact)	Electronics, printed graphics refrigerator liners	1.04
Acrylonitrile butadiene styrene	Musical instruments, printers, computer monitors, drainage pipes, protective equipment	1.06–1.08
Polyamide (Nylon)	Textiles, toothbrush bristles, fishing lines, automotive	1.12 - 1.14
Polycarbonate (bisphenol-A)	CDs, DVDs, glass alternative, electronics, lenses	1.2
Polyurethane	Foams	1.2
Metacrylate (acrylic)	Alternative for plate glass	1.19
Cellulose acetate	Cigarette filters, fabric fiber	1.28
Cellulose nitrate	printing inks, nail polish, foil	1.35
Polyvinyl chloride	Pipes, window frames, flooring, shower curtains, film	1.38
Polylactic acid (biodegradable)	Packaging, cups	1.21 - 1.43
Polyethylene terephtelate	Soft drink bottles, food packaging, thermal insulation, blister packs	1.34 - 1.39
Polyester	Textiles	1.40

Type of polymer	Typical use	Density (g/cm^{-3})
Melamine	Flooring, dinnerware, dry boards	1.57

LDPE, HDPE and PP have lower densities than fresh water (~1000 kg/m^3 density) and seawater (~1025 kg/m^3), while PVC and PET have higher densities. Some polymers vary in density depending on their state. For example, PS has a solid density of 1050 kg/m^3, while PS foam, extruded polystyrene, and extruded PS foam are light, with densities of 10 to 40 kg/m^3 (Zhou et al. 2020). However, despite its buoyancy, low-density plastic is present in the water column and sediments (Schwarz et al. 2019).

2.2 Plastics as an emerging environmental threat

In the early 1900s, plastic was made for military use, but soon found its way into industry and homes. Since the early 1950s, the amount of plastic produced has increased exponentially (Nikiema et al. 2020). In 2016, global annual production was 330×10^6 tons, with the potential to quadruple over the next 20 years due to projected global population growth (Chassignet et al. 2021). As a result, plastic is such a widespread material that geologists now regard its fragments in bottom sediments as a leading indicator of the current geological period (Anthropocene), in which the growing plastic debris has attracted global attention (Ramaremisa et al. 2025). The first reports of polluting plastic waste date back to the 1970s (Worm et al. 2017). The environment contained about 60% of the total amount of plastic produced in 2017 (8,300 million tonnes), of which 95% ended up in landfills and 50% in oceans and other land areas (Worm et al. 2017). By 2050, the global accumulation of plastic waste in landfills and/or in the environment is projected to reach nearly 12,000 Mt (Geyer, Jambeck, and Law 2017; Chamas et al. 2020). As a result, the same properties that make plastics so adaptable to humans have also led to growing concerns about the environment.

2.3 Degradation of plastics

Plastic waste can take hundreds of years to degrade (Table 2.2), depending on the physio-chemical properties of polymer materials and environmental conditions. During this process, their properties change, including crystallinity, hydrophobicity, functional groups, and mechanical properties (Chamas et al. 2020a). In nature, the degradation process of plastics starts with photo-degradation, followed by hydrolysis and thermal oxidation. These processes lead to the breakdown of plastic waste into low molecular weight compounds, which can then be metabolized by microbial activity (Sameh Samir Ali et al. 2021).

According to research, photo- oxidation, thermo-oxidation, hydrolysis and biodegradation are the main mechanisms of degradation of plastics in the environment (Ali et al. 2021c).

Table 2.2: Degradation period of major thermoplastic polymer (Ojeda 2013)

Polymer	Crystallinity (%)	Life span (year)
PS	0	50-80
PVC	0	50-150
PET	0-50	0-50
PP	50	10-600
LDPE	50	10-600
HDPE	70	> 600

2.3.1 Physicochemical properties

The physical and chemical properties of plastics play an important role in the degradation process. Skeletal carbon chains such as PP, PE, PS, and PVC make polymers resistant to degradation as they reduce the susceptibility of plastics to abiotic and biotic degradation (Ali et al. 2021c). However, the addition of heteroatoms, as in PET and PU (oxygen-containing polymers), makes the plastic susceptible to thermal and biological degradation (Wayman and Niemann 2021). Furthermore, the degradation efficiency is influenced by the hydrophobicity of the polymer and the rate of degradation increases with increasing hydrophilicity.

In addition, the degradation rate depends on the chemical structure, degree of crystallinity, and molecular weight. Polymers are large molecules that have both regular crystals (crystalline region) and irregular crystals (amorphous region), the latter imparting flexibility to polymers. Highly crystalline polymers such as PE (95%) are stiff and have low impact strength (Chamas et al. 2020a). Because the more crystalline the polymer structure, the more water and oxygen are required for degradation. For example, increasing the molecular weight or the degree of crystallinity reduces the rate of degradation (Sameh Samir Ali et al. 2021). On the other hand, water and oxygen can attack the structure of the amorphous polymer. In addition, the amorphous areas of the polymer are considered more suitable for thermal oxidation (Hawkins 1984). In addition, high molecular weight polymers show a slower rate of degradation due to their smaller relative surface area. Interestingly, the manufacturing processes and additives used in the manufacture of plastics have a significant impact on the degradation rate of plastics. For example, PP made by Ziegler-Natta catalyst or bulk polymerization is more prone to photo-degradation than PP made by copolymerization (Chamas et al. 2020b). Also, PS polymers formed by radical polymerization were less resistant to photo-oxidation than PS prepared by anionic polymerization due to the presence of functionalizing groups (peroxy moieties). In addition, various additives are used, including stabilizers to reduce the rate of degradation and chromophores (carbonyl and hydroperoxy groups). For example, the presence of chromophores causes the initiation of photo-degradation by photo-chemically generated radicals due to the presence of multiple photo-oxidation sites (Puls et al. 2011). Furthermore, the presence of metal-to-metal bonds can accelerate photo-degradation as homolytic bonds are broken down during irradiation (Dimassi et al. 2022). It should also be considered that the morphological properties of plastics, which tend to increase with rough surfaces, are more suitable for the formation of biofilms than smooth surfaces (Song et al. 2020).

2.3.2 Environmental conditions

The mechanisms and speed of plastic degradation are influenced by external factors such as ultraviolet (UV) radiation, wind, waves, seawater, bacteria, etc. These interactions lead to an increase in the concentration of polymeric alkyl radicals, which increases the rate of cleavage and cross-linking products. Furthermore, the availability of water plays a key role in the biotic and

abiotic degradation processes due to the hydrolysis process leading to functional group cleavage and polymer chain cleavage (Hawkins 1984).

Plastics are generally grouped into macro, micro- and nano-plastics in plastic pollution studies, with other subgroups sometimes added. Due to different methods of data collection, tracking and identification, size classifications are not standardized and a variety of size ranges and different terms have been used in the studies (Hidalgo-Ruz et al. 2012; Aragaw 2021).

2.4 Microplastics

Plastics can be divided into five size classes: megaplastic (> 1 m), macroplastic (≥ 25 mm), mesoplasty (5 to < 25 mm), microplastic (1 to < 5 mm) and nanoplastic (< 1 μm) (J. Lee et al. 2013) Thushari and Senevirathna 2020). MPs is a diverse group of plastics with different chemical compositions, origins, shapes, sizes, colours, and densities (Bessa et al. 2018; Blankson et al. 2022). They cover a wide range of sizes. Most often they are defined as small particles less than 5 mm in diameter in the largest dimension (Dehghani et al. 2017). There is practically no lower limit, and the nano-plastics definition often includes 0.1 μm (Peng et al. 2020).

However, the range of sizes reported in different studies may vary, for example, due to the use of different analytical instruments and methods (different sampling devices and methods, sample preparation techniques, and different methods of visual identification) (Hanvey et al. 2017). Recent studies by Gago et al. (2019) describe MP as solid particles or polymer matrix with regular or irregular shapes and dimensions from 1 μm to 5 mm, primary or secondary origin, insoluble in water. MPs are broadly classified based on their physiological characteristics, including size, colour, and shape. Size is an important categorizing factor for MPs, as it largely determines their mobility, placement, exposure, and fate. Small particles have a large surface area to volume ratio, which means they have a high potential for filtration and chemical adsorption (Velzeboer et al. 2014). According to Lorenzo-Navarro et al. (2020), MP can be divided into five groups according to their shape, as shown in Fig 2.2: (a) fibers (fibrous or thin and uniform plastic threads); (b) micropellets (hard and rounded particles); (c) fragments (hard particles with jagged edges); (d) foam a polystyrene-like material; and (e) film (thin two-dimensional plastics) Main plastics, the original shape, erosion and erosion processes of the particle surface (such as biodegradation, photo-degradation, physical forces, etc.), and the residence time of the particle surface in the environment are factors affecting the shape of the partial MP. Smooth edges indicate long

occupation, while sharp edges indicate recent entry (Sharma et al. 2021). Identification of these morphologies can give some indication of potential sources, such as textiles or ropes for fibers, as well as their behavior inside an environmental compartment (Brosset et al. 2015).

The plastics industry has incorporated a variety of colours into polymer blends to make plastics more visually appealing or to enhance mechanical strength against UV deterioration or flame retardancy (Ambrogi et al. 2017). The fact that the MP is coloured indicates that it is enriched with trace amounts of organic compounds. The colour can provide important information, such as the conditions the object has been exposed to or the preferred feeding habits of aquatic life (e.g. weathering and biofilm formation). Care should be taken when sorting coloured particles, as some can become transparent due to discolouration from atmospheric processes (Pfaff 2017).

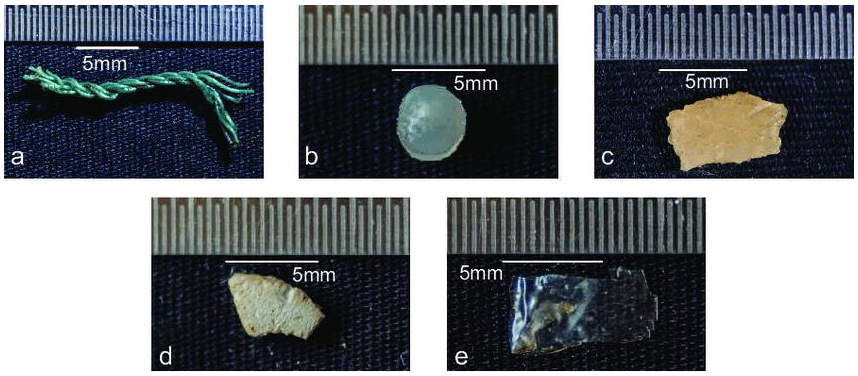


Fig 2.2: Shapes of MPs: (a) fiber, (b) pellet, (c) fragment, (d) foam and (e) film (Alvarez-Zeferino et al. 2020)

2.5 Sources of MPs

Member identification is essential to minimize negative social, environmental, and economic impacts. Based on their origin, MPs are further divided into primary and secondary, as shown in Figure 2.3.

2.5.1 Primary MPs

Primary MPs are made in millimeter (microscopic) size. These include (1) pre-production resin granules, typically 3-5 mm in diameter, used as raw material for plastic production; (2) projectiles used to blast surfaces; and (3) microspheres used in toothpaste, exfoliating creams, and other cosmetic and personal care products, and to a lesser extent in medicine, as drug delivery systems

(Thushari and Senevirathna 2020). For example, an estimated 6% of liquid skin cleansing products sold in the European Union, Norway, and Switzerland contain MP (Galafassi et al. 2019). Particles from cosmetic products typically enter the environment via wastewater, while particles used in the manufacture of raw materials may inadvertently enter the environment during transportation and handling or as a result of factory runoff or processing. According to press releases from the European Parliament, they account for 15% to 31% of fine dust in the oceans (Abiodun and Ayeleru 2025).

2.5.2 Secondary MPs

Secondary MPs result from gradual breakdown into smaller fragments due to mechanical forces, by photolysis, thermo-oxidation, and biodegradation processes (Saad et al. 2024a). They are generated by using plastic products (such as paints, tyres, and textiles) or after they have been disposed of into the environment, which are most MPs present in the environment. Identification of secondary MPs is difficult due to the variety of sources and pathways, they can enter the environment through a variety of routes including (1) fragmentation of items in landfills as well as tyre abrasion, which may introduce MPs into the rivers, ocean, and atmosphere, by surface runoff or\and wind; (2) Textile particles (fibers) can enter the environment via wastewater after washing or air during drying; (3) weathering of plastic litter on beaches and in coastal areas, may end up in coastal sediments or be transported further offshore and; (4) Weathered plastics used in agricultural applications may enter the environment through surface runoff from the soil. Temperature, UV light exposure, and abrasion are the main environmental factors linked to secondary MP production. However, according to news reports from the European Parliament, secondary MPs account for 69-81% of MPs in the oceans (Kiran, Kopperi, and Venkata Mohan 2022; Ge et al. 2023; Li et al. 2025).

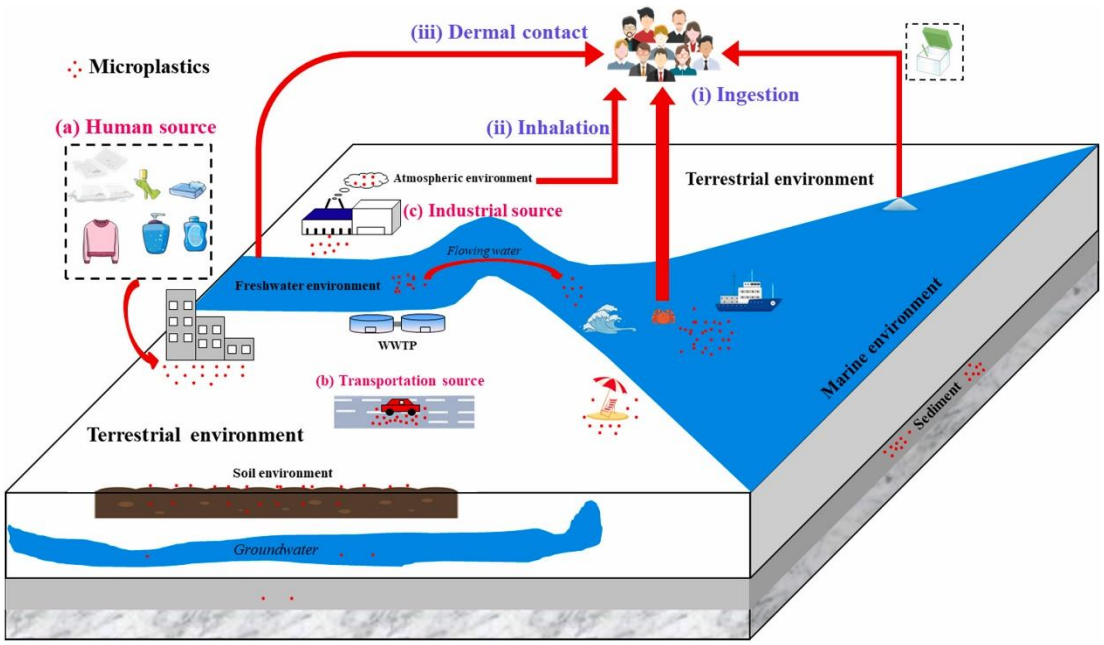


Fig 2.3: Microplastic source (Wang et al. 2021)

2.5.3 Fisheries as a source of MPs

As fisheries and aquaculture are highly dependent on plastics, aquatic activities (such as fishing, shipping, tourism etc.) introduce plastics directly into the aquatic environment (Chen et al. 2021). Aquaculture systems use equipment such as feeders, waterproof membranes, cages, floats, boats, fences, ropes, oxygenators, and materials to pack and transport the fish. These materials have low wind and wave resistance, and some gears are designed to catch species deep in the water column and on the seabed that expose the gear to irregularities such as rocks and crevasses, leading to damage and losses. Gillnets, trawls, hand lines, and long lines are important elements of Abandoned, Lost or Discarded Fishing Gear (ALDFG) that are the main source of plastic waste in the aquatic environment from fisheries and aquaculture, but there is considerable heterogeneity in their distribution and abundance (Lovell 2023).

In addition, MP fibers can be manufactured in cleaning equipment used in aquaculture environments to remove fouling organisms. In addition, environmental conditions such as weather and seabed irregularities, operational pressures (e.g. using too much fishing gear for limited periods) and illegal fishing can also contribute. According to Lejars, Margaillan, and Bressy (2012), ships can also be a source of MP, which can come from shipping traffic and peeling paint.

2.6 Potential impacts and risks of MPs

The concerns over MPs are due to the potential harms that induce negative impacts on both, the environment and human health. MPs are a heterogeneous class of pollutants with a broad range of individual properties such as particle size, particle shape, and material type (Scherer et al. 2018). These diverse material properties make them potentially available to a broad range of benthic species (sedimenting materials, density >1 g cm^{-3}), pelagic (materials in suspension) and neustonic (buoyant materials, density <1 g cm^{-3}). As a result, MPs can penetrate various trophic levels and ecological niches in aquatic food webs (Hidalgo-Ruz et al. 2012).

Other characteristics, such as shape, may influence the handling and capacity for ingestion (Botterell et al. 2019a). Many previous laboratory studies have used polystyrene microspheres, which have been proven to be readily ingested by a variety of species, demonstrating that this shape is bioavailable to a wide range of taxa (Cole and Galloway 2015; Botterell et al. 2019 ;Botterell et al. 2020). Nonetheless, several studies from the field investigating MPs ingestion in zooplankton found that microfibers were most commonly ingested (Desforges, Galbraith, and Ross 2015; Steer et al. 2017; ;Sun et al. 2017; Zheng et al. 2020; Goswami et al. 2023). It's unclear whether this shape is more bioavailable or if it was the most common MPs in the areas examined. Large plastic pieces consumed by aquatic species can cause internal or external wounds, decreased ability to feed, a false sense of satiety, debilitation, obstructed digestive tracts, ulcerating sores, choking, and even death. Though preliminary studies provided an indication of physical impacts, the actual impact of MPs-sized particle ingestion on aquatic organisms are largely unknown (Gall and Thompson 2015). However, some studies found that MPs ingestion by aquatic organisms can affect their feeding behaviour, reproduction, and growth. In addition to their possible health risks, MPs behave as a medium of transfer to concentrate persistent bioaccumulative toxic substances (PBTs) and chemicals to organisms (Simon et al. 2013). MPs can be carriers of two types of chemicals: (i) chemicals, which are integrated during the manufacturing process to improve product performance, the so-called additives, such as the flame retardants (polybrominated diphenyl ethers), catalysts (organotin), antioxidants (nonylphenol) and antimicrobials (triclosan), and (ii) pollutants, due to their hydrophobic properties and large surface area to volume ratio, MP have the potential to act as micro-vectors for toxic chemicals and organic pollutants, adsorbing and desorbing them depending on the prevailing environmental condition. Ultimately, when aquatic organisms ingest MPs, they can transmit these chemicals and pollutants to other aquatic

organisms, resulting in serious and adverse health impacts (Browne et al. 2013). More than 200 organic chemicals which are related to MPs have been identified, among which, dichlorodiphenyltrichloroethanes (DDTs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and hexachlorocyclohexanes (HCHs) are the most commonly detected (Gauquie et al. 2015).

Polymers such as polyvinylchloride and polystyrene are made of monomeric repeating subunits which can induce reproductive abnormities and release toxic monomers associated with cancer in humans, rodents, and invertebrates (Vom Saal and Hughes 2005).

The sorption behavior of MPs is highly influenced by their properties and the surrounding environmental conditions. For instance, it has been discovered that older MPs are more likely to absorb contaminants. Environmental factors, including salinity and pH, are known to impact how contaminants adhere to MPs (Guo and Wang 2019). According to research, the sorption of Pb, Ni, Co, and Cd on PE pellets rose with a rise in pH values, while the affinity of PE for PFOS increased as salinity increased (Holmes, Turner, and Thompson 2014; Fei Wang, Shih, and Li 2015).

The size of MPs has an important role in the sorption of pollutants on MPs (Guo et al. 2018). Organic pollutants sorb more efficiently on smaller particles than on larger ones. However, MPs with the same or similar sizes, differences in their specific surface area caused by their chemical composition, particle structure, and surface properties could lead to distinct sorption capacities (Rivers et al. 2019). For example, (Hüffer and Hofmann 2016), investigated the sorption of seven aromatic and aliphatic organic compounds by particles made of four types of polymers, all of which had diameters smaller than 250 μm PS > PVC > PE > polyamide (PA) were absorbed in that sequence according to their unique surface areas. In addition to affecting the specific surface area of plastic particles, crystallinity, spatial organization of the polymer units, and degree of cross-linking can lead to different sorption mechanisms (Guo et al. 2012).

2.7 Distribution of particulate matter in the aquatic environment

MPs are widespread throughout the world's habitats and persist for long periods in different aquatic compartments and environments, from the surface down to deep layers, including freshwater (lakes and rivers), groundwater, and marine water (Klein et al. 2018). Several studies have attempted to identify the occurrence, sources, and distribution of MPs (Cole et al. 2011). Ocean

estuaries and other coastal areas that are significantly impacted by anthropogenic influences were reported to be the most contaminated locations of MPs, according to Frere et al (Frere et al. 2017). The distribution of MPs in aquatic systems is influenced by various factors, some relate to: (i) environmental conditions, such as exposure to sun light, temperature, weathering, etc.; and (ii) others relate to their physical and chemical properties (size, shape, colour, density, and chemical composition) (Kane and Clare 2019).

The physical properties (size, shape, colour, and density) of MPs are essential for understanding their distribution and behavior in aquatic environment (Aragaw 2021). The main factor influencing MP distribution, transport, pathway, and fate in aquatic environments is size. The size distribution of plastic particles in the aquatic environment is impacted by a variety of factors, including biofouling and aggregation, fragmentation, transportation routes, flocculation, and the time of residence (Ryan 2015). The size of MPs is expected to gradually decrease over time due to natural degradation mechanisms in the riverine environment. Implying that, the longer plastic waste is exposed to the degradation mechanisms, the more abundant and smaller the size. Notably, size affects dispersal in the water column, consumption by sea species, and sediment accumulation. Furthermore, the particle size determines the efficiency of wastewater treatment plants (WWTPs) in removing MPs (Kurniawan et al. 2023). Additionally, the different size distribution was observed, and different techniques were adopted by different scholars. The MP sizes that are recorded are influenced by the sample methods, pretreatment techniques, and identification procedures that are used (Dong et al. 2023). Moreover, the sampling site (polluted and less polluted areas), the sample type (sediment, water, and biotas), and the water systems (freshwater and marine water) all have significant implications.

Identifying the shapes of plastic particles is very important for the subsequent establishment of policies on plastic waste management. It has been suggested that the shape of MPs is determined by residence time and fragmentation processes. Sharp edges may be the result of either the recent break-up of larger parts or the recent entry into the environment, While smooth edges may represent older fragments that have been continuously honed by certain particles or sediments (Chubarenko et al. 2016). The shape of MPs could affect their transport in the environment e.g., certain shapes are distributed differently; fragments are dominantly reported in water surface, while fibers and pellets are mostly found in the sediment (He et al. 2021). Also, the high percentage of fibers and fragments, particularly were recorded within larvae, fish, and insect samples

(Adeogun et al. 2020; Akindele, Ehlers, and Koop 2020). Foam MPs percentage abundant were observed in a small proportion as compared with other shapes. According to certain investigations, the amount of fiber in the total MPs samples differed depending on the region. The urban water bodies had more fibers than the coastal region, because of their release from textile products (Duis and Coors 2016; Dunne et al. 2016). However, previous studies have rarely compared the concentration of plastic fragments or non-fibrous MPs with fibers on a global scale.

Colour is another important physical characteristic that play significant role in determining the fate of MPs in the aquatic environment and their impacts on aquatic biota (Fen Wang et al. 2018). Due to the colour resemblance to the original prey, aquatic organisms may unintentionally ingest coloured MPs (Carreras-Colom et al. 2018). In addition, the colour of the plastic materials may indicate their potential sources. Black fragments could be originating from vehicles tires, and colored fibers could be from clothing. White and transparent MPs are commonly originated from fishing lines, plastic carry bags, and packing materials. Many of the reports from the literature reviewed confirmed that different colour MPs in different locations and sample types in the same water system were found (McNeish et al. 2018; Hossain et al. 2019).

MPs that are less dense than water can go quite a distance in surface water. As a consequence, the proportion of buoyant particles rises in direct proportion to the distance from the shore. On the contrary, high-density MPs are more likely to be trapped in setting sand grains and remaining in the sediment (Duis and Coors 2016). The two most diverse polymers found in marine sediments are PS (50%) and PVA (25%). These two types of polymers have relatively high density, about 1.2 g/cm^3 and 1.4 g/cm^3, respectively (Graca et al. 2017). Interestingly, the density of plastics can change during their residence in aquatic environments due to biofouling and degradation. In aquatic environments, MPs are easily colonized by a variety of organisms, such as algae and microbes. This biofouling will make the plastic denser, which could change its buoyancy. As a result, buoyant plastics can sink below the water's surface and disperse through the sediment (Auta, Emenike, and Fauziah 2017). The distribution of MPs in freshwater ecosystems is geographically heterogeneous, and the findings from several research studies have varied by several orders of magnitude. Sediments generally show higher concentrations than water samples and living organisms.

The distribution of MPs in freshwater systems was unclear until recently. To date, limited studies have investigated the presence of MPs in freshwater ecosystems such as estuaries, lakes, and rivers

(Dris, Imhof, et al. 2015). Table 2.3 below shows some recent studies on MPs in freshwater worldwide in the past ten years. Based on the available data, low to average levels were observed, and higher MP levels have been reported in China, the US, Africa and some European countries.

Table 2.3: Published work on MPs in freshwater

Location	Water body	Substrate	MP abundance	Reference
America				
Canada/USA	Lakes Huron	Water	0–0.093 particles/m^3	(Eriksen et al. 2013)
Canada/ USA	Lakes Erie	Sediment	0.18–8.28 particles/m^2	(Zbyszewski et al. 2014)
USA	Chesapeake Bay (Estuarine)	Sediment	5.53–297.93 particles/g dw	(Yonkos et al. 2014)
Canada/USA	Lake Ontario	Sediment	0.02–27.83 particles/g dw	(Ballent et al. 2016)
Mexico	Atoyac River	Sediment	1133.33 ± 72.76 particles/kg dw	(Shruti et al. 2019)
Canada	Lawrence River	Sediment	65–7562 particles/g dw	(Crew et al. 2020)
Canada/USA	British Columbia lakes	Water	0.607 ± 0.153 particles/m^3	(Klasios and Tseng 2023)
England	Tamar Estuary	Water	0.028 particles/m^3	(Sadri and Thompson 2014)

Location	Water body	Substrate	MP abundance	Reference
Europe				
France	River Seine	Water	3–108 particles/m^3	(Dris, Gasperi, et al. 2015)
Italy	Lake Chiusi	Water	0.82–4.42 particles/m^3	(Fischer et al. 2016)
Germany	River Rhine	Sediment	0.228–3.763 particles/g dw	(Bessa et al. 2018)
Scotland	Kelvin River	Sediment	296.5 particles/g dw	(Blair et al. 2019)
Russia	Altai lakes	Water	0.007 - 11,000 particles/m^3	(Frank et al. 2022)
England	Thames River	water	0.33 - 127.33 particles/m^3	(Devereux 2023)
Siberia	Lake Baikal	Water	175 particles/m^3	(Yang et al. 2025)
Asia				
Mongolia	Lake Hovsgol	Water	0.0002–0.0089 particles/m^3	(Free et al. 2014)
China	Gorges Dam	Water	0.038–2.73 particles/m^3	(Zhang et al. 2015)
China	Lake Taihu	Sediment	16–258 408 particles/g dw	(Fok et al. 2020)

Location	Water body	Substrate	MP abundance	Reference
Asia				
China	Haihe River	Water	0.69 to 74.95 particles/m^3	(Liu et al. 2020)
China	Koshi River	Water	202 ± 100 particles/m^3	(Yang et al. 2021)
South Asia	Ganges River	Water	0.038 ± 0.004 particles/m^3	(Singh et al. 2025)
Vietnam	Mekong River	Water	64-0.182 particles/m^3	(Mendrik et al. 2025)
Africa				
South Africa	Bloukrans River	Sediment	160.1 ± 139.5 particles/kg	(Nel et al. 2019)
South Africa	Vaal River	Sediment	463.28 ± 284.08 particles /kg dw	(Saad, Ndlovu, et al. 2022)
Ghana	Volta Lake	Sediment	(19.9 ± 3.8 particles/50g	(Boateng et al. 2024)
Egypt	River Nile	Water	2.24 ± 0.6 to 3.76 ± 1.1 particles/L	(Khedre et al. 2024)
Nigeria.	River Benue	Sediment	28.33 ± 3.51 particles/g	(Doherty et al. 2024)

2.8 MPs in freshwater fish

In recent years, increasing attention has been given to studying MPs in biotic components of freshwater environments, allowing us to understand more about the species' susceptibility to ingestion, and dietary transport of MPs (Adeogun et al. 2020). Due to their correlation with water and sediment, aquatic biota are recommended "as bio-indicators" to study the levels of MP

contamination in water bodies. The abundance of MPs is reported in biota samples in several freshwater studies. (Dahms et al. 2020) confirmed this by detecting and correlating MPs from three separate samples (sediment, water, and larval species) collected at the same sampling site.

Fish are a fundamental biological component of the freshwater systems with substantial economic and nutritional value worldwide. These are the most investigated species accounting for 49 % of biota research, due to the higher trophic level of freshwater fish and the potential for trophic transfer of MPs. The majority of studies have assessed the occurrence of MPs in gastrointestinal (GI) tracts (O'Connor et al. 2020). Some studies, however few at the moment, have looked at liver and muscle tissue for MP translocation, but probable translocation routes and consequences have not been evaluated (Collard et al. 2018).

In most of the examined species, MPs were shown to be consumed either directly or as a result of secondary ingestion (i.e., trophic transfer) (Hurley, Woodward, and Rothwell 2017; Collard et al. 2018). Due to various encounter rates or various types of MPs (morphology, color, size, and density) found in the benthic sediment and water column, feeding habits, i.e., whether fish are benthic feeders or pelagic predators, may influence MPs ingestion (Bessa et al. 2018). Trophic roles may also influence the exposure to MPs in fish and other organisms, meaning, MPs can be consumed via trophic transfer when predators indirectly ingest MPs by consuming prey or scavenging detrital matter (Horton et al. 2018).

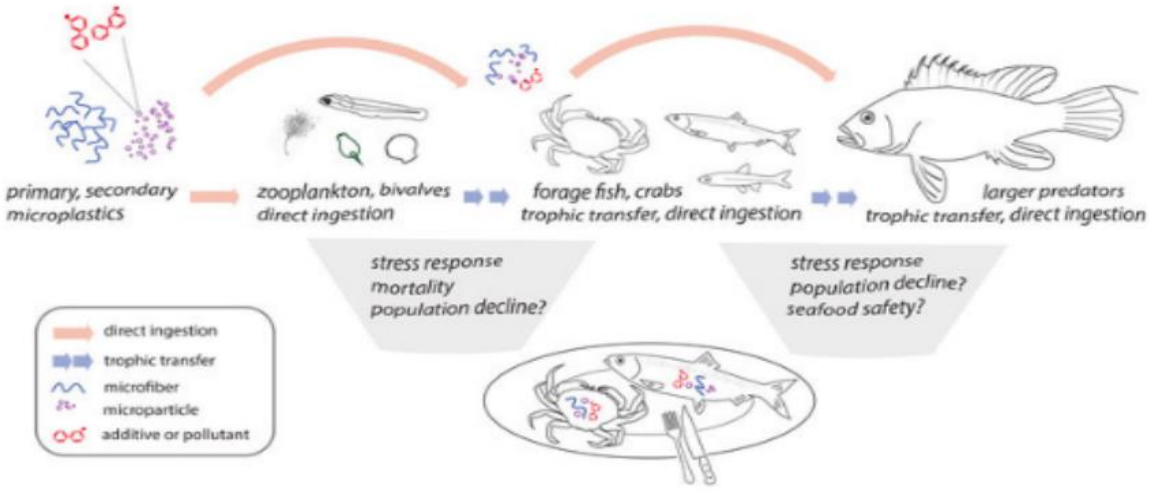


Fig 2.4: MPs in the food chain (Baechler, et al. 2019)

Fish consumption of MPs has been the subject of 267 peer-reviewed studies worldwide, the first of which was published in 1972 (Carpenter and Smith Jr 1972). Studies on MPs in fish have steadily increased over the past ten years, with 87 studies published in 2020 as opposed to one in 2010. Regionally, the majority of studies on MPs ingestion by fish have been conducted out in the northern hemisphere, particularly in Asia (27% of all studies), North America and Europe (35% of all studies) (Pawlak, Koziol, and Polkowska 2021; Honorato-Zimmer et al. 2022).

Less than 14% of all research conducted worldwide is contributed by the Middle East, Oceania, and Africa combined (Biginagwa et al. 2016; Naidoo, Smit, and Glassom 2016).

The first confirmation that freshwater fish consume MPs was presented by Sanchez et al. in a preliminary field report in 2014. They studied wild gudgeons (Gobio gobio) that were taken in 11 French streams that had a variety of environmental conditions, and they discovered MPs in the digestive tract of 12% of the fish (Sanchez et al. 2014). Jabeen et al. also documented the occurrence of meso- and micro-plastic degradation in freshwater and sea fishes from China, finding MPs in 95.7% of freshwater fish (Jabeen et al. 2017). They also revealed for the first time the abundance of MPs in the intestines suggesting that the abundance of MPs in the intestines was even higher than in the stomachs in some fish species.

In African continent, MPs were found in fish species samples in South Africa in (Jukskei River, Bloukrans River of Grahamstown, Vaal River and Durban Harbor estuaries), as well as Nigeria in (Lake Eleyele, Osun River), Tanzania (Lake Victoria), Egypt (River Nile), and Eastern seashore (Ghana) (Aragaw 2021). In study of Lake Eleyele, MPs were detected in a total of 109 fish samples from eight different species, with an average of 69.7%. The disparity in abundance was attributed to the species' organs, maturity, and ingesting capacity (McGoran, Clark, and Morritt 2017). Similarly, the abundance of MPs in Lake Victoria was investigated using two different fish species (Nile perch and Nile tilapia), in which MPs were recovered from the gastrointestinal tracts of 11 perch (55%) and 7 tilapia (35%) (Biginagwa et al. 2016). Study in River Nile (Egypt), examined the digestive tracts of two fish species, catfish *(Bagrus bayad)* and Nile Tilapia *(Oreochromis niloticus)*. MPs were found in over 75% of the total examined fish, with a prevalence of 78.6% and 75.9 % for catfish and Nile Tilapia, respectively. The most common MP shape was fibers (65%), followed by films (26.5%), and the remaining MPs were fragments (Khan et al. 2020). In another study by Saad et al. (2022), 100 % of common carp fish (*Cyprinus carpio*) contained MPs in gastrointestinal tracts with an average of 26.23 ± 12.57 particles/fish, and an average abundance

of 41.18 ± 52.81 particles/kg (Saad et al. 2022). As a consequence, the use of gastrointestinal tracts in the preparation of fish meal is a possible route for MP contamination of fish meal.

2.9 Nile Tilapia *(Oreochromis niloticus)*

Nile Tilapia is the most significant species of fish in tropical and subtropical freshwater and a frequent component of commercial fisheries in many African countries (El-Sayed and Fitzsimmons 2023b). It is economically viable because to its high environmental tolerance and ability to consume both formulated and natural feeds. Also, Nile Tilapia has been used in toxicological studies (Hamed et al. 2019). The Nile Tilapia has a diverse feeding pattern that includes both generalist and opportunistic feeding (Canonico et al. 2005). Depending on a wide range of seasonal and spatial environmental conditions, its diet composition can change (Karl et al. 2001). The food composition may also vary according to the fish's maturity, size, habitat kinds ,and environmental conditions (Kamal et al. 2010).

2.9.1 Feeding behavior of Nile Tilapia

An organism absorbs and assimilates nutrients through the process of nutrition to support growth, tissue repair, migrating, and reproductive activities. Any living thing must therefore consume food in order to meet all of its basic needs (Gandega and Ghorbel 2009).

Fish feeding habits vary by species; some are known to be herbivores (which eat just plants), carnivores (eat only meat), and omnivores (which consume both plants and meat) (Salsabila and Affandi 2019). Many authors have classified Nile Tilapia as an omnivore species since it feeds a variety of plant and animal foods, including phytoplankton, diatoms, and plant species such as leaf segments, plant tissues, dead leaves, flower buds, as well as some zooplankton, insect larvae, protozoa, and detritus (Tesfaye et al. 2021; Temesgen et al. 2022; Mandal and Bera 2025). For instance, the studies carried out in some rift valley lakes e.g. Lake Hawassa, Lake Chamo, Lake Ziway, Lake Langeno and Koka Reservoir, and River Omo ,indicated that phytoplankton is the most consumed food prey by Nile Tilapia (Tadesse etal. 1999; Teferiet al. 2000; Negassa and Prabu 2008; Schoellner et al. 2016; Temesgen et al. 2017). However, zooplankton is the most popular food type ingested by Nile Tilapia in Lake Hashenge (found in roughly 60% of stomach studies), and this is an exceptional finding (Asfaw, Bogale, and Teame 2016). In some of the rift valley lakes, macrophytes were also found to be slightly more prevalent in the diet of Nile Tilapia

(Negassa and Prabu 2008; Engdaw, Dadebo, and Nagappan 2013; Temesgen et al. 2017; Tesfahun and Temesgen 2018). Also, the contribution of animal origin was very small as compared to the food of plant origin except in Lake Hashange (Asfaw, Bogale, and Teame 2016).

The studies showed that contribution of other food items were generally very low throughout the water bodies. This indicates the changing of food items composition in the diet of Nile Tilapia based on the diet composition in the aquatic environment, which may change according on the season (water level), environment condition, and habitat types of the aquatic environment (Shuai et al. 2023).

2.10 Extraction of MPs from biological samples

According to (Alprol et al. 2021), the number of research studies on MPs that were published in the previous ten years more than doubled. In this interim, it has also been noted that there has been a parallel rise in the variety of procedures and techniques for the qualification and quantification of MPs, both in in biological and environmental samples which contain complex organic and inorganic substances (Enfrin et al. 2021). However, the complexity of comparing different studies has increased as research diversifies and expands the academic community, where, certain difficulties with methodological standardization, such as identifying, characterizing, and evaluating MPs, remain largely unresolved (Tsangaris et al. 2021).

MPs can be found in a variety of environmental matrices, including freshwater, saltwater, wastewater, sediments, soils, food, and biota. The evidence ingestion of MPs by biota has been achieved using several methodological approaches (Amy Lusher, Hollman, and Mendoza-Hill 2017b; Barboza et al. 2018; Weber et al. 2018). However, its uses have not been standardized among studies. lack of harmonization in isolation procedures for MPs internalized in body tissues/cavities or entangled (externally) in biota is currently recognized as a foremost concern across all MP research (Biginagwa et al. 2016; Adeogun et al. 2020). Which is means it is difficult to facilitate comparisons or assess MPs exposure levels between similar species.

In recent years, an increasing number of extraction protocols of MPs from biological matrices have been developed and modified according to the sensitivity of the characterization techniques used (Horton et al. 2024). The routine processes used to investigate MPs have developed from simple processing (extract MPs directly), such as dissection followed by rinsing GI tracts with water followed by microscopy, to elaborated protocols for the removal of organic and inorganic

compounds. That involve enzymatic or chemical digestion of biological samples with the aim of extracting, identifying and quantifying the MPs (Lusher et al. 2017b).

Simple procedures result in an under-representation of MPs, due to MPs might be lost during this step and difficulties enumerating and identifying smaller particles that may adhere to dietary remains and other biological materials within an organism's digestive tract (Adika et al. 2020).

Because MPs are organic and degrade in case the treatment is too drastic, a number of isolation protocols have been observed damage polymers (Garcia et al. 2020). As a result, the digestion process must be gentle, and the digestive step must (i) avoid contamination, (ii) not alter the shape and size distribution of MPs, and (iii) be both quick and inexpensive (Enfrin et al. 2021).

To prevent contamination all equipment and setups should be constructed of non-polymer materials Procedures should always include procedural blank samples and recovery tests because contamination cannot be completely avoided (Cai et al. 2021).

Since there are now no standardized protocols for the digestion of organic matter, it is more appropriate to use chemicals to digest biotic material in order to get MPs from biological samples because they are inexpensive and have relatively high recovery rates. This include; chemical digestion (e.g., oxidizers, acids, bases) and enzymatic digestion which is mild and non-destructive and can be used for tissue digestion (e.g., proteinase, cellulose, lipase, chitinase) (Hossain et al. 2019; Baalkhuyur et al. 2020; Yin et al. 2022).

Strong mineral acids, such as nitric acid (HNO_3), have been reported to cause degradation and damage to delicate polymers (e.g., PS, PE, PA), and are often substituted for oxidizers (e.g., 30% H_2O_2) or bases (e.g., 10% KOH), which are easy to execute on soft tissue organisms like GI tracts of fish, and can be achieved at room temperature (Santana et al. 2022; Akbulut et al. 2024). Potassium hydroxide (KOH) is being as the most successful protocol for extracting MPs from biotic samples in present MP research (Rani et al. 2023). Due to requires a simple procedure, cost efficient and utilizes accessible chemicals. Blankson et al. (2022), used alkaline method (KOH 10%) to digest the GI tracts of Black-chinned Tilapia *(Sarotherodon melanotheron) and* Bagrid Catfish *(Chrysichthys nigrodigitatus)* from the Densu River in Ghana, and reported high digestion efficiency (Blankson et al. 2022).

For organisms containing hard parts, such as benthic macro invertebrates, wet peroxide oxidation (WPO) utilizing Fenton's reagent (H_2O_2 in the presence of a Fe (II) catalyst) is the recommended approach that was developed by the National Oceanic and Atmospheric Administration Marine

Debris Program, which requires to be heated in order to degrade the chitin in their exoskeleton. However, this method has also been used to digest the soft tissue of fluminea, tadpoles, and the digestive tracts of fish (Kallenbach et al. 2021; Tore and Yilmaz 2022; Zong et al. 2025). Similar findings have been made with WPO, which has been reported to be a successful technique for breaking down biogenic material despite bleaching (dyeing) MPs particles. This could have an affect the colour that is determined, instead than underestimating the particle abundance (dos Santos Galvão et al. 2022).

According to Luscher et al. (2020), post-digestion treatments for biological specimens frequently involve density separation and filtration stages (vacuum pump), followed by washing of the digested solution using distilled water. Studies by Collard et al. (2015), Dehghani et al. (2017), and Lusher & Hernandez-Milian (2018), provide examples of the use of these procedures (Collard et al. 2015; Dehghani, Moore, and Akhbarizadeh 2017; Lusher and Hernandez-Milian 2018).

In general, the digestion stage is ineffective at breaking down partially inorganic material, like the shards of bones. Hence, a density separation step can be used to separate the undigested biomass and other undesired abiotic components, from the MPs (Wakkaf et al. 2020). However, this steep primary drawback is its reduced applicability to plastics with higher densities, like polytetrafluoroethylene (2.2 g/cm^3), in addition, the requirement of sample transfer, resuspension, and retrieval of MPs during the density separation step is another problem with this process, which may raise the risk of sample loss or contamination (Dellisanti et al. 2023).

2.11 Analytical techniques for MP analysis

Physical identification and chemical characterization are achievable using a variety of analytical techniques. For physical identification, microscopes such as stereoscope, ordinary optical microscopes, phase contrast microscopes, and fluorescent microscopes, are used to classify and quantify MPs with unique colours and morphologies (Garcia et al. 2020). As well as, Scanning electron microscopy (SEM).

Microscopy alone offers information about the morphology of the particles, including shape and size, while imaging software in conjunction with microscope produces the average particle size and the number of particles (Athulya and Chandrasekaran 2023).

Stereo (or dissecting) microscopy has wide application in the identification of MPs whose size falls between the hundreds of micron range, however colorless, irregularly shaped MPs with sizes <100 μm are difficult to characterize with certainty (Dellisanti et al. 2023).

SEM is a powerful technique for the study of micro-scale physical composition. It has been shown to be helpful in identifying between MPs and other organic/inorganic component, in addition to being good for revealing physical degradation such pits and cracks. Integrating Energy-Dispersive X-ray (EDS) and scanning electron microscopy (SEM) provides information about MPs morphology and surface roughness. SEM-EDS aids in the rapid screening of plastics spheres vs non-plastic spheres and can be used to differentiate MP particles <1 mm, which are missed in visual detection. Furthermore, used to characterization of inorganic plastic additives (Agnieszka, 2019). Thus determining their eco-toxicological impact (Martinez-Tavera et al. 2021; Vo and Pham 2021).

For a comprehensive analysis, physical identification must be followed by chemical characterization, this involves one or more types of the following techniques; (i) thermal analysis techniques such as Pyrolysis–Gas Chromatography–Mass Spectrometry (Pyr-GC/MS), Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GC/MS) and Differential scanning calorimetry (DSC), (ii) spectroscopic techniques which is the most used chemical identification method for MPs, e.g. Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), Near-infrared spectroscopy (NIR) and Nuclear Magnetic Resonance (NMR) (Hanvey et al. 2017). For the investigation of MPs, thermo-analytical techniques have been devised. These techniques measure changes in the physical and chemical properties of polymers based on their temperature stability. Due to the high molecular weights of polymer the analysis is difficult to carry out which, they typically dissolve limited and have very low vapor pressure. However, the breakdown of a polymer typically results in the formation of smaller molecules, which then allow for the use of volatile analysis to help characterize the breakdown or decomposition mechanism. DSC is frequently employed to investigate the thermal properties of polymeric materials. Being a tried-and-true technique, it has the benefits of being affordable and simple. However, because each plastic product has unique DSC characteristics, reference materials are needed to distinguish between different polymer kinds. Furthermore, due to their mass to surface ratios are larger than those of smaller particles, large particles can produce interferences.

Mass spectrometry is used in conjunction with pyrolysis gas chromatography or thermal extraction desorption gas chromatography to determine the mass of polymer present in the sample. Both enable the determination of the mass of MPs in a sample as well as the chemical identification of the various polymer types. Last year's Py-GC/MS has been used to get information on the composition of polymers with some applications on MPs identification, by releases gaseous components into samples that are then transported to gas chromatography-mass for the determination of chemical compositions. The accurate identification of numerous polymer types is made possible by Pyr- GC/MS, which also offers information on potentially harmful organic plastic additives. but the major disadvantages that are MPs must be manually inserted into the pyrolysis tube, resulting in an approximate 70-minute sample preparation for one particle every run. Thus, it is not appropriate for regular analysis when there are large sample amounts (Xu et al. 2019).

In TDS-GC/MS the polymers can be heated from ambient temperature to around 300 °C. Small volatile compounds having a molecular mass between 350 and 400 m/z will first trapped using cryogenic heating, then reheated under strict control, before being finally separated using a chromatographic column and identified using mass spectrometry. As a result, the species and quantities of the complex thermal or thermo-oxidative breakdown products produced may be accurately determined (Dümichen 2017).

The most common techniques used for chemical characterization of MPs are FTIR and Raman spectroscopy. These vibrational techniques are accurate, low sample amount requirement, not destruction the sample, possibility for high throughput screening and environmental friendliness, and produce a unique spectrum as a result of the molecule's interaction with light (Xu et al. 2019; Dodo, Fujita, and Sodeoka 2022). When used with automated chemical imaging processing to gather data on abundance, size, and type of polymer. FTIR analysis is feasible for larger particles of 20 μm, while Raman spectroscopy has the capacity to detect MPs as small as 1 μm, when Raman and FT-IR spectroscopy are coupled with microscopy.

FTIR one of the most successful technique for detecting suspended MPs in the environment since it is direct, cost-efficient, non-destructive, and widely used (Zhang and Chen 2020). It is however limited to measuring particles of sizes around 25 μm and thickness <100 μm. Also, it's possible that polymer bands will overlap, which will make it challenging to tell one polymer from another (Cooper and Corcoran 2010; Jeong et al. 2015). 23 of the 43 investigations reviewed by Hanvey

et al. (2017) employed FTIR (Hanvey et al. 2017b). FTIR has three optimizing technologies: Attenuated total reflection (ATR), micro-FTIR and Focal –plane array (FPA). Harrison et al. (2012), tested the usefulness of micro- FTIR and reflectance micro-FTIR techniques for the characterization of MPs in marine sediments and successfully demonstrated the potential of such technique through molecular mapping based on their spectral characteristics (Dellisanti et al. 2023). ATR-FTIR is another reliable and fast detection technique, and has been used for polymer characterization isolated from water and biological samples and can identified small MPs of size <50 μm (Hanvey et al. 2017; Xu et al. 2019). FPA-FTIR offers analysis and identification of MP particles less than 20 μm by scanning the isolated MPs residues on a filter paper with a high degree of lateral resolution without pre-sorting the filter area (Hanvey et al. 2017).

Raman spectroscopy is a spectroscopy technique that delivers information in the form of vibrational spectra, on the basis of inelastic light scattering that provides information upon the molecular vibrations of a system in the form of a vibrational spectrum (Yin et al. 2022). Similar to a fingerprint of chemical structure, the Raman spectrum enables identification of the components present in the sample. Raman spectroscopy is therefore appropriate for the detection of nano-plastics partials, provided that other obstacles be overcome (such as the samples preparation, purification, and the adequate control of blanks) (Dąbrowska et al. 2022).

Although, FTIR spectroscopy has been around longer than Raman spectroscopy as a common analytical technique, and it evolved as the polymer industry grew, Raman techniques show better spatial resolution (down to 1 mm), larger spectral coverage, stronger sensitivity to non-polar functional groups, fewer water interference, and shorter spectral bands as compared to FTIR spectroscopy (Xu et al. 2019). Raman spectroscopy has some drawbacks, including the potential for fluorescence interference, an inherent low signal-to-noise ratio, high cost, and the potential for sample heating due to the use of a laser as the light source. These drawbacks can occasionally result in background emission and polymer degradation.

For NIR, pretreatment is not required and can detect particles as small as 1mm in an environmental sample (Hanvey et al. 2017). NMR analysis provides quantity of MPs present in the sample. Application of 1HNMR spectroscopy (qNMR) can be applied for quantitative and qualitative analysis of MPs. However, up to date, very few studies have reported the use of NMR for quantification of MPs (Adeogun et al. 2020).

Chapter 3

MATERIAL AND METHODS

This chapter illustrates a detailed account of the methods followed and equipment used from the study area, sampling collections, measuring of biometric parameters, sample processing, characterization techniques, Contamination control, and statistical analysis

3 Materials and methods

To date, there are no established standarised methods for for the monitoring of microplastics. Nevertheless, in general, MP identification necessitates fast and reliable analytical methods that are usually divided into two phases: (i) sampling and extraction from environmental matrices, and, (ii) detection, quantification, and characterization (Fu et al. 2020).

The fish samples in this study were collected from the River Nile (Khartoum, Sudan). The analysis of MPs was performed in the MP laboratory at the University of the Witwatersrand, Johannesburg, South Africa. Before identification and characterization, samples underwent a series of laboratory procedures, including (1) measurement of biometric parameters of each fish sample, (2) sample processing, which included (i) dissection, (ii) digestion, and (iii) density separation and filtration. The method followed in this study is detailed in the below sub-sections and reported in a journal article (Saad and Alamin 2024).

3.1 Study area

The River Nile (known as **Baḥr Al-Nīl** or **Nahr Al-Nīl** in Sudanese Arabic dialect) is located in central Sudan, immediately south of the junction of the Blue Nile and the White Nile, the two main tributaries of the River Nile. The Blue Nile flows west from Lake Tana in Ethiopia, while the White Nile flows north from Lake Victoria (Zerboni et al. 2021). The Nile continues north from that point towards Egypt and drains into the Mediterranean Sea (Fig 3.1). For thousands of years, the Nile has provided water for irrigation to transform the dry area surrounding it into lush agricultural lands. Nowadays, the river continues to support the economic growth of several communities in different African countries.



Fig 3.1: Google Earth™ satellite imageries of River Nile including the path of the Blue and White Niles

3.2 Fish collection

In August 2021, thirty Nile Tilapia fish samples were bought (freshly caught) from a fisherman at Al-Mawrada fish market along the River Nile in Omdurman, Khartoum. Fish were frozen and transported to the microplastic laboratory at the University of Witwatersrand in South Africa and kept frozen at − 20 ◦C until processing.



Fig 3.2: Nile Tilapia fish

3.3 Measurement of biometric parameters

In the laboratory, fish were defrosted and thoroughly rinsed with filtered distilled water to remove sediments and other impurities. A routine physical examination of the fish samples was conducted before dissecting the digestive tract. This includes measuring the total length, weight, and identifying the sex of the fish. The total length was measured from the tip of the snout (mouth closed) to the extreme end of the caudal fin by using a measuring tape (0.1 cm precision), and the weight was measured with a digital scale (0.1 g precision).

The external and internal reproductive structures were used to identify the sex of the fish. Males were identified by the presence of two openings; one opening is the anus which is situated immediately in front of the anal fin, and the other opening is the urethral, which is located at the end of the genital papilla (an oval-shaped lobe directly beyond the anus) and is where urine and milt are discharged. Females, on the other hand, possess three body openings; of which one is the oviduct (a crescent-shaped slit) and from which eggs are released, the second is the urethra, and the third is the opening of the anus (Nandlal and Pickering 2004). The presence and absence of testes and ovaries seen during dissection later confirmed this classification.

3.4 Sample processing

3.4.1 Dissection

A total of 30 fish samples were dissected to remove the digestive tract, where food is processed, and nutrients are absorbed. Tilapia, being herbivores, have long intestines to aid in digesting plant matter, which is tough, fibrous in shape, and more difficult to break down into usable elements (Gordon and Prins 2023).

A longitudinal incision was made in the abdomen from the anus to the lower jaw using forceps and a scalpel by following the standard procedure reported in previous studies (Rocha-Santos and Duarte, 2015; Primpke et al. 2017).

The digestive tracts of each fish were weighed and placed in a glass beaker covered with aluminum foil to prevent contamination in subsequent digestion. For the analysis of MPs, modified National Oceanic and Atmospheric Administration (NOAA) laboratory procedures were adopted, both for sample preparation and extraction of MPs (Masura et al. 2015).

3.4.2 Digestion

Digestion is an important pre-treatment step to degrade biological, organic, and inorganic particles that would otherwise hamper MP analysis. Alkaline digestion using potassium hydroxide (KOH) has been reported in several studies (Foekema et al. 2013; Besseling et al. 2015; Karami et al. 2017; Saad et al. 2022). KOH is a prototypical strong base, it forms strong alkaline solutions in water and other polar solvents, reacts with organic components, and destroys biological materials by hydrolyzing chemical bonds and denaturing proteins (Lusher et al. 2020). It is reported that KOH digests organic matter with an efficiency of >90% (Zhao et al. 2014).

In this study, a strong basic KOH solution was used to digest the digestive tract of Nile Tilapia fish. The solution was prepared by dissolving 10 grams of KOH (supplied by Sigma-Aldrich) in 100 ml of distilled water to prepare KOH (10% w/w). Digestion was accomplished by adding a 10% KOH solution to the digestive tract in 250 mL beakers at a volume ratio of 1:3, covered with aluminum foil, and the glass beakers were placed in a pre-heated incubator at 40 °C and rotated at 125 rpm for 24 h (Saad et al. 2022; Saad and Alamin 2024). Blank samples were run concurrently with the digestion procedure.

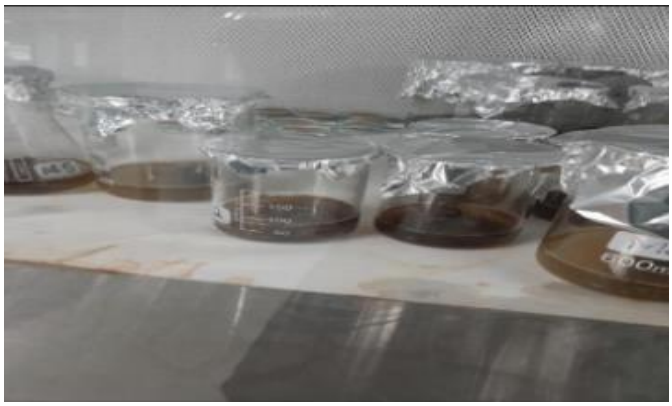


Fig 3.3: Samples in KOH digestion

3.4.3 Density separation and filtration

Salt-based density separation uses saturated salt solutions to enhance the flotation of low-density MPs as part of the supernatant and thus separate them from high-density organic and inorganic materials. In this study, a high-density (1.8 g cm^{-3}) solution of Sodium Iodide (NaI) (supplied by Ace Chemicals South Africa ≥ 99.5%) was used to extract MPs. The density separation solution was prepared by dissolving 80 g of NaI in 100 mL distilled water. The solution was then added to

each sample in a volume ratio of 1:2, and the mixtures were shaken and left overnight to settle. After density separation, samples were filtered through 47 mm diameter with 1.6 µm pore size Whatman GF/A glass microfiber filters using a Nalgene vacuum filter. To prevent cross-contamination, the sides of the filter funnel were thoroughly rinsed with filtered RO water to ensure that any particles sticking to the sidewalls were washed into the filter papers (Dehaut et al. 2016). The filter papers were air-dried and stored in covered Petri dishes for further analysis.

3.5 Characterisation techniques

Due to the complexity and the wide range of physio-chemical properties of MPs, including size, colour, additives, and polymer types, it is not advised to apply a single analytical approach. In 26% of studies, identification is performed entirely by physical observation, which raises the risk of misidentification (Fernández-González et al. 2022; Murugan et al. 2023). In this study, the physical identification of MPs was further confirmed by chemical characterization. Firstly, potential MP particles were identified according to their size, shape, and colour using a stereomicroscope. The microscope was also used to count their numbers. Secondly, Chemical characterization was performed using Raman spectroscopy to identify the polymer type.

3.5.1 Physical characterisation

3.5.1.1 Stereomicroscope

An optical microscope known as a "stereoscopic" or "dissecting" microscope provides three-dimensional pictures of a specimen by typically employing light reflected from an object's surface rather than light transmitted through it (Sharpe et al. 2002). The two different light routes that the dissecting microscope's objectives and eyepiece employ determine its operating principle. Each light path presents a different angle of view. Stereomicroscopes have a bottom light for viewing the images and a top light for dissecting. This illumination is made possible by the design of two eyepieces (binocular stereoscope), each of which displays a distinct sort of light pathway and offers a viewing comfort region. The images are then streamed live in three dimensions on a computer monitor (Gustafsson et al. 2008).

All suspected particles on the filters were visually observed, measured, and photographed using a Nikon stereomicroscope (Nikon MET SMZ745T, Japan). Visual criteria reported in literature were used to identify MPs, this includes the absence of cellular or biological components, organic

structure and metallic luster, unnatural shapes, and homogeneous texture, clear colours (Ramaremisa, Ndlovu, and Saad 2022; Yang et al. 2023). MPs were sorted to a maximum size of 5 mm and five shapes, namely, fragments, pellets, fibers, films, and foams. A USB 3.0 imaging source camera (TIS) was used to capture the particles identified as potential MPs using NIS Elements-D imaging software Version 5.20 (Nikon Cooperation, Japan).

3.5.1.2 Scanning electron microscope (SEM) analysis

Scanning electron microscopy (SEM) provides images with exceptional clarity and magnification using high-resolution images of the surface texture of the particles (Joshi et al. 2008). Thus, SEM images can be used to identify MPs from organic particles (Zarfl 2019). Selected particles were placed on a carbon tape affixed to aluminum stabs; samples were coated with a 25 nm carbon layer with a vacuum coater. TESCAN Vega SEM with a tungsten emission system and a resolution of 2 nm at 20 kV was used to generate high-resolution images of morphological surface structures of some MP shapes detected under the optical microscope. Particles with different shapes were randomly selected and analyzed.

3.5.2 Chemical characterisation

3.5.2.1 Raman spectroscopy measurements

Raman spectroscopy is an efficient non-destructive technique with high accuracy in identifying MPs. It has a high numerical-aperture objective and a short excitation wavelength with a monochromatic laser source (with a wavelength that depends on the available lasers, normally between 500 and 800 nm). This light interacts with the molecules and atoms of the sample, producing a backscattered light with a frequency that differs from that of the irradiating laser (Araujo et al. 2018).

Raman spectrum serves as a chemical fingerprint that gives specific information on a sample's chemical structure (including its molecular structure, cellular structure, and physiological state) (Dodo et al. 2022). Plastic polymers exhibit distinctive Raman spectra, which makes it possible to identify the polymer by comparing it to reference spectra. Also, the ability to describe MPs with sizes less than 1 µm. In this investigation, a 514.5 nm (green) line of a Lexel Model 95-SHG argon ion laser was employed in conjunction with a Horiba Lab RAM HR Raman spectrometer with an Olympus BX41 microscope attachment. The chemical composition of the polymer for each

particle analyzed was identified by comparing the detected polymers' reference spectra to those in the Know.

3.6 Quality control measures

Several precautions were taken to avoid or minimize cross-contamination of the samples during pretreatment and analysis. These include covering the samples and utilizing glass or metal equipment and apparatus instead of plastics. All work was performed in a laminar-flow hood, and after each experimental procedure, glassware and containers were thoroughly cleaned with deionized water and sterilized with 70% ethanol. 47 mm microfiber filter papers (Whatman GF/A) were used to filter out all the chemicals used for processing and preservation. Cotton lab coats and powder-free nitrile gloves were worn throughout the experimental work. Furthermore, blank tests with ultrapure water were carried out to test for potential cross-contamination. However, no contamination-related evidence was observed in the blanks.

3.7 Statistical analysis

The prevalence of MPs in the fish samples was calculated using the following formula:

$$\mathbf{P\% = (Ni / N) \times 100}$$

Where P% = the prevalence of MP particles; Ni = the number of digestive tracts that contained MP particles; N = the total number of digestive tracts investigated.

Also, the Condition factor (K) was computed using Microsoft Excel, for each individual the formula is as follows:

$$\mathbf{K=W/L^3 \times 100}$$

Where K is the condition factor, W is the weight body of fish in grams, and L is the total length in centimeters (Ighwela et al. 2011).

Additionally, the variation in the abundance and the characteristics of MPs in the thirty samples of Nile tilapia were tested using one-way ANOVA. Statistical tests were considered significant at p values < 0.01. Correlation analyses between the fish`s morphological features (e.g., body mass

and total length) and the abundance of MPs ingested were also tested. All analyses were performed using IBM SPSS 20.

Chapter 4

RESULTS AND DISCUSSION

This chapter presents the findings of the study and discusses them in terms of abundance, shape, and colour. Potential sources of MPs in the River Nile and the potential impact of the pollution are also discussed.

4.1 Body measurements

The data for weight, total length, condition factor (K), and sex for thirty Nile Tilapia fish samples are recorded in Table 4.1. According to their size, fish samples were grouped into three categories: small (< 0.25 kg), medium (0.25 - 0.5 kg), and large (> 0.5 kg) to study the relationship between the size of fish and MP abundance, if any. It should be noted, however, that this classification was relative to the sizes of the fish caught in this study and does not reflect the size range and variation of Nile Tilapia species.

The fish weight ranged from 0.11 to 1.39 kg, with an average of 0.48 ± 0.38 kg, while the total length was 19.10 cm to 45.00 cm, with an average of 30.05 ± 8.10 cm. The condition factor values of individual fish ranged from 0.68 g/cm^3 to 1.62 g/cm^3 with an average of 1.43 ± 0.20 g/cm^3. Of the thirty specimens, twenty samples were identified as males, and ten samples were identified as females.

Table 4.1: The biometric data of Nile Tilapia fish

Sample Code	Sex	Body Mass (Kg)	Total Length (cm)	Condition factor (K) (g/cm^3)
S_1	Male	0.120	26.0	0.68
S_2	Female	0.147	21.0	1.59
S_3	Male	0.118	20.8	1.31
S_4	Female	0.114	20.7	1.29
S_5	Male	0.132	22.6	1.14
S_6	Male	0.107	19.1	1.54
S_7	Male	0.116	19.5	1.56
S_8	Male	0.128	20.0	1.60
S_9	Female	0.160	21.5	1.61
S_{10}	Male	0.159	21.4	1.62
S_{11}	Male	0.307	27.6	1.46
S_{12}	Male	0.353	28.0	1.61

Sample Code	Sex	Body Mass (Kg)	Total Length (cm)	Condition factor (K) (g/cm^3)
S_{13}	Male	0.310	30.1	1.14
S_{14}	Female	0.380	31.5	1.22
S_{15}	Female	0.305	28.1	1.37
S_{16}	Male	0.321	29.7	1.23
S_{17}	Female	0.304	28.0	1.38
S_{18}	Female	0.365	28.4	1.59
S_{19}	Female	0.325	27.6	1.55
S_{20}	Male	0.389	29.0	1.59
S_{21}	Male	0.738	37.5	1.40
S_{22}	Male	0.969	40.7	1.44
S_{23}	Female	1.132	42.1	1.52
S_{24}	Male	1.154	41.8	1.58
S_{25}	Male	1.388	45.0	1.52
S_{26}	Male	0.776	37.1	1.52
S_{27}	Male	0.720	37.2	1.40
S_{28}	Male	0.871	38.7	1.50
S_{29}	Male	0.946	40.2	1.46
S_{30}	Female	0.945	40.6	1.41

❖ *S_1-S_{10} = small fish, S_{11}-S_{20} = medium fish, S_{21}-S_{30} = large fish*

In this study, the condition factor is calculated to assess the fish's growth, habitat conditions, and the general well-being of the aquatic environment, in this case, the River Nile in Khartoum, Sudan. Low K values (less than one) normally reflect unfavorable environmental conditions and/or insufficient nutrition, whereas favorable conditions such as habitat and prey availability are indicated by high K values that are greater than one (Shahabuddin et al. 2015; Townhill et al. 2023; Bhatkar et al. 2025).

As the values in Table 4.1 show, all K values except for sample S_1 were found to be greater than 1 indicating that fish were in good health condition and a good general well-being. Furthermore, no

significant differences in condition indexes were found between groups, suggesting that they received equivalent nutrition.

4.2 MP prevalence, abundance, and intensity in the digestive tracts of Nile Tilapia

In this study, MP particles were 100% prevalent in all the Nile Tilapia specimens. A total of 567 particles were recovered from the digestive tracts of the fish, with an average intensity (number of MPs per fish) of 18.90 ± 9.17 particles/individual. The intensity of MPs in fish samples varied with a minimum number of 5 particles/individual (sample S_{24}) and a maximum number of 47 particles/individual (sample S_{11}). However, some samples had a considerably higher intensity than others (S_{11}, S_{19}, S_{21}, and S_{27}), as shown in Fig 4.1.

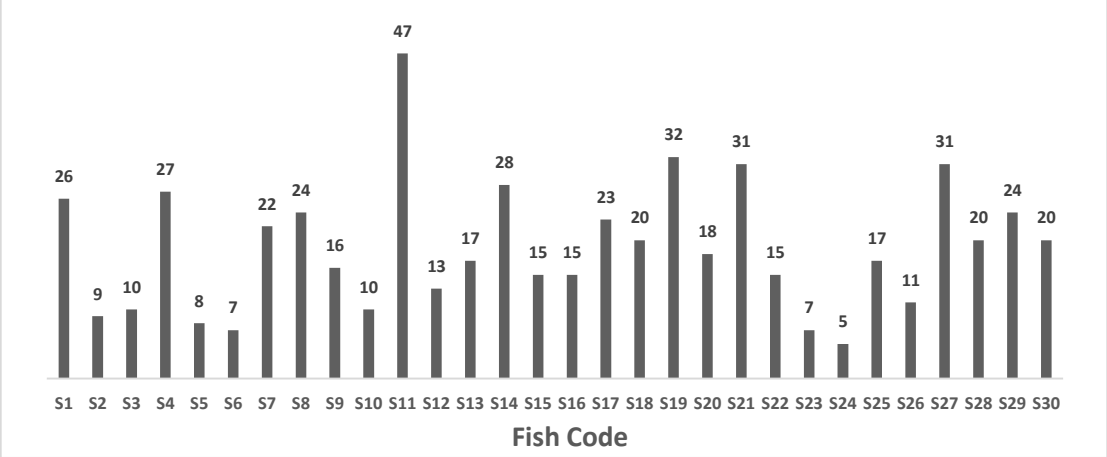


Fig 4.1: MP intensity in Nile Tilapia fish sampled from the River Nile

The abundance of MPs ranged from 04.33 particles/kg to 236.84 particles/kg, with an average of 72.02 ± 62.06 particles/kg. A relatively high abundance of MPs was observed in S_1(216 particles/kg) and S_4 (237 particles/kg), both were classified as small fish. On the other hand, a minimum abundance of MPs was found in S_{23} (6 particles/kg) and S_{24} (4 particles/kg), both are large fish.

The high prevalence of MPs in fish, which may reflect the extent of plastic pollution in the River Nile ecosystem, shows that the aquatic biota of the Nile is exposed to significant amounts of bioavailable MPs.

Rivers play a significant role in the transportation of plastic waste from land-based activities to the river systems depending on several factors such as river hydrology and weather conditions (Van Emmerik et al. 2022). Atmospheric sedimentation, direct dumping along riverbanks, agriculture, boat discharge, urban and rural runoff, artificial discarding, and wastewater treatment plant effluents are some potential sources of riverine litter (Demsie and Yimer 2023). The high abundance of MPs is not only a reflection of the widespread utilization of plastics and MPs but also, a result of their buoyancy and extreme persistence. This is often determined by land use, social, and economic activities in the stream area. It is therefore expected that each river exhibits a characteristic profile of plastic litter composition, based on the influencing factors in its course (McNeish et al. 2018).

MP ingestion by aquatic biota is frequently reported in different aquatic environments (Jabeen et al. 2017; Abbasi et al. 2018; Bordós et al. 2019; Adeogun et al. 2020; Nikki et al. 2021; Saad et al. 2022; Lestari rt al. 2023; Liu et al. 2024). The prevalence and intensity of MPs in Nile Tilapia in this study are significantly high compared to those reported in other studies (Table 4.2).

Table 4.2 Nile Tilapia studies around the world

Freshwater system	Region	Number of fish	Number of MP	prevalence %	Abundance particles/Kg	Reference
Lake Eleyele	Nigeria	43	1–4	34.0	-	(Adeogun et al. 2020)
Lake Victoria	Tanzania	07	-	35.0	-	(Biginagwa et al. 2016)
Lake Hawassa	Ethiopia	30	147	36.67	4.03 ± 3.19	(Demsie and Yimer 2023)

Freshwater system	Region	Number of fish	Number of MP	prevalence %	Abundance particles/Kg	Reference
Atoyac River	Mexico	15	139	53.7	-	(Martinez-Tavera et al. 2021)
River Nile	Egypt	29	164	75.9	-	(Khan et al. 2020)
Volta Lake	Ghana	30	84	80	2.8 ± 0.6	(Boateng et al. 2024)
Lake Amatitlán	Guatemala	65	631	96.9	65.74 ± 57.55	(Oliva-Hernández et al. 2021)
Lake Ranu Grati	Indonesia	25	576	-	-	(Aunurohim et al. 2024)
River Nile	**Sudan**	**30**	**567**	**100**	**72.07 ± 63.10**	**Present study**

The differences observed in MP prevalence among these studies, including this present study, could be attributed to the various conditions of the environments as well as preferences in the trophic web (Alnail et al. 2013; Dris, Gasperi, et al. 2015). Important to note, is that direct comparisons between different studies are limited and could be inconsistent due to the dissimilarities in extraction methods, quantification, and analysis techniques (Avio, Gorbi, and Regoli 2015; Haberstroh 2021).

In general, the findings of this study reveal that the River Nile in Khartoum is considerably contaminated with MPs, and that, the fish in the Nile may be threatened by MP contamination. However, given that the abundance of MPs in the surface waters of the River Nile has not yet been assessed, it is not possible to solely relate the ingestion of MPs by the fish to the actual abundance of MPs in the river. In other words, other biotic factors such as feeding habits may contribute to the high abundance and intensity of MPs in the fish.

4.3 Physical characteristics of MPs recovered from Nile Tilapia fish

Understanding the physical properties of MPs is crucial for determining their distribution, transport, path, behavior, and fate in aquatic environments (Kurniawan et al. 2023). Size is the main factor influencing MP distribution, transport, pathway, and fate in aquatic environments (Saad et al. 2024b). However, the shape of MPs also affects their transport in the environment e.g., certain shapes are distributed differently. For instance, fragments are dominantly reported on the water surface, while fibers and pellets are mostly found in sediment (He et al. 2021).

4.3.1 Size of MPs in Nile Tilapia

The size of MPs recovered from the digestive tract of the thirty Nile Tilapia specimens ranged from 0.04 to 4.94 mm (Fig 4.2). According to the distribution of sizes, it is observed that smaller MPs were more abundant, showing the following order: < 0.5 mm (30.51%) followed by 1-2 mm (27.69%), 0.5-1 mm (25.93%), 2-3 mm (9.35%), 3-4 mm (4.94%) and 4-5 mm (1.59%).

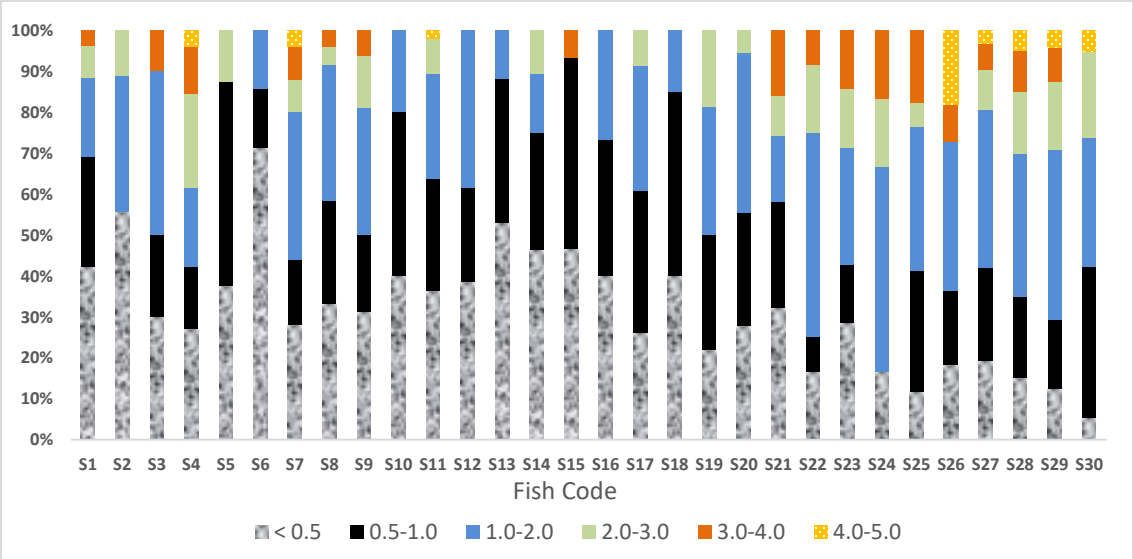


Fig 4.2: Distribution of MP sizes per individual fish

The prevalence of small-sized MPs was also observed by Demsie et al., (2023), who investigated the occurrence of MPs in the digestive tracts of Catfish *(Clarias gariepinus)* and Nile Tilapia (*Oreochromis niloticus*), collected from three sampling sites of lake Hawassa. They reported that

84.6% of the MPs found in the two species were smaller than 1 mm. Similar observations were reported in different fish species in several freshwater bodies around the world (Dris, Gasperi, et al. 2015; Andrade et al. 2019; Haberstroh 2021; Oliva-Hernández et al. 2021; Demsie and Yimer 2023; Siddique, Tahsin, and Das 2025).

The prevalence of smaller MPs (< 0.5 mm) is of special concern, due to their high surface area-to-volume ratios, these tiny particles can adsorb other contaminants from the surrounding environment, which pose a greater threat to aquatic organisms (Bradney et al. 2019). The absorption of contaminants has been reported to be more pronounced in MPs less than 1mm. Several studies indicated that small MPs could transport contaminants and are considered a sink for persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbon (PAHs), and dichloro diphenyl trichloroethane (DDT) along with heavy metals (e.g., Al, Zn, Pb, Cu, Ag, and Pb) (Vo and Pham 2021; Martinez-Tavera et al. 2021). Consequently, they have a higher potential to serve as contamination vectors and increase the bioavailability of toxicants (Campanale et al. 2020).

4.3.2 Shape of MPs in fish

In this study, five shapes (fibers, fragments, films, foams, and pellets) were detected under the microscope. Following a definition made by Goral et al. (2023), particles with a slender and elongated appearance were categorized as fibers; particles of irregular shapes were categorized as fragments; very thin particles were categorized as films; and round particles with spherical shape were categorized as pellets. Fig 4.3 (a–e) shows microscopic images of the detected shapes.

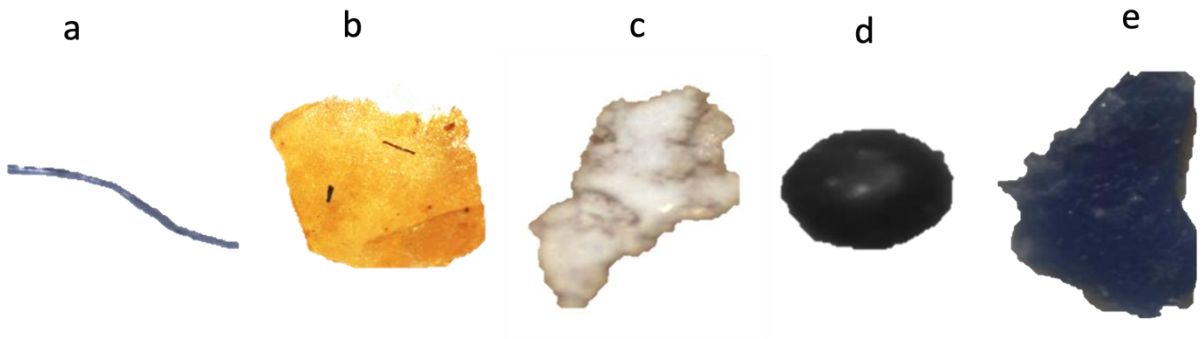


Fig 4.3: MP images captured under the optical microscope (a) fiber, (b) film, (c) foam, (d) pellet, (e) fragment.

Fibers were the most abundant (n = 483) and prevalent (100%) shape detected in fish samples, accounting for 85.04% of the total MPs. Fragments (n = 54) and films (n = 27) were present in small percentages of 9.5% and 4.6%, respectively. While foams (n = 2) and pellets (n = 2) were detected in a minor percentage of 0.35%, both. The distribution of sizes is shown in Fig 4.4. The ANOVA test revealed a significant difference (P=< 0.001) between MP abundance and shape, further supporting the finding that fibers are the most prevalent

Fibers and fragments are commonly reported to be the most abundant shapes in several studies such as Great Lakes tributaries (USA), Danang Bay(Vietnam), Lake Hawassa (Ethiopia), and Vaal River (South Africa) (Cable et al. 2017; Dang et al. 2022; Demsie and Yimer 2023; Saad et al. 2024).

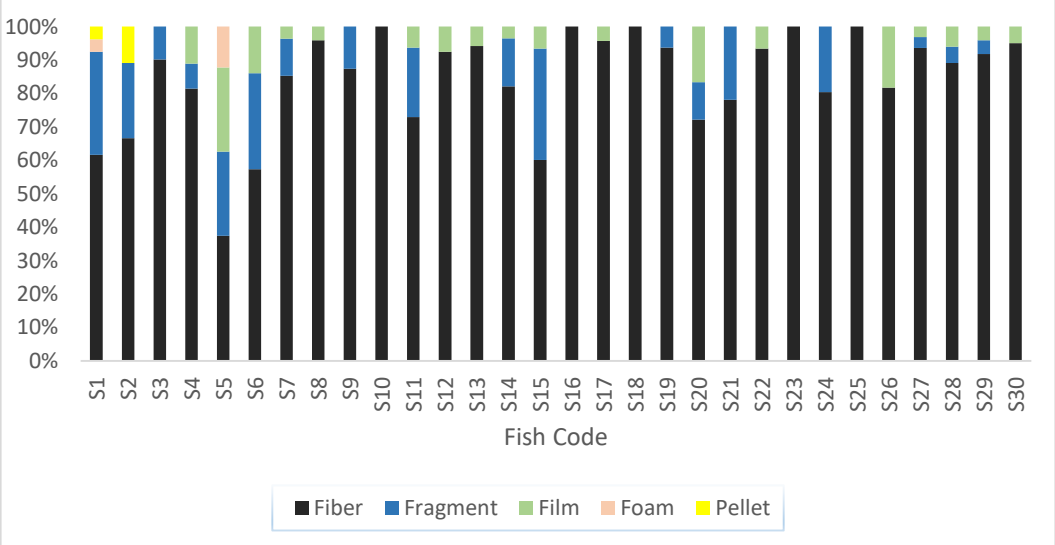


Fig 4.4: Distribution of MP shapes per individual fish.

Fibers have been reported to have a longer intestinal residence time and higher mortality in the digestive tract than other shapes of MP particles (Qiao et al. 2019; Elizalde-Velázquez et al. 2021; Rebelein et al. 2021; Saad et al. 2024). The ingestion of fibrous MPs by aquatic biota has frequently been reported in several studies (Rezania et al. 2018; Rebelein et al. 2021). For instance, Mizraji et al. (2017) reported a high abundance of fibers in the digestive tract of omnivorous fishes (99%) in the central coast of Chile. Similarly, Lusher et al. (2013) reported the dominance of fibrous MPs in ten fish species from the English Channel in Plymouth, UK .

In this study, foam and pellets were found to be less prevalent forming 0.35%. Which indicates probable primary MP sources. However, the dominance of fibers and fragments over pellets clearly indicates that MP contamination in the River Nile is mostly of secondary sources.

As shown in Fig 4.5, fibers were observed across all size ranges, with the greatest proportion (24.34%) observed in the size range of 1.0 - 2.0 mm, followed by (23.81%) in < 0.5 mm, (19.22%) 0.5-1.0 mm, (8.82%) in 2.0 - 3.0 mm, (4.76%) in 3.0 - 4.0 mm, and (1.41%) in 4.0 - 5.0 mm. Fragments were observed in three size ranges: < 0.5 mm (6.53%), 0.5-1.0 mm (5.11%), and (1.41%) in 1.0-2.0 mm. Films were observed in two size ranges 0.5 - 1.0 mm and 1.0 - 2.0 mm with 3.35% and 0.53%, respectively. While pellets and foams were observed in only one size range of 0.5-1.0 mm with (0.35%).

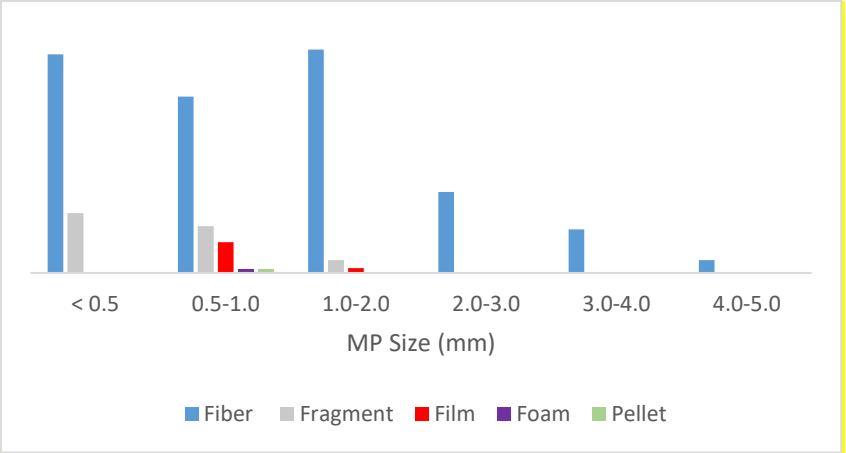


Fig 4.5: Distribution of MP shapes per size class

Fibers may originate from clothing material, ropes, and fishing lines; and fragments from effluent discharge and degradation of large debris (Santos et al.2009; Dunne et al. 2016). Film and foams may come from waste generated from boat trips, food containers, fish storage/box, handicrafts, and ornaments (Cordova et al. 2020). Pellets, on the other hand, come from personal care products in wastewater (Mason et al. 2016).

In this study, the high abundance of fibers may be attributed to domestic effluent from the residential areas near the river as there are no controls that prevent direct discharge into the river. Due to their greater length-to-diameter ratio, fibrous MPs remain suspended in the water column for extended periods and are less likely to sink easily compared to other shapes. This characteristic

makes them more bioavailable for ingestion by Nile Tilapia and other organisms. However, Nile Tilapia may preferentially consume fibrous MPs because they resemble fiber-like prey (Xie et al. 2010). For instance, Horton et al. (2018) found that the guts of roach fish contained a significant amount of fiberous MPs alongside filamentous algae due to their visual similarity.

In addition, the predominance of fibers in comparison to other MP morphologies may potentially indicate extended digestive tract retention. Unlike hard shards and pellets, which cannot be easily bent into smaller sizes, fibers are more pliable to fit into the narrow alimentary canals, and ingested synthetic fibers can tangle and group together to create agglomerates that may clog the GI tract, leading to the buildup of plastic fibers in fish (Neves et al. 2015). According to Au et al. (2017), fibers stayed in the crustacean *(Hyalella azteca's)* digestive system for a longer time than pieces and natural food.

4.3.3 Colour of MPs in fish

A great variety of colours were observed, of which, green was the most abundant (26.63%), followed by black (18.69%), blue (14.29%), red (8.82%), grey (7.23%), and white (3.53%). Other less frequently observed colours (purple, yellow, pink, and brown) were grouped under one category "others" and represented (4.41%), altogether. Transparent MPs constituted 16.40% of the total MPs (Fig 4.6).

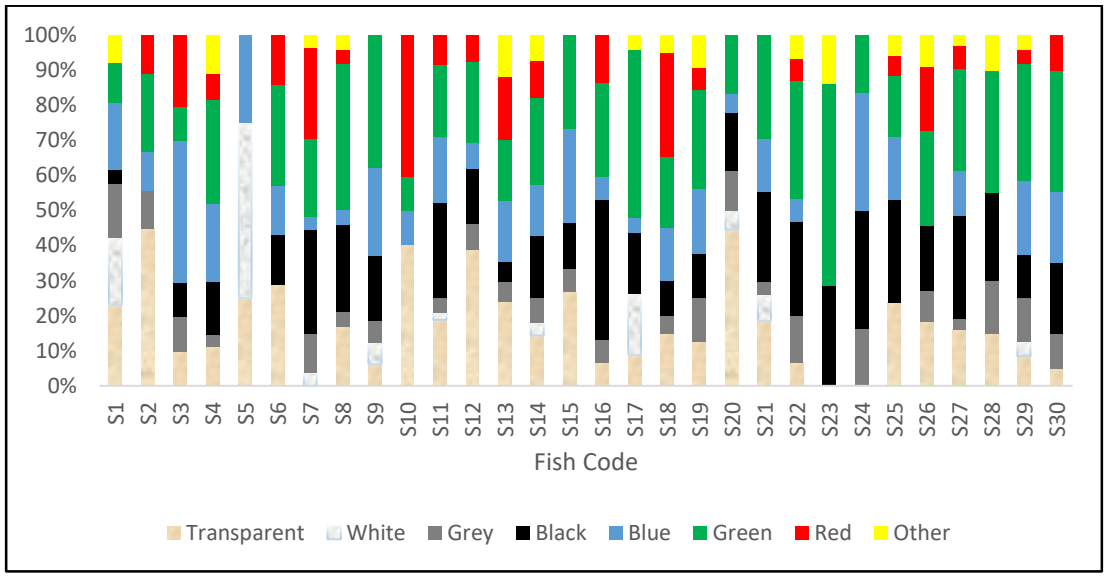


Fig 4.6: Distribution of MP colours per individual fish

As shown in Fig 4.7, there was a higher abundance of coloured fibers (73.02%) compared to transparent fibers (11.82%). Similarly, coloured fragments were more abundant (9.70%) compared to transparent fragments (0.18%). The variety of colours indicates the diversification of MP sources (Xu et al. 2020). For instance, black fragments may come from car tires, whereas, translucent and white fragments may result from packaging materials and plastic carry bags (Xu et al. 2020). Transparent and white fibers are known to originate from fishing robes, and coloured fibers come from clothes (Burgess and White 2019). Wastewater effluent could be the primary source of these MPs (Schwarz et al. 2019).

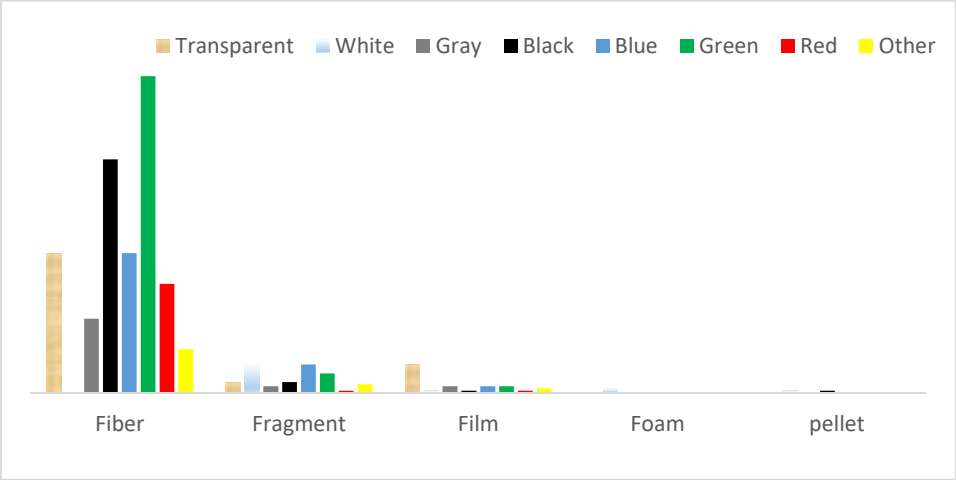


Fig 4.7: Distribution of MP colours per shape

Several freshwater studies conducted around the world have regularly noted the prevalence of coloured MPs in aquatic species. Aquatic biota are thought to consume MPs that resemble their natural prey in terms of colour (McNeish et al. 2018; Castro-Castellon et al. 2022).

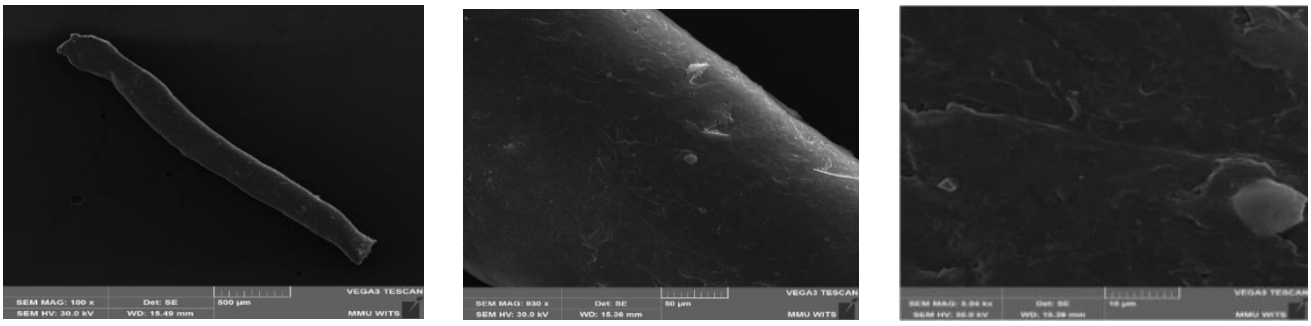
Green was the most common colour in the present study, which could be consumed intentionally by the fish due to its resemblance to natural food. This was evident in the small fish that feed on phytoplankton.

Transparent MPs presented a relatively high portion of the total MPs (16.40%). It should be noted, however, that KOH used for digestion may cause discolouration of some polymers, possibly resulting in an overrepresentation of white or/and transparent MPs in the sample (Battaglia et al. 2020). Bråte et al. (2018) observed bleaching of some MPs when using 10 KOH at 60 °C for 24 h. Also, it is uncertain how degradation in the environment as well as in the digestive tract of Nile

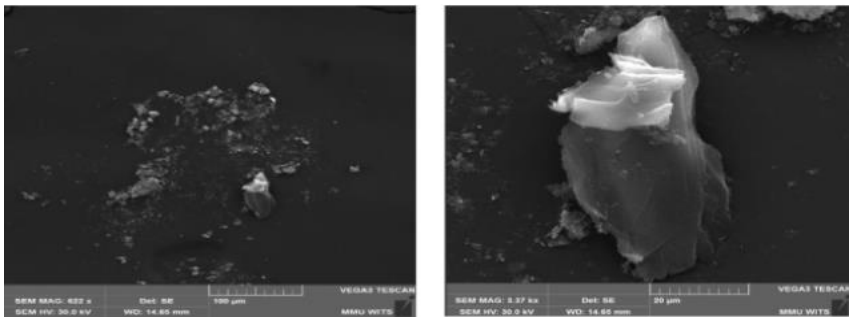
Tilapia can affect the leaching of dyes from MPs. As noted by Pfaff and Gerhard (2021), other factors that affect whether the colour is retained include the chemical composition of the colourants that are added to the plastic particles during manufacturing, and, the techniques by which they are incorporated (i.e., dissolution vs dispersion).

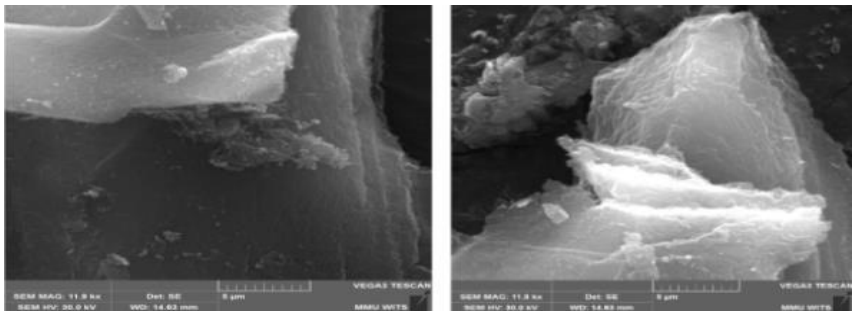
4.3.4 Surface texture

Figure 4.8 shows SEM images of selected MPs of different shapes. The fiber (Fig 5.8a), fragment (Fig 4.8b), and foam (Fig 4.8c) particles have shown significant disintegration and/or fragmentation signs (cracks, pores, pits, and scratches). The resultant roughness of these particles` surfaces increases the surface area, thus facilitating the adsorption of chemicals and microorganisms (Chouchene et al. 2022; Yang, Li, and Boraschi 2023; Liu et al. 2025). The film particle (Fig 4.8d), had tear marks and its surface was somewhat smooth similar to that of the pellet, both are devoid of weathering processes.

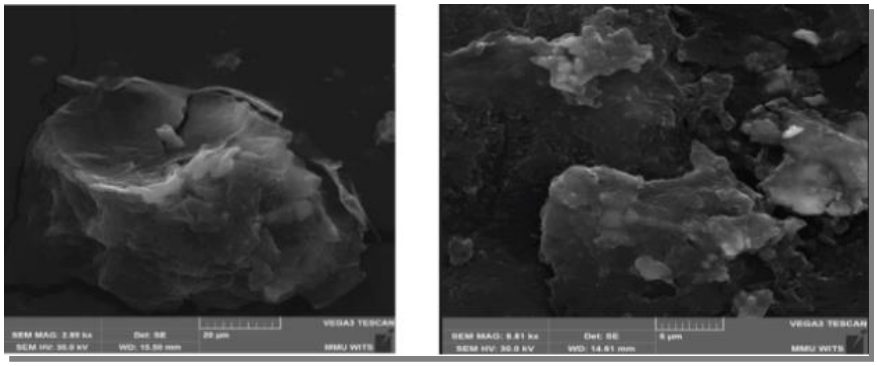


(a)

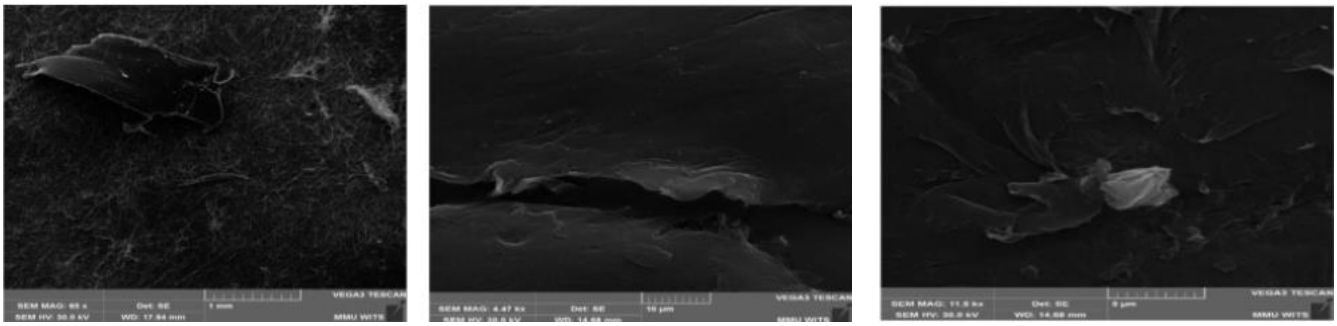




(b)



(c)



(d)

Fig 4.8: SEM images of selected MPs (a) fiber, (b) fragment, (c) foam, and (d) film.

It is well known that the interaction of MPs with their surroundings is greatly influenced by their surface texture (Dąbrowska et al. 2022). In addition to the impact of surface morphology on the

adsorption/desorption of other pollutants and the development of biofilms, surface morphology can also affect the transport, distribution, fate, and bioavailability of MPs (Ding et al. 2019).

In addition, deteriorated MPs are reported to emit dimethyl sulfide (DMS) stench. Where fish that feed on primary consumers (like zooplankton), are induced to engage in foraging behavior as a result of DMS and its chemical precursor. Hence, Nile Tilapia may intentionally consume weathered MPs due to confusing the particles for natural prey (Savoca 2017; Sarkar 2018; Song 2024).

In this study, the presented observations may have been caused by mechanical abrasion, exposure to chemical, and biological activities in the River Nile. Which emphasise that the majority of MPs in the Nile are from secondary sources. Similar findings were reported in the Hashilan wetland, Iran, and in wastewater treatment plants in Ireland (Abbasi 2021; Razeghi et al. 2024; Mahon et al. 2017). All studies observed grooves, flakes, blistering, and fracturing on the surfaces of the MP particles.

4.4 Chemical characteristics of MPs in Nile Tilapia fish

The type of polymer is a crucial characteristic to consider because it provides information on potential contamination sources. Further, the physical and chemical characteristics of polymers, such as density and chemical additives determine the distribution, transport, fate, and effects of MPs on aquatic ecosystems.

4.4.1 Polymer Types

The chemical characterization of MPs in this study showed only two types of polymers, high-density polyethylene and polypropylene, which is indicative of both the ubiquitous usage of these polymers and their persistence in the environment. The Raman spectra for the two polymers are given in Fig (4.9 & 4.10), the sample spectra are shown in blue while the reference spectra are in red.

The transport and distribution of polymers in aquatic environments are influenced by the physical properties of polymers such as plastic density and residence time (Rodríguez-Seijo and Pereira 2017; Sooriyakumar et al. 2022; Li et al. 2023). The relatively low densities of PE and PP enable spatial dispersal in aquatic environments. As a result, these two polymers are among the most

frequently identified polymers among all aquatic compartments (surface water, sediment, and biota) (Garcia et al. 2020).

Their high prevalence can also be related to their widespread use in several applications including storage containers, food packaging, water bottles, plastic bags, fishing nets, and boat ropes (An et al. 2020; Dambrosio et al. 2023; Megha et al. 2025). According to Büngener et al. (2024), PE and PP are the two most common polymers in the Rhine-Main River in Germany. The authors contend that given that PE and PP account for 57.9% of the demand for plastic in Europe, their availability can be linked to their high economic worth (Cafiero et al. 2025).

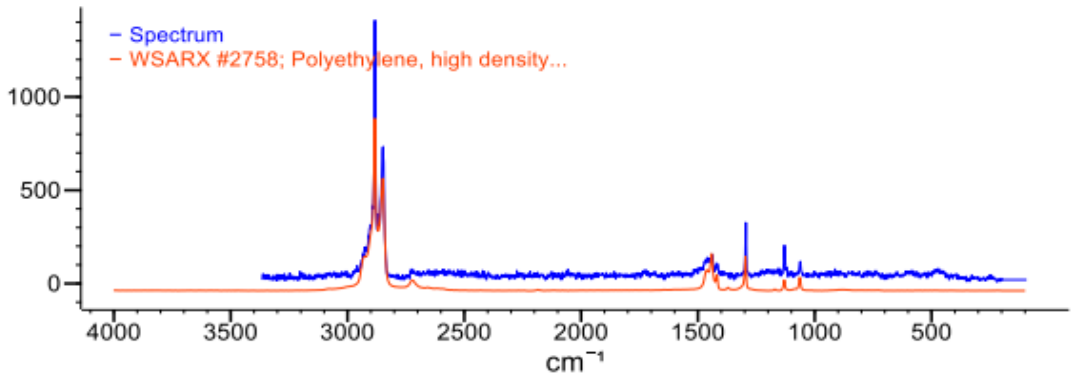


Fig 4.9: Raman spectrum of high-density polyethylene

PE is virtually defined by its very name as a polymer of ethylene produced by addition polymerization, which is divided according to its density, melting point, and molecular structure as either LDPE or HDPE. HDPE is a highly crystalline polymer produced from a low-pressure polymerization using Ziegler–Natta catalysts, HDPE has a linear polymeric chain, high density, and high melting point. Applications for HDPE include large containers, drums, fuel tanks, bottles, pipes, crates, and wrapping film (Martin 2022; Dai et al. 2023).

HDPE is the most inert polyolefin, yet it can still degrade gradually in the environment, where the C−C backbone does not readily undergo hydrolysis and photooxidation due to the lack of UV-visible chromophores. Nevertheless, impurities or structural defects can act as chromophores. HDPE might have a few unsaturated carbon bonds, such the vinyl groups. These sites are readily

oxidized by tropospheric radicals to unstable hydroperoxides, which are then converted to more stable UV-absorbing carbonyl groups.

Mazurais et al. (2015) reported high mortality rates in European sea bass (Dicentrarchus labrax) exposed to PE microspheres, which was attributed to possible blockage of the intestinal lumen.

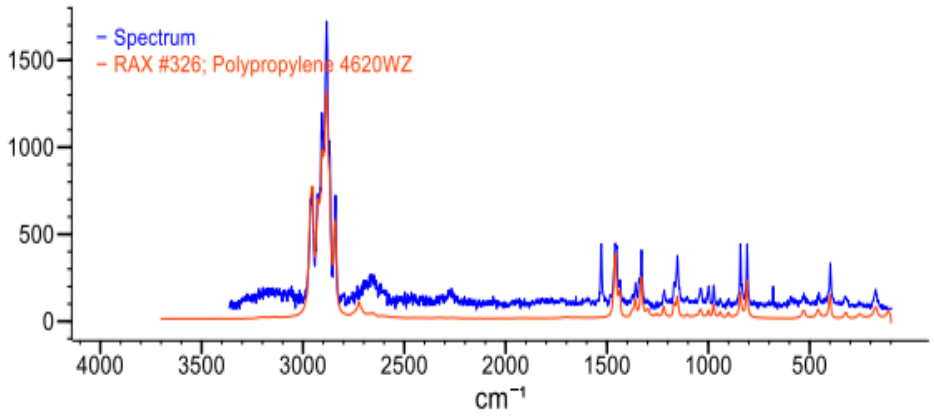


Fig 4.10: Raman spectrum of polypropylene

Propylene is semi-rigid crystalline, translucent, tough, and exhibits good heat and chemical resistance, with a density range of 1.04 to 1.06 g/cm^3 (Maddah 2016). PP has a higher softening point, higher rigidity, and is harder than HDPE. Three differing grades of PP are commercially available: homopolymers, block copolymers containing 5-15 % ethylene, and random copolymers incorporated with randomly arranged co-monomer units. Hence, it is one of the most used plastics in a variety of applications in the polymer industry. Examples include flexible packaging, rigid packaging, piping, ropes, carpets, concrete reinforcement and food containers, and clear plastic bags. PP fibers are used in clothing and diapers (Gahleitner et al. 2019). PP MPs have been widely detected in the gastrointestinal tracts of fish, mussels, tadpoles and sea turtles (Ozturk and Altinok 2020; Hou and Rao 2022; GÜNDOĞDU et al. 2024).

The relatively low densities of PE and PP enable spatial dispersal in aquatic environments. As a result, these two polymers are among the most frequently identified polymers among all aquatic compartments (surface water, sediment, and biota) (Lorenz et al. 2019).

Their high prevalence can also be related to the widespread use in several applications including storage containers, food packaging, water bottles, plastic bags, fishing nets, and boat ropes (Maddah 2016; Lusher, Hollman, and Mendoza-Hill 2017; Kozioł, Paso, and Kuciel 2022).

4.4.2 Other chemical compounds detected

In addition to the potential health hazards posed by MP particles, there is also the risk posed by the additives that are added to plastics during manufacturing, such as stabilizers, colourants, and flame retardants. Furthermore, MPs are known to adsorb and desorb toxic elements and organic contaminants from the surrounding environment, depending on the prevailing environmental conditions. It is therefore, expected that other compounds associated with MPs will be detected when studying MPs. In this study, in addition to the two polymers presented in section 4.4.1, two colourants (Pigment Black 7 and Pigment White 6), were identified. In addition to carbon.

Raman spectrum for carbon is shown in Fig 4.11. The detection of carbon in these samples could be due to burning green bricks in ambushes near the River Nile, a common practice in several locations on the riverbank in Khartoum and other cities in Sudan. This may result in MPs being coated with carbonaceous matter. Additionally, soot from fires and incineration of plastics may also contribute to that effect.

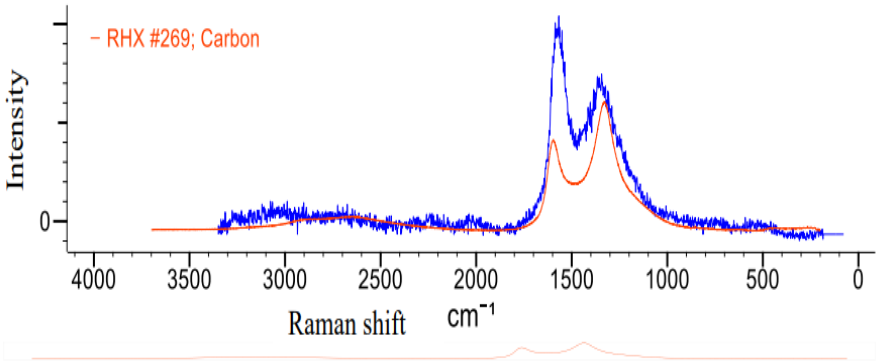


Fig 4.11: Raman spectrum of carbon

The Raman spectrum in Fig 4.12 had carbon bands similar to those of Ivory black (Pigment Black 7). Vine black is made of carbonaceous materials, hence it has a spectrum that is almost identical to that of carbon black (Liang et al. 2018; Lluveras-Tenorio et al. 2019).

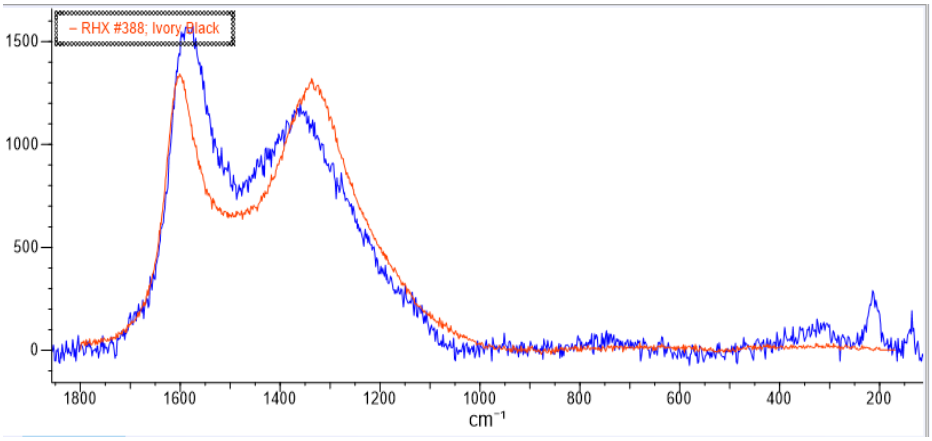


Fig 4.12: Raman spectrum of ivory black

Carbon black (ivory black) is a carbon-based pigment and reinforcing agent used in polymers and polymer blends. It is the second most widely used colourant in the plastic industry (Vaidya 2011; Muthuvairavasamy 2022; Berrie et al. 2024). In nearly all applications, carbon black is incorporated into a polymer matrix. However, according to Key et al. (2024), carbon black particles generally do not leach from plastics into food. Consequently, carbon blacks are not considered a direct genotoxicant or reproductive toxicant (Achawi et al. 2021; Sanjuan-Navarro, Moliner-Martínez, and Campíns-Falcó 2022).

Titanium dioxide, known as titanium (IV) oxide or titania, is an inorganic compound with the chemical formula TiO_2 known as Pigment White 6, and is the most widely used plastic colourant in the world (Wu 2022). The sample and reference spectra in Figure 4.13 had main peaks at 203.03 cm^{-1} (multiple phonon scattering processes), 447.75 cm^{-1} (Eg vibrational mode), and 606.22 cm^{-1} (A1g vibrational mode) assigned to TiO_2 (Mohammed et al. 2022; Trinh et al. 2024).

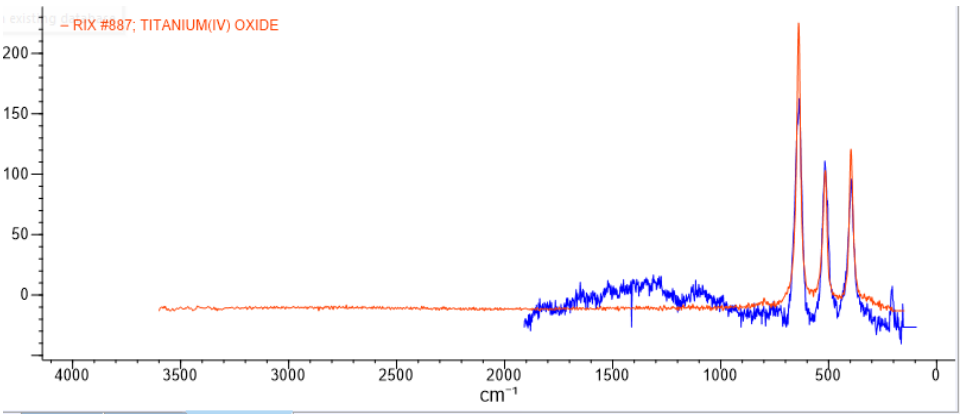


Fig 4.13: Raman spectrum of titanium dioxide

Titanium dioxide is chemically inert and insoluble in polymers which prompted its replacement of white lead as the principal white colourant. Titanium dioxide is used as a pigment and optical agent, particularly in PVC, PE, and alkyd resins. It is extensively used in white carrier bags, window profiles, and others (Pfaff 2021; Christie and Abel 2021; Andrady et al. 2023).

These findings show that MP particles in the River Nile contained black and white pigments added into polymers during production. Similar observations were made for MPs from the deep sea (Di Giacinto et al. 2023), as well as in the tissue of bivalves from the North Sea and the Atlantic ocean (Silva 2024).

4.5 Potential source of MPs in the River Nile

To date, no studies are reporting the presence of MPs in the sediment and water of the River Nile in Sudan or other countries. However, the high prevalence of MPs in the fish samples in this study indicates their presence in the water and sediment of the river. This is a result of increasing pollution loads arising from economic growth and high population density in the region. Hence, the river has been exposed to nonpoint source pollution, including chemicals and sedimentation from agricultural practices and recreational activities. This is in addition to domestic and industrial discharge. Due to the lack of efficient wastewater treatment facilities, coupled with the high population density in Khartoum, there has been extensive discharge of untreated effluent into the river from domestic, industrial, and agricultural activities. Consequently, the river is subjected to MP pollution from overflowing sewage drains and litter from the three largest towns in Khartoum state, i.e., Omdurman, Khartoum, and Khartoum North.

Urban towns of Khartoum, Khartoum North, and Omdurman have high population densities. Local sources of MPs include industries, WWTPs, storm water runoff, informal and formal settlements, and recreational activities. Storm drainage and urban runoff may carry MPs from landfill sites, road paint, and tire wear from vehicles into the River Nile. Several studies indicate a decrease in MP abundance with distance from urban centres. The proximity of a freshwater body to urban areas with highly intensive anthropogenic activities is positively correlated with an increased number of MPs (Torres-Agullo et al. 2022; Latcheman 2023; Österlund et al. 2023). For instance, lower MP abundances were detected further away from the Wuhan City Centre in China (Li et al. 2024).

The areas adjacent to the River Nile have vibrant scenes of life, as there are many recreational activities along the river, for example, water sports, restaurants, and tourist ferries as well as tea ladies' sellers (a common phenomenon in Sudan, especially in Khartoum). All these could be potential sources of macro-plastics that eventually degrade into MPs. Fig 4.14 shows Field pictures from the study area.



Fig 4.14: photo shown plastics pollution around study area

Considering the state of the wastewater system in the three large cities of Khartoum, WWTPs could very much be the main source of MPs. Omdurman/Karary, Wd-Daffiaa, and Soba WWTPs are characterised by a history of breakdown, outdated equipment, operating beyond design capacity, and rarely treating wastewater to acceptable standards (Gorani and Ebraheem 2012; Bakhit and Eltayeb 2021).

The sugar industry in the White Nile State region could also be a significant potential source of MP pollution reaching the River Nile. Near the banks of the Blue Nile and White Nile, there are sugar cane processing companies (Assalaya) that release their effluent directly into the rivers (Alnail et al. 2013).

4.6 Biotic factors

4.6.1 Fish feeding habits

Feeding behavior has been shown to play an important role in the ingestion of MPs by fish (Jabeen et al., 2017). While some fish ingest MPs accidentally, others may intentionally consume them due to similarity with their natural prey. This is dependent on their sensory capacities to locate food, their ability to catch, ingest, and manage food items, as well as their biochemical and

physiological capacities to digest and transform the ingested nutrients (Kestemont and Baras 2001).

Nile Tilapia have a versatile feeding behavior, which induces variation in nutrient composition (Temesgen et al. 2022b). They consume a variety of organisms including phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, and bacterial films, as well as other fish and fish eggs (Pauly and Froese 2012). Several studies have reported the importance of plankton as the main food for the Nile Tilapia (Genanaw et al. 2021; Tesfaye et al. 2021; Tikue and Workagegn 2022; Abo-Taleb, El-Tabakh, and Hendy 2023).

Planktivorous fishes swallow large amounts of water to filter enough food, thus, they may accedentially ingest non-dietary items, such as MPs (Lima et al. 2014). Moreover, planktivorous fish have longer and more twisted intestines, which also allow them to amass MPs with a wider range (Yin et al. 2022). The composition of the Nile Tilapia diet may vary in a wide range of seasonal and spatial conditions, fish size, and maturity. In this regard, Ashour et al. (2025) hypothesized that Nile Tilapia mostly consume phytoplankton species, but they also devour zooplankton species (omnivorous).

Fish with diverse feeding habits such as Nile Tilapia are at higher risk of MPs` ingestion due to their foraging behavior across a wide range of food types and throughout the water column (Garcia et al. 2020).

The formation of biofilms on MPs may further increase the ingestion of MPs, even in species that have chemosensory foraging habits (Onda and Sharief 2020).

In this study, it was noted that small fish (<0.25 kg) ingested a higher number of MPs compared to larger fish. Some studies indicate that small Nile Tilapia are generally omnivorous and mainly feed on zooplankton, insect larvae, and phytoplankton (Tesfahun and Temesgen 2018; Tesfaye, Fetahi, and Getahun 2020). This is because small fish need high protein intake to support a high growth rate and metabolism. Additionally, the variation in habitat preference between different size groups of fish can result in a difference in their food composition (Benavides et al. 1994). In contrast, larger fish instead rely on food from plant origins, such as macrophytes and detritus. In addition, larger fish are more capable of digesting cell wall materials and therefore can be less selective in their feeding pattern. Many investigators also reported similar feeding variations in different size groups of Nile Tilapia in different water bodies (Assefa and Getahun 2015b; El-Saidy and Gaber 2005). However, in addition to the influence of feeding behaviurs, differences

in body size may also contribute to variations the MP concentration differences in fish guts (Sun et al. 2019).

4.6.2 Body size - age

The mean abundance of MPs in the small fish samples was 126.56±72.14 particles/kg. The mean abundance of MPs in medium fish was 68.95 ± 34.72 particles/kg, whereas, the lowest was observed in large fish (20.70 ± 13.34 particles/kg). Body size (mass and total length) of Nile Tilapia fish were negatively correlated with MP abundance (ANOVA, p=<0.001), as shown in fig 4.15 and 4.16 below, confirming that smaller individuals contained higher MPs, with maximum abundances of 236.84 particles/Kg and 216.67 particles/kg in S_1 (0.120) and S_4 (0.114) Kg, respectively.

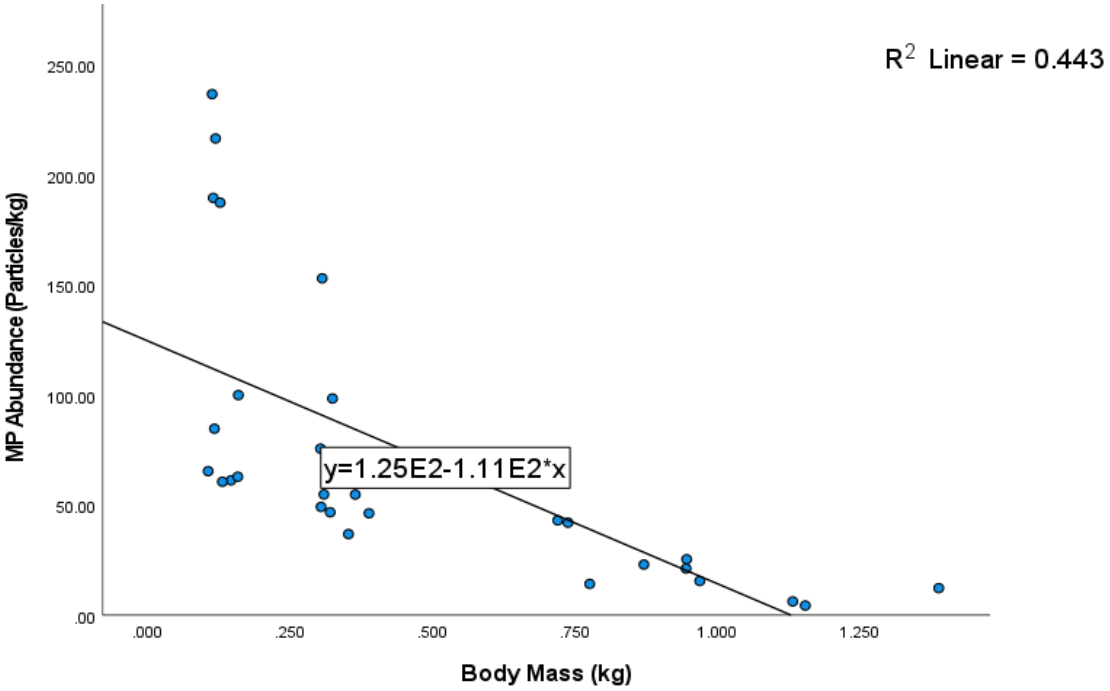


Fig 4.15: Correlation between MP and body mass of fish

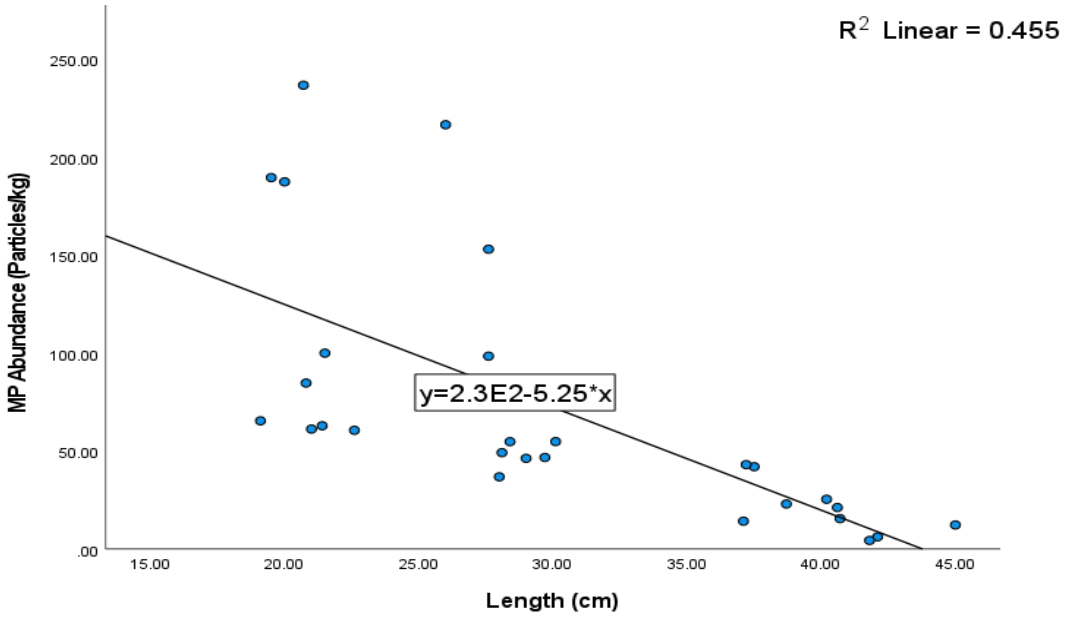


Fig 4.16: Correlation between MP and total length of fish

The calculated p-value for both the body mass and total length of the fish in relation to MP Abundance was less than 0.001. A p-value below 0.05 is considered statistically significant, indicating strong evidence against the null hypothesis. Therefore, based on the p-values, the null hypotheses are rejected. The conclusion is that there is sufficient evidence to suggest a negative correlation between MP abundance and the body size of the fish (which may indicate the fish's age). This can be explained by one or more of the following factors: (i) The difference in feeding habits discussed in the previous section (6.4.1), (ii) smaller Nile Tilapia may be able to access areas that have higher sediment plastic concentrations, (iii) large fish, have ontogenetic developmental factors that may affect their capacity to distinguish between genuine food and MPs, (iv) smaller Nile Tilapia may retain more plastic particles in their digestive tracts due to their less developed digestive systems, which may take longer to process the particles. However, it is important to note that the sample size in this study was not wide enough to draw definitive conclusions.

Other studies examining the relationship between fish size/age and MP ingestion have reported carrying resulys. Some report significant positive correlations between MP ingestion and fish size/age (Schmid et al. 2018; Hossain et al. 2019; Alomar et al. 2021; Parvin, Jannat, and Tareq 2021; Saemi-Komsari et al. 2023). The increased potential for MP ingestion by larger fish is often attributed to their higher energy demands and greater food intake capacity (Alberghini et al. 2022).

On the other hand, some studies have not found a significant correlation between MP abundance in fish and their body size (Zhang et al. 2022).

4.6.3 Fish sex

Initial assumptions regarding potential differences between sexes in MP ingestion and subsequent responses can be attributed to variations in features, such as body size and/or behaviour (Prokić et al. 2021)**.** Of the thirty fish specimens analysed, ten were identified as females (33.3%), and twenty as males (66.7%). Both males and females exhibited considerable variation in terms of the number of MP ingested. To better understand the effects of sex on MP uptake, a comparison of medium-sized was made, shown in Fig 4.17. This size category includes an equal proportion of males and females, with five individuals of each sex.

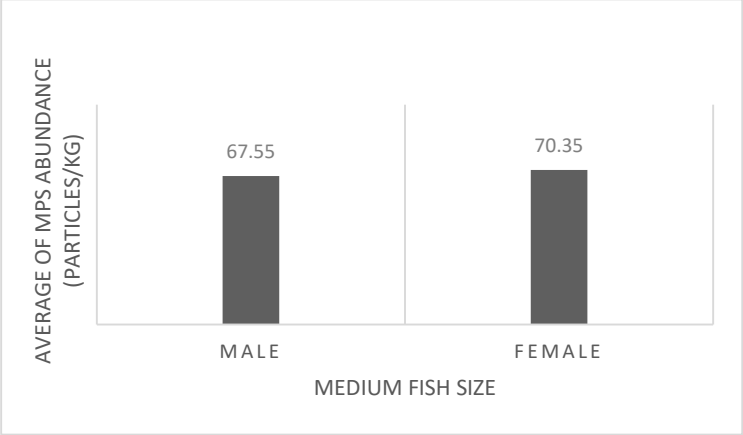


Fig 4.17: Average of MP abundance in medium size fish of both male and female

In this study, IBM SPSS Statistics V 28 was used to examine whether there is a difference in MP abundance between male and female fish. An Independent-Samples T-test was conducted to assess this. Before performing the T-test, the variances between the two groups (males and females) in terms of MP abundance was examined, using an F-test first. The significance level (p-value) obtained was 0.629, indicating that the results were not statistically significant. Therefore, could not reject the null hypothesis that the variances between the two groups were equal. Having established that the variances were equal, proceeded to conduct the Independent-Samples T-test. The two-sided p-value we obtained was 0.735, which indicates that there is no statistically

significant difference between the sexes in MP ingestion. in other words, no sex-dependent differences were found in MP ingestion between male and female fish.

These findings are consistent with some field studies (Alomar and Deudero 2017; Bernardini et al. 2018; Lee et al. 2021), but, contradicts with others (Welden and Cowie 2016; Su et al. 2019; Lee et al. 2021; Chen et al. 2025).

4.7 Environmental and health risks of MPs

Microplastics have a detrimental impact on the environment, eco-systems, and human health. Studies have shown that fish can accumulate between 0 to 2.9 MPs/g of MP, with a maximum annual uptake of about 55,000 MP particles (Rana and Gupta 2025; Grifoni et al. 2025). The ingestion of MPs can block or damage the digestive tract, decrease individual fitness, and ultimately lead to the death of marine animals (Yin et al. 2021; Pingki et al. 2025). In addition to their direct impacts, MPs may also disrupt aquatic life by acting as carriers for other toxic substances, such as heavy metals and persistent organic pollutants (POPs) (Andrady and Zhu 2021; Ding et al. 2022).

In this study, the higher abundance of MPs, found in the Tilapia fish of the Nile may contribute to the decline of fishery resources and pose significant risks to the long-term sustainability of fisheries. Investigations and reviews on the impact of MPs on human health are currently underway. While evidence regarding the adverse effects of MPs on human health is limited, MPs could affect human health via their direct or indirect toxicity (Sangkham et al. 2022). Several reports have focused on identifiying and characterising MPs in various aquatic environments to assess the extent and severity of plastic pollution. These concerns, along with the toxic impacts of MPs on aquatic organisms and human health, are supported by the fact that adsorbed pollutants may leach out upon ingestion, providing routes for secondary toxicity due to bioaccumulation and biomagnification along the food web (Ziccardi et al. 2016; Endo and Koelmans 2016; Datta 2023; Tyagi 2024).

Chapter 5

CONCLUSION

This chapter provides concluding remarks on the study.

5.1 Conclusion

This study addresses the limited research on MP contamination in African freshwater biota, focusing on the River Nile in Khartoum, Sudan. MPs were found in all samples (100% prevalence). Nile Tilapia fish exhibited a mean MP intensity of 18.90 ± 9.17 particles/individual, of which fibers were the most abundant shape.

The dominance of fibrous, coloured, and fine particles reflects both the feeding habits of Nile Tilapia and the specific characteristics of MP particles that influence their distribution, mobility, and bioavailability.

Polyethylene (PE) and polypropylene (PP) were the most prevalent polymer types typically used in packaging and disposable products. These findings suggest that MP contamination in biota could have widespread implications for Sudan's population, potentially affecting the food chain, as well as recreational, industrial, agricultural, and domestic activities dependent on the River Nile.

A review of existing literature revealed a major gap in research on MP contamination in Sudanses freshwater systems and other environmental compartments. Investigating MPs in water and sediment, alongside biota, is essential for understanding their pathways and fate within the river ecosystem.

Additionally, the effectiveness of wastewater treatment plants in removing fine particulates, including MPs, must be evaluated. Determining the extent of MP contamination will provide valuable data for local communities, environmental agencies, and policymakers to make informed decisions.

Overall, this study provides essential baseline data on microplastic pollution in Sudan, highlighting the urgent need for comprehensive monitoring, risk assessment, and the development of biodegradable polymer alternatives to mitigate long-term environmental impacts.

5.2 Study limitations

One limitation of this study is the small sample size of fish analysed, which may not fully represent the broader Nile Tilapia population. Consequently, the findings should be interpreted as preliminary baseline data that highlight important research gaps rather than definitive trends. Additionally, the weight range of sampled fish in the study did not encompass the entire size spectrum of Nile tilapia, which may have influenced the accuracy and generalizability of the

study's conclusions. Further, the uneven sex distribution among the sampled fish also limits the generalizability of sex-related interpretations.

5.3 Future work and recommendations

Building on the findings and acknowledging the study's limitations, the following recommendations are proposed for future research:

- **Predicted Ecological Risk Assessment**

  Future studies should apply predictive ecological risk models to complement empirical findings and estimate potential environmental impacts of MPs. Such models can reduce the need for experiments involving live organisms, promoting more ethical and sustainable research practices.
- **Sample Expansion and Species Diversity**

  Broader sampling across rivers, lakes, and coastal systems in Sudan is needed to assess spatial variation and potential pollution sources. Including multiple fish species representing different trophic levels will help evaluate microplastic bioaccumulation and trophic transfer.
- **Temporal and Seasonal Monitoring**

  Long-term and seasonal monitoring is recommended to capture temporal variations in MP contamination and assess the influence of environmental factors such as rainfall and temperature.
- **Ecological and Toxicological Studies**

  Further research should examine the physiological and biochemical impacts of MP ingestion on fish health and the implications for human consumers.
- **Public Awareness and Policy Development**

  Enhanced public education and stronger policy frameworks are required to improve plastic waste management and reduce MP release into aquatic environments.

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Appendices

Appendix A1- optical microphotograph of blue fragment



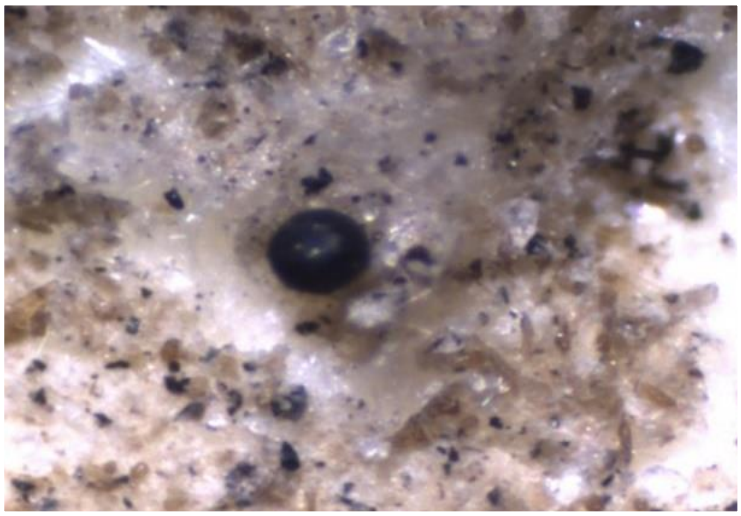
Appendix A2- optical microphotograph of transparent flim



Appendix A3- Raman microphotograph of transparent fibre



Appendix A4- optical microphotograph of black pellet



Appendix A5- Nikon stereomicroscope



Appendix A6- Raman spectrometer

