An in-depth identification and characterisation of microplastics in the Vaal River, South Africa

by

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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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This dissertation is submitted after examination and approval by the following supervisors.

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Signed on the 22nd of February 2023 at the University of the Witwatersrand, Johannesburg.

ABSTRACT

Microplastics (MPs), polymer additives, and their associated contaminants have drawn significant attention in recent years as emerging pollutants. MPs pose a myriad of threats to the environment, freshwater biota, and terrestrial organisms. In South Africa, as is the trend internationally, the body of knowledge on the presence of MPs in freshwater is remarkably less than in marine systems. This study was dedicated to investigating the level of microplastic (MP) pollution in the surface waters of the Vaal River.

The occurrence, abundance, distribution, and chemical composition of MPs were assessed. MP abundances ranged from 0.13 - 2.5 particles/m^3 (1.6×10^4 - 2.2×10^5 particles/km^2) with an average abundance of 0.61 ± 0.57 particles/m^3 (66×10^3 ± 54×10^3 particles/km^2) (mean value ± stdev). MPs were observed in four shapes, predominantly, fibers (41.6%) and fragments (39.9%), with films (10.9%), and pellets (7.7%) seldomly observed. The sizes of MPs ranged from 0.06 - 5 mm, with an average of 0.96 ± 0.88 mm (mean ± stdev). Microplastics with sizes less than 2 mm were most prevalent representing 80% of the total particles. The average sizes (mean value ± stdev) of fibers, fragments, films, and pellets were, 1.2 ± 0.86 mm, 0.64 ± 0.59 mm, 1.7 ± 1.3 mm, and 0.32 ± 0.19 mm, respectively. There was a significantly higher abundance of coloured (82.7%) MPs compared to transparent MPs (17.3%). A variety of colours were observed including green (22.3%), black (19.1%), blue (18.3%), white (11.1%), and red (6.5%); in addition to other colours in minor percentages (5.4%, altogether).

Chemical characterisation of MPs revealed that 85.2% of identified polymers were polypropylene (PP) and polyethylene (PE) (Low-density polyethylene and high-density polyethylene). A minor proportion of poly(ethylene-co-vinyl acetate) (PEVA) (7.4%), poly(ethylene-co-acrylic acid) (PEAA) (5.6%), and polystyrene (1.9%) were also detected. Additionally, a few colourants were also identified including Pigment Blue 15, Pigment Yellow 83, Pigment Black 6, and Pigment White 6.

The high prevalence of fibers and fragments indicates that microplastic pollution is from secondary sources. These shapes are formed from the fragmentation of macroplastics. Their potential sources include domestic and industrial effluent, tributaries, and surface run-off from urban centres. This study has provided a benchmark for guiding future monitoring studies and identifying further research gaps.

DEDICATION

I would like to dedicate this work to my family and friends, in particular my adoring mother, Ms. Julia Mashudu Ramaremisa, and my partner. You have been a source of strength and inspiration.

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LIST OF ABBREVIATIONS AND ACRONYMS

ARB	Antibiotic-resistant bacteria
CO_2	Carbon dioxide
Cm	Centimeter
CBD	Central business district
CCD	Charge-coupled device detectors
^oC	Degrees Celsius
DDT	Dichlorodiphenyltrichloroethane
EPSs	Extracellular polymeric substances
FT-IR	Fourier Transform Infrared spectroscopy
GPS	The global positioning system
g/cm^3	grams per cubic centimetre
HDPE	High-density polyethylene
HGT	Horizontal gene transfer
HQI	Hit quality index
h	Hours
L	Litre
LDPE	Low-density polyethylene
LLDPE	Linear low-density polyethylene
µm	micrometres
MPs	Microplastics
MDPE	Medium-density polyethylene
Mm	millimetres
Mins	Minutes
Nm	nanometres
OCPs	Organochlorine pesticides
POPs	Persistent organic pollutants
PBDE	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls

PAHs	Polycyclic aromatic hydrocarbons
PE	Polyethylene
PEAA	Poly(ethylene-co-acrylic acid)
PET	Polyethylene terephthalate
PEVA	Poly(ethylene-co-vinyl acetate)
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
Pyr-GC-MS	Pyrolysis-Gas Chromatography-mass spectrometry
RMS	Raman microspectroscopy
SAHRC	The South African Human Rights Commission
SEM	Scanning Electron Microscope
TED-GC-MS	Thermal extraction desorption-gas chromatography-mass spectrometry
UV	Ultraviolet
WWTPs	Wastewater treatment plants

CHAPTER ONE-INTRODUCTION

This chapter gives historical background on MPs, the formation, and the toxicological effects of MPs on which the study is predicated. It highlights knowledge gaps in MP research in African freshwater systems, thus providing a basis for the motivation and purpose of the study.

1.1 What are plastics?

The word plastic originated from the Greek word "*plastikos*", which is used to describe materials capable of being moulded and shaped. Plastics are organic polymers mainly synthesised from fossil feedstocks (crude oil, natural gas, and coal). A minor portion is manufactured from bio-based feedstocks (corn, cassava, sugarcane, and wood waste). The discovery of the first synthetic polymer, Bakelite, dates to the early 20th century, in 1907. Polyvinyl chloride (PVC) and polyvinyl acetate (PVA) were discovered five years later (Amaral-Zettler et al., 2020; Chamas et al., 2020; Gourmelon, 2015).

Although many plastics generally consist of only one monomer such as ethylene or propylene, some consist of multiple monomers or co-polymers to address specific needs. Plastics are categorised as either thermoplastics or thermosets. The former is a class of plastics that are reversible and whose form can be easily altered by altering temperatures, whereas the latter are plastics that cannot be reversed when heated (Nicholson, 2017; Verster and Bouwman, 2020; Verla et al., 2019).

Plastics were commercialised in the 1950s and, by the 1970s, they were the most widely utilized material in the world. Plastics have replaced concrete, glass, steel, and paper in some applications. Their popularity is due to many advantageous properties. They are cheap to produce, water-resistant, durable, corrosion-resistant, and have high mechanical strength, low thermal, and electrical conductivity. Since their industrialization, plastics have had a key role in the world's economic expansion, innovation, and production of low-cost goods. Plastics are forecasted to continue to be an essential growth industry (Amaral-Zettler et al., 2020; Gourmelon, 2015; Hahladakis et al., 2018).

1.2 The extent of global plastic production and pollution

It has been estimated that the global plastics industry generated over ZAR 9 trillion from the production of over 360 million tons in 2020. A quarter of this is currently produced by China. The quantity of plastic produced is estimated to double in the next

20 years. In South Africa, 2 284 000 tonnes of plastic products were consumed, and this amount is expected to increase by 3% annually (Sadan and De Kock, 2020; Walker, 2021). Plastic consumption is dominated by thermoplastic types of polypropylene (PP) (19.7%), low-density and linear low-density polyethylene (LDPE and LLDPE) (17.4%), medium-density and high-density polyethylene (MDPE and HDPE) (12.9%), PVC (9.6%) and polyethylene terephthalate (PET) (8.4%). Thermoplastics such as polybutylene terephthalate, polycarbonate, and polytetrafluoroethylene, together account for 10.7%. Polystyrene and other plastics make up the remaining 21.3% (PlasticsEurope, 2021; Yeung et al., 2021).

Commercial and industrial packaging are the main outlets for plastics, contributing approximately 40.5% of the total plastic output, followed by industries such as construction (20.4%), automotive (8.8%), electrical and electronics (6.2%), household, leisure, and sports (4.3%), agriculture (3.2%), and others (16.7%) (PlasticsEurope, 2021; Yeung et al., 2021). Although plastics offer many benefits to society, it is evident that they have a detrimental impact on the environment.

Plastics are engineered to be durable and stable under various conditions due to the incorporation of various additives. More than 80% of additives produced are used in the three most mass-produced and consumed polymers: PVC, PP, and PE. Reports suggest that it takes an average piece of plastic approximately 450 years to degrade, while some may take over 1 000 years (Chamas et al., 2020; Murphy et al., 2016; Murphy, 2001).

Despite significant advances in management, treatment, and recycling in the last three decades, only 18% of plastic waste is recycled, and 24% is burned. The remaining 58% constitutes anthropogenic waste materials released into the environment. Once hailed as a revolutionary material, it is now synonymous with being labelled as a global environmental threat once degraded or disposed of as plastic waste (Chamas et al., 2020; Hahladakis et al., 2018).

1.3 Concerns about plastics

Most of the plastic waste produced is trapped in terrestrial ecosystems, where it accumulates and subsequently pollutes inland freshwater systems. Plastic emissions from rivers into the world's oceans are estimated to be between 1.15 and 2.41 million tonnes per year globally (Cable et al., 2017; Park and Park, 2021). Their presence in aquatic systems poses a myriad of threats. At a macroscopic level, physical interactions with plastic waste can induce asphyxia, entanglement, and ghost fishing of aquatic organisms. Macroplastics were considered the biggest environmental threat until the discovery of microplastics (MPs) in the early 21st century. Degraded plastics pose a greater ecological and toxicological threat on a microscopic scale (Cera et al., 2020; Hoellein et al., 2014; Meijer et al., 2021).

1.4 What are microplastics?

In 1972, Carpenter and Smith reported the first evidence of small pieces of floating plastic on the Sargasso Sea Surface. However, the term "microplastics" was initially coined by Richard Thompson in 2004 to describe microscopic plastic debris (Carpenter and Smith, 1972; Thompson, 2004). In 2009, Arthur and his co-authors proposed that 5 mm (5000 µm) be the upper size limit for MPs (Arthur et al., 2009). Cole et al. (2011) refined the definition of MPs to include classification according to their source, as either primary or secondary MPs. There is currently no defined lower size limit. The detection limit is determined by the sensitivity of the sampling method employed as well as the detection limit of the analytical equipment (Cole et al., 2011; Gago et al., 2016; Hartmann et al., 2019).

1.5 Sources of MPs

Microplastics are ubiquitous, having been reported in air and aquatic environments worldwide (Alimi et al., 2018; Bianco and Passananti, 2020). Microplastics in the environment are introduced through two main pathways: as primary MPs synthesised in microscopic dimensions, such as virgin plastic pellets and microbeads in personal hygiene products, cosmetics, industrial abrasives, and cleaning products. Secondary MPs are formed through the fragmentation of macroscopic debris

under the influence of mechanical forces, biological and photochemical processes. However, these processes may also work in tandem. For instance, photochemical degradation results in the production of carbonyl groups, which renders polymers brittle and more susceptible to mechanical degradation. Furthermore, degradation mechanisms are not uniform for all plastics; for instance, PS and PE are more susceptible to photochemical degradation compared to other plastics (Chamas et al., 2020; Dris et al., 2015).

MPs are present in various shapes such as fragments, spheres, fibres, films, flakes, foams, beads, pellets, sheets, granules, nurdles, and foils (Koelmans et al., 2019; Onoja et al., 2022). Their distribution, fate, abundance, and type in aquatic compartments are determined by their physical-chemical properties (shape, size, and density) as well as riverine environment variables such as river length, pH, and organic matter concentration. All these variables, alone or synergistically, influence the transport and behaviour of MPs in the environment (Campanale et al., 2020; Kumar et al., 2021).

The contribution of various sources of MPs to the environment includes synthetic textiles (35%), vehicle tires (28%), city dust (24%), road markings (7%), marine coatings (3.7%), personal care products (2%), and plastic nurdles (0.3%). Synthetic textiles are the single greatest contributor to secondary MPs, with vehicle tires in second place. Around 1412.6 million vehicles are in use globally. A tire consists of synthetic rubber, a plastic polymer, and natural rubber. Tyre shed MPs when they erode through heat and friction on roads. City dust includes losses from the abrasion of objects like synthetic soles of footwear and plastic cooking utensils (Boucher and Friot, 2017).

Microplastics have many potential pathways into riverine ecosystems, such as wastewater effluent, surface runoff containing MPs from roads, fibres from textiles released during the washing of clothes, and land application of biosolids from wastewater treatment plants (WWTPs). Additional sources include agricultural run-off,

incidental releases from industrial processes, and atmospheric deposition. Consequently, MP pollution has been acknowledged as the dark side of industrialization for the riverine ecosystems (Cole et al., 2011; Kumar et al., 2021).

The large surface area-to-volume of MPs enhances their propensity to sorb toxic chemicals. Consequently, MPs can act as vectors for water-borne hydrophobic pollutants and pathogens. Microplastics can adsorb them from or desorb them to the surrounding environment depending on the polymer properties and surrounding media conditions (Chen et al., 2021; Rochman, 2015b; Verla et al., 2019).

1.6 Toxicity of MPs

Microplastic-related toxicity may be divided into two categories, viz., physical effects of particles (i.e., particle-induced ulceration, abrasion, and decreased lipid production) and chemical effects (i.e., toxicity induced by released endogenous compounds additives and adsorbed contaminants) (Gao et al., 2019; Wang et al., 2021a). Polymers are rarely used in their pure form. Most polymers produced contain fillers, reinforcements, colorants, flame retardants, lubricants, plasticizers, and other additives. These additives are added during manufacturing to enhance the polymer's physical and mechanical properties (Table 1.1) (Barrick et al., 2021; Fred-Ahmadu et al., 2020; Hahladakis et al., 2018; Murphy, 2001).

Once fragmented due to environmental forces, MPs are of great environmental concern as they may leach hazardous unreacted residual monomers and additives (Koelmans et al., 2014; Luo et al., 2020; Wright and Kelly, 2017). Several studies have reported the migration of plastic monomers into food. Health risks could potentially arise if the concentration of these compounds reaches a certain limit in the human body (Hahladakis et al., 2018; Khaksar and Ghazi-Khansari, 2009).

Table 1.1 Polymer additives and their applications

Type of additives	Examples of additives	Applications
Fillers and reinforcements	Clay, glass, and carbon fibres.	Enhances flexibility and durability of polymeric films.
Colourants	Cobalt diacetate, titanium dioxide, cadmium, chromium, and lead compounds.	Add specific colours to polymers.
UV resistance	Oxanilides, Octylphenol benzophenones, and benzotriazoles.	Slow down the oxidation of plastics exposed to sunlight.
Heat resistance	Cadmium and lead compounds, nonylphenol, and bisphenol-A.	Preventing thermal degradation of polymers.
Flame retardants	Polybrominated diphenyl ethers and tetrabromobisphenol-A.	Reduce flammability.
Plasticizers	Phthalic esters, aliphatic dibasic acid esters, and benzoate esters.	Render the material pliable, flexible, and processable.

1.6.1 Association of metals and MPs

Microplastics can either adsorb or desorb metals from their surfaces. Metals are added to polymers during manufacturing to perform functions such as being inorganic pigments, flame retardants, heat stabilisers, and catalysts can desorb from MPs into freshwater systems (Brennecke et al., 2016; Catrouillet et al., 2021; Rochman, 2015a). Photo-oxidation, weathering, and biofilms may change the surface structure of MP

particles and make it easy for particles to obtain an electric charge, thus adsorbing metal ions to achieve charge balance. Micro-organisms form biofilms, which grow within a matrix of extracellular polymeric substances (EPSs). EPSs can attach to MPs through bonds with amide, carbonyl, carboxyl, phosphoric, phenolic, and hydroxyl groups. EPSs may be partially responsible for the association of MPs and heavy metals (Amaral-Zettler et al., 2015; Lobelle and Cunliffe, 2011; Richard et al., 2019).

Metals can accumulate on MP surfaces up to eight hundred orders of magnitude higher than metal concentrations in the surrounding media (Gao et al., 2019). Iron (Fe), cadmium (Cd), cobalt (Co), manganese (Mn), aluminium (Al), lead (Pb), copper (Cu), silver (Ag), and zinc (Zn) are among the metals adsorbed by MPs (Catrouillet et al., 2021; Gao et al., 2019; Godoy et al., 2019; Richard et al., 2019; Turner and Holmes, 2015). Microplastics were reported to increase cadmium accumulation in the liver, intestine, and gills of zebrafish (*Danio rerio*), which may cause oxidative damage and inflammation (Lu et al., 2018). The correlation between exposure to Cd, Cr, Co, Cu, and Pb and cancer incidence in humans is extensively documented (Tchounwou et al., 2012).

1.6.2 Association of pathogens and MPs

Bacteria linked to polymer breakdown and biofilm formation from the *Klebsiella*, *Pseudomonas*, and *Sphingomonas* genera have been detected on MP surfaces. Thus, biofilm formation causes MPs to be less hydrophobic and buoyant, thus making them bioavailable to benthic organisms. Additionally, they can bio-transform MPs into harmful compounds that pose a risk to human health and food security. Antibiotics produced from agricultural runoff and sewage discharge can contaminate freshwater habitats. These antibiotics can stimulate horizontal gene transfer (HGT) and the evolution of antibiotic-resistance genes (Kelly et al., 2021; Nnadozie and Odume, 2019).

Microplastics may enrich pathogens by elevating their metabolic pathways and providing a colonizable matrix or microcosm "plastisphere" for more effective HGT.

Interactions within the plastisphere have been reported to enhance antimicrobial, antibiotic, or heavy metal resistance (Amaral-Zettler et al., 2020, 2015; Gao et al., 2019; Yang et al., 2019; Zettler et al., 2013). A study by Zhang et al. (2020c) investigated the enrichment of antibiotic-resistant bacteria (ARB) on the surface of MPs. The counts of cultivable ARB in MP samples were up to 5×10^3 times higher than those in water samples. Consequently, antibiotics attached to MP surfaces induce the emergence and enrichment of antibiotic-resistant bacteria, multi-antibiotic-resistant bacteria, and superbugs.

Microplastics facilitate the transport of pathogens through WWTPs and aquatic systems by providing buoyant surfaces for attachment. Microplastics have the potential to increase the mobility of wastewater-associated bacterial taxa (*Vibrio*, *Campylobacter*, and *Arcobacter*) throughout rivers, thereby exerting deleterious consequences on freshwater biota (Kelly et al., 2021). Rotjan et al. (2019) proved the pathogenic transfer of E. coli (*Escherichia coli*) from MPs into the gut of a northern star coral. The increase of pathogens with enhanced resistance transfer poses a significant threat to organisms and humans. When attached to MP surfaces, the pathogens can be transported over long distances.

1.6.3 Association of persistent organic pollutants (POPs) and MPs

The hydrophobic nature of MPs enables their hydrophobic interactions with POPs such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), dichlorodiphenyltrichloroethane (DDT), and polycyclic aromatic hydrocarbons (PAHs). Through sorption interactions, MPs might influence the environmental distribution and movement of POPs by concentrating them at a specific point and therefore influencing the environmental risks posed by the sorbent and sorbate pair (Bakir et al., 2014; Puckowski et al., 2021; Verla et al., 2019).

Persistent organic pollutants are lipophilic, have low solubility, are resistant to biochemical and photolytic degradation, and can bioaccumulate in ecosystems. Persistent organic pollutants adsorbed on MPs can reach up to 1×10^6 higher

concentrations than ambient concentrations, and these compounds may be desorbed inside organisms. The leaching of toxic chemical compounds and the desorption of pathogens may be more pertinent in species with longer gut retention times, such as fish (Botterell et al., 2019; Desforges et al., 2015; Koelmans et al., 2014; Wright and Kelly, 2017). Persistent organic pollutants are well known for their carcinogenic, mutagenic, and neurological disruption activities (Browne et al., 2013; Kinigopoulou et al., 2022; Mato et al., 2001).

1.6.4 Potential health risks of MPs in biota

The prevalence of MPs in aquatic environments has raised concerns about their bioavailability and health risks. The wide variety of material types, sizes, shapes, and physicochemical properties provides a myriad of ways in which they can interact with biota. For instance, due to their small size and resemblance to natural food items, MP particles are accidentally or intentionally consumed by aquatic biota (Acquah et al., 2021; Cole et al., 2013; Romeo et al., 2015; Saad et al., 2022).

The uptake of MPs by aquatic organisms can occur indirectly via the consumption of other organisms that contain MPs. For example, MPs were detected in the stomachs of black-mouth catsharks (*Galeus melastomus*) from the Mediterranean Sea, which could be attributed to bioaccumulation from their prey that had been contaminated with MPs (Alomar and Deudero, 2017).

Ingested MPs are mostly retained in the digestive systems of biota, including the stomach and intestine (Bessa et al., 2018; Cordova et al., 2020; Jabeen et al., 2017). However, several studies have reported the possibility of adherence to the skin or translocation to other tissues (i.e., gills, liver, and muscle), the circulatory and lymphatic systems (Abbasi et al., 2018; Daniel et al., 2020; Deng et al., 2017; Kolandhasamy et al., 2018; von Moos et al., 2012). Ingestion of MPs by aquatic biota provides a potential pathway for introducing hazardous substances and pathogens into aquatic food webs.

Humans are exposed to MPs by inhalation, ingestion, and dermal contact. Microplastics from the atmosphere have the potential to accumulate on the surface of food items. Thus, inadvertent consumption of indoor dust may expose people to MPs (Ageel et al., 2022; Dris et al., 2015; Zhang et al., 2020a). The projected yearly ingestion of MPs is 1 000 particles from salt, 5 800 particles via tap water, 11 000 particles from shellfish, 68 415 particles from atmospheric fallout, and 90 000 from bottled water. Research suggests that bottled water is the greatest source of MPs to humans. It is projected that drinking exclusively bottled water will result in daily consumption of 246 MPs (Cox et al., 2019; Schwabl et al., 2019).

Microplastics have been detected in a variety of drinks consumed by humans, such as tap water (Mintenig et al., 2019; Pivokonsky et al., 2018); bottled (mineral) water (Mason et al., 2018; Schymanski et al., 2018; Weisser et al., 2021; Zuccarello et al., 2019); soft and energy drinks (Shruti, 2020); white wine (Prata et al., 2020); beer (Kosuth et al., 2018; Liebezeit and Liebezeit, 2014); milk (Kutralam-Muniasamy et al., 2020); salt (Karami et al., 2017; Kosuth et al., 2018); and tea bags (Hernandez et al., 2019).

Microplastics have also been found in freshwater biota (Abbasi et al., 2018; Bessa et al., 2018; Cordova et al., 2020; Saad et al., 2022), canned fish (Akhbarizadeh et al., 2020), commercially cultured or sold biota (Bessa et al., 2018; Daniel et al., 2020; Farrell and Nelson, 2013; Neves et al., 2015; Van Cauwenberghe and Janssen, 2014; von Moos et al., 2012).

Microplastic particles have also been detected in human stool and placentas, confirming their presence inside humans (Ragusa et al., 2021; Schwabl et al., 2019; Zhang et al., 2021b). Microplastics were found in 77% of human blood samples. PET was the most widely encountered polymer, accounting for 50% of MPs in all tested donors. Drinking water bottles are mainly manufactured from PET. The high abundance of this polymer further supports the notion that bottled water may be a

significant source of MPs. Scientific evidence suggests that MPs may be transported to various organs in the body via the bloodstream (Leslie et al., 2022; Ragusa et al., 2021).

Microplastic exposure may cause inflammatory lesions, particle toxicity, endocrine disruption, growth inhibition, chronic inflammation, neoplasia, and preeclampsia (Bhuyan, 2022; Ragusa et al., 2021). Polystyrene nanoparticles were introduced to Crucian carp (*Carassius carassius*) trophic transfer, from algae (*Scenedesmus sp.*) to zooplankton (*Daphnia magma*) and then the fish. Ingestion of the nanoparticles altered the metabolism, feeding, and shoaling behaviour of Crucian carp. Humans may also be exposed to MP particles through ingesting contaminated fish (Forte et al., 2016; Mattsson et al., 2015). Although plastics have been essential to human evolution, their degradation to MPs and eventually nanoplastics have harmful ramifications. Each continent and country need to provide tailor-made solutions to its unique MP pollution problems.

1.7 The extent of plastic pollution in Africa

Africa is the second-most populous continent, and many countries have been undergoing rapid development. Thus, there is a high demand for plastic products due to their low cost and availability. However, a lack of state-of-the-art recycling facilities, poor plastic waste management, and pollution awareness has left freshwater ecosystems at risk of MP pollution (Akindele et al., 2019; Aragaw, 2021; Babayemi et al., 2019; Jambeck et al., 2018; Khan et al., 2020). Africa has the lowest number of freshwater systems in the world. These must be sustainably managed to support its development. In South Africa, as elsewhere in the globe, freshwater systems (i.e., rivers, groundwater reservoirs, ponds, streams, and wetlands) have essential economic and social values. Freshwater systems are extensively used for traditional practices, electricity generation, transport, agriculture, breeding livestock, and recreation (Akindele et al., 2019; Biginagwa et al., 2016; Khan et al., 2020; Saad et al., 2022). The risks posed by MP pollution may be enhanced in these developing countries where many communities remain dependent on land and freshwater systems (Rochman et al., 2015a).

Considering its national importance and the risks posed to its water quality, it is essential to investigate the abundance, physical, and chemical characteristics of MPs in the Vaal River. This is essential to assess the extent of MP pollution and address the socio-economic implications through evidence-based recommendations, informed decision-making, policy formulation, and comprehensive risk assessment and management.

CHAPTER TWO-LITERATURE REVIEW

This chapter presents an in-depth review of the literature on the study region, with an emphasis on the importance of the Vaal River catchment to its host community and country. Furthermore, it underlines the value of Africa substantially contributing to the conversation regarding MPs. It then discusses various sampling and sample preparation techniques, as well as physical and chemical characterization. It culminates with a review of the health effects of MPs, reinforcing the need for characterisation.

2.1 The Sedibeng District Municipality

The Sedibeng District Municipality in Gauteng province draws inspiration from Sesotho, which means "the place of the pool", because of the Vaal River. The district is home to 1.8% of South Africa's total population and it is comprised of three local municipalities: Emfuleni, Midvaal, and Lesedi. There are bigger towns under this district municipality such as Vereeniging, Vanderbijlpark, Heidelberg, Devon, and Meyerton (Figure 2.1). The "Vaal triangle" refers to a region located between Emfuleni Municipality (Vereeniging and Vanderbijlpark) and Metsimaholo Municipality (Sasolburg) in the Free State province. The municipality is characterised by substandard infrastructures, potholes, sewage spillages, a lack of waste disposal facilities, and aging road infrastructure (Gauteng Province, 2019; Cooperative Governance and Traditional Affairs [COGTA], 2020).

Figure 2.1 The Sedibeng District Municipality (Gauteng Province, 2019)

The construction of formalised housing with water and water-borne sanitation as part of the Reconstruction and Development Programme has led to an increase in the wastewater load. This has led to the establishment of comprehensive WWTPs to service the growing population in the district. The Sedibeng District Municipality has four main WWTPs: the Rietspruit, Midvaal, Sebokeng, and Leeuwkuil WWTPs.

According to Sedibeng District Municipality, wet industries ranging from battery industries, chemical manufacturing, ferrous and non-ferrous industries, food processing industries, and various small-to-medium industries discharge their effluent into the Vaal River. Consequently, the Vaal River is the natural receiver of treated domestic and industrial wastewater from the Sedibeng District Municipality (Iloms et al., 2020; Remilekun et al., 2021; Tempelhoff, 2001, 2009a; Wepener et al., 2011). Carr et al. (2016) reported that WWTP outlets are among the most significant sources of MPs.

2.2 Significance of the Vaal River

The Vaal River gets its name from the muddy colour of its water, with "Vaal" meaning "gray water". The Vaal River is the second-largest river in South Africa. It rises on the Drakensberg escarpment in Mpumalanga. It then flows approximately 900 km west-south-west and feeds into the Orange River in Douglas, Northern Cape. After joining the Orange River, it forms part of South Africa's largest freshwater system, the Orange-Vaal River system. It flows through Gauteng, Free State, Northern Cape, and Mpumalanga provinces. The Vaal River catchment has a catchment area of approximately 196, 000 km^2. The catchment has a sub-tropical dry savanna climate. (Braune and Rogers, 1987; Wepener et al., 2011).

The Vaal River is the backbone of the largest urban economy in sub-Saharan Africa and has been Africa’s most economically valuable aquatic ecosystem for over 100 years. The Vaal River has essential economic and social benefits for approximately 19 million people in Gauteng, Mpumalanga, the North-West, and the Free State provinces. It is used for traditional practices, water sports, recreational activities, electricity generation, transport, agriculture, and breeding livestock. According to the National Treasury, 98% of the raw water used by Rand Water is extracted from the Vaal River. Once purified, it supplies water to about 926 industries, 40 mines, and 13 municipalities. Essentially, it contributes more than 50% to the gross domestic product and at least 80% to the electricity supply (Wepener et al., 2011).

For more than nine decades, the Vaal River barrage has been an essential component of the hydrological landscape of South Africa's most populated and economically active province. It is situated 64 km downstream of the Vaal Dam and forms part of the southeastern border of Gauteng Province. The Vaal River barrage was constructed in 1923 and has a catchment area that covers a surface area of about 900 km^2. However, after the completion of the Vaal Dam in 1938 to further secure water for development in the Witwatersrand-Vaal Triangle, the Vaal River barrage was to a large extent redundant. The water between the barrage and Vaal dam was once owned by South Africa's largest portable water utility, Rand Water, supplying an average of 3.7 million litres of water daily (Tempelhoff et al., 2007; Tempelhoff, 2009b, 2001).

2.3 The Vaal River's tributaries

Tributaries have significant input on the pollution in the main river. Understanding the potential sources of anthropogenic litter in the tributaries is essential to identifying sources of pollution in the main river (Zhang et al 2015; Dahms et al., 2020). The Suikerbosrand, Klip, Taaibosspruit, Leeuspruit, and Rietspruit Rivers are notable tributaries of the Vaal River.

2.3.1 The Klip River

The Klip River has its source from a myriad of small streams or spruits (e.g., Klipspruit and Bloubos Spruit) that trickle through the suburbs of Johannesburg. The Klip River drains the Witwatersrand plateau's southern slopes until it joins the Vaal River at Vereeniging. The upper Klip is characterised by a concentration of formal and informal townships, industries, mines, and tailings dumps, whilst the lower Klip is typified by agricultural activity. These various activities pollute the river through acid mine drainage, wastewater effluent, agricultural and surface run-off from settlements (Davie, 2018; Dzwairo and Otieno, 2014; Marara and Palamuleni, 2019; McCarthy et al., 2007; Weideman et al., 2020).

2.3.2 The Rietspruit, Suikerbosrand, and Leeuspruit Rivers

The Leeuspruit River drains the Sasolburg area, and the Rietspruit River originates from Peter Wright Dam in West Rand, Gauteng. The Rietspruit River is predominantly characterized by industries and WWTPs. It flows past Vanderbijlpark and enters the Vaal River a short way above the Barrage. Loch Vaal is the arm of the lake that extends up the Rietspruit River. The Suikerbosrand River has its source at Suikerbosrand Nature Reserve and joins the Vaal River at Vereeniging. The Blesbokspruit River is a tributary of the Suikerbosrand River, and its water quality has suffered greatly due to acid mine drainage and other pollutants related to urbanization (du Plessis et al., 2014; Raji et al., 2021). Despite mounting water quality issues in the Vaal River and its tributaries, little is known about the concentration of MPs throughout the Vaal River system, a knowledge gap that should be addressed (Weideman et al., 2019; Wepener et al., 2011).

2.4 Challenges and opportunities in MP research

There are several challenges that researchers face. For instance, there is no consensus as to which materials constitute plastics. Some people in the scientific community consider paint particles, rubber, and cellophane as plastics, but some argue that they are not according to polymer chemistry definitions. Moreover, some have advocated that natural polymers made from regenerated cellulose, such as cotton and rayon can be considered as MPs since they pose similar threats in various environments (Hartmann et al., 2019).

There are also suggestions that since "micro" is a System International (SI) descriptor, MPs should be defined as particles less than 1 mm and not less than 5 mm. This is based on the notion that the SI metric system has historically provided internationally accepted basic units of measurement. The search for a unified upper and lower size limit for MPs is a very controversial topic. A failure to reach a consensus has contributed to the lack of standardisation in sampling, sample purification, and quantification methods (Frias et al., 2019; Gigault et al., 2018; Hartmann et al., 2019).

However, discrepancies in defining MPs have resulted in the development of novel methods for sampling, sample pre-treatment, and characterization.

2.5 Microplastic sampling methods

Sampling procedures differ in terms of sampling time, equipment, sample size, and depth of sampling. Depending on the nature of the freshwater body, dynamic or stationary sampling is performed. The volume filtered through the nets or trawls is measured by a flow metre. The area sampled can be calculated by multiplying the distance trawled by the width of the trawl mouth (Adomat and Grischek, 2021; Campanale et al., 2020; Prata et al., 2019a; Stock et al., 2019).

During dynamic or volume-reduced sampling, plankton nets, manta trawls, and neuston nets are generally used for sampling surface water, bongo nets are used for mid-water, and benthic trawls for seabed or riverbed (Figure 2.2) (Park and Park, 2021). The sampling apparatus is towed by boats using a rope, keeping them away from the wake of the vessel. Volume-reduced sampling is more suitable for sampling large bodies of water and is recommended by the United States of America's National Oceanic and Atmospheric Administration for sampling in the oceans. On the other hand, smaller rivers, streams, and creeks with changing water regimes are sometimes not entirely navigable all year round, making dynamic sampling difficult, if not impossible in certain conditions. In such cases, the sampling apparatus is held stationary over a bridge or affixed to the riverbed using poles (Campanale et al., 2020; Park and Park, 2021; Stock et al., 2019; Zhang et al., 2021c).

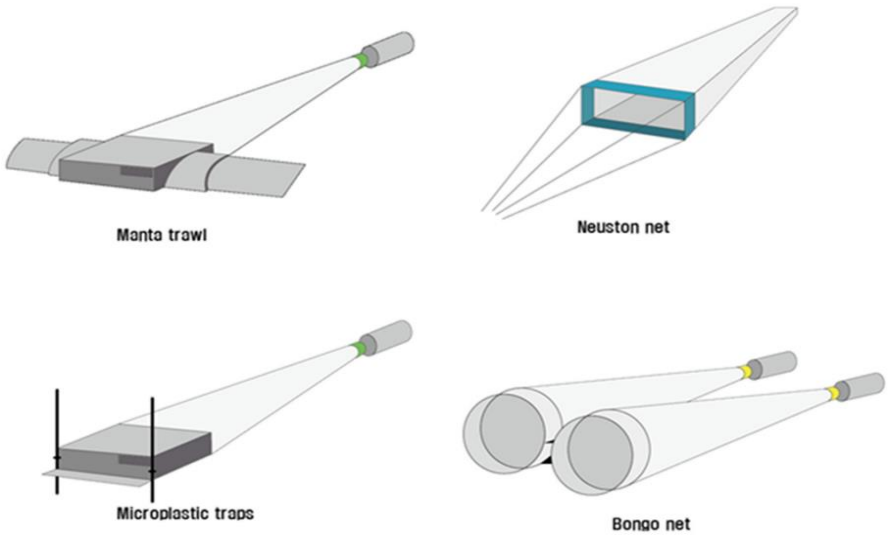


Figure 2.2 Sampling equipment (Park and Park, 2021)

Sampling apparatus and methods are not limited to the above-mentioned; they are often determined by the research needs and preferences. Generally, 333-335 µm mesh apertures are the most used, but these may underestimate smaller-sized MPs. To avoid underestimating MPs, it is advised to use the smallest mesh size available. Sampling using different mesh sizes can produce variations in the classification and quantification of MPs. For instance, a 100 µm nylon net can have MP concentrations almost a hundred times higher than a 333 µm manta net due to its ability to trap smaller MPs. The lack of standard net types and mesh sizes has significantly impeded data comparison on MP concentrations (Campanale et al., 2020; Dusaucy et al., 2021; Uurasjärvi et al., 2020).

The sampled area can be measured using the global positioning system (GPS) start and stop positions. A flowmeter can be used to determine the volume filtered. GPS and flowmeter readings can give vastly different results. These two techniques can potentially halve (or double) MP abundances (Campanale et al., 2020; Dusaucy et al., 2021; Frias et al., 2019). Therefore, Frias et al. (2019) suggested the use of both methods during net sampling, so that differences between the two techniques can be taken into consideration.

2.6 Sample digestion

This is an essential step as it removes organic, inorganic, and biological matter that may interfere with qualitative and quantitative analyses of MPs. This step must proceed without altering the physical-chemical properties of MPs and the generation of artificial generation of secondary MPs. Digestion can be categorised as either chemical or enzymatic degradation. Digestion protocols can be used in isolation or combination with one another. In recent literature, scientists have investigated the main digestive protocols to seek the optimal protocol. The four main protocols included oxidation using hydrogen peroxide (H_2O_2), alone or as Fenton's reagent (H_2O_2 + Fe (II)), acidic, alkaline, and enzymatic digestion (Cutroneo et al., 2020; Prata et al., 2019b; von Friesen et al., 2019).

2.6.1 Oxidation digestion

Initially, an oxidation digestion protocol was developed by Nuelle et al. (2014), using only H_2O_2, it was then augmented by Masura et al. (2015), by elevating temperatures and using Fe (II) to accelerate the reaction in a method known as wet peroxide oxidation. Prata et al. (2019b) suggested that the efficiency of degradation of matter by H_2O_2 had increased almost two-fold when Fe (II) was added. However, recent optimization studies suggest that high temperatures may damage some MP particles and that room temperature is optimal. However, if the temperature decreases to below 15°C, it forms a yellow precipitate. Wet peroxide oxidation is pH sensitive and if pH exceeds 5 or 6, an iron hydroxide precipitate will form (Hurley et al., 2018; Masura et al., 2015; Rueda Márquez et al., 2018). Hence, oxidation digest is sensitive to variations in temperature, pH, and concentration.

2.6.2 Acid digestion

Acid digestion involves the use of mineral acids such as hydrochloric acid (HCI) and nitric acid (HNO_3). Acids are highly effective in digesting organic matter. However, they can also damage MPs, especially those with a low pH tolerance, such as polyamide (PA), polyurethane (PU), nylon, and polystyrene (PS). The structure of PET may become fused during acid digestion. Acids can cause degradation and melting

of MPs, even when low concentrations of acids are used. Hence, acids are considered not to be suitable for isolating MPs from sample matrices (Dehaut et al., 2016; Qiu et al., 2016; Van Cauwenberghe et al., 2015). Acids have been used to cause the leaching of metals adsorbed by MPs (Gao et al., 2019; Godoy et al., 2019; Turner and Holmes, 2015).

2.6.3 Alkaline digestion

This method utilises bases such as NaOH or KOH. A solution of 1 M NaOH at room temperature has been used to digest marine samples with 90% efficiency. Increasing concentration and temperature enhances digestion efficiency, such as using 10 M of NaOH or 10% KOH at 60°C. However, there are reports of MPs such as cellulose acetate (CA), PVC, and PET being damaged under these conditions (Foekema et al., 2013; Ghosal et al., 2018; Mintenig et al., 2017). It has been reported that when 10% KOH was used to digest the guts of the Japanese medaka (*Oryzias latipes*), it caused the degradation of MP particles and affected the Raman analyses (Ghosal et al., 2018).

2.6.4 Enzymatic digestion

Initially, it is common practice to introduce a detergent to the samples. The detergent makes biological material susceptible to enzymatic degradation. Subsequently, the sample is treated with various enzymes, depending on the sample matrix. Examples of highly efficient enzymes include Proteinase-K and Trypsin, with efficiencies of 97% and 88%, respectively. Although enzymatic degradation is highly effective and does not damage MPs, it is costly and time-consuming due to the long exposure time required even when cheaper enzymes are used (Cole et al., 2015; Hurley et al., 2018; Karlsson et al., 2017; Othman et al., 2021).

2.7 Density separation

Density separation involves mixing a salt solution of a specific density with the sample. The density of common consumer plastic polymers ranges from 0.8 g/cm^3 (Silicone) to 1.4 g/cm^3 (PVC). Microplastic particles can thus be extracted from

higher-density matrices, such as sediments (2.5 g/cm^3), by flotation with high-density salt solutions. This process enables the low-density MPs to float to the upper layer of the ionic solution, where MPs can be recovered from the supernatant (Hurley et al., 2018; Xu et al., 2019).

Saturated NaCl solutions (1.2 g/cm^3) are widely used to achieve separation due to their cost-effectiveness and safety. However, MPs may contain additives that increase their density, or they are made from highly dense polymers such as polyvinyl chloride, polytetrafluoroethylene, and polyoxymethylene, whose densities are higher than saturated NaCI. In such cases, density separation through NaCI becomes a challenge. This has been addressed by using salts such as sodium iodide (NaI, 1.6 - 1.8 g/cm^3), and zinc chloride ($ZnCl_2$, 1.5 - 1.7 g/cm^3) to extract MPs of higher densities (Han et al., 2019; Hurley et al., 2018; Qiu et al., 2016; Rodrigues et al., 2018). After density separation, the supernatant is filtered, dried, and stored in Petri dishes until analysis.

2.8 Analysis of MPs

The characterisation of materials is a core principle of analytical chemistry. The complexity and variety of physical-chemical properties of MPs, such as the wide range of sizes, variable shapes, presence of additives/sorbed contaminants, and the variety of polymer types, as well as complex environmental matrices, make it difficult to use a single analytical method. Consequently, a combination of analytical techniques has been widely used in environmental analysis. Generally, MP analysis can be separated into two broad areas: a) morphological and physical characterization; and b) chemical characterization (Bumbrah and Sharma, 2016; Cheng et al., 2022; Ghosal et al., 2018).

2.8.1 Morphological and physical characterization

Visual inspection of the samples for possible MPs with or without the use of a microscope is routinely used. Visual sorting is necessary to separate MPs from other materials, such as sediments, animal parts, dried algae, and glass. Stereoscopes, scanning electron microscopes, and fluorescent microscopes, can be used to identify

and characterize MPs according to their shape, size, and colour (Lenz et al., 2015; Xu et al., 2019).

2.8.1.1 Optical microscopy

Microscopes such as stereoscopes, scanning electron microscopes, ordinary optical microscopes, and fluorescent microscopes are used to classify and quantify MPs with unique morphologies. Stereomicroscopy (or dissecting microscopy) is commonly used to identify MPs. However, colourless, bio-fouled, and irregularly shaped MPs with sizes < 100 μm are difficult to characterise with certainty and are prone to biases. Plastic-like natural fibers are often mistaken for MP fibres (Cheng et al., 2022; Eriksen et al., 2013; Wang et al., 2017b).

2.8.1.2 Electron microscopy

Scanning electron microscopy (SEM) provides better magnification and superior imaging capabilities than optical microscopy; its resolution may reach up to 0.5 nm. A concentrated electron beam is used in SEM to scan the surface of MPs to generate surface images. It may give excellent resolution and magnification of surface characteristics, which is quite useful for MP identification (Ghosal and Wagner, 2013; Rocha-Santos and Duarte, 2015).

The combined use of optical microscopy and scanning electron microscopy, plus scanning electron microscopy (SEM) and energy dispersive spectroscopy (SEM-EDS) or energy dispersive X-ray spectroscopy (SEM-XEDS), provides high-resolution images of particle surface structures and elemental composition signatures. SEM-EDS can be used to differentiate carbon-containing MPs particles, including multi-colored plastic spheres of inorganic substances. SEM-EDS can be used to characterize inorganic plastic additives in MP fragments. Chlorinated MPs of PVC can be identified with SEM-EDS due to their unique elemental signatures (Ghosal and Wagner, 2013; Gniadek and Dąbrowska, 2019; Sullivan et al., 2021; Wang et al., 2017b).

2.8.1.3 Fluorescence microscopy

The lipophilic fluorescent dye Nile Red was introduced to MP analysis by Andrady in 2011 and has since then been modified to provide a rapid and cost-effective alternative for MP identification (Andrady, 2011; Erni-Cassola et al., 2017; Shim et al., 2017). However, Nile Red may not exclusively stain polymers and may be prone to co-staining natural and synthetic polymers (Corami et al., 2020; Tamminga et al., 2019).

To address this, Stanton et al. (2019) proposed the co-application of Nile red with 4′,6-diamidino-2-phenylindole (DAPI), which is a fluorescent dye that only stains biological materials. Gbogbo et al. (2020) highlighted the potential of organic matter dye, 4,5,6,7-tetrachloro-2,4,5,7-tetraiodofluorescein (Rose Bengal) to identify particles of biological origin that are stained by Nile red. DAPI and Rose Bengal exclusively stain organic particles facilitating visual differentiation between plastic and non-plastic particles (Gbogbo et al., 2020; Stanton et al., 2019).

Reporting MP abundances

After morphological and physical characterization, MP abundances can be reported in the unit of particles per volume (i.e., particles/L or particles/m^3) or particles per unit area (i.e., particles/m^2or particles/km^2). There is no consensus about the appropriate data reporting units. Data reporting units will depend primarily on available apparatus, adopted methodologies, research aims, and objectives (Campanale et al., 2020; Dusaucy et al., 2021; Frias et al., 2019).

2.8.2 Chemical characterisation

Chemical characterization and quantification involve the use of two types of techniques, thermoanalytical techniques such as thermal extraction desorption-gas chromatography-mass spectrometry (TED-GC-MS), and pyrolysis–gas chromatography-mass spectrometry (Pyr-GC-MS)**,** and vibrational spectroscopic (i.e., Raman spectrometry and Fourier-transform infrared (FT-IR)) (Alimi et al., 2021; Käppler et al., 2016; Xu et al., 2019).

2.8.2.1 Thermoanalytical techniques

TED-GC-MS

Samples are heated in an inert atmosphere in a TGA balance connected to a solid phase-adsorber (polydimethylsiloxane), which is subsequently analysed by TED-GC-MS. For polymer identification, there must be at least two compounds identified unambiguously. The major advantage of TED-GC-MS over Pyr-GC-MS is the ability to analyse relatively large (100 mg) complex and heterogeneous samples without the need for pre-treatment. Sample contamination and degradation state do not interfere with particle detection; hence it is suitable for fast environmental analysis. However, there are drawbacks, such as a lack of adsorption of highly polar gaseous markers (e.g., PET) and limited application in MP analysis (Dümichen et al., 2017, 2015; Elert et al., 2017).

Pyr-GC-MS

Pyr-GC-MS is based on similar principles to TED-GC-MS in the sense that pyrolyzed polymer products are analysed by GC-MS and identification is carried out by matching the retention time and mass spectrum with that of standards of polymers or the use of spectral libraries. Instead of using a solid adsorber, the pyrolyzed products are cryo-trapped and subjected to a programmed temperature at controlled heating before being analysed. Pyr-GC-MS can be used to simultaneously identify polymer types of MP particles and associated organic plastic additives (Fries et al., 2013; Käppler et al., 2016; Vilakati et al., 2021).

The major disadvantages of Pyr-GC-MS are that MPs must be pre-treated and digested before being manually placed into the pyrolysis tube. The analysis of one MP particle may take approximately 70 min. Hence, it is not suitable for routine analysis in the situation of large sample quantities. Pyr-GC-MS has a risk of misinterpretation because different polymers have similar pyrolysis products and pyrogram databases are available for few a polymers, which is not ideal for routine environmental analysis. Pyr-GC-MS requires extensive equipment maintenance because the relatively heavy moieties resulting from polymer breakdown might condense in the capillary between

the pyrolysis chamber and the GC, causing blockages and cross-contamination (Elert et al., 2017; Fries et al., 2013; Xu et al., 2019).

2.8.2.2 Vibrational spectroscopy

Vibrational spectroscopic methods have a long history of being used to characterise synthetic organic polymers. Both Raman and infrared (IR) spectroscopy are based on the interaction of radiation with molecular vibrations, but they differ in the way the photon energy is transferred to the molecule and alters its vibrational state. Vibrational microscopy techniques are complementary in the sense that compounds that are IR inactive are Raman active and vice versa. Raman spectroscopy provides a better response for nonpolar compounds, while FT-IR allows clearer identification of polar compounds. Spectroscopic analysis in chemical imaging mode can acquire enriched information covering both spatial and spectral features, enabling morphological and chemical characterization of MPs (Elert et al., 2017; Lenz et al., 2015; Xu et al., 2019).

Fourier-transform infrared (FT-IR)

FT-IR has been mainly used in the qualitative analysis of MPs most commonly, in μ-FT-IR and ATR-FT-IR modes. ATR-FT-IR mode produces stable spectra from irregular MP surfaces while μ-FT-IR in reflectance mode produces unstable spectra. However, μ-FT-IR in transmission mode gives high-quality spectra, unfortunately, it is only applicable to sufficiently thin MPs (<100 mm) on an IR-transparent substrate such as Aluminium oxide and Silicon filters. Currently, μ-ATR–FT-IR offers a useful method to identify MPs in environmental samples. Nevertheless, complete filter analysis requires extremely long measurement times when using a single-element detector. However, by applying modern focal plane array (FPA) detectors, this time is reduced. Each element on the FPA is an independent IR sensor that allows the fast acquisition of thousands of spectra in a single measurement with high resolution. Photo-oxidation in polyolefins can result in the formation of hydroxyl-and carbonyl groups and can impede accurate IR spectra acquisition. Common polymer fillers such

as glass, clay, and silica can lead to considerable obscuration of spectra in FT-IR (Elert et al., 2017; Löder et al., 2015; Xu et al., 2019).

Raman microspectroscopy (RMS)

Raman spectroscopy is a non-destructive technique used for chemical analysis. It provides specific information about chemical make-up, phase, crystallinity, and molecular interactions. Raman spectroscopy can be used with an optical microscope, thus facilitating resolution up to 0.5 µm (Xu et al., 2019). Raman microspectroscopy (RMS) has the advantage of simultaneous identification of organic and inorganic additives (fillers, pigments, and dyes) and polymers. However, light intensities emitted by additives can be orders of magnitude stronger than Raman scattering. As a result, they overlay the polymeric signals, resulting in the modification of the spectra (Käppler et al., 2016; Lenz et al., 2015).

The presence of micro-biological, organic (humic substances), or inorganic (clay minerals, unperfected crystal structures) compounds on the MP particles often results in a broad fluorescent band that overshadows the polymer peaks. Moreover, the heterogeneous distribution of flame retardants within polymers results in localised concentrations that can be detected by RMS. Microplastic degradation makes matching degraded polymer spectra to reference polymer spectra in library databases more difficult. For instance, the photodegradation of PVC initialises the elimination of HCl and the subsequent formation of a carbon-carbon double bond. This so-called "unzipping" reaction changes the Raman spectra, which makes the characterization of PVC difficult (Ghosal et al., 2018; Käppler et al., 2016; Lenz et al., 2015; Xu et al., 2019). To mitigate against high fluorescence background and improve polymer peak signals, adequate acquisition parameters, pre-treatment agents, photobleaching, and automated methods must be used (Elert et al., 2017; Käppler et al., 2016).

CHAPTER THREE-RESEARCH AIMS AND OBJECTIVES

The chapter commences with the rationale for the study and concludes with the aims and objectives that the project seeks to meet or address.

3.1 Problem statement and motivation

While the circles of life and water are inextricably linked, MPs represent a detrimental new addition to these intertwined circles. Over the past decade, there has been a greater emphasis on MPs as emerging contaminants and as a novel research subject, with an increase in global investments. However, MP research in African freshwater systems is a fledgling research field (Alimi et al., 2021; Babayemi et al., 2019). Microplastic research has been conducted in only 9.3% (five countries) of African countries in both freshwater and marine systems. This scientific gap must be addressed for each country and by extension the whole continent to develop tailored solutions to MP pollution.

Freshwater systems serve as the primary mode of transport for MP particles from terrestrial to marine environments. Understanding microplastic pollution concentrations and transport in rivers is essential for determining the full extent of MP pollution and is a key part of ensuring the effectiveness of future management initiatives.

The Vaal River is an ideal case study as it is under extreme pressure as both a source of water and a means of waste removal, practices which are diametrically opposed. As per a South African Human Rights Commission (SAHRC) report on the Vaal River, pollution is "beyond acceptable levels," affecting natural ecosystems and compromising people's health and violating constitutional rights. The report also stressed that “In the absence of a timely and effective response from the multiple spheres of government, Gauteng’s most vital water resource may very well have been irreparably damaged” (SAHRC, 2021).

Malfunctioning WWTPs are suspected to be a major cause of the Vaal River’s deteriorating water quality. Municipal WWTPs around the Vaal River have a history of operating beyond design capacity, breakdowns, and dilapidated infrastructure. Some WWTPs around the Vaal rarely treat their wastewater to acceptable standards and are often ill-equipped to remove substantial amounts of non-biodegradable waste while

others participate in direct industrial wastewater discharge, contaminating receiving surface freshwater sources (Dikio, 2010; Herbig, 2019; Iloms et al., 2020; Vilakati et al., 2021; Wepener et al., 2011).

Data on MPs in the Vaal River will pave the way for science-based environmental risk assessment of MPs in other freshwater systems. This will facilitate a societal and political discussion at a national level on the extent of MP pollution in South Africa. Several entities stand to benefit, such as government agencies, legislative frameworks at the national, and regional levels, and non-profit organisations dedicated to preserving freshwater systems and basic human rights to clean and safe water.

3.2 General objectives and specific objectives

3.2.1 General objectives

The central aim of this project was to address the knowledge gap in MP research in the African context and the lack of data on MP pollution in African freshwater systems. More specifically, the study aimed at generating new data about the presence of MPs in the Vaal River around Gauteng province.

3.2.2 Specific objectives

The specific objectives for achieving the above-mentioned aim are:

- To screen the presence of MPs in the Vaal River using water samples.
- To determine the concentration of MPs in the Vaal River.
- To characterise and identify the MPs (shape, size, and polymer type).
- To compare the obtained results to those reported elsewhere.
- To provide evidence-based recommendations and risk-informed standards to influence and guide policymaking.

3.2.3 Research questions

The proposed research seeks to address the following fundamental questions:

- What is the level of MP pollution in the Vaal River?
- What are the most abundant colours, shapes, and sizes of MPs present in the Vaal River?

- What are the causes of MP pollution in the Vaal?
- How does the level of MP pollution compare to previous freshwater studies in South Africa, Africa, and the world?

3.2.4 Hypothesis

- The Vaal River is polluted with MPs.
- Microplastic abundance in the Vaal River is expected to be high, considering recent reports about the pollution profile of the river.
- MP pollution in the Vaal River is expected to be of secondary sources.
- Microplastics made from low-density polymers are expected to be most abundant in surface water samples.

CHAPTER FOUR-MATERIALS AND METHODS

This chapter describes the geographical location of the sampled area as well as the equipment, chemicals, procedures, quality control, and assurance measures followed in the extraction of MPs. It also accentuates the analytical procedures that were employed to qualify and quantify the MP particles.

4. Methodology

The study was conducted in two phases.

- phase one: sampling, pre-treatment, and purification of the samples.
- phase two: visual sorting and characterization of MPs.

Phase one

This involved the collection of samples from the Vaal River, extraction, and purification of MPs for analysis.

4.1 Study area

Surface water samples will be annotated as L1 to refer to the sample collected at location 1. Each is the endpoint of the sample collection, where the plankton net was taken out of the river and the sample was collected. Each sample was collected over an average distance of 2.30 km.

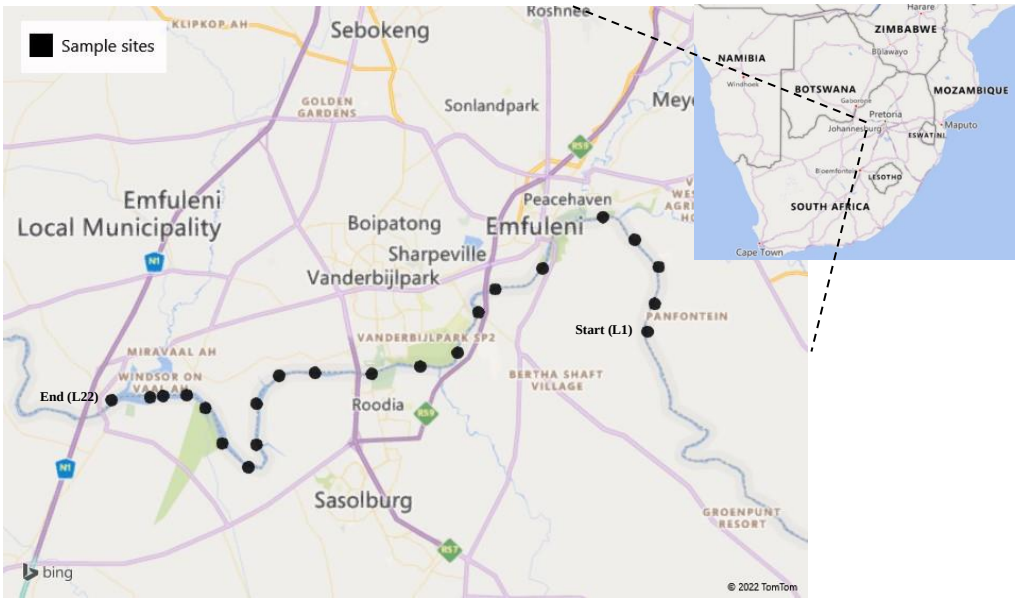


Figure 4.1 The Vaal River sampling area

Surface water samples were collected over approximately 50.4 km between the Lethabo weir (Panfontein) and the Vaal River barrage (near the N1) (Figure 4.1) and each location is described in detail in Table 4.1. The Lethabo weir is located between

the Vaal Dam and the barrage, it is a wall protruding just above the surface of the Vaal River close to the Eskom-Lethabo power station and Rand Water-Lethabo pump station. This region of the Vaal River has water flowing in from tributaries such as the Suikerbosrand, Klip, Taaibosspruit, Leeuspruit, and Rietspruit Rivers (Tempelhoff et al., 2007; van Vuuren, 2014).

Table 4.1 Description of sample locations

Sample code(s)	Description
L1 and L2	L1 and L2 are at the edge of the Lethabo weir.
L3 and L4	L3 and L4 are approximately 2.06 km apart, close to the Makouvlei Dam and Rand water Zuikerbosch pump station in Klipplaatdrift, Vereeniging.
L5 and L6	L5 is at Three Rivers, Vereeniging between the confluences of the Klip, Suikerbosrand, and Vaal Rivers. L6 is close to Arcelor Mittal Vaal works and Riviera aquatics club. It is about 1.53 km from the Rand water Vereeniging pump station. The water was dark grey, and sewage was draining into the Vaal River.
L7 and L8	Located near the R59 and Ascot on Vaal road. There are informal human settlements under the R59. L8 is near the North-West University Vaal Triangle Campus and the Vaal University of Technology in Vanderbijlpark.
L9 and L10	L9 is located between the North-West University Vaal Triangle Campus and Emerald Resort animal world. L9 and L10 are approximately 1.35 km and 1.18 km away from the confluence

	of the Vaal River and Taaibosspruit River, respectively. The Aquadome and Eligwa boat club are located close to L10.
L13 and L14	L13 is very close to the Vaal River Lodge and Spa and Compu-Kart Raceway. L14 is 2.05 km downstream of L13.
L15 and L16	L15 is close to Duck Point Venue and about 0.287 km from the confluence of the Vaal River and Leeuspruit River. L16 is 1.36 km downstream of L15. The Leeuspruit River drains Sasolburg, Free state.
L17 and L18	L17 and L18 are close to the residential areas of Adenworld AH and Malbank River estate AH in Vanderbijlpark.
L19, L20, L21, and L22	All are in a region between the Waterfront Country lodge and Vaal barrage. They are located on the edge of the Vaal barrage near the confluence of the Rietspruit and Vaal Rivers.

4.2 Sample collection

Surface water samples were collected to provide an indication/representation of the freshwater system being investigated while trying to avoid the generation of secondary MPs and contamination. Between 29-30 June 2021, twenty-two surface water samples were collected using a 55 μm plankton net (0.25 m in diameter, 0.50 m in length), and a detachable cod-end (Hydro-bios, Germany). To avoid contamination and ensure undisturbed sampling conditions, the plankton net was positioned outside the wake of the boat. The plankton net was towed using a 4 m rope, skimming the river's surface (Baldwin et al., 2016). The net was towed from the Lethabo weir to Vaal Barrage at a speed of 12 km/h.

The sampled material was concentrated at the cod-end inside a removable net bucket with sieve gauze. Samples were collected at 15-minute intervals and between

sample collections, the sample was retrieved from the cod end and the net was rinsed with river water. Global Positioning System (GPS) readings at the beginning and end of each tow were recorded to determine the sampled area. Volume filtered through the net was calculated using a flowmeter (General Oceanics model 2030R) attached to the inside of the net frame (Baldwin et al., 2016; Egessa et al., 2020; Frias et al., 2019; Lam et al., 2020; Tamminga et al., 2019). The procedure will be explained below. Samples were stored in 325 cm^3 pre-cleaned glass jars, transferred to the laboratory in cooler boxes and kept at 4℃.

Determining the volume sampled

The distance covered was determined by converting the number of flowmeter rotations obtained from the difference between the initial and final flowmeter readings to metres using the formula:

$$\text{Distance travelled (m)} = \frac{\text{flowmeter revolutions x 26873}}{999999}$$

The volume sampled was calculated by multiplying the distance trawled by the area of the net opening (Lam et al., 2020; Tamminga et al., 2019; Weideman et al., 2019). The filtered volume was calculated as follows:

$$\text{Area of net opening} = \pi r^2 = \pi \times (0.125\ \text{m})^2 = 4.9 \times 10^{-2}\ \text{m}^2$$

$$\text{Volume filtered} = \text{area of net opening} \times \text{distance travelled}$$

$$\text{Volume filtered (L1)} = 4.9 \times 10^{-2}\ \text{m}^2 \times 1.8 \times 10^{3}\ \text{m}$$

$$\therefore \text{Volume filtered (L1)} = 88.5\ \text{m}^3$$

Determining the area sampled

Vincenty's inverse formula was used to calculate the distance between two GPS coordinates. The distance between any two samples was determined using the Australian government geoscience geodetic calculator available at: https://geodesyapps.ga.gov.au/vincenty-inverse. The area sampled was obtained by

multiplying the geographical distance sampled with the width of the plankton net opening (Egessa et al., 2020; Zhang et al., 2015).

$$\text{Area sampled} = \text{Width of the net opening} \times \text{distance travelled}$$

$$\text{Area sampled (L1)} = 2.5 \times 10^{-4}\ \text{km} \times 1.63\ \text{km}$$

$$\therefore \text{Area sampled (L1)} = 4.1 \times 10^{-4}\ \text{km}^2$$

4.3 Samples pre-treatment

Due to the presence of co-enriched biological, organic, and inorganic particles that hamper MPs analysis, pre-treatment is usually suggested before analyses. The key aspect of pre-treatment is to remove materials such as biofilms, sand, wood, and other particles while avoiding the artificial generation of secondary MPs (Hurley et al., 2018; von Friesen et al., 2019).

Chemicals

All chemicals were obtained from Sigma Aldrich, and Ace Chemicals South Africa without further purification. Sodium iodide ACS reagent ≥99.5%, sodium chloride ACS reagent, ethanol 96% AR reagent, 50% (v/v) hydrogen peroxide, sulfuric acid ≥95%, and iron (II) sulfate heptahydrate ACS reagent ≥99.5%.

Preparation of solutions

Milli-Q® water was used to prepare all solutions, 0.07 M of $FeSO_4 \cdot 7H_2O$ catalyst was prepared by dissolving 20 g of $FeSO_4 \cdot 7H_2O$ in 1 000 cm^3 of water. 30% (v/v) H_2O_2 was prepared by adding 600 cm^3 of 50% (v/v) H_2O_2 and filling 1 000 cm^3 volumetric flask to the mark. To prepare a 1.8 g/cm^3 NaI solution, 80 g NaI salt was dissolved using Milli-Q® water in a clean 100 cm^3 Erlenmeyer flask. To prepare a 1.2 g/cm^3 NaCI solution, 45 g NaI salt was dissolved using Milli-Q® water in a clean 100 cm^3 volumetric flask.

Quality assurance and quality control

In the laboratory, a series of measures were taken to avoid/reduce background and cross-contamination of samples. Powder-free nitrile gloves and a cotton lab gown were worn during the whole process. All laboratory equipment and containers were thoroughly cleaned with Milli-Q® water and rinsed with ethanol. All work was done in a laminar flow cabinet. Solutions were subsequently filtered through a glass microfiber filter (Whatman®, 1.6 µm, 47 mm Ø) to decrease the chance of contamination. Method blank experiments were conducted with Milli-Q® water to check potential contamination. No MPs were detected in the blank samples.

4.3.1 Sample digestion

The use of Fenton's reagent was preferred as it eliminates the extensive exposure periods required by other methods. Furthermore, various sample preparation processes such as desiccation, physical homogenization, sonication, or several incubation phases used in enzymatic degradation were not required (Hurley et al., 2018; Prata et al., 2019b; Rueda Márquez et al., 2018).

To each sample, 30 cm^3 of 0.07 M $FeSO_4 \cdot 7H_2O$ and 30 cm^3 of 30% (v/v) H_2O_2 were added in 5 cm^3 increments. A few drops of concentrated sulphuric acid were added to lower the pH to 3.0 and 5.0 if necessary. The samples were subsequently swirled to allow the solutions to soak into the samples. The reagents were allowed to react at room temperature for 24 - 48 h until the fizzing had completely stopped, or a small amount of fizzing was observed (Figure 4.2). The subdued fizzing is an indication that the organic matter has been digested and the reaction is complete. An ice bath was made and use to cool the reaction if necessary (Egessa et al., 2020; Hurley et al., 2018; Yan et al., 2019).

Figure 4.2 Samples being digested

4.3.2 Density separation

Many samples require high amounts of heavy-density salt solutions (e.g., NaI and $ZnCI_2$) that are generally expensive. Solutions of NaI have densities close to those of zinc-based salts but with lower toxicity and have the potential to be recycled, making it a cost-effective efficient option (Campanale et al., 2020; Kedzierski et al., 2017). Depending on the sample compositions, samples were either filtered without density separation or subjected to a two-step density separation protocol developed by Nuelle et al. (2014) with some modifications. This was done to ensure the high extraction of MPs while avoiding excessive use of NaI (Di and Wang, 2018). Samples with little inorganic and organic matter were completely filtered without any density separation.

Once digested, the samples with high organic and inorganic matter, as shown in Figure 4.3, were subjected to density separation. To reduce the amount of NaI solution used, 45 cm^3 of NaCl (1.2 g/cm^3) was added to the samples, allowing 24h to density separate, the supernatant was then vacuum filtered through a glass microfiber filter (Whatman®, 1.6 µm, 47 mm Ø). To ensure the extraction of high-density polymers, 45 cm^3 of NaI (1.8 g/cm^3) was then added and the sample was allowed to density separate for 24h (Figure 4.4). The supernatant was vacuum filtered through a glass

microfiber filter (Whatman®, 1.6 µm, 47 mm Ø). The filter papers were dried overnight and stored in glass Petri dishes until analysis.

Figure 4.3 Samples with high inorganic and organic matter

Figure 4.4 Sample undergoing density separation

Phase two

The purpose of this phase was to analyse MPs qualitatively and quantitatively from water samples.

4.4 Physical analyses

Visual identification of particles was conducted using a Nikon MET SMZ745T stereomicroscope (Nikon Cooperation, Japan), equipped with the imaging source camera USB 3.0 (The Imaging Source Europe GmbH, Germany) with up to 50x magnification. Particle size was determined using NIS Elements-D imaging software Version 5.30 (Nikon Cooperation, Japan).

To mitigate against misidentification and subjectivity, the "break test" was used. Tweezers were used to probe individual particles. Plastic particles are flexible and do not break; instead, they bounce or spring when prodded. Particles that broke when touched were excluded (Marine and Environment Research Institute (MERI), 2015; Egessa et al., 2020). Furthermore, visual criteria proposed in the literature were adopted to identify MPs in this study. In-order for particles to be considered MPs, they had to meet the following criteria (Hidalgo-Ruz et al., 2012a; Norén, 2007; MERI, 2015).

a. The longest dimension of the particle should be less than 5mm.
b. Cellular or biological structures were not present on the particle. However, MPs may have organic material on their surface due to biofouling. Care was taken to note if organic material was present throughout a portion of the particle surface.
c. The thickness of fibers should be constant along their whole length. However, sometimes splitting or fraying can occur, and particles were subjected to the break test to confirm their identification.
d. Particles should exhibit a clear and homogeneous colour throughout. However, it should be noted that patterns or stripes may occur, and biofouling can potentially disguise the colour, or part of the particle may be bleached.

TESCAN Vega Scanning Electron Microscopy (SEM) was used to examine the surface structure of the detected MPs. Particles were extracted using tweezers from the filters and mounted on double-sided adhesive tape and coated with one layer of carbon and gold-palladium and eventually analysed.

The size of MPs was determined from the longest dimension of the particle. Microplastics were classified into six size classes or categories per previous studies: <0.5 mm, 0.5 - 1 mm, 1- 2 mm, 2 - 3 mm, 3 - 4 mm, and 4 - 5 mm (Wang et al., 2017a; Yan et al., 2019; Zhang et al., 2021c). Microplastic particles were grouped into various colour categories, such as blue, black, red, green, white, and transparent, as these colours were the most abundant. All other colours (pink, purple, yellow, and brown) in low quantities were classified as "others" (Frias et al., 2019; Lots et al., 2017; Yan et al., 2019). Microplastics were also categorized according to their shapes as fragments, pellets, fibres, and films (Huang et al., 2021; Su et al., 2016; Yuan et al., 2019).

Fragment particles were plastic particles from degraded hard plastic materials. Films were soft and thin sheet-like pieces of plastic from plastic carry bags and wrappers. Fibres were slender and elongated plastic pieces, with one dimension significantly longer than the other two. Pellets were round plastic particles with little or no signs of fragmentation. Particles were classified as fragments when they couldn’t be classified as fibre, pellet, or film (Egessa et al., 2020; Rodrigues et al., 2018; Su et al., 2016).

4.5 Chemical analyses

Spectroscopic identification offers insights into the chemical nature of the particles, thereby minimizing the potential misidentification of MPs based on visual inspection alone. Spectroscopic identification enabled the identification of the polymer type and any associated additives. Complete sample analysis is time-consuming, costly, and labour-intensive. Subsequently, a subset of the filter was examined, to provide statistical information on the whole filter (Bumbrah and Sharma, 2016; Ghosal et al., 2018; Harrison et al., 2012; Song et al., 2015).

Particle characterization was performed on a subsample due to the substantial time required to analyse the entire sample (Lenz et al., 2015; Song et al., 2015). An average of twenty-one particles per sample were analysed, thus, 54% of particles were analysed. Chemical characterization was performed using the Horiba LabRAM HR Raman spectrometer, equipped with gratings ruled at 600 lines per mm and a liquid nitrogen-cooled charge-coupled device detector. Wavelength calibration was performed each day of analysis by focusing on a silicon wafer and analysing the first phonon band of silicon at 520.7 cm^{-1}. The 514.5 nm radiation of an argon ion laser and a 100× objective of an Olympus BX41 microscope (NA = 0.80) were used for spectra acquisition. The confocal pinhole was set at 100 μm. Raman spectra were recorded in the wavenumber range of 100 - 3500 cm^{-1}, with laser power between 0.04 and 0.4 mW. Due to high background fluorescence in MPs, particles were subjected to photobleaching for 5 - 60 s before acquisition depending on the intensity of the background fluorescence. The incident laser power and the acquisition conditions were varied between particles to avoid polymer degradation due to laser-induced heating and to acquire a better response signal (Prata et al., 2020).

After spectra acquisition polynomial baseline correction was performed on the raw Raman spectrum, to reduce noise and enhance spectrum quality using LabSpec version 6 software (Horiba Scientific, Japan). The chemical composition of polymers was identified by comparison with reference spectra in the SLOPP Library of Microplastics and polymer databases of KnowItAll® Informatics system software 2021, spectroscopy edition (John Wiley & Sons, Inc.). In cases where the spectra could not be assigned to a polymer at the set threshold, a two or three-component search was done on KnowItAll® Informatics system software to identify the polymer and additives present. The threshold or hit quality index (HQI) of more than 80% similarity between the sample and reference spectra was used to confirm polymer identification (Egessa et al., 2020; Ragusa et al., 2021; Song et al., 2015).

CHAPTER FIVE-RESULTS AND DISCUSSIONS

This chapter presents and evaluates the outcomes of the research work. The findings include the physical and chemical classification of MPs and related additives.

5.1 Microplastic abundance in the sampled area

As previously indicated in section 4.2, the volume and area sampled were calculated from the distance trawled by the area of the net opening and the width of the plankton net opening, respectively. The initial and final flowmeter readings, flowmeter revolutions, distance towed, and volume sampled at each location are given in Appendix A. The GPS coordinates of each location, the distance sampled, and the area sampled per location are provided in Appendix B.

As shown in Figure 5.1 there was a 100% MP prevalence in all surface water samples from the Vaal River. A total of 848 particles were identified as MPs with an average of 38.5 ± 35.5 particles per sample. Microplastic abundances ranged between 0.13 - 2.5 particles/m^3 (1.6×10^4 - 2.2×10^5 particles/km^2). The mean abundance of MPs was 0.61 ± 0.57 particles/m^3 (6.6×10^4 ± 5.4×10^4 particles/km^2) (mean value ± stdev). Microplastic abundance was higher at six locations, L5 - L10 with 0.73, 1.2, 2.5, 0.74, 1.6, and 1.3 particles/m^3, respectively. These samples were collected from a region between Vereeniging and Vanderbijlpark (Figure 4.1).

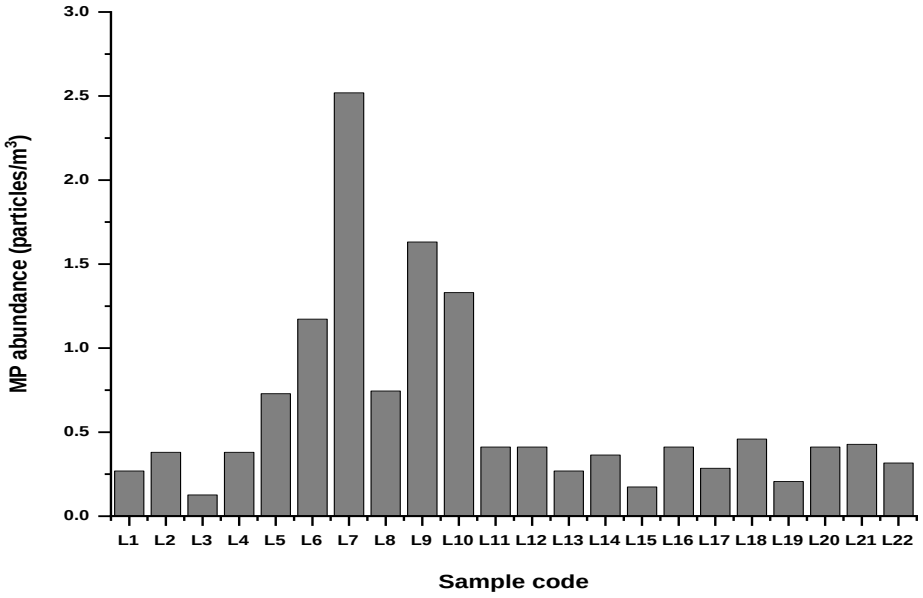


Figure 5.1 Microplastic abundance per sample

5.1.1 Sources of MPs in the Vaal River

5.1.1.1 Urban centres

Urban towns of Vereeniging and Vanderbijlpark have high population densities. Local sources of MPs include industries, WWTPs, stormwater runoff, informal and formal settlements, and recreational activities (Wepener et al., 2011). Storm drainage and urban runoff may carry MPs from landfill sites, road paint, and tire wear from vehicles into the Vaal River (Boucher and Friot, 2017; Horton et al., 2017).

Microplastic abundance generally decreased with distance from urban centres, which suggests there was a short-distance transport of plastic particles from these 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 (Cable et al., 2017; Egessa et al., 2020; Huang et al., 2021; Lestari et al., 2020; Meijer et al., 2021). Lower MP abundances were detected further away from the Wuhan City Centre in China (Wang et al., 2017a). The Vaal River has a vibrant tourism industry, there are various tourist attractions such as Emerald Resort animal world, Aquadome, Eligwa boat club, Vaal River lodge and Spa, and Compu-Kart Raceway (Table 4.1). All these may be a source of macroplastics that eventually degrade into MPs.

5.1.1.2 Wastewater treatment plants

Considering the state of the wastewater systems in the Sedibeng District Municipality, WWTPs could very much be a potential source of MPs. The Leeuwkuil, Sebokeng, Midvaal, and Rietspruit WWTPs are characterised by a history of breakdown, outdated equipment, operating beyond design capacity, and rarely treating wastewater to acceptable standards (Wepener et al., 2011; Iloms et al., 2020; SAHRC, 2021). In 2016, a feasibility study indicated several of the biofilters and anaerobic digesters at the Leeuwkuil and Rietspruit WWTPs were not operating. Only four of approximately eleven biofilters at the Rietspruit WWTPs were operational. Consequently, by late 2018, the Emfuleni waste treatment system was beyond repair and eventually collapsed. In 2020 there were reports that Sebokeng and Leeuwkuil WWTPs were operating above their hydraulic capacity. The breakdown of wastewater

treatment infrastructure in the Sedibeng District Municipality caused sewage to flow into the Vaal River which led to the death of thousands of fish (Ash., 2022; Evans., 2021; SAHRC., 2021). Sewage was observed flowing into the Vaal River near L5 and L6 during sampling. According to the SAHRC, wastewater and sewage spillages into the Vaal occur regularly as a result of a lack of preventative maintenance, vandalism, and dilapidated infrastructure (SAHRC., 2021).

Provided that WWTPs can extract all MPs and concentrate them in wastewater sludge. Microplastics may eventually be released into the environment through wastewater sludge (Carr et al., 2016; Estahbanati and Fahrenfeld, 2016; Murphy et al., 2016; Talvitie et al., 2017). Thus, the presence of Rand Water's Panfontein sludge disposal site less than 3 km from the Vaal River could be another potential source of MPs. Approximately, 1300 tons of wastewater sludge produced daily is pumped to Rand Water's Panfontein sludge disposal site in the Midvaal Local Municipality. The sludge disposal site is less than 3 km from the Vaal River. A portion of the wastewater sludge is stored in a drying paddock, while some are sprayed onto the land using irrigation equipment. MPs in the paddock-dried sludge may be resuspended and become airborne and end up in the Vaal River (Dris et al., 2015; Mokonyama et al., 2017; Wu et al., 2016). The land around the Vaal triangle is predominantly used for agricultural purposes. MPs in the sludge used for agricultural beneficiation may potentially become remobilized during flash flooding, resulting in the contamination of the Vaal River (Corradini et al., 2019; Ubomba-Jaswa and Kalebaila, 2020; Remilekun et al., 2021).

5.1.1.3 The Vaal River's tributaries

The Vaal barrage catchment receives copious amounts of wastewater effluent through the Rietspruit, Taaiboschspruit, Leeuspruit, Klip, and Suikerbosrand Rivers. These tributaries carry stormwater and wastewater from southern Gauteng and northern parts of the Free State (Tempelhoff et al., 2007; Weideman et al., 2019). Tributaries have been observed to be a major source of MPs in the Qiantang River, China (Zhao et al., 2020), Yangtze River, China (Zhang et al., 2015a), and Braamfontein Spruit, South

Africa (Dahms et al., 2020). Fischer et al. (2016), investigated MP abundances in two freshwater Lakes in Italy. Although Lake Chiusi has a smaller catchment area than Lake Bolsena, a greater MP abundance was reported in Lake Chiusi. Lake Chiusi had an MP abundance of 2.7 - 3.7 particles/m^3 and Lake Bolsena had 0.82-4.4 particles/m^3. Lake Chiusi is fed by two tributaries while Lake Bolsena has no tributaries (Fischer et al., 2016).

However, similar observations were not made at the confluences of the Leeuspruit-Vaal River and the Rietspruit-Vaal River. The water at this confluence is slow-moving and could enhance the sedimentation of MPs. Moreover, the confluence of the Vaal River and Rietspruit River is stretched out into Loch Vaal Lake (Figure 5.2**)**. Thus, MPs may be more abundant further away from the area sampled. The Rietspruit River is a potential source of MPs to the Vaal River, burst sewerage pipes were detected on the banks of the Rietspruit River, resulting in raw sewage flowing into the Rietspruit River (SAHRC, 2021).

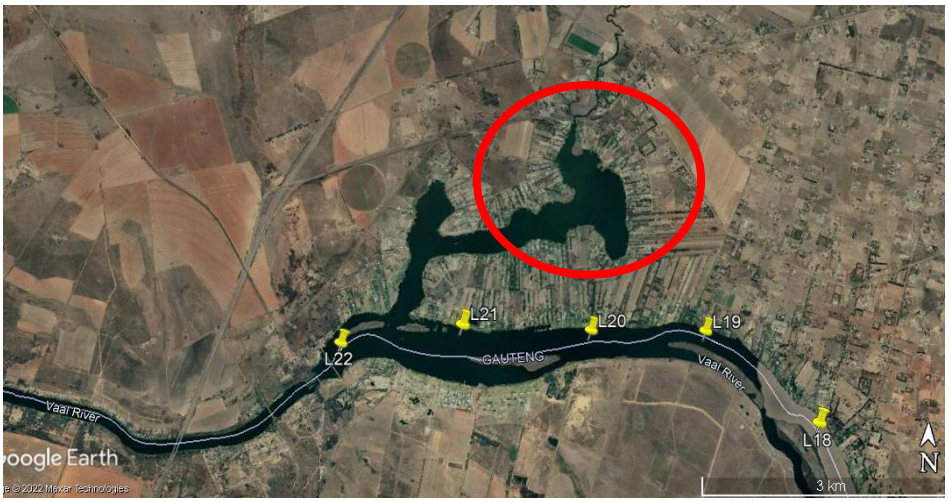


Figure 5.2 Map of Vaal-Rietspruit River confluence

5.1.1.4 Industries

Sedibeng District Municipality is currently faced with serious water pollution challenges that have affected the Vaal River and its tributaries. Most notably that the

Klip River and Blesbokspruit are polluted by runoff from industrial areas (e.g., Arcelor Mittal Vaal works, Riviera aquatics club Sasol, Rand Water, and Eskom) (Table 4.1). Potential MPs sources in this region may include effluent from industries that releases effluent below the Vaal Barrage. Most of the industries areas are heavy/noxious industries and sewage spills from these industries could potentially have a negative environmental impact on the Vaal River (Dzwairo and Otieno, 2014; COGTA, 2020; McCarthy et al., 2007; Tempelhoff, 2009b).

5.1.2 Comparison of microplastic abundance in selected freshwater systems

Since abundance levels with different units cannot be compared, MP abundance in this study was only compared with studies that used the same units of abundance. As shown in Table 5.1, MP abundances were generally lower in artificially and naturally regulated freshwater systems such as Lake Michigan, Lake Hövsgöl, Lijiang River, Feilaixia Reservoir (Beijiang River), and Laurentian Great Lakes.

In the above-mentioned freshwater systems sampling apparatus with higher mesh sizes were used which could also account for their lower abundance. Sampling with nets of different mesh sizes and different types of sampling methods may lead to different results (Feng et al., 2021; Zhang et al., 2020b). Dris et al. (2015) and Song (2014) both reported a steep increase in MP concentrations when using smaller 80 µm mesh nets than bigger 330 µm mesh nets.

Similar abundances were reported when a 75 µm plankton net was used in the Lijiang River, China. Greater abundances were reported in freshwater systems from China, the USA, South Korea, South Africa, and Uganda. An important factor to consider in the Qiantang River and Braamfontein Spruit is that bulk sampling was employed. Generally, bulk sampling leads to smaller sample volumes which causes a higher estimation of MPs (Liu et al., 2020b; Zhang et al., 2021a; Zhang et al., 2021c). The average volume sampled for this study was 63.1 m^3 (6.3×10^4 L) which is at least three orders of magnitude higher than the volumes sampled in the other two studies.

Higher MP abundances were also reported in the Lijiang River, a 12-V DC Teflon pump with a 25 μm sieve was used to collect samples. Pump-based water sampling generally results in small volumes filtered and may not accurately reflect the characteristics of MPs in the surface water. However, trawl or net-based volume-reduced sampling filters larger volumes of water (several hundred m^3), which may be used to estimate the large-scale distribution of MPs. Some studies also found that the MP abundances reported for pump samples can be several orders of magnitude greater than net or trawl samples (Liu et al., 2020b; Zhang et al., 2021a). Discrepancies brought about the use of different sampling methods to highlight the need for a unified sampling method.

However, other factors may also influence MP abundances such as population densities and human activities. For instance, higher abundances were reported in the Pearl River, Great Lakes tributaries, Haihe River, the Han River, Lake Victoria, and Yangtze River (Table 5.1), although volume-reduced sampling was employed with bigger mesh sizes (Egessa et al., 2020; Wang et al., 2017a; Zhao et al., 2020).

Lake Victoria is the second-largest freshwater lake in the world and is exposed to greater sources of anthropogenic waste due to being shared by Kenya, Uganda, and Tanzania. Lake Victoria may be more significantly impacted by human activities such as fishing, agriculture, and tourism than the Vaal River. The fishery at Lake Victoria is the world's largest inland fishery with over 200 000 people engaged in direct fishing (Egessa et al., 2020; Nyamweya et al., 2016).

On the other hand, in the Vaal River, only a handful of fishermen engage in reactional fishing activity. Similar arguments can be made for the Yangtze River, the longest river in Asia. China is the world's largest producer and consumer of plastic materials. Thus, the Yangtze River is expected to have a higher total amount of MP pollution (Feng et al., 2021; Hu et al., 2018; Xiong et al., 2019).

Table 5.1 Comparison of microplastic abundance in selected freshwater systems

Freshwater system	Sampling apparatus	Number of samples	Range	Mean abundance (mean value ± stdev)	Reference
Lake Michigan (USA)	300 μm manta trawl	59	1.3×10^4 - 2.2×10^4 particles/km^2	1.7×10^4 particles/km^2	Mason et al. (2016)
Lake Hövsgöl (Mongolia)	333 μm manta trawl	9	997 - 4.4×10^4 particles/km^2	2.0×10^4 particles/km^2	Free et al. (2014)
Lijiang River (China)	300 μm plankton net	13		0.15 ± 0.15 particles/m^3	Zhang et al. 2021a
Feilaixia Reservoir, Beijiang River (China)	112 μm plankton net	8	0.28 - 1.1 particles/m^3	0.56 ± 0.45 particles/m^3	Tan et al. (2019)
Laurentian Great Lakes (USA)	333 μm manta trawl	21	0-4.7×10^5 particles/km^2	4.3×10^4 ± 1.2×10^5 particles/km^2	Eriksen et al. (2013)

Lijiang River (China)	75 μm plankton net	13	0.13 - 1.5 particles/m^3	0.67 ± 0.41 particles/m^3	Zhang et al. (2021a)
The Pearl River (China)	333 μm manta trawl	11	1.1×10^5 - 1.3×10^5 particles/km^2 (0.69 - 8.2 particles/m^3)	3.8×10^5 particles/km^2 (2.4 ± 0.70 particles/m^3)	Lam et al. (2020)
Great Lakes tributaries (USA)	333 μm neuston net	107	0.05 - 32 particles/m^3	4.2 particles/m^3	Baldwin et al. (2016)
Haihe River (China)	333 μm manta trawl	61	0.69 - 75 particles/m^3	14.2 ± 14.6 particles/m^3	Liu et al. (2020b)
Lijiang River (China)	12-V DC Teflon pump with a 25 μm sieve	13		67.5 ± 65.6 particles/m^3	Zhang et al. (2021a)
The Han River (South Korea)	100 μm manta trawl	21	0 - 42.9 particles/m^3	7.0 ± 12.9 particles/m^3	Park et al. (2020)
Qiantang River (China)	20 L container	37	50 - 3.2×10^3 particles/m^3	1183 ± 269 particles/m^3	Zhao et al. (2020)

Braamfontein Spruit (RSA)	25 L container	9	160 - 2.0×10^3 particles/m^3	705 particles/m^3	Dahms et al. (2020)
Lake Victoria (Uganda)	300 µm manta trawl	24	2.8×10^3 - 3.3×10^5 particles/km^2	1.2×10^5 particles/km^2	Egessa et al. (2020)
Yangtze River (China)	333 µm manta trawl	15	2×10^5 - 9×10^5 particles/km^2	4.9×10^5 particles/km^2	Xiong et al. (2019)
The Vaal River (RSA)	**55 µm plankton net**	**22**	**0.13 - 2.5 particles/m^3 (1.6×10^4 - 2.2×10^5 particles/km^2)**	**0.61 ± 0.57 particles/m^3 $6.6\times10^4 \pm 5.4\times10^4$ particles/km^2**	**This study**

5.2 Physical characterisation

The size, colour, and shape of MP particles were visually identified using a stereomicroscope. The surface morphology of MPs was determined using SEM. The implications of the physical characteristics of MPs in this study are discussed in the following sections.

5.2.1 Distribution of MP shapes

MPs were classified into four morphotypes as indicated in section 4.4. Typical MPs shapes observed are shown in microscopic images in Figure. 5.3a-d. The major types of MPs were classified as fibers (41.6%), and fragments (39.9%) with films and pellets seldomly observed, with abundances of 10.9% and 7.7% respectively (Figure 5.4). Fibres and fragments were previously detected at near equal proportions in the Vaal River and Suikerbosrand River (Verster and Bouwman, 2020). They are commonly reported to be the most abundant shapes in several studies such as Western Lake Superior, USA (Hendrickson et al., 2018), Great Lakes tributaries, USA (Baldwin et al., 2016), and Poyang Lake, China (Yuan et al., 2019).

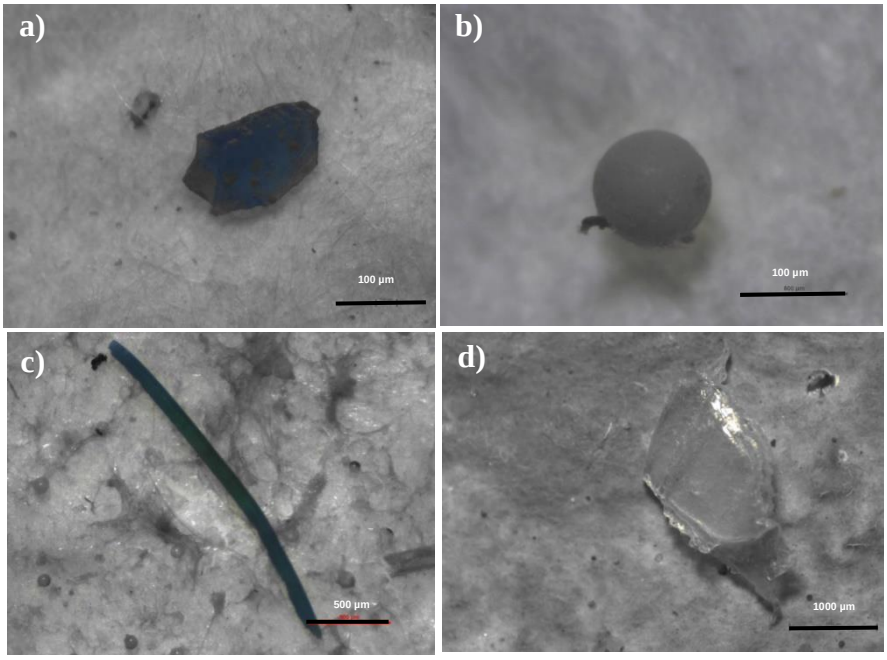


Figure 5.3 Microplastic shapes: a) fragment, b) pellet, c) fiber, and d) film

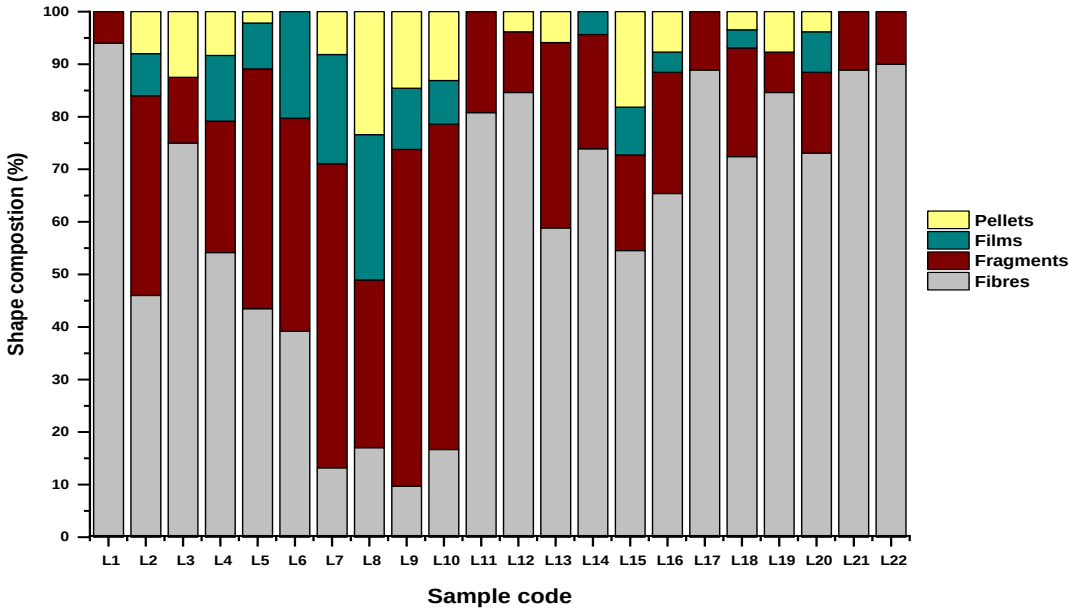


Figure 5.4 Composition of MP shapes per sample

The relatively high abundance of fibres may be attributed to domestic effluent. Approximately 92.8% of households in the Sedibeng District Municipality have access to flush toilets (COGTA, 2020). The SAHRC has reported that domestic sewage from the Sedibeng district has been leaking into the Vaal River. It has been reported that around 6×10^6 fibers can be released per 5 kg of clothing (Napper and Thompson, 2016). As the natural receiver of domestic and industrial effluent from WWTPs in the Sedibeng District Municipality, the Vaal River may be at risk of high MP fibre input. The proximity of the sampling sites to residential areas suggests that surface run-off could be another source of fragments and fibers. (SAHRC, 2021). In addition to their myriad potential sources, fibrous MPs may be retained longer in the water column due to their physical properties. Their longer sizes ratio enables them to resist sinking and remain in surface water rather than in sediment (Zhang et al., 2021c).

Agricultural plastic films used for crop growing are a source of plastic contamination in agricultural soils. They become fragile and prone to deterioration as a result of weathering. Disintegrated particles may be washed into agricultural soils and subsequently accumulated there. Thus, agricultural drainage is another potential

source of MPs in the Vaal River (Corradini et al., 2019; Li et al., 202b; Remilekun et al., 2021; Zubris and Richards, 2005).

Vereeniging and Vanderbijlpark are located on the banks of the Vaal River, which serves as popular recreation areas. Along these banks are guesthouses, hotels, and golf courses. Along the river, visitors can embark on cruises and engage in water sports. Plastic litter discarded by tourists and nearby residents could contribute to the high presence of fragments and minimal presence of films in the water body (Eerkes-Medrano et al., 2015; de Witt, 2014).

The main sources of pellets could be various industrial activities around the Vaal triangle, these include plastic resin powder from plastic production industries, microbeads from personal hygiene and cosmetic products, and pellets from air blasting machines. Personal care and cosmetic products used in guesthouses and spas could be another potential source of pellets. Facial scrubs are reported to release approximately 1×10^5 microbeads or pellets into domestic sewage systems (Eerkes-Medrano et al., 2015; Horton et al., 2017; Mao et al., 2020).

5.2.2 Distribution of MP sizes

Microplastics were classified into six categories according to their sizes as indicated in section 4.5. Overall MPs ranged in length from 0.06 to 4.95 mm, with an average of 0.96 ± 0.88 mm (mean ± stdev). MPs less than <0.5 mm accounted for 38.2 %, 0.5-1 mm (28.5%), 1–2 mm (22.3%), 2–3 mm (7.1%), 3–4 mm (2.2%), and 4-5 mm (1.7%). MPs with a size of less than 2 mm accounted for 89% of the plastic particles in all samples (Figure 5.5).

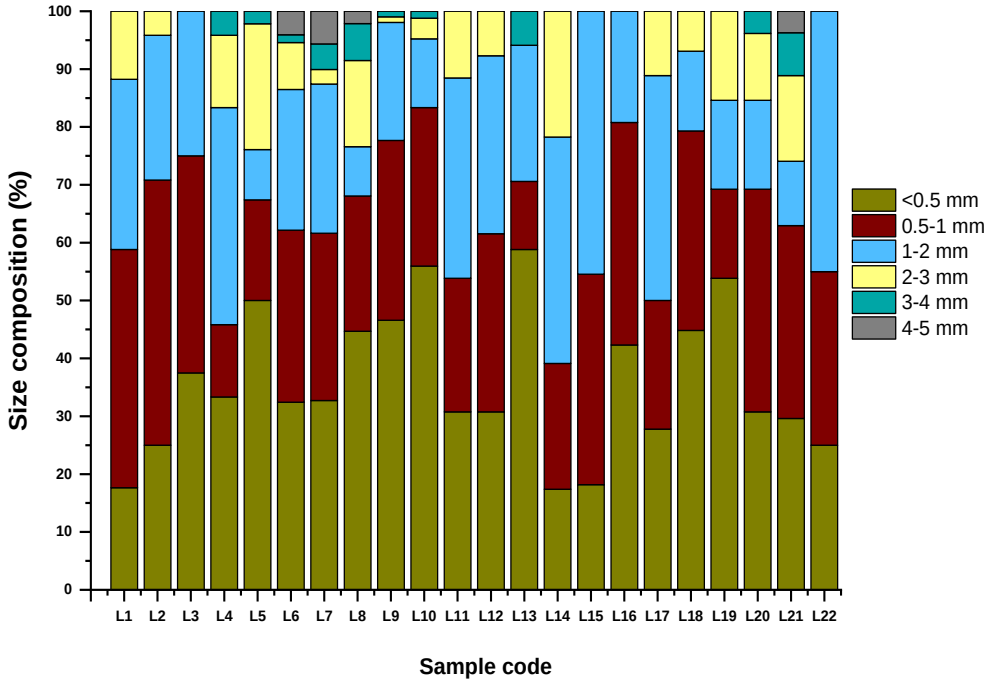


Figure 5.5 Composition of MP size classes per sample

The average lengths (mean ± stdev) of MPs differed. Fibers ranged in length from 0.11-4.95 mm, with an average length of 1.2 ± 0.86 mm. The shortest fragment was 0.06 mm and the longest was 3.96 mm, fragments had an average length of 0.64 ± 0.59 mm. Films had an average length of 1.7 ± 1.3 mm, with a minimum of 0.13 mm and a maximum of 4.94 mm. Pellets ranged in length from 0.07-1.23 mm with an average length of 0.32 ± 0.19 mm. MPs had the trend: films>fibres>fragments>pellets.

As shown in Figure 5.6, the greatest proportion (>85.2%) of fibres were in the ranges <0.5 mm (20.3%), 0.5-1 mm (32.6%), and 1-2 mm (32.3%). Lower quantities were in higher size ranges 2-3 mm (11.1%), 3-4 mm (2%), and 4-5 mm (1.7%). Fifty-four percent of fragments were <0.5 mm and 28% were in the range of 0.5-1 mm. Microplastics were detected in smaller quantities in the 2-3 mm (3%), and 3-4 mm (1%) size ranges. There were no fragments in the range of 4-5 mm. Films were distributed as follows: <0.5 mm (15.6%), 0.5-1 mm (25%), 1-2 mm (31.3%), 2-3 mm

(11.5%), 3-4 mm (9.4%), and 4-5 mm (8.3%). Approximately 85.9% of pellets were <0.5 mm. The remaining 14.1% were in the 0.5-1 mm (12.5%), and 1-2 mm (1.6%).

The dominance of small-sized plastics has been found in many freshwater studies, such as Laurentian Great Lakes, USA (Baldwin et al., 2016; Cable et al., 2017), Lake Hövsgöl, Mongolia (Free et al., 2014), Lake Victoria, Uganda (Egessa et al., 2020), and Taihu Lake, China (Su et al., 2016).

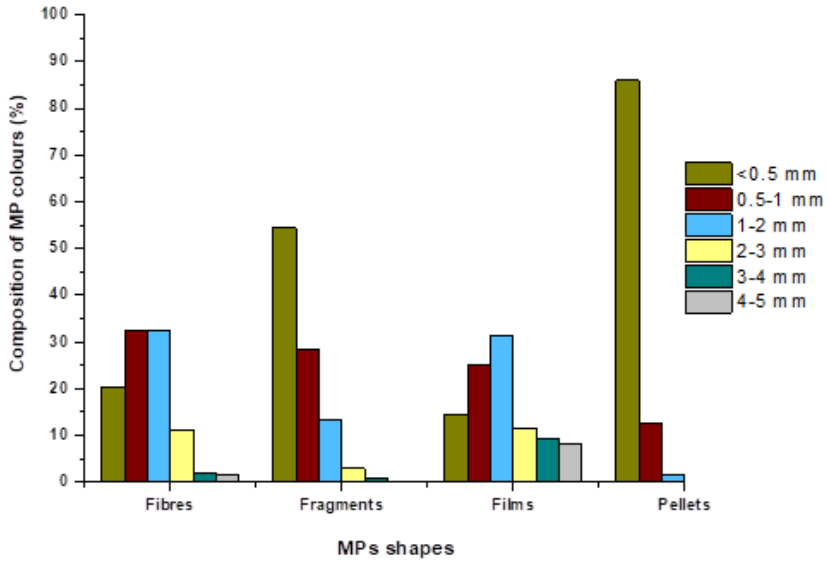


Figure 5.6 Size class distribution of MPs per shape

The size distribution across fibres, fragments, and films indicate that large plastic debris undergoes successive fragmentation of when exposed to photo-oxidative, thermal, chemical, microbial, and mechanical forces in the Vaal River. The size of plastic particles may gradually decrease due to degradation mechanisms in the water systems (Boucher and Friot, 2017a; Emmerik and Schwarz, 2020; Lestari et al., 2020; Mason et al., 2016; Onoja et al., 2022).

The high abundance of pellets <0.5 mm is due to them being manufactured in microscopic dimensions (0.06-0.8 mm) for application in personal care and cosmetic products. Pellets or microbeads are found in approximately 72% of sunscreens (Chang, 2015; Cheung and Fok, 2017; Kleida, 2020). These can easily enter the Vaal River as

people engage in different water activities/sports or are released through wastewater effluent from spas.

The relative abundance of MPs generally increased with decreasing MP size, which is consistent with previous studies (Eriksen et al., 2014; Feng et al., 2021). The Spearman correlation test revealed a weak/insignificant correlation ($r = 0.12$; $p = 0.60$) between MP abundance and size. This could be explained by the fact that the relationship between MPs abundance and size can be influenced by other variables. This includes environmental degradation processes such as photo-oxidative, thermal, chemical, microbial, and mechanical forces.

The size of MPs affects their transport, distribution, and fate in water columns by affecting the magnitude of buoyant, gravitational, and drag forces (Hoellein et al., 2014; Kowalski et al., 2016; Kumar et al., 2021). The higher surface-to-volume ratio enables small plastic particles to have lower relaxation time and lower settling velocity. Thus, small MPs are more likely to remain suspended in the water column rather than sink into sediments (Lestari et al., 2020; Zhang et al., 2015). However, small MPs may have faster sinking rates when bio-fouled which could increase their availability to benthic biota in the water column (Cole et al., 2013; Kowalski et al., 2016).

The average size of MPs from the Vaal River was less than 1 mm. This raises several concerns. Plastic particles <1 mm are considered to pose a greater threat to aquatic organisms. For instance, the adsorption capacity of nanoplastics has been reported to be at least two orders of magnitude greater than that of MPs. Moreover, the desorption of plastic additives has been reported to increase with a decrease in the sizes of MPs. Their high surface area-to-volume ratios facilitate the desorption or leaching of toxic chemicals. The desorption of additives has been reported to be more pronounced in MPs less than 1 mm. For instance, Liu et al. (2020a) investigated the effect of MP sizes on the leaching of cadmium pigments into water under simulated sunlight. The authors reported that there was greater leaching of cadmium pigments in MPs less than 0.85 mm than those with sizes above 0.85 mm (Koelmans et al., 2014;

Liu et al., 2020a; Luo et al., 2020; Velzeboer et al., 2014). Consequently, small particles have a greater potential to act as vectors for sorbed contaminants and enhance the bioavailability of plastic-derived toxicants along the food chains (Bakir et al., 2014; Vethaak and Leslie, 2016). Small-sized MPs have a higher probability of being accidentally ingested by aquatic biota since their sizes are similar to zooplankton (Ageel et al., 2022; Cordova et al., 2020).

Once ingested, these particles have the potential to cause adverse health effects to biota by obstructing airways, inflammation, and/or accumulation in organs after translocation. MPs have been detected in human feces, placenta, lung tissues, colon, nasal mucous, and blood (Ibrahim et al., 2021; Leslie et al., 2022; Ragusa et al., 2021; Vethaak and Leslie, 2016). Smaller particles may accumulate rapidly and more efficiently in human gastric cells (Forte et al., 2016). A combination of MP shape and size may influence the toxicity of MPs, due to the differences in retention time, accumulation, and extent of physical damage.

Microplastic fibers have been reported to have longer intestinal residence time and higher mortality in freshwater amphipods (*Hyalella azteca*) than other shapes of MP particles (Au et al., 2015; Gray and Weinstein, 2017). Similarly, Gray and Weinstein, (2017), reported fibers had a higher residence time and stronger acute toxicity than beads/pellets and fragments in grass shrimp (*Palaemonetes pugio*). Similar observations were made by Qiao et al. (2019) in the guts of zebrafish (*Danio rerio*) where fibers had greater residence times and potential toxicity than microbeads/pellets and fragments. The greater propensity of fibres to be retained in biota was also corroborated by an investigation of MPs in human colon tissue. Microplastics were detected in all colon tissue samples, with fibers accounting for 96.1% of the particles detected (Ibrahim et al., 2021; Schwabl et al., 2019).

Non-spherical MPs may have higher residence times biota as they are harder to egest and can easily be embedded in tissues than spherical MPs. Microplastic fibres may become entangled within the intestinal tract and eventually cause a blockage,

resulting in death. Internal structures in the biota may also become damaged as entangled fibers pass through them (Au et al., 2015; Gray and Weinstein, 2017; Qiao et al., 2019). The intake of tiny particles can cause great harm to organisms, including reduced growth rate, altered shallowing behaviour, oxidative stress, reproductive complications, and metabolite changes in the liver, muscles, brain, and heart (Ageel et al., 2022; Mattsson et al., 2015; Ragusa et al., 2021).

As indicated above distribution of MPs in the water column, accumulation in sediment, and ingestion by aquatic biota, are all size and shape-dependent. Furthermore, the efficiency of wastewater treatment plants (WWTPs) in removing MPs is determined by particle size and shape. Smaller-sized MPs are reported to bypass the fine mesh filters in WWTPs. Due to water being extracted from the Integrated Vaal River System by potable water utilities, MPs may be present in drinking water and bottled beverages which could pose a direct threat to public health.

5.2.3 Distribution of MP colours

Green (22.3%), black (19.1%), blue (18.3%), and transparent (17.3%) MPs were the most abundant. White (11.1%), red (6.5%), and other coloured MPs (5.4%) were less prevalent as shown in Figure 5.7. As shown in Figure 5.8, there was a higher abundance of coloured fibres (94.3%) than transparent fibres (5.7%). Similar observations were made for fragments with 85.6% of coloured fragments and 14.4% of transparent fragments. Coloured plastic fragments and fibres could result from clothing and other plastic materials. Hence, wastewater effluent may be the primary source of these MPs (Andrady, 2017; Fendall and Sewell, 2009).

Unlike fibres and fragments, transparent films (66.3%) were the most abundant types of films and the remaining 33.7% were coloured. Similar to fibres and fragments there was a higher abundance of coloured pellets (85.7%) and only 14.3% were transparent. Coloured pellets could originate from cosmetic products. The wide variety of colour distribution across MP shapes suggests that MPs in the Vaal River are from various sources.

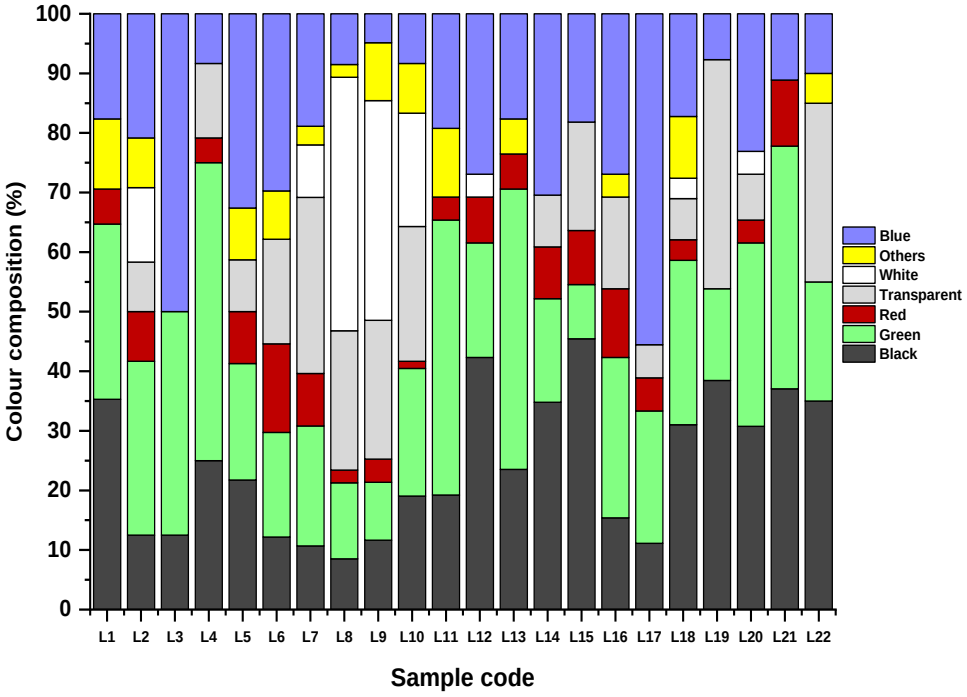


Figure 5.7 Size class distribution of MPs per shape

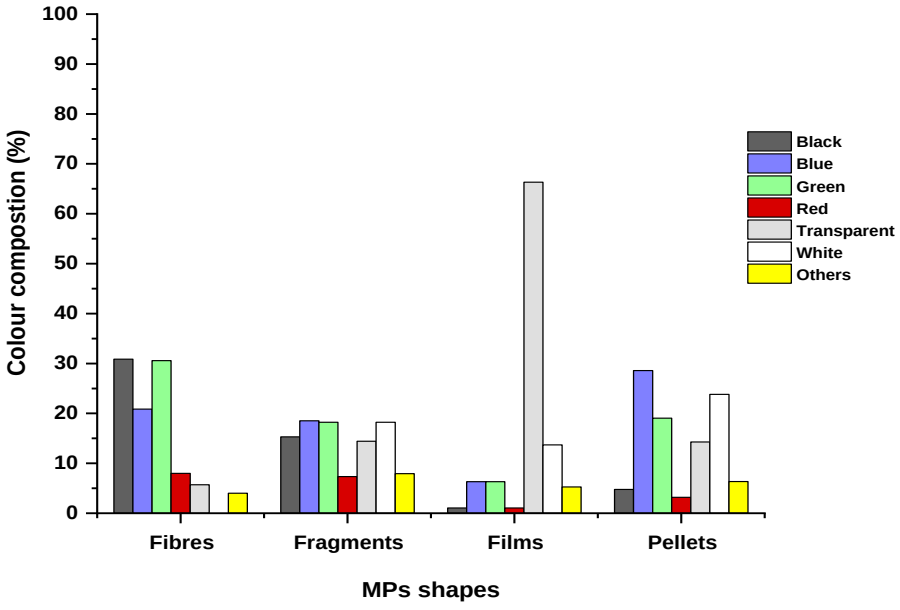


Figure 5.8 Colour distribution of MPs per shape

The high abundance of coloured MPs is of particular concern to aquatic organisms within the Vaal River. Aquatic biota may mistakenly ingest MPs that are visually similar to their prey or food source (Acquah et al., 2021; Nan et al., 2020). Certain colourants can accelerate the degradation, oxidation, and stabilisation processes of polymers (Allen, 1994). Thus, chemical compounds used as colourants may leach from MPs into the water and potentially have adverse ecotoxicological effects on humans and freshwater biota that depend on the Vaal as a water and food source.

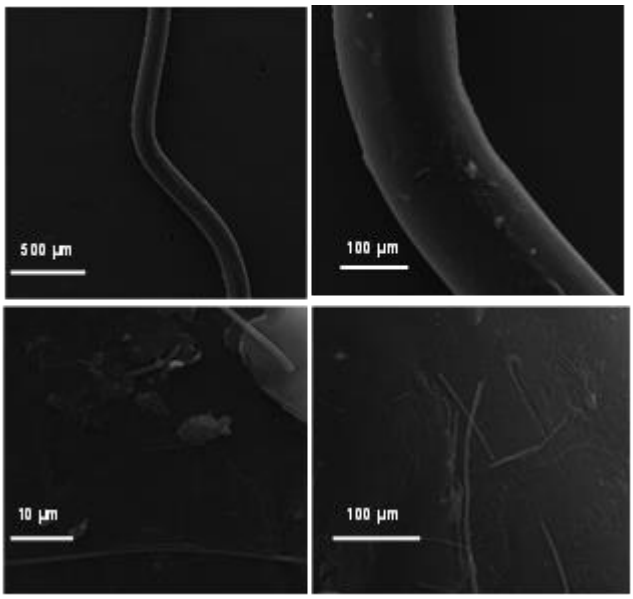
5.2.4 Surface morphology

SEM images of selected MPs are depicted in Figure 5.9. The images clearly show cracks, scratches, and pores on the surfaces of the particles, all of which, are signs of degradation. Such signs include the rough surface of the MP fragment (Figure 5.9b), the cracks and the porous surface of the pellet in Figure 5.9d, and the fine particles pitting on the surface of the fibre and film particles (Figure 5.9a and Figure 5.9c).

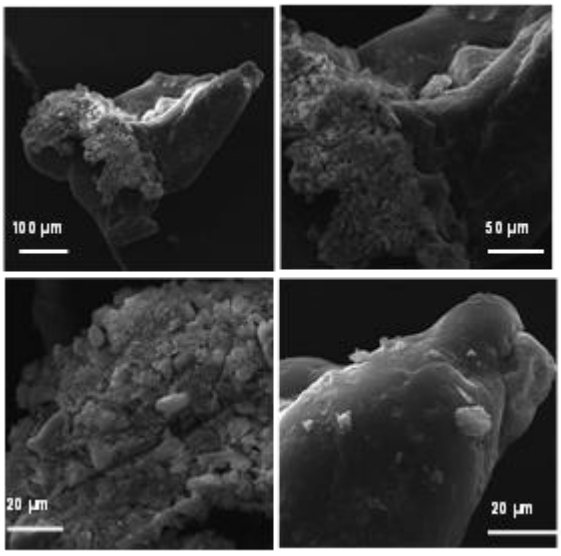
In the Vaal River, plastics/MPs are exposed to different weathering and degradation processes, which increase the surface area of MPs resulting in an increased adsorption capacity. This enhances their potential to act as vectors for chemicals and microorganisms (Liu et al., 2020a; Mao et al., 2020; Sekudewicz et al., 2021; Wang et al., 2021b). This explains the presence of small particles on the surface of the fibre and film.

Degraded MPs are therefore of great concern due to their higher adsorption capacity of co-existing pollutants. Although MPs have a high affinity toward hydrophobic organic pollutants, weathering/degradation of MPs introduces oxygen-containing functional groups which increase their polarity and affinity towards hydrophilic pollutants. Thus, they may act as vectors for organic and inorganic pollutants.

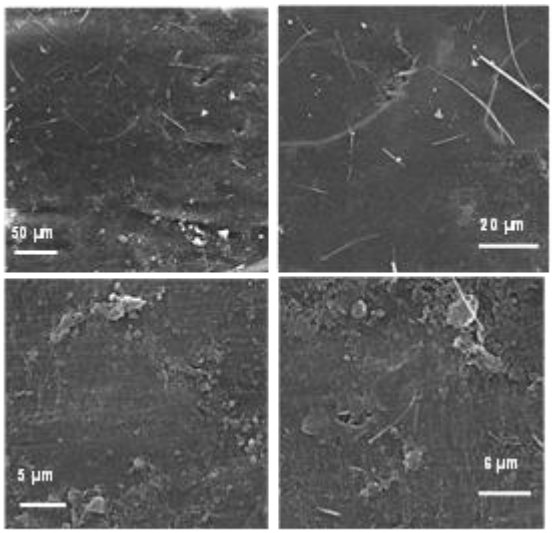
a)



b)



c)



d)

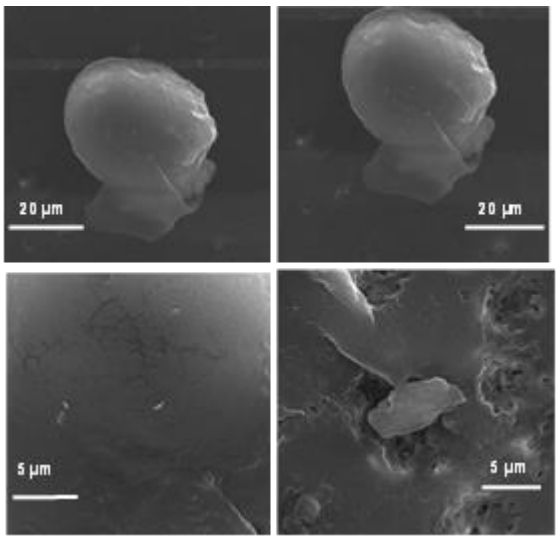


Figure 5.9 SEM micrographs of a) Fibre b) Fragment c) Film, and d) Pellet

5.3 Microplastic chemical composition

5.3.1 Polymer types

In Figures 5.10 - 5.15 below the reference spectra are presented by a red line and the sample spectra are presented by a blue line. Selected KnowItAll® Informatics HQI lists are provided in the tables below. The names of the databases are abbreviated, for instance, the Raman-Forensic-Horiba database is abbreviated as RAX and WSARX for the Raman-Sigma-Aldrich Library of Raman Spectra-Wiley database. The polymer identity number (ID) and name of the reference polymer in the database are also given. Six polymers were identified, namely: polyethylene (PE) (both low-density polyethylene (LDPE) and high-density polyethylene (HDPE)), polypropylene (PP), poly(ethylene-co-vinyl acetate) (PEVA), poly(ethylene-co-acrylic acid) (PEAA), and polystyrene (PS).

The Raman spectra of HDPE and LDPE had noticeable differences. In the CH_2 bending and CH_2 twisting regions, the intensity of the CH_2 bending mode at around 1414.40 cm^{-1} and 1440.51 cm^{-1} was higher for HDPE than the intensity for LDPE at 1418.34 cm^{-1} and 1439.55 cm^{-1}. Due to its crystalline state, PE had a more pronounced doublet of narrow bands in the region 1400-1500 cm^{-1} (Ibrahim and He, 2017; Jumeau et al., 2013; Strobl and Hagedorn, 1978). The differences between HDPE and LDPE are also pronounced in the C-H stretching region. Additionally, the intensity of the symmetric CH_2 stretching mode at 2849.50 cm^{-1} (relative to the asymmetric CH_2 stretching mode at 2882.29 cm^{-1}) is higher for LDPE compared to HDPE with symmetric and asymmetric CH_2 stretching modes at 2881.10 cm^{-1} and 2848.32 cm^{-1}, respectively (Figure 5.10a and Figure 5.10b). Since the C-H stretching (2800-2900 cm^{-1}) and the CH_2 bending regions (1400-1500 cm^{-1}) are sensitive to the density of PE, these regions were used to differentiate between the different crystalline and amorphous phases of PE (Adar, 2019; Ibrahim and He, 2017).

The sample Raman spectra in Figure 5.10c had a 96.8% HQI to the reference spectra of PEVA (Table 5.2). The spectra shared the main peaks at 1062.78 and

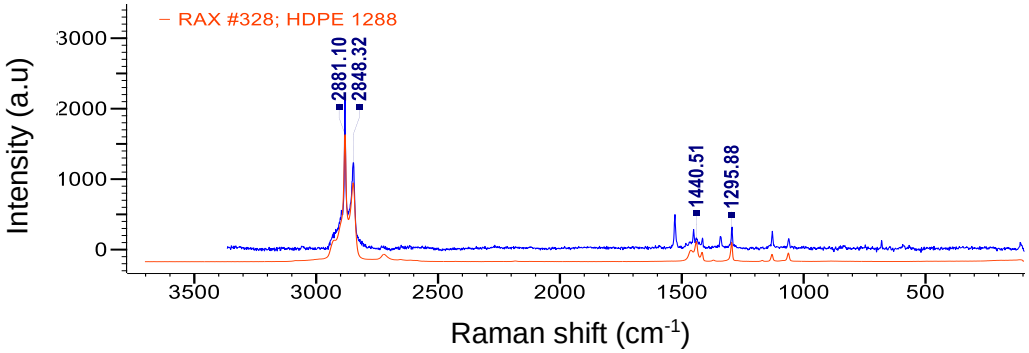
1128.35 cm^{-1} (C-C stretch), 1296.16 cm^{-1} (CH_2 twist), 1418.64-1439.86 cm^{-1} (CH_2 bend), and 2848.85-2881.64 cm^{-1} (C-H stretch) assigned to PEVA (Adar, 2019; Ibrahim and He, 2017).

The sample Raman spectra in Figure 5.10d had a 95.5% HQI to the reference spectra of PEAA (Table 5.2). The vibrations of the C=O bond generate the weak band at 1064.63 cm^{-1}. The spectra also shared the main peaks at 1297.02 cm^{-1} (vibrations of the C-C-H aliphatic moieties), 1440.70 cm^{-1} (bending of CH_2 moieties), and 2851.43-2883.25 cm^{-1} (C-H stretching moieties) were assigned to PEAA (Adar, 2019; Murli and Song, 2010; Rúan-Esparza et al., 2002).

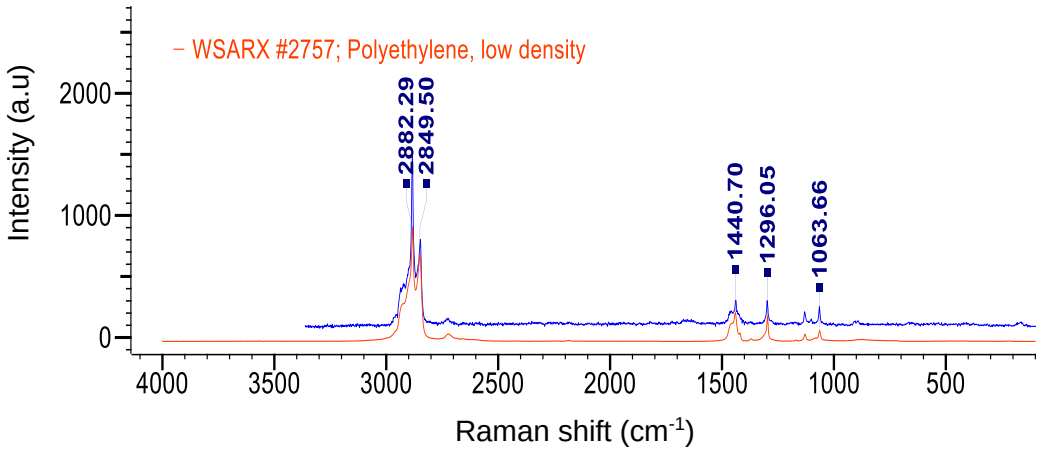
Table 5.2 KnowItAll database HQI values for HDPE, LDPE, PEVA, and PEAA

HQI	Database	ID	Name
93.9	RAX	328	HDPE 1288
93.2	WSARX	2757	Polyethylene, low density
96.8	QRX	1551	Ethylene/vinyl acetate copolymer*resin
95.5	WSARX	2721	Poly(ethylene-co-acrylic acid), 20 wt.% acrylic acid

a)



b)



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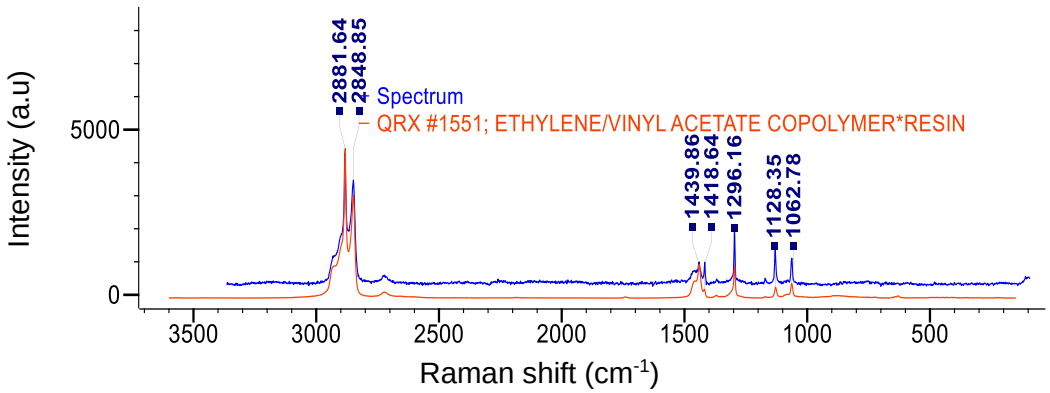

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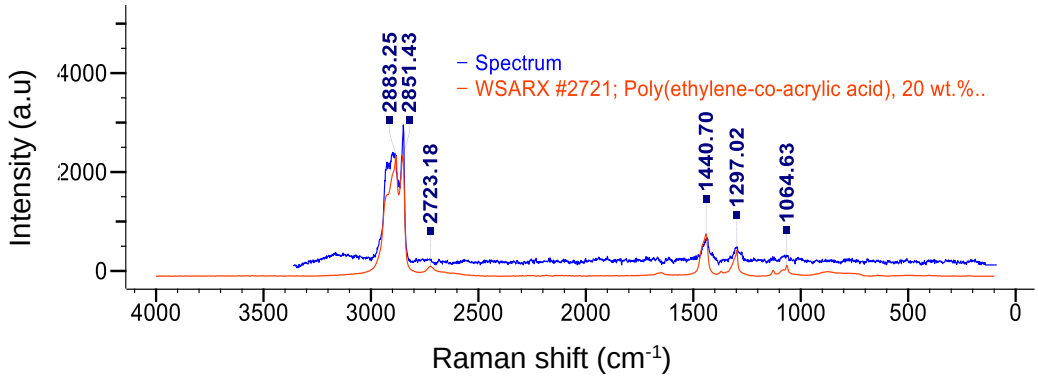


Figure 5.10 Raman spectra of a) HDPE, b) LDPE, c) PEVA, and d) PEAA

PE is the world's most produced polymer, PEs are divided according to their density, melting point, and molecular structure as either LDPE or HDPE. LDPE is a product of a non-catalyzed high-pressure polymerization and is characterized by its highly branched chain structure. As a result of this, their density and crystallinity are reduced while flexibility increases. Applications for LDPE include packaging, bottles, plastic bags, clothes, water tanks, plastic furniture, and films. 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 (Novotna et al., 2019; Zohuri, 2012).

Polymer blending is an economically effective way of developing polymers with distinct properties from those of each constituent. Modifications to PE and PP can be made by changing catalysts, molecular weight, and incorporating linear α-olefin comonomers, and/or blending with other polymers. A lack of reactive groups on the saturated organic backbone has limited the applications of PEs due to the limited compatibility of polyolefins with other polymers. The addition of block or grafting copolymers increases the compatibility and adhesion of polyolefins to other materials (Borsig, 1999; Park et al., 2005; Tham et al., 2015).

PEAA copolymers are typically synthesized through high-pressure free radical polymerization. Increasing the acrylic acid content increases sealing properties and adhesion to polar substrates, aluminum foil, steel, and glass. PEAA plastics have a wide range of applications in flexible packaging material, compatibilizer in multi-layer films, condiment sachets, sports equipment, electronic and electrical cables, and cosmetic bottles (Baughman et al., 2007; Wiggins and Bielawski, 2013).

PEVA is a copolymer of ethylene and vinyl acetate. PEVA is synthesised through free radical polymerisation with different acetate contents. The polar vinyl acetate group increases the plastic’s flexibility, filler compatibility, and fracture toughness. However, it can have low tensile strength and high flammability. PEVA is

used in applications such as agriculture films, footwear, flexible packaging, and electronic and electrical insulation (Bidsorkhi et al., 2014; Meng, 2014; Tham et al., 2015; Wang and Deng, 2019).

Although PE is the most inert of the polyolefins, however, it can slowly degrade in the environment. The C−C backbone does not readily undergo hydrolysis and photo-oxidation due to the lack of UV-visible chromophores. However, impurities or structural defects can act as chromophores. PE may contain a small number of unsaturated carbon bonds such as vinyl groups in HDPE and vinylidenes in LDPE. These sites are readily oxidized by tropospheric radicals to unstable hydroperoxides, which are then converted to more stable UV-absorbing carbonyl groups. An increased rate of photo-oxidation has been reported for LDPE, relative to HDPE, due to the higher frequency of reactive branch points in LDPE (Chamas et al., 2020).

Various sample Raman spectra were superimposable to reference spectra of PP as shown in Figure 5.11. The sample and reference spectra had a 98% HQI and shared main peaks at 399.13 cm^{-1} (wagging of CH_2 moieties or bending of C-H moieties), 807-842.68 cm^{-1} (the rocking of CH_2 and CH_3 moieties, stretching of C-C and C-CH_3 moieties), 1151.24 cm^{-1} (stretching of C-C and bending of C-H moieties), and 1459.80 cm^{-1} (bending of CH_3 and CH_2 moieties), all assigned to PP (Nielsen et al., 2002; Ragusa et al., 2021).

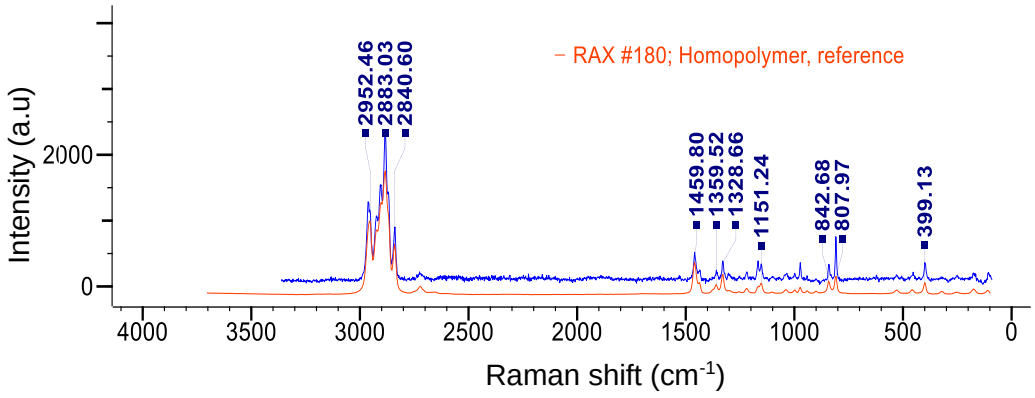


Figure 5.11 Raman spectra of PP

Commercial PP polymers contain endogenous additives added to enhance physical-chemical properties. Conjugated rings present in colourants are highly polarizable and their Raman signals can completely overlay those of the polymeric matrix. Raman spectroscopy can be sensitive to both colourants and polymers (Frère et al., 2016; Imhof et al., 2016; Käppler et al., 2016; Oßmann et al., 2018).

As shown in Figure 5.12, the sample Raman spectrum resulted superimposable to a composite spectrum of Pigment Blue 15 (Copper (II) phthalocyanine) and polypropylene with an 86% HQI. The composite spectra shared the main peaks at 397.32 cm^{-1} (wagging of CH_2 moieties, bending of C-H moieties), 808.06-840.32 cm^{-1} (the rocking of CH_2 and CH_3 moieties, stretching of C-C and C-CH_3 moieties), 1152.14 cm^{-1} (stretching of CC and bending of C-H moieties) and 1459.66 cm^{-1} (bending of CH_2 moieties), which is due to PP and Pigment Blue 15. The main pigment peak was at 1527.40 cm^{-1} due to pyrrole group stretching. Other pigment peaks (679, 746 and 1450 cm^{-1}) were overshadowed by the polymer peaks (Aguayo et al., 2010; Anghelone et al., 2015; Nielsen et al., 2002; Ragusa et al., 2021; Scherrer et al., 2009).

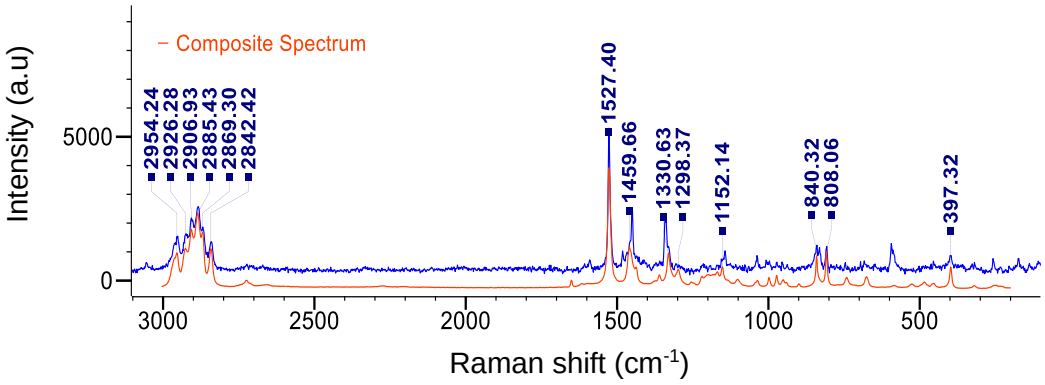


Figure 5.12 Composite Raman spectra of PP-Pigment Blue 15

PP is a semi-crystalline, linear hydrocarbon polyolefin and one of the most versatile polymers available, it is semi-rigid, translucent, tough, and exhibits good heat and chemical resistance. PP has a higher softening point, higher rigidity, and is harder than PE. 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. Applications for PP include automotive, appliances, patio furniture, bottles, films, and straws (Borsig, 1999; Zohuri, 2012).

Phthalocyanines are macrocycles formed by four isoindole units that can coordinate different metal atoms. However, there are metal-free phthalocyanines (Pigment Blue 16) used as pigments. Phthalocyanines are the most used polycyclic synthetic organic pigments. However, Pigment Blue 15 is the most used variant and is available in reddish-blue and green-blue shades used for staining plastics (i.e., PVC, LDPE, HDPE, PP, and PET) (Anghelone et al., 2015; Christie, 1994). Phthalocyanine pigments have been identified in MPs from sediments (Imhof et al., 2016; Van Cauwenberghe et al., 2013), commercially sold salt (Karami et al., 2017), cultured bivalves (Van Cauwenberghe and Janssen, 2014), seawater (Frère et al., 2016), bottled mineral water (Oßmann et al., 2018), and the placenta (Ragusa et al., 2021).

The Raman spectrum of a white pellet was superimposable to the one of a PS as shown in Figure 5.13. The sample and reference spectra had a 97.6% HQI and shared main peaks at 618.98 cm^{-1} (ring deformation mode), 794.82 cm^{-1} (C-H out-of-plane deformation), 1001.26 cm^{-1} (ring breathing mode), 1030.31 cm^{-1} (C-H in-plane deformation) 1155.12-1198.29 cm^{-1} (C-C stretch), 1601.77 cm^{-1} (C=C moieties) and 3053.99 cm^{-1} (C-H stretching), all assigned to PS (Mazilu et al., 2010; Palm, 1951).

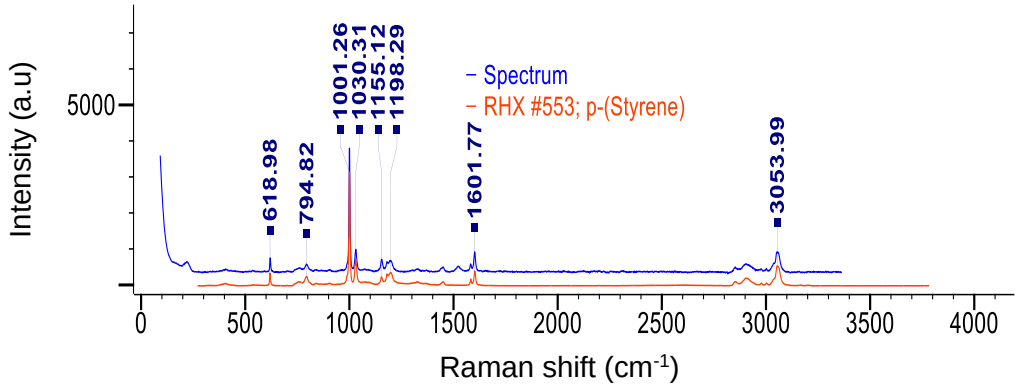


Figure 5.13 Raman spectra of PS

PS is an amorphous thermoplastic polymer used in various applications. PS comes in two forms either expanded PS (EPS) or high-impact PS (HIPS). Commercial HIPS generally contains fractions of polybutadiene (3–10%) and rubber (10–40%) to facilitate greater flexibility and durability. Applications include disposable cutlery, compact disk cases, meat trays, disposable plates, small household appliances, computers, and toys (Grigorescu et al., 2019; Li et al., 2021a). The presence of polystyrene particles is concerning to aquatic biota and by extension humans due to their potential to inhibit cell viability, gene expression, induce pro-inflammatory, alter mitochondrial depolarization and Adenosine triphosphate synthesis when ingested (Forte et al., 2016; Wu et al., 2019).

As shown in Figure 5.14, PP and PE made up 85.2% of identified polymer types. A minor proportion of PEVA (7.4%), PEAA (5.6%), and PS (1.9%) were also detected. The polymer type of MPs is another essential part of determining sources of MPs. The relatively high abundance of PE and PP is consistent with global demands and previous studies (Feng et al., 2021; Lestari et al., 2020; Mao et al., 2020; Singh et al., 2021). In South Africa, Sasol Polymers and Safripol polymerise the ethylene and propylene into polyolefins (PE or PP) (Sadan and De Kock, 2020). Polyolefins are the most ubiquitous synthetic commercial polymers accounting for 50% of the total global plastic demand in 2021 (PlasticsEurope, 2021).

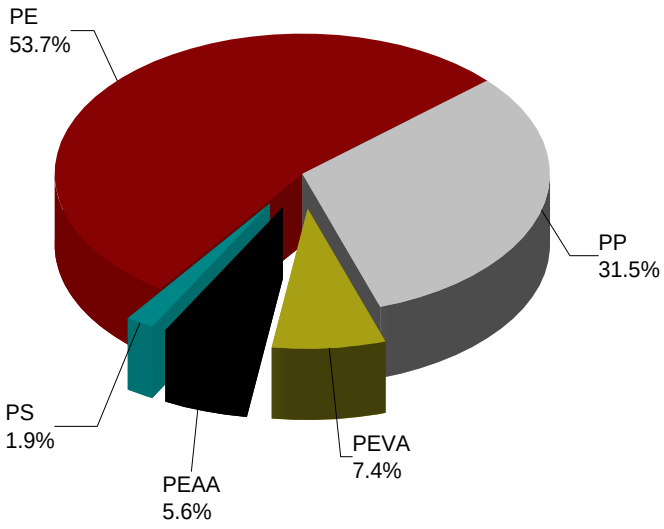


Figure 5.14 Composition of MP polymers

Additionally, the polymer types of the MPs affect and determine their vertical distribution and abundance in the water column due to their different specific densities. LDPE, HDPE, PP, PEAA, and PEVA are categorized as low-density polymers (Table 5.3). Thus, they tend to be buoyant on the water's surface which accounts for their high abundance in the surface waters of the Vaal River. PS is categorized as an intermediate-density polymer which accounts for its low abundance (Choong et al., 2020; Grigorescu et al., 2019; Huang et al., 2021; Lestari et al., 2020; Schwarz et al., 2019). However, its EPS form has a density lower than freshwater and is positively buoyant and may float in surface waters of the Vaal River. Similar assertions were made by Egessa et al. (2020) and Huang et al. (2021).

Table 5.3 Density ranges and classification of polymers

Polymer type	Density range (g/cm^3)	Density class
LDPE	0.89-0.93	Low
HDPE	0.94-0.98	Low
PP	0.90-0.92	Low
PEVA	0.92-0.95	Low
PEAA	0.93-0.96	Low
PS	1.04-1.09	Intermediate

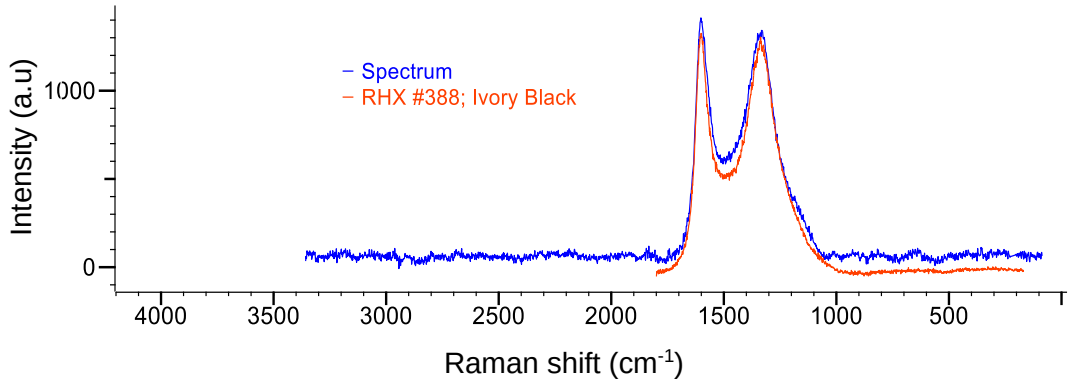
5.3.2 Colourants

Three colourants (Pigment Black 7, Pigment White 6, and Pigment Yellow 83) were identified via Raman analysis. The Raman spectrum in Figure 5.15a had characteristic carbon bands of Ivory black (Pigment Black 7) at 1337.14 cm^{-1} and 1534.30 cm^{-1}. The sample Raman spectra were superimposable to other carbon blacks such as Lamp black, Vine black, and Van Dyck brown. Carbon blacks are carbon-based pigments and reinforcing agents used in polymers and polymer blends. In terms of the

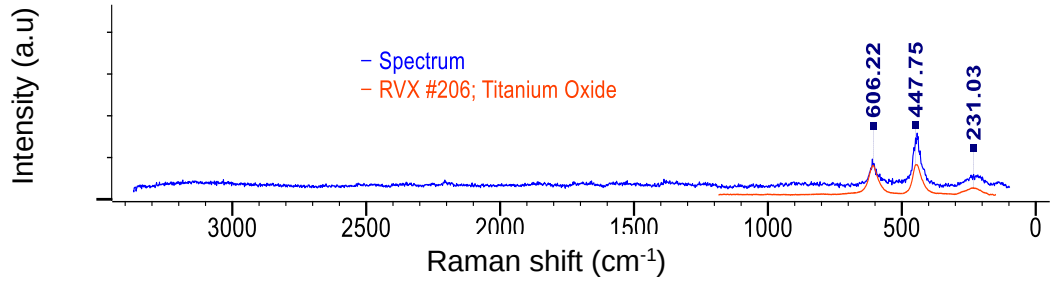
volume of all pigments used by the plastics industry, carbon blacks are the second most widely used colourant in plastics (Coccato et al., 2015; Scherrer et al., 2009; Tomasini et al., 2012). All carbon blacks had HQI values greater than 97%. Black particles generally contain carbon black colourants which exhibit carbon-like Raman spectra and their polymer types are sparsely confirmed via RMS (Coccato et al., 2015; Huang, 2002; Käppler et al., 2016; Lenz et al., 2015).

Titanium dioxide (TiO_2) (Pigment White 6) is the most widely used plastic colourant in the world (Christie, 1994). The sample and reference spectra in Figure 5.15b had an 85.6% HQI and shared main peaks at 203.03 cm^{-1} (multiple phonon scattering processes), 447.75 cm^{-1} (E_g vibrational mode), and 606.22 cm^{-1} (A_{1g} vibrational mode) assigned to TiO_2 (Balachandran and Eror, 1982; Challagulla et al., 2017; Lim et al., 2018). The sample and reference spectra in Figure 5.15c had a 92.45% HQI and shared main peaks at 1259.71-1293.73 cm^{-1} (stretching of C=C moieties), 1406 cm^{-1} (stretching of N=N moieties), and 1601.04 cm^{-1} (stretching of C=C and bending of C-H moieties), all assigned to Pigment Yellow 83 (Frère et al., 2016; Scherrer et al., 2009; Yakes et al., 2017).

a)



b)



c)

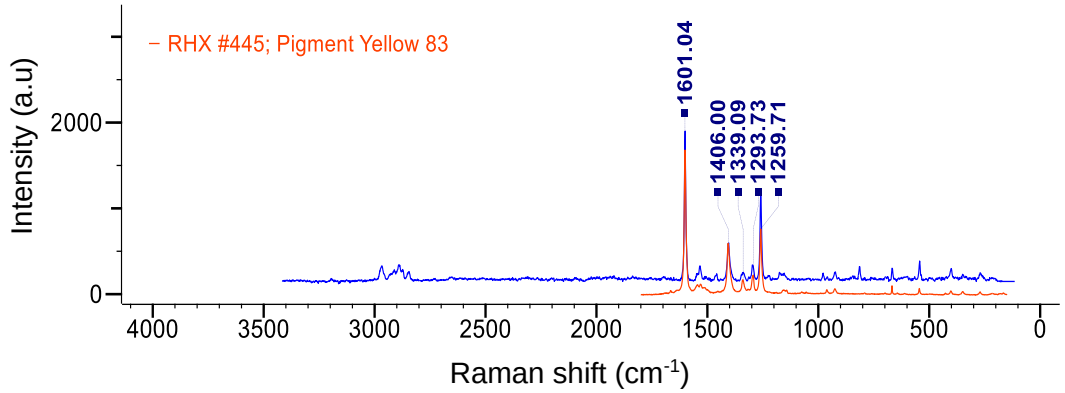


Figure 5.15 Raman spectra of a) Titanium dioxide, b) Ivory black, and c) Pigment Yellow 83

In nearly all applications, carbon black is incorporated into a polymer matrix. However, according to Bott et al. (2014), carbon black particles generally do not leach from plastics into food. Consequently, carbon blacks are not considered a direct genotoxicant or reproductive toxicant (Bott et al., 2014; Chaudhuri et al., 2018).

Titanium dioxide exists in three crystalline structures: two tetragonal phases (anatase and rutile), and an orthorhombic phase (brookite) (Ramos-Corella et al., 2019). Titanium dioxide and titanium mixed-phase oxide compounds are widely in the paint and polymer industry. Titanium dioxide is chemically inert and insoluble in polymers which prompted its replacement of white lead as the principal white colorant. Titanium dioxide is used as a pigment and optical agent, particularly in PVC, PE, and

alkyd resins. Titanium dioxide is extensively used in white carrier bags, window profiles, and others (Kemp and McIntyre, 2001; Lim et al., 2018; Schubert et al., 2019).

Colourants can affect the photostability of the polymer matrix. Generally, most colourants are added increase photostability in addition to the primary function of imparting colour. However, polymer degradation and prolonged exposure to sunlight lead to the generation of reactive oxygen species. This may include superoxides ($O_2^{\bullet-}$), hydroxyl radicals (OH•), and perhydroxyl radicals ($HO_2^{\bullet}$). These reactive oxygen species are responsible for polymer matrix degradation. The two tetragonal phases of TiO_2 have opposite photoactivity when incorporated into polymers. When incorporated into polyolefins, rutile is relatively inactive. However, anatase has photocatalytic behaviour and leads to the degrading of the polymer. Copper (II) phthalocyanine exhibits similar photocatalytic and polymer degradation behaviour (Allen, 1994; Christie, 1994; Kemp and McIntyre, 2001; Liu et al., 2020a).

The compound 2,20-[(3,30-dichloro[1,1-biphenyl]-4,40-diyl)bis(azo)bis[N-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutanamide] (Pigment yellow 83) is a part of group of yellow classical organic pigments known as Diarylide yellows. Diarylide yellows are important yellow azo pigments, used in polyethylene, polypropylene, flexible polyvinyl chloride, printing inks, and industrial coatings (Barrow et al., 2003; Christie, 1994). Pigment Yellow 83 has previously been detected in MPs samples from Lake Gard (Imhof et al., 2013). Pigment Yellow 83 was found to be insoluble in the body and in phagolysosomal solution *in vitro*, which simulates the lung environment. Pigment Yellow 83 is classified as carcinogenic (Hartwig and Commission, 2021). Thus, MPs containing the pigment yellow 83 could pose a threat to a wide variety of freshwater biota in the Vaal River and people that ingest them as a food source.

This study was able to show that MP particles from the Vaal River contained pigments added into polymers during production. Similar observations have been made in MPs from the deep sea (Van Cauwenberghe et al., 2013), as well as in the tissue of

bivalves from the North Sea and the Atlantic Ocean (Van Cauwenberghe and Janssen, 2014).

CHAPTER 6-CONCLUSION AND REFERENCES

The chapter gives concluding remarks on the overall project and assesses whether it was able to achieve the main aims and objectives that the project seeks to meet or address. It then lists literature references used to compile the study. Finally, the appendices containing supplementary data are provided below.

6.1 Conclusions

This study provided new data on the presence and characteristics of MPs in the surface waters of the Vaal River. Methods frequently reported in the literature for sampling, digestion, density separation, and filtration were applied and, in some instances, modified.

Microplastics were detected in all samples. There was a relatively higher abundance in samples close to urban centres and confluences of the Vaal and its tributaries. Wastewater effluent from WWTPs with a history of releasing poorly treated effluent into the river was suspected to be a major source of MPs. The abundance of MPs in the sampled area of the Vaal River was found to be comparable to the findings of other studies.

The prevalence of fibres and fragments is consistent with other studies and indicates secondary sources. The high abundance of smaller MPs (with an average size of less than 1 mm), coupled with the dominance of coloured particles is of particular concern, considering the greater ecological and toxicological implications of coloured MPs of small size on freshwater biota. These implications may extend to a significant portion of the South African population that depends on the Vaal River for food sources.

Four colourants (Pigment Blue 15, Pigment Black 7, Pigment White 6, and Pigment Yellow 83) and five polymers (PE, PP, PEVA, PEAA, and PS) were identified via Raman microspectroscopy. The prevalence of PE and PP is consistent with previous studies, which identified these two polymers as the most abundant in freshwaters. PE and PP polymers are less dense than water, which makes buoyant and more abundant in surface waters. Their abundance also reflects the national production and consumption of plastic, which is dominated by these thermoplastics.

The limitations of this study were mostly related to the challenges associated with microplastic analysis, particularly, the determination of their physical-chemical

properties. For instance, due to the lack of automated instruments that can precisely determine the different shapes, sizes, and colours of MPs, the physical identification of each particle on every filter is often laborious and time-consuming. Additionally, the identification of colours and shapes is often subjective. For example, different analysts may classify different shades of colours differently (lighter shade of red vs pink and darker shade of blue vs green); similarly, pellets and films can be classified as fragments. In chemical characterisation, Raman spectroscopy was hindered by the presence of background fluorescence which interferes with analysis, however, this was overcome by photobleaching and using LabSpec to adjust the spectra's baseline.

Future studies may focus on the characterisation of polymer additives in MPs, results from such a study can be used to narrow down potential sources and evaluate their potential toxicity to biota. Such a study will provide the scientific community with a full perspective of the threats posed by MPs. The Vaal River has essential economic and social value and supports the country's economic heartland. Hence, more efforts should be made to ensure that it continues to support a rapidly developing country. The findings presented in the previous chapter revealed that the Vaal River is considerably polluted, mostly by secondary MPs. Which emphasises the need to strengthen the existing practices of plastic waste management This should also be supported by legislation to integrate MPs as a descriptor of water quality and as a harmful substance within wastewater sludge.

In-order to reduce the extent of MP pollution in South African rivers, more efforts should be directed at creating public awareness about the presence of these ubiquitous pollutants and their harmful impacts on the ecosystem and human health. This may encourage the recycling of plastics and reduce illegal dumping close to freshwater systems. The government should also implement new policies to reduce the extensive use of single-use plastics and explore the feasibility of biodegradable alternatives. Although South Africa has relatively high recycling rates compared to other African countries, more investments should be made to build state-of-the-art

recycling facilities. Additionally, investments should be made to overhaul the country's ageing wastewater infrastructure and upgrade wastewater treatment technologies.

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Appendix A: Volume sampled calculated from flowmeter readings

Sample code	Initial flowmeter reading	Final flowmeter reading	Revolutions	Distance (m)	Volume sampled (m^3)
L1	287571	354598	67027	1801.2	88.5
L2	354798	357245	2447	65.8	3.2
L3	357734	359521	1787	48.0	2.4
L4	359522	361323	1801	48.4	2.4
L5	361236	362026	790	21.2	1.1
L6	362049	392056	30007	806.4	39.6
L7	392051	453944	61893	1663.3	81.7
L8	453936	532428	78492	2109.3	103.6
L9	523439	553779	30340	815.3	40.0
L10	713303	774960	61657	1656.9	81.4
L11	597773	656054	58281	1566.2	76.9
L12	646195	712994	66799	1795.1	88.2
L13	553689	597782	44093	1184.9	58.2
L14	774984	847238	72254	1941.7	95.4
L15	847238	921274	74036	1989.6	97.7
L16	921270	860807	60463	1624.8	79.8
L17	860807	801595	59212	1591.2	78.1
L18	32095	76380	44285	1190.1	58.4
L19	76393	114374	37981	1020.7	50.1
L20	114374	148194	33820	908.9	44.6
L21	148202	216716	68514	1841.2	90.4
L22	216716	313116	96400	2590.6	127.2

Appendix B: Area sampled calculated from GPS coordinates

Sample code	Latitude	Longitude	Distance (km)	Area sampled (km^2)
L1	-26.72876	27.99241	1.63	4.1×10^{-4}
L2	-26.71453	27.99648	2.11	5.3×10^{-4}
L3	-26.69557	27.9988	2.06	5.2×10^{-4}
L4	-26.68169	27.985	2.22	5.6×10^{-4}
L5	-26.67002	27.96685	2.64	6.6×10^{-4}
L6	-26.69635	27.93224	2.45	6.1×10^{-4}
L7	-26.70701	27.90505	2.95	7.4×10^{-4}
L8	-26.71888	27.89533	1.63	4.1×10^{-4}
L9	-26.73957	27.88345	2.58	6.5×10^{-4}
L10	-26.74665	27.86203	2.27	5.7×10^{-4}
L11	-26.75035	27.83427	2.79	7.0×10^{-4}
L12	-26.74984	27.80157	3.25	8.1×10^{-4}
L13	-26.75127	27.78104	2.05	5.1×10^{-4}
L14	-26.76563	27.76807	2.05	5.1×10^{-4}
L15	-26.7866	27.768	2.32	5.8×10^{-4}
L16	-26.79814	27.76349	1.36	3.4×10^{-4}
L17	-26.78595	27.74847	2.01	5.0×10^{-4}
L18	-26.767881	27.738719	2.23	5.6×10^{-4}
L19	-26.761625	27.714467	2.51	6.3×10^{-4}
L20	-26.763786	27.684917	2.95	7.4×10^{-4}
L21	-26.762375	27.707019	2.20	5.5×10^{-4}
L22	-26.761311	27.728019	2.09	5.2×10^{-4}