ECOLOGICAL AND HUMAN HEALTH RISK ASSESSMENT OF MICROPLASTICS IN THE DIEP AND PLANKENBURG RIVERS, CAPE TOWN, WESTERN CAPE

Report to the
Water Research Commission

by

BO Opeolu[1*], O Pereao[1], KM Apetogbor[1], AB Khan[1], S Mlonyeni[1], C Sparks[1], B Brooks[2], D Jungmann[3] and H Boernick[3]

[1] Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, South Africa
[2]Department of Environmental Science, Baylor University, Waco Texas USA
[3]Technische Universität Dresden, Germany

* Project Leader

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Obtainable from
Water Research Commission
Bloukrans Building, Lynnwood Bridge Office Park
4 Daventry Street
Lynnwood Manor
PRETORIA

orders@wrc.org.za or download from www.wrc.org.za

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EXECUTIVE SUMMARY

BACKGROUND

The global annual plastic demand has exponentially increased with the growth and accumulation of plastic waste in the freshwaters becoming a critical concern due to the increase in the plastic mass usage. The global plastic production is controlled by mostly six types of polymers such as polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PU) and polyethylene terephthalate (PET) while materials used for packaging are the main application constituting nearly 40% of the plastic market. Microplastics (MPs) are polymers with different densities classified into two different types which include large microplastics range from 1 to 5 mm, and small microplastics from 20 µm to 1 mm. Microplastic sources include virgin pellets, weathering and wear of plastic goods, mismanaged plastic waste on land or at sea, leakage from transport of goods, fishing equipment, and wastewater effluents all contribute to macro- and microplastics in the environment. The nature and location of plastic sources as well as environmental conditions are influencing and affecting the occurrence and amount in the aquatic environment. The potential hazards of plastics and the toxic impacts are still not well characterized and may have contributed to the microplastic's complex nature. The intake of microplastics have been demonstrated for a broad range of filter organisms, invertebrates, fish, mammals, and birds. Physical stress can originate from microplastics by entanglement or intake causing gastrointestinal obstruction, suffocation, or even starvation. Plastics usually contain additive chemicals, giving the product certain properties, which can cause biological effects in the host organism. Monomers used to synthesize the polymer and by-products can potentially also be released from plastics. Besides carrying additive chemicals and monomers, chemicals from the surrounding environment, like persistent organic pollutants, polyaromatic compounds, and metals, can sorb to the particle surface or partition into the bulk polymer. Plastics can also carry invasive species to new environments and act as a hard substrate for organisms. The assessments of the amount of plastic are scarce but important and the introduction of plastics into the environment can come from production plastics (called virgin pellets), after-use products and landfilled plastic waste. To fully assess the risks of microplastics in Cape Town, Western Cape freshwaters, it is important to consider the fate of microplastic particles which enters the environment in the Diep and Plankenburg Rivers including the ecological and human health risk assessment of the microplastics.

AIMS

The following were the aims of the project:

1. Quantify the amount of plastics present in the Diep and Plankenburg Rivers the contribution of wastewater treatment plant to plastic pollution in the Diep River.
2. Determine ecological and human health risks of microplastics in aquatic ecosystems.
3. Engage with stakeholders (Government, citizens, academia) through workshops, symposia, conferences, and public enlightenment campaigns.

METHODOLOGY

The sampling points were selected based on possible routes of microplastic entry, including human and industrial activities. Sampling was undertaken over four seasons (summer, spring, autumn, and winter) for both the Diep and Plankenburg Rivers. For the Diep River, 100 L water sample was filtered in five replicates of 20 L per site while 30 L samples in three replicates of 10 L were processed onsite for the Plankenburg River. The onsite water samples were filtered in through 250 µm stainless steel sieves. The residual particles on the sieve was transferred and stored in a small glass jar at 4ºC and taken to the laboratory for further analysis. Another 20 L sample of the surface water was collected at each sampling site and taken to laboratory for further analysis. The 20 L brought into the laboratory was filtered in five replicates of 4 L per site through 20 µm mesh. Five sampling points were selected for the Diep River and four sampling points were selected for the Plankenburg River based on the land use practices determining the occurrence of microplastic items at the different sites. Sediment samples were collected using metal scoops to approximately 5 cm depth at each sampling site to achieve three replicate samples per site. Sediment samples were transferred into aluminium foil, stored in sealed plastic bags, placed in an ice chest, and transported to the Cape Peninsula University of Technology Microplastic Laboratory. It was then air dried in white dissecting trays prior to analysis and covered to avoid contamination. The collected samples were processed to isolate the microplastics. MPs was extracted from the 20 L offsite sample taken to the laboratory by filtration through a vacuum pump system with 20 µm mesh. Alkali digestion was used to degrade organic matter prior to the isolation process. Residues of samples processed onsite was already stored in glass jars and labelled appropriately. The residues was transferred into 500 ml glass beaker with 10% Potassium hydroxide (KOH) in a ratio of 1:3 (w/v) and covered with aluminium foil for 24 h in the oven at a temperature of 50°C. Hypersaline solutions (NaCl 360 $g \cdot \ell^{-1}$) was added to the digested sample, stirred vigorously for 2 min, and was allowed to settle for 15 min and then filtered through a vacuum pump system with 20 µm mesh. This extraction process was repeated three times for each sample. The filters were then placed in clean petri dishes for further analysis. The dried meshes in the pre-cleaned petri dishes were examined for microplastics based on physical appearance under a Stereo microscope (BS-3060CT, Bestscope, China). Particles were categorised based on their morphological characteristics (size, shape, and colour). Microplastics particles larger than 500 µm were analysed using Fourier transform infrared (FTIR). Biotests were used to determine the ecological health of the Diep and Plankenburg Rivers. Information obtained from ecotoxicity tests was used to determine the microplastic pollution load indices of the rivers. Ames testing was used to screen water samples for mutagenicity.

RESULTS AND DISCUSSION

Spatial and temporal distributions showed variable patterns of abundance of MPs in the Diep and Plankenburg Rivers. Sampling sites with various anthropogenic activities showed an increase in MP occurrence. Spatial and temporal distributions showed substantial differences based on the proximity to urban/industry areas and wastewater treatment plantsThe observations were due to differences in seasonal conditions, water flow and volumes, and anthropogenic activities in the vicinities of the sampling sites. Polystyrene and polyethylene are the most common polymers detected in Diep River, while polyethylene terephthalate was the most common in Plankenburg River. Temperature increases negatively affected exposed organisms. The correlation analysis of the physicochemical properties of water samples and microplastics suggested critical adverse implications

with climate change. Toxicities were observed on organisms exposed to environmental water samples with and without microplastics. Both rivers were classified as polluted using the pollution load index model.

GENERAL

The occurrence of microplastics in freshwater systems is less understood than in marine environments in South Africa. Hence, the present study aimed at providing new insights into microplastics abundance and distribution in the Diep and Plankenburg Rivers, Western Cape, South Africa. This study provided an understanding of microplastics' spatial and temporal distribution in water and sediments by evaluating different sites of the Diep and Plankenburg Rivers. This study emphasized the importance of rivers as a pathway for microplastics exposure to man and the environment.

CONCLUSIONS

This study provided new insights into MPs abundances and distribution in water and sediments of the Diep and Plankenburg river in Western Cape. Spatial and temporal distributions showed substantial differences based on the proximity to urban/industry areas and wastewater treatment plants. MPs were also detected in freshwater systems near the wastewater treatment plants as well as freshwater environments in cities with dense population and large agricultural practises, and industrial activities. MPs with particle size less than 1 mm were most abundant and fibre was a common polymer shape in surface waters and sediments of several typical freshwater systems sites in the Diep and Plankenburg river in Western Cape. Among all the detected MPs samples, polymer types of Polyethylene terephthalate (PET) were the most common in Plankenburg river. Pre-treatment methods used include density separation, filtration, acidic/alkaline digestion, which efficiently removed impurities from MPs samples. Advanced characterization technologies, such as FTIR, were employed to analyse MPs' chemical composition and other properties. Water acidification will further exacerbate microplastics effects in water bodies considering the negative correlation between microplastics occurrence and pH values. Higher values of temperature may also increase the sediment burden of microplastic pollution with implications for filter feeders and benthic organisms. The battery of biotests showed variation in the levels of toxicity of river water samples over the four seasons. This affirms the need for routine biotest analyses in addition to the physicochemical assessment of rivers. This is the first study to screen the mutagenicity potential of the Diep and Plankenburg Rivers in Western Cape in Western Cape, South Africa.

RECOMMENDATIONS

A 20 L sample per site filtered through a 20 µm mesh is sufficient for excellent MP recovery from water. This is ideal for microplastic sampling of surface water, which is especially useful for developing countries with limited equipment. The mutagenicity is an indication of the possible mutagenic effects that can be experienced by human exposure to freshwater. Currently, the South African Department of Water and Sanitation does not require ecotoxicology testing, it is important to regularly include ecotoxicological (acute and chronic) bioassays in national environmental monitoring programmes for water quality management. The tests can used to broaden the detection capacity and to evaluate holistically, ecological and human health risks of freshwater ecosystems especially in developing countries where indiscriminate disposal of wastes is on the increase.

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Reference Group	Affiliation
Dr Eunice Ubomba-Jaswa	Water Research Commission
Mrs Bettina Genthe	Independent Consultant
Dr Brenda Moodley	University of KwaZulu-Natal
Dr Thabo Nkambule	University of South Africa
Dr Holly Nel	University of Birmingham
Prof Joshua Olowoyo	Sefako Makgatho University
Ms Khaya Mgaba	Rhodes University
Mr Peter Manyara	IUCN
Dr Bridget Shaddock	WSP Group Africa (Pty) LTD
Prof. Henk Bouwman	North-West University
Dr Paul Mensah	Rhodes University
Ms Penny Jaca	Water Research Commission

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ACRONYMS & ABBREVIATIONS

FTIR	fourier transform infrared
HD	high density
LD	low density
mm	millimetre, 10^{-3} metre
nm	nanometre, 10^{-9} metre
PE	Polyethylene
PES	Polyester
PET	polyethylene terephthalate
POPs	persistent organic pollutants
PS	Polystyrene
PVC	Polyvinylchloride
UV	Ultraviolet
WWTPs	wastewater treatment plants
µm	micrometre, 10^{-6} metre

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Global population continues to rise at exponential rates especially in the poorer countries of the world resulting in greater demand for food, shelter, goods, and services. This, in turn, leads to more pressure being exerted on the earth's natural resources such as land, water and minerals. Agricultural and manufacturing sectors need several inputs that range from land to agrochemicals, high-yielding, and disease-resistant crops/animals to toxic industrial chemicals, extracted natural resources, water and much more. All these activities generate wealth, improved quality of life but with deleterious effects on man and ecological systems.

Although water is the most abundant resource on earth, a limited amount is available for human use. Water covers about 73% of the earth surface and significant amounts of water are also components of the atmosphere and terrestrial ecosystems. About 97% of global water is in the marine system with only about 3% freshwater resources. The 3% freshwater can further be grouped into polar icecaps and glaciers (79%), groundwater (20%) and accessible water 1%. And the 1% accessible water is distributed into lakes (52%), soil moisture (20%), atmospheric water vapour (8%), water in biomass (1%) and river water (1%). Therefore, the most visible and most available part represents less than 0.02% of the total water supply on earth.

South Africa is a water-scarce country; climate change has further exacerbated the stress exerted on freshwater resources. Climate change is the result of a combination of global warming due to greenhouse gases emissions from fossil combustion. It is characterized by melting glaciers, sea level rises, erratic rainfall patterns, flooding, and extreme weather events, among others (Lelieveld et al., 2019). Anthropogenic activity coupled with industrialisation has been identified as the main cause of water quality degradation globally. Water availability and quality are part of the most complex problems of the 21st century due to urbanisation, population explosion, drought, flooding, conflicts, and several other issues associated with climate change.

Ecological health is therefore threatened by both natural and man-made factors. Natural issues that negatively affected ecosystems include volcanoes, mega-tsunamis and regional climate change in addition to anthropogenic factors from industrial, agricultural and domestic activities. In coming years microplastics pollution and infectious diseases, among others were identified as future threats to ecosystems (Sutherland et al., 2012). Emerging contaminants were defined as compounds that were not usually considered as important but capable of causing adverse ecological and human health risks. Microplastics, an emerging contaminant which has been associated with endocrine disruption, is of interest due to recent global awareness and concerns. Plastic materials play an essential role in the modern society and this has increased worldwide synthetic polymer production in the last decade to about 359 million tonnes in 2018 (PlasticsEurope, 2019). Plastic progressively fragments into smaller pieces (Ryan et al., 2009) and the occurrence of these plastic fragments has been well documented with a large quantity growth in all freshwater and marine environments (Dris et al., 2015; Eerkes-Medrano et al., 2015). Microplastics (MPs) are considered as particles lesser than 5 mm in size and have become an emerging worldwide issue in aquatic environments (Eerkes-Medrano et al.,

2015). MPs are easily ingested and have the potential to accumulate in both aquatic food web and biota (Carbery et al., 2018; Pittura et al., 2018).

Microplastics in the environment exist in various sizes, and the majority of microplastics found in the environment have irregular shapes: they often exist in the form of fragments, fibres or films (Nan et al., 2020). MPs and NPs generate different types of environmental problems compared to macro- and meso-plastics pollution due to their size difference (Llorca et al., 2020). The unique characteristics, such as high durability, light weight and low manufacturing costs, make these materials indispensable in our daily lives, but they have also increased environmental contamination. Once disposed, plastics undergo morphological and chemical transformations due to the action of physical factors (wave and wind), photo (UV radiation), and biodegradation (microorganism) (Lara et al., 2021). MPs and NPs are made of different polymers and can contain many additives that are added during plastic production, and their leaching can significantly alter their ecotoxicological profile (Schrank et al., 2019). They also differ in terms of the degree of degradation, associated microorganisms, and the number and concentrations of pollutants that are adsorbed from their surroundings onto their surface (Acosta-Coley et al., 2019; G. Liu et al., 2019). All these factors affect the behaviour, fate, and ecotoxicity of microplastics (Kalčíková et al., 2020). The pervasiveness of microplastics, which can absorb pollutants, has a certain impact on pollutant migration in natural waters (Bao et al., 2020). Due to the highly hydrophobic properties, microplastics can adsorb substantial hydrophobic organic contaminants (HOCs) such as polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) (Pereao et al., 2020b; Zuo et al., 2019), thus strongly affecting the distribution and fate of organic pollutants in environments. When microplastics are mistakenly ingested, those HOCs might be released in the body of the biota, whose growth, metabolism, sexual development and function could thereafter be affected (Ziajahromi et al., 2017a).

Microplastics have a strong mobility due to their low density, thus they could desorb persistent organic pollutants (POPs) to the surrounding marine compartments and act as the source of organic contaminants (León et al., 2018). The sorption of contaminants on particles that are <5 mm in size (referred to as microplastics) is particularly high. Within a few weeks microplastic particles can accumulate pollutants on the particle surface at concentrations that are orders of magnitude greater than in the surrounding water (Hirai et al., 2011) and following the sorption onto the particles, the contaminants are carried along with the plastics from their original source (Van et al., 2012). Thus, microplastics have a large capacity to facilitate the transport of contaminants through the aquatic environment.

Plastic-associated toxicity may be caused by the leaching of chemicals, including residual monomers, starting substances, solvents, and catalysts, as well as additives (e.g. antioxidants, dyes, biocides, plasticizers) incorporated during compounding and processing (Andrady, 2015; Lambert et al., 2017). MPs can be ingested by a variety of organisms, such as zooplankton, bivalves, worms, crustaceans, fish, and mammals (Bejgarn et al., 2015; Browne et al., 2013). Ingestion of MPs causes physical damage, including blockages or internal abrasions. In addition to the direct exposure, researchers have reported that the additives leached from MPs may also cause toxic effects (Koelmans et al., 2014). The need for regulatory and management framework (Analytical methods needed for qualitative and quantitative assessment of microplastics in water samples) was identified as a gap (Geissen et al., 2015).

MP data for South African aquatic environments are very scarce but developing (Naidoo et al., 2019) and there is a growing understanding of microplastic pollution in South Africa but the focus has been more on the marine environment (Lamprecht, 2013; Nel and Froneman, 2015) and only a few reports are currently available on freshwater microplastics including WWTP. A significant number of other available research on plastic particles in the aquatic environment had focussed on particles greater than 5 mm (Fazey and Ryan, 2016) while a few of the studies evaluated plastics along the coastline of different parts of South Africa (Naidoo et al., 2015). The effects of microplastics on the aquatic ecosystems of South Africa are still largely speculative because of the limited reports on the toxicological risks of microplastics on aquatic organisms. A significant body of knowledge on the qualitative and quantitative analysis of microplastics has been generated elsewhere and a few in South Africa. There is however a dearth of data on ecological and human health risks of microplastics. A recent work already identified the need for data on human and ecological implications of microplastics in South Africa (Naidoo and Rajkaran, 2020). This study is therefore very timely, important, and relevant for the South African water sector. It will provide empirical information on the microplastics pollution status of the Diep and Plankenburg Rivers as well as potential associated risks to man and the environment for policy decisions. It will also contribute to capacity development in microplastics analysis and risk assessment competencies in South Africa.

Recent studies have demonstrated that urban drainage channels transport conventional (e.g. physical, chemical and microbiological) and emerging pollutants (e.g. pharmaceuticals and illicit drugs) to the ocean (Roveri et al., 2020b, 2020a) via river. Therefore these complex mixtures may contain compounds such as microplastics with ecotoxicological (Gosset et al., 2016) and genotoxicity (Baršiene et al., 2012) potential which could cause a detrimental and harmful effect on the aquatic biota (Kalmykova et al., 2013). One way of assessing the mutagenic potential of chemical compounds is by the use of bioassays (in vitro) such as the Ames (Salmonella/microsome) test (Khallef et al., 2019). The Ames test is a quick and convenient assay specifically designed to detect a wide range of chemical substances that can produce gene mutations, such as polychlorobiphenols (PCBs) and polycyclic aromatic hydrocarbons (PAH) (Baršiene et al., 2012). These assays are easy to implement and require few financial resources, and are therefore suitable for developing countries (Roveri et al., 2021). There is scarcity of studies focusing on the surface waters to detect the mutagenic potential of these complex mixtures which carry microplastic load into the ocean via the river. One of the objectives of this study is to characterize and investigate the mutagenicity (Ames Salmonella/microsome test) of the river which carry microplastic load through the river into the ocean. This new knowledge will allow us to understand the potential risk for the human and environmental health arising from microplastic in rivers.

1.2 PROJECT AIMS

The following are the aims of the project:

1. Quantification of microplastics in the Diep and Plankenburg Rivers and the contribution of wastewater treatment plant to plastic pollution in the Diep River
2. Evaluation of ecological and human health risk potentials of microplastics in the Diep and Plankenburg Rivers
3. Engagement with stakeholders (Government, citizens, academia) through workshops, symposia, conferences, and public enlightenment campaigns

1.3 SCOPE AND LIMITATIONS

This study was carried out on the Diep within (Milnerton) and Plankenburg (Stellenbosch) Rivers and in Western Cape Province. The study emphasis was on only plastic particles in the size range of ≤ 5 mm. The mesh size was limited to 250 µm mesh for on-site filtration due to sampling and time constraints. *Daphnia magna* (crustaceans), *Raphidocelis subcapitata* (algae) and *T. thermophila* (protozoan) were used as models for ecotoxicological studies. Mutagenicity potential of microplastics was assessed using Ames test. Two master's degree dissertations were authored, and three articles will be published in DHET accredited peer-review journals. Information generated from this study will be available for government agencies and other stakeholders for policy development, policy decisions, policy implementation and other mitigation strategies.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Microplastics (MPs) are polymers with different densities classified into two different types which include large microplastics range from 1 to 5 mm, and small microplastics from 20 μm to 1 mm. They have their origin in cosmetics, cleanser products and nurdles (primary MPs) and in the fragmentation and erosion of plastics pieces and debris (secondary MPs) (Auta et al., 2017; Pereao et al., 2020b). Microplastics, come from a range of sources such as the degradation of plastic wastes, synthetic fibres released during textile washing processes, plastic microbeads in personal care products and industrial plastic pellets. Microplastics presence in the freshwater system has raised concern over its ecological and toxicological effects on freshwater organisms and the ingestion of microplastics by biota have been identified to have some physical and hormonal effect. Different studies also suggest that ingested microplastics can release chemical substance to freshwater organisms with adverse implications. Despite the different research on microplastics effect in marine and freshwater, its eco-toxicological effect in freshwater is still barely understood. This review is a summary of the source, occurrence, and distribution, characterization as well as the ecological and human health effects of microplastics in freshwater systems.

2.2 ENDOCRINE-DISRUPTING CHEMICALS AND EMERGING CONTAMINANTS IN WATER

Microplastics have become the focus of several studies recently due to MP detection in groundwater, surface water, air, oceans, sediments, and soils (Alam et al., 2019; K. Liu et al., 2019; Mintenig et al., 2019). Plastics break down into smaller microplastic pieces with size range between 0.001 mm to 5 mm through different processes (Nel et al., 2018; Shim et al., 2017). These particles are considered an emerging threat to human health, since they have already been found in the human lung (Amato-Lourenço et al., 2021; G. Chen et al., 2019) and due to their morphological and chemical properties, such as relative high surface area, hydrophobic characteristics and high mobility, MPs have been reported to sorb and transport contaminants through freshwater and marine systems as well as leaching chemicals incorporated during manufacturing to the environment (Q. Chen et al., 2019).

A particular class of environmental contaminants, endocrine disrupting compounds (EDC) are commonly found in water and can cause great harm even at low concentrations affecting neurological, immune, and reproductive systems of animals such as rats (Zhou et al., 2021), tadpoles (Corrie et al., 2021), and daphnia magma (Rodrigues et al., 2021), and human beings (Duarte et al., 2021). EDCs are substances that alter the function of the endocrine system. They are very significant at ultra-trace levels due to their potential adverse effects on the biotic components of ecosystems (Segner, 2005). They include estrogenic and androgenic chemicals (such as alkylphenols, polychlorinated compounds, polybrominated diphenyl ethers, phthalates, and steroid sex hormones), etc. which bind to oestrogen or androgen receptors interfering with the action of endogenous steroid hormones (Ferraz et al., 2007). Other EDCs include heavy metals (As, Cd, Hg and Pb), organotin compounds used in PVC for water pipe manufacture, detergent breakdown products such as nonylphenol, bisphenol A, a compound employed in the manufacture of polycarbonate plastics, used in baby bottles and teats (Falconer et al., 2006; Ferraz et al., 2007).

EDCs exhibit disruption activities by either of three ways. First, because of their molecular structure, they may bind to hormone receptors thereby mimicking or antagonising the action of the natural ligand (Barceló and Kettrup, 2003; Vogel, 2005). It is also possible for the chemicals to indirectly affect concentrations of hormones by altering their synthesis or metabolism. And lastly, by interfering with signals between different components of the hypothalamus-pituitary-endocrine gland axes (Dawson, 2000). The modes of action of these chemicals through the endocrine system trigger toxicity in specific endocrine target organs (Gelbke et al., 2007). Common sources of exposure that contribute to our total toxic load of endocrine disruptors include drinking water, lawn chemicals, consumer products, household insecticide use, plasticizers, etc. (Scippo et al., 2004; Vogel, 2005). Domestic and industrial wastewaters are significant sources of EDCs to the receiving surface, coastal waters and regional environments as well as the uncontrolled domestic and industrial discharge to waterways in developing countries (Arditsoglou and Voutsa, 2008; Falconer et al., 2006). Surface runoff from cities, roads and waste landfills also contribute to these chemicals in the aquatic environment (Dmitruk et al., 2008). The effects of EDCs on human body differ substantially from chronic or acute exposures. They may cause cancer, physiological birth defects, gene mutation, cell damage or acute health effects such as nausea, vomiting and even, death. Exposure to EDCs during critical stages of development can lead to gross birth defects or an increase in cancer susceptibility, but also, more subtle and possibly more pervasive problems such as infertility in an adult, attention-deficit/hyperactivity disorders, immune system deficiencies, the feminization of males, masculinization of females and bioaccumulation in animals near the top of the food web (Fenner-Crisp et al., 2000; Vogel, 2005). Chemicals may exhibit endocrine disruption only over longer periods, or selectively during certain stages of development, or in later generations.

Microplastics (MPs) could adsorb endocrine disrupting compounds (EDCs) (Guedes-Alonso et al., 2021). Even though the interaction mechanisms involving microplastics and several chemical compounds remained poorly understood, however, studies on ten types of microplastics indicated that polyamide microplastics had a greater affinity to 17β-estradiol (E2) suggesting that, besides microplastic hydrophobicity, hydrogen bonding also played an important role in the sorption process (F. fei Liu et al., 2019) and the same conclusion was found by Jia Li et al., (2018) on studying the sorption of different antibiotics on polyamide microplastics. The adsorption and desorption of 17β-estradiol and 17α-ethynylestradiol on MPs in seawater by Lu et al., (2021) determined that the highest adsorption rates were reached at pH 8.0, which is a serious ecological problem because the steroid hormones adsorbed on MPs can be transported through aquatic systems, which would increase the combined harmful effect of both pollutant types.

2.3 CLASSIFICATION OF MICROPLASTICS IN THE AQUATIC ENVIRONMENT

Plastics have numerous benefits within society and had registered a production increase from 0.5 million tonnes in 1950 to over 360 million tonnes in 2018. However, the accumulation of plastic debris in the terrestrial environment is considered as one of the contributors to water pollution. The presence of plastics in the environment is currently a priority research area in environmental sciences globally. Plastics' occurrence in the environment is currently a global concern (Alomar et al., 2016; J. Li et al., 2016) and this growing fear is due to evidence from available empirical data that supports occurrence and negative effects of plastics in aquatic systems. About 50% of all plastic products manufactured

globally are estimated to be disposable (J. Li et al., 2016) and more than half of the world's ocean litter has been reported to be plastic fragments (Nobre et al., 2015).

Large plastics waste items undergo fragmentation under physicals action, ultraviolet radiation, oxidative properties of the atmosphere and hydrolytic properties of water bodies (Andrady, 2011; Auta et al., 2017; Webb et al., 2012) and are progressively broken down into smaller particles known as microplastics. Engineered microplastics may also be produced for use in household cleaning and personal care products, among others. Microplastics are defined as polymers with different densities and are classified into two different types which include large microplastics range from 1 to 5 mm (Figure 1), and small microplastics from 20 µm to 1 mm (MSFD, 2013; Wagner et al., 2014) in the environment. They occur as macroplastics, mesoplastics, microplastics and nanoplastics in the aquatic environment based on particle size but there is currently no specific international standard (SI) size definition of microplastics (Pagter et al., 2018).

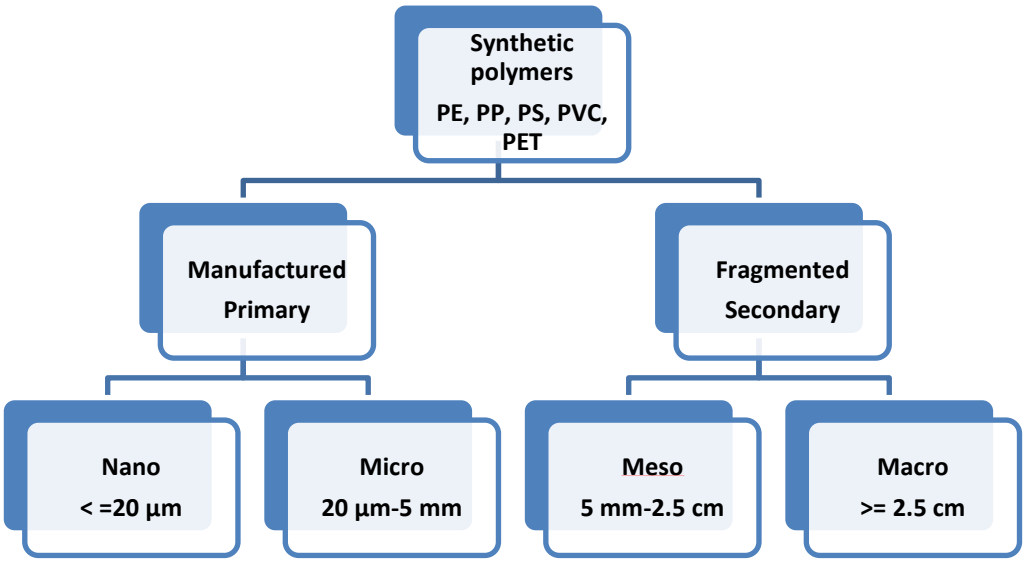

Figure 2.1: Size classification of microplastics in environmental samples

Microplastics can further be categorised into primary and secondary microplastics (Lei et al., 2018; Ma et al., 2016). Primary microplastics are intentionally manufactured as small size with small spherical pellets which are mainly used in textiles, medicines and other personal care products such as facial and body scrubs (Cole et al., 2011; Pereao et al., 2020b), and pre-production pellets (nurdles) (Storck, F. R. and Kools, 2015). Secondary microplastics, such as fibres, fragments, and flakes, are derived from large plastic debris fragmentation due to photo-degradation, physical, chemical and biological interactions (Galgani et al., 2013; Meeker et al., 2009), and from other mechanisms, such as ultraviolet (UV) light and hydrolysis (Guo et al., 2020). Other sources of secondary MPs may be mismanaged plastic litter, industrial resin pellets, washing of synthetic textiles (Boucher and Friot, 2017; Browne et al., 2011; Eerkes-Medrano et al., 2015), tyre debris, and road marking paints (Boucher and Friot, 2017). Eriksen et al., (2013) reported that the majority of microplastics in the freshwater environment are secondary MP and this number would increase along with an increase in

the input of large plastic debris from different origins due to continuous transformation of secondary microplastics (Cole et al., 2011).

Microplastics are defined by their size, origin, shape, polymer composition, and colour (Wagner et al., 2014) and are usually reported as fragments (rounded, angular), pellets (cylinders, disks, spherules), filaments (fibres), and granules (MSFD, 2013) (Figure 2.2). Microplastics shapes include fragments (rounded, angular), pellets (cylinders, disks, spherules), filaments (fibres), and granules (Wagner et al., 2014) while the most identified microplastics in the environment are polypropylene (PP), polyethylene (PE, high and low density), Polyvinylchloride (PVC), polyethylene terephthalate (PET) and polystyrene (PS) (Kershaw, 2015; PlasticsEurope, 2019) as well as polyamide fibres (nylon) (Wagner et al., 2014).

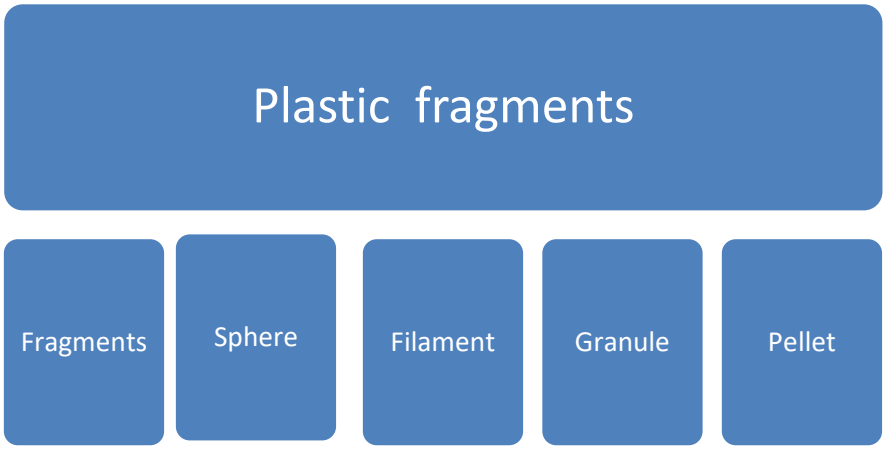

Figure 2.2: Morphological classification of microplastics found in environmental samples

2.4 MICROPLASTICS IN FRESHWATER

Freshwater represents streams, rivers, lakes, dams, and ponds. It represents the most complex system with regards to microplastic transport and retention. Freshwater resources receive microplastic from the terrestrial environment. Rivers are dynamic freshwater bodies and acts as conduits for microplastic to the marine environment. Freshwater serve as a means for microplastic production through breakup of larger plastics, and as sinks that retain microplastic in sediment (Horton and Dixon, 2018). Martinez-Tavera et al., (2021) noted that marine settings contain the highest percentage of plastic contamination, however, most plastics are used and disposed of inland and its adjacent freshwater systems, with nearly 1,15-2,41 million tons of plastic waste that enters the ocean every year from river systems. There is much less knowledge on the accumulation and effects of plastics in freshwater and terrestrial systems relative to marine ecosystems (Eerkes-Medrano et al., 2015). Freshwater resources may accumulate numerous microplastic particles and fibres, but less efforts have been made to monitor microplastic in freshwater bodies(Jingyi Li et al., 2018). However, some studies have been done on microplastic abundance in freshwater matrices but the results revealed that average values of microplastics abundance varied greatly from almost none to several million pieces per cubic metre (Jingyi Li et al., 2018).

Microplastics can quickly be transferred to the aquatic environment; however, in remote and isolated freshwater bodies, microplastics are trapped in water and sediment (Jingyi Li et al., 2018). Additionally, studies

were done on microplastic concentrations in marine and freshwater environments in Viet Nam (Strady et al., 2021) and results revealed that microplastic concentrations are related to the surrounding anthropogenic activities such as fishing, aquaculture, households, landfills, urban pressure on the environment and the direct release of treated and untreated wastewater (Strady et al., 2021). Wagner and Lambert, (2018) posited that plastics will enter the freshwater environment from various sources via many routes. Many plastic materials that enter the environment will not remain stationary but will be transferred between environmental compartments. Plastics will be disintegrated into microplastics as transportation and distribution continues. Microplastics undergo different rates of degradation and could be transported and distributed at quicker rates than larger plastics (Wagner and Lambert, 2018). Depending on the body of freshwater, a substantial amount of microplastic contamination comes from the degradation of disposable plastic, industrial abrasives and the breakdown of certain synthetic clothing fibres from washing (Allen et al., 2019). Both primary and secondary microplastics are mainly released to the aquatic environment through wastewater effluents, of domestic or industrial origins (Strady et al., 2021) which emphasizes that rivers are indeed dynamic freshwater bodies.

There are several microplastic sources released into the aquatic environment. It can be from waste origin during solid waste collection, processing, land-filling or transportation, from tyre particles, vehicle-derived debris, agricultural plastic, runoff, wind and atmospheric effects (Strady et al., 2021) while river channels can be heavily contaminated with microplastics and fluvial transport is a major supplier of microplastics to the oceans (Woodward et al., 2020). Wagner and Lambert, (2018) stated that degradation rates are limited when microplastics are transported to sediment and when biofilms form on microplastic surfaces due to reduced light exposure. Riverbeds are generally well-oxygenated. It provides an important habitat for macroinvertebrates such as stoneflies, caddisfly larvae and shrimps. Woodward et al., (2020) further discusses that the fine channel bed sediments contain important food sources for this ecosystem, which includes algae and decaying organic matter. This zone (riverbed) is of importance because it is a feeding zone for many aquatic species and they pointed out and argued that the fine sediments on a river bed are the most ecologically relevant sampling environment and the most appropriate context to establish the extent of microplastic contamination and any potential threat to the aquatic ecosystem (Woodward et al., 2020). Freshwater and soil systems are subject to both point and non-point source inputs of plastics, and great research effort is needed to understand transport, exposure and ecological effects of microplastics in these systems (Horton et al., 2017b). According to Ghayebzadeh et al., (2021), there are differences in the abundance and spatial distribution of microplastics in water and sediments and it is primarily influenced by environmental and anthropogenic factors.

2.5 SOURCES AND OCCURRENCE OF MICROPLASTICS IN THE AQUATIC ENVIRONMENT

Plastics have become the largest component of marine debris and thus, a major threat to aquatic ecosystems (Green, 2016). Microplastics are ubiquitous in the environment (Hu et al., 2019); they are widely used because they are relatively cheap and durable but persistent in the environment (Lin et al., 2020). They get into rivers through the wind, storm sewers and wastewater treatment plants. Rivers have become sinks for microplastics since most wastewater treatment plants do not effectively remove microplastics from effluents. Plastic waste ubiquity in marine and the freshwater environment has detrimental impacts on the ecological systems, biodiversity, and human health. Several studies on microplastics have been conducted on marine environment

but there is limited information on the freshwater system in Africa and South Africa (Migwi et al., 2020; Nel et al., 2018; E. A. Weideman et al., 2020).

Most plastics are manufactured, used and deposited on terrestrial environment, with rivers as a primary pathway (source) of microplastics to the ocean (Chowdhury et al., 2021; Phuong et al., 2022). Mani et al., (2015) identified the closeness of urbanisation to rivers as sources of microplastics from activities such as effluent discharge, road runoff, littering and atmospheric deposition to aquatic ecosystems. The most significant source of freshwater pollution is wastewater effluent from sewage treatment plants and runoff from road surface caused by the breakdown of road-marking and tyre debris (Eriksen et al., 2013; Horton et al., 2017b). Wastewater treatment plants (WWTPs) is another important point source of freshwater environmental secondary microplastics due to domestic waste (in the form of microbeads from personal care products and microfibers from the laundry), industrial input and stormwater (Leslie et al., 2017; Mintenig et al., 2017). Though WWTPs are reasonably efficient to remove between 96-99.9% of microplastics (Talvitie et al., 2017; Ziajahromi et al., 2017b) from the wastewater stream (Wardrop et al., 2016), the remaining microplastic particles that are not effectively removed will still be discharged to the freshwater environment via effluent. Microplastics particles removed from wastewater are retained within the sludge which contained more microplastics than the effluent (Magnusson and Norén, 2014; Mintenig et al., 2017) and are considered as a source of microplastics pollution when used as agricultural land fertilizer and/or deposited on landfills via surface runoff or drainage.

Microplastics may be transported to water bodies (rivers, lakes and ultimately river basins) and into the marine system (Leslie et al., 2017; Wagner et al., 2014). Microplastics also enter rivers through storm drains systems during storm events and periods of high rainfall allowing runoff from roads and urban areas to enter directly to rivers. In periods of heavy rainfall, microplastics enter rivers through combined sewage overflows that are designed to discharge untreated sewage overflow directly to rivers (Horton et al., 2017b). Litter represent another source of microplastics to the rivers either directly into the water or washed in from the bank or surrounding land (Horton et al., 2017a).Eleanor A.Weideman et al., (2020) study on litter loads in urban stormwater run-off from an urbanised city (Cape Town) in South Africa showed a vast variation in the number of plastics loads in stormwater. Microplastics burden in industrial (78%), residential/commercial (49%) and residential area (40%) established industrial areas as a major source of plastics pollution. The fragmentation of these plastics into secondary microplastics represent a huge amount of microplastics transport in freshwater systems. Microplastic pollution burden in aquatic ecosystems continue to grow with increasing production and consumption of plastic materials. The presence of microplastics is, therefore, worrying due to the potential threats to the suitability of water for human use (Bouwman and Minnaar, K., Bezuidenhout, C. & Verster, 2018).

Several studies have been conducted on the MP pollution in both marine and freshwater ecosystems to understand the occurrence of microplastics on waterbodies. In the past decades, marine environmental microplastics had received the attention of scientists globally (Maryani et al., 2020) and drove the need for an understanding of microplastic impact as a marine pollutant (Lusher, 2015). The presence of microplastics was reported globally in all marine systems including oceans, from the Arctic to the Antarctic, water columns and seabed sediments, and at sea surfaces (Abayomi et al., 2017; Isobe et al., 2017; Lusher, 2015; Zhang et al., 2017). Many of the studies reported the accumulation and persistence of microplastics, for instance, Sharma

and Chatterjee, (2017) reported the accumulation of microplastics in the Mediterranean Sea in the range of 1.000 and 3.000 tonnes, Law et al., (2010) reported that human activities were driven by wind and geostrophic circulation and that turbulence and oceanographic effects (Turra et al., 2014) accounted for the plastic pollution load of the investigated ecosystems. Microplastics are therefore potentially bioavailable to various organisms like zooplankton, lobsters, worms, fishes, birds and even mammals while the ingestion by organisms is a concern due to microplastics potential for increased bioaccumulation with decreasing size (Sharma and Chatterjee, 2017; Wright et al., 2013).

Freshwater is a primary human need for survival and was identified by (Lebreton et al., 2017) and (Schmidt et al., 2017) as one of the pathways to marine microplastics pollution. Horton reported microplastics in different freshwater ecosystems including rivers and lakes (Horton, 2019). Freshwater hydrologic system (flow rate, deep and topography) contribute to the accumulation of microplastics in freshwater ecosystems (Tibbetts et al., 2018) which leads to more questions about microplastics source apportionment and the occurrence of ecological adverse effect in the freshwater systems. The variation in occurrence and abundance of microplastics in freshwater systems is dependent on sampling points, anthropogenic activities, sampling approaches (Eerkes-Medrano et al., 2015; Jingyi Li et al., 2018), water surface area, depth, wind, currents and density of particles (Eriksen et al., 2013; Fischer et al., 2016). Weideman et al., (2020) investigation reported variable values for microfiber presence in wet and dry seasons suggested that seasons might play an influential role in microplastics abundance and occurrence through water flow in the river system. Microplastics particles were found to be 65% higher in the populated urban section of the River Tame compared to populated rural sites of the River Tame (Tibbetts et al., 2018). However, Weideman et al., (2020) reported the abundance of microplastics in the upper, middle and lower reaches of the Orange-Vaal River in South Africa. Microplastics were identified in the surface water samples of Yongjiang River (China) with a mean concentration of 2345 ± 1858 particles per cubic metre (n/m^3) while the midstream of Yongjiang river had an average concentration of 3675 ± 2361 n/m^3 microplastics particles, the upstream and downstream had 1300 ± 477 n/m^3 and 1617 ± 560 n/m^3 respectively (Zhang et al., 2020).

Microplastics studies in freshwater have increased rapidly and this indicates the importance to the water sector. Reported microplastics values in freshwater ecosystems are equivalent to the observed amounts in the marine environment (Ma et al., 2019; Peng et al., 2017) with a highly heterogeneous distribution in different areas (Jingyi Li et al., 2018) as shown in table 1.

Table 2.1: Examples of microplastics distributions in the freshwater systems

Location	Sampling Point/Region	MP Occurrence Average/range	References
Yongjiang River, South China	Midstream	3675 ± 2361 n/m^3	(Zhang et al., 2020)
	Upstream	1300 ± 477 n/m^3	
	Downstream	1617 ± 560 n/m^3	
Mega-cities, Shanghai, China	Park, Caohejing river	1535 ± 771 kg^{-1}/ dw	(Peng et al., 2018)
	Residential, Beishagang river	1600 ± 191 kg^{-1}/ dw	
	Rural, Jiangjiagang river	1120 ± 56 kg^{-1}/ dw	
	Park, Yujiabang river	410 ± 127 kg^{-1}/ dw	

Location	Sampling Point/Region	MP Occurrence Average/range	References
River Tame, Birmingham, UK	urban section, River Tame	350 particles/ kg^{-1}	(Tibbetts et al., 2018)
	rural section, River Tame	20 particles/ kg^{-1}	
Naivasha Lake, Kenya	Malewa River mouth	0.633± 0.067 particles/m^2	(Migwi et al., 2020)
	Hippo Point	≅ 0.17±0.2 particles/m^2	
River Kelvin sediment, UK	SE1 (December 17, 2015)	220±448 items /kg dw	(Blair et al., 2019)
	SE2 (February 15, 2016)	161±432 items /kg dw	
Poyang Lake sediment, China	Upstream reaches of Raohe	3153 items/kg dw	(S. Liu et al., 2019)
	Najishan National Nature Reserve	11 items/kg dw	
Rhine-Main, Germany	R3 (Mainz-Kastel)	30106 particles/m^2	(Klein et al., 2015)
	R6 (Walluf)	1784 particles/m^2	
Kallavesi Lake, Finland	Site 7 (city harbour)	0.66 MPs/m^3	(Uurasjärvi et al., 2020)
	Site 2 (highway bridge)	0.037 MPs/m^3	
Great Lakes, US	Detroit River plume	1,910,562 particles km^{-2}	(Cable et al., 2017)
	Lake Huron	126,933 particles km^{-2}	
Subalpine Lakes, Italy	Lake Iseo	40000 particles/km^2	(Sighicelli et al., 2018)
	Lake Maggiore	39000 particles/km^2	
	Garda Lake	25000 particles/km^2	
Orange-Vaal Rivers, SA	Upper Orange (Wet/ Dry)	0.6 ± 0.4 N. L^{-1}/ 1.0±1.2 N. L^{-1}	(E. A. Weideman et al., 2020)
	Lower Orange (Wet/ Dry)	17.1±17.4 N. L^{-1}/ 1.3±1.3N. L^{-1}	
	Upper Vaal (Wet/ Dry)	0.4 ± 0.3 N. L^{-1}/ 2.3±3.2 N. L^{-1}	
	Lower Vaal (Wet/ Dry)	0.7 ± 0.7 N. L^{-1}/ 0.8±0.8 N. L^{-1}	

Peng et al., (2018) study on Shanghai rivers observed that urban freshwater river sediments are reservoirs for land-based microplastics with 802 ± 594 MP items per kilogram of dry weight (items/ kg^{-1} dw) in the river sediment samples (Peng et al., 2018). The average microplastics found in Poyang Lake were 1134 (items/ kg^{-1} dw) (G. Liu et al., 2019). These studies demonstrated that the occurrence of microplastics in freshwater (rivers and lakes) systems were mainly due to the population density in the vicinity of the freshwater resource and the accompanying intense anthropogenic activities. Wind, runoff through stormwater and seasons contributed to uneven distribution of microplastics in freshwater ecosystems.

2.6 MICROPLASTICS DISTRIBUTION IN AQUATIC ENVIRONMENT

Plastics have high volume usage and usually end up in the marine environment. High density particles will sink to the bottom and accumulate in sediment, whereas low density particles will float at the sea-surface (Van Cauwenberghe and Janssen, 2014). Hence, the density of plastic particles will determine its distribution in the aquatic environment. Similarly, it can be assumed that in an aquatic environment, high density particles will sink and accumulate in sediment and low-density particles will float on the surface. Transport and distribution of plastic debris to the marine, terrestrial and aerial environments are influenced by the environmental conditions and physical properties of plastics (Patil et al., 2021). Studies on abundance and fate analysis in freshwater, especially in rivers, are still in the early stages (Sarkar and Kumar Manna, 2020). Plastic debris transported from riverine areas to land systems account for 80% of debris into the marine environment (Sarkar and Kumar Manna, 2020). Van Cauwenberghe and Janssen, (2014), noted that due to the persistent nature of microplastics, their abundance in the marine environment will only increase. Plastics have a stable physical and chemical structure, and for this reason, they will not decompose in the natural environment for tens to hundreds of years (Jiang et al., 2018). This suggests that plastics, or microplastics, are likely to remain in the environment for a very long period due to their persistent property. The distribution, abundance and occurrence of microplastics in the environment are affected by many factors such as the environment type, properties of microplastic (type, density, size and shape), climate regions, industrialisation, urbanisation, waste management, development and societal standards (Miloloža et al., 2021). According to Pereao et al., (2020), the introduction of plastic fragments into terrestrial and aquatic ecosystems are due to anthropogenic actions such as industrial and domestic activities in coastal areas and these sources are diverse and usually consist of both primary and secondary plastic classification.

2.7 EFFECTS OF PLASTIC POLLUTION IN THE AQUATIC SYSTEMS

Plastic products are widespread in the environmental matrices with wide application in industrial production systems, commercial entities, and domestic products. Lozoyaa et al., (2015) reported that plastics are widely used in many products. The ubiquity of microplastics coupled with its adverse impact on the ecological system makes it important to have more knowledge on the risk assessment but there are very few and limited researches on both the environmental exposure and ecotoxicology of microplastics in freshwater (Zhang et al., 2020). Plastic pollution in an aquatic ecosystem may exert either or all physical stress, biochemical processes disruption through leachate of chemicals additives of the breakdown of the plastic polymer. Adverse effects of plastic wastes in aquatic ecosystems include damage to energy intake, hormone secretion, growth rate, and their reproductive capacity (Qu et al., 2018; Su et al., 2018). They have been identified as EDCs and may also cause choking, internal or external wounds, ulceration, blocked digestive tracts, false sense of satiation, debilitation and death (Eerkes-Medrano et al., 2015). Negative consequences of plastics include adverse effects on aquatic organisms, bioaccumulation with resulting biomagnification in the food web, loss of aesthetic value of water bodies, among others. Land-based plastic wastes were also reported to contribute to the plastic pollution burden of aquatic systems (Lozoyaa et al., 2015). Several studies in the marine environment have reported ingestion of microplastics in the range of 250-1000 µm by the Wild Gudgeon fish (Sanchez et al., 2014) and by fish larvae (Steer et al., 2017).

Microplastic is of great concern due to the increased potential of bioaccumulation with decreasing size (Wagner et al., 2014). The size of microplastic enable bioaccumulation or the uptake of microplastics to occur in organisms higher up in the food chain. Microplastics may be ingested by various organisms and can range from plankton and fish to birds and mammals. The small size of microplastics and large specific surface area makes them available to a wide variety of biota in benthic and pelagic habitats (Wagner et al., 2014) and hence microplastics are biologically available to aquatic organisms (Ghayebzadeh et al., 2021). Ingestion of microplastics have been reported for species such as mussels, lugworms, crabs, sea birds and fish (Klein et al., 2015). It was reported that less dense and floating microplastics are mainly associated with lower-level organisms whereas denser microplastics are related to benthic invertebrates (Sarkar and Kumar Manna, 2020). Wagner et al., (2014) opined that microplastics may be ingested by various organisms with the potential for bioaccumulation at all trophic levels.

The ingestion of microplastics by marine organisms may cause physical and chemical damage to organisms (Zhao et al., 2017); it may affect growth, mortality rate, metabolism, reproduction and health (Lusher et al., 2013; Von Moos et al., 2012; Xu et al., 2017). Bioaccumulation of microplastics in the food chain could further affect human health as the highest trophic level consumer (Barboza et al., 2018; Wang et al., 2018). Green, (2016) reported altered assemblages in a marine habitat mesocosm of organisms repeatedly exposed to high concentrations of microplastics. A Mediterranean fish species – *Boops boops (L)* was assessed for spatial variability and influence of biological parameters for microplastics contamination and microplastics were detected in the gastrointestinal tract of 56% of the samples with exposures playing a major role in the number of microplastics in individual samples. It was widely reported that microplastics are ingested by some aquatic species (Anbumani and Kakkar, 2018; Eerkes-Medrano et al., 2015) such as pelagic fish (Rummel et al., 2016), shrimp, mussels, polychaete larvae and ciliates (Setälä et al., 2016), and invertebrates, as well as, filter feeders, lugworms and detritivores (Besseling et al., 2013). Ingested microplastics may translocate to tissue and liver, causing adverse effects such as inflammation and lipid accumulation (Lu et al., 2016), reduced growth (Au et al., 2015), immobilisation (Rehse et al., 2016), and mortality (Jemec et al., 2016).

Scherer et al., (2018) reported that 39 freshwater species (4 species of fish and 35 species of an invertebrate) ingested microplastics in their study (Figure 2.4). Other studies made similar observations for *Tubificid worms*, *Gammarus pulex* and *Hyalella Azteca* (Hurley et al., 2017; Redondo-Hasselerharm et al., 2018; Weber et al., 2018). The concern over the ecological effects of microplastics was raised and demonstrated by Farrell and Nelson, (2013); Remy et al., (2015); Setälä et al., (2014) reported that microplastics can be ingested and transferred through all of the trophic chains, where they may bioaccumulate, affect the gastrointestinal tract (Gall and Thompson, 2015) or transport toxic substance and metal (Andrady, 2011), and also act as a vector for microbial and pathogens transport to the environment (Zettler et al., 2013). Organisms that have ingested microplastics may experience some physical harm, blockage of the gut, internal or external abrasion or inflammation or suffocation caused by blockage of gills (Von Moos et al., 2012; Wright et al., 2013).

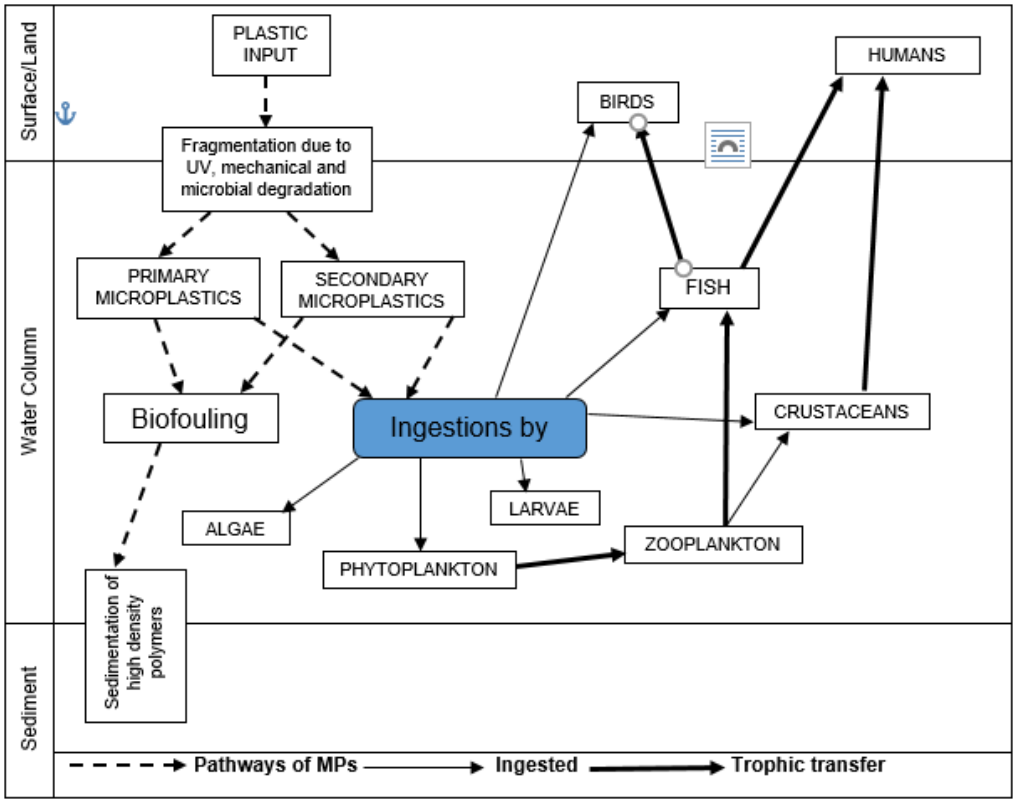

Figure 2.3: Microplastics ingestion by freshwater organisms

Most studies on the effects of microplastics on aquatic ecosystems are on marine organisms with a dearth of information on the microplastics fate in freshwater ecosystems (Elizalde-Velázquez et al., 2020). *Daphnia magna* was observed to ingest microplastics (Besseling et al., 2014; Rehse et al., 2016), at a rapid rate under laboratory conditions (Rosenkranz et al., 2009). Microplastics was reported in the gastrointestinal tracts of both *D. magna* and *Pimephales promelas* with a low probability of bioconcentration and bioaccumulation in environmental conditions (Elizalde-Velázquez et al., 2020). According to Horton et al., (2017b), consumption can take place by direct ingestion from water or via prey that ingested microplastics which was transferred and accumulated along food chains (Cole et al., 2013; Setälä et al., 2014). The ingestion and transfer of microplastics may harm ecosystems and human health (Wright et al., 2013), because microplastics may leach and transfer toxic additives (Phthalates, nonylphenol, polybrominated diphenyl ethers (PBDE)), and adsorbed persistent organic pollutants (POPs) from the environment to the biota (Anbumani and Kakkar, 2018). Microplastics ingestion may also release pollutants initially sorbed on the surface of microplastics with potential availability to human beings through bioaccumulation and biomagnification often exacerbated at low pH and high-temperature values (Jingyi Li et al., 2018). Another study showed that the desorption rate of sorbed contaminants in organisms was accelerated when compared to the marine system (Bakir et al., 2014).

Studies have shown adverse effects of microplastics on juvenile and adult fish (Klein et al., 2015) and some studies observed physical damage of microplastic exposure on organisms (Guo et al., 2020). The presence of biofilms on the plastic surface has increased the likelihood of microplastic ingestion, which triggers the sensory responses in organisms (Chen et al., 2021). Biofilms are formed when organisms attach to a plastic, and that triggers organisms to ingest food which include the plastic material. When biofilm attach to microplastics, the

feeding behaviour of organisms are adversely affected due to chemical secretions, resulting in changes to microplastics (Liu et al., 2021). Organisms might feed directly on microplastics, mistaking it for food or selectively feeding on microplastics instead of prey (Cormier et al., 2021). The feeding activity in zooplankton is also affected by microplastics (Patil et al., 2021). Moreover, as mentioned by Klein et al., (2015), smaller particles can be consumed by organisms at the bottom of the food chain like zooplankton, isopoda, or mysid shrimps, and biomagnification is expected. Microplastics have been detected in a number of food products such as drinking water, beverages, honey, fish, mussels, table salt and sugar (Shopova et al., 2020). In fact, mussels which were cultured for human consumption were also discovered to contain microplastic particles (Klein et al., 2015). Therefore, the ingestion of microplastic particles in various types of organisms can occur and bioaccumulation can take place throughout the food chain.

There is mixed evidence on impacts of microplastics on reproductive rates in individual species. For example, a decrease in reproduction rates were observed in freshwater amphipod crustaceans whereas no evidence of impact on reproductive rates in *Daphnia magna* were observed. However, effects of phthalates on *Daphnia magna* include a reduction in reproductive rates, body size, and lifespan, and effects of bisphenol A (BPA) include damage to genetic information triggering metabolic stress responses and decreased rates of reproduction (Akdogan and Guven, 2019; Wu et al., 2020). Furthermore, microplastics can be carriers of two types of chemicals, namely, chemicals which are integrated during the plastic manufacturing to improve plastic production; and chemicals which are sorbed onto the microplastic surface from the aquatic environment (Guo et al., 2020). These chemicals consist of large quantities of unreacted raw materials or additives which may leach into the surrounding environment during usage or after disposal (Pereao et al., 2020b). According to Cormier et al., (2021) , polymerisation is rarely complete and residual monomers and additives can leach out from plastic into the environment.

Additionally, polymer additives used to manufacture plastics are contaminants such as flame retardants and softeners (Klein et al., 2015). Leaching of additives and monomers from consumer plastics to water and toxicity to freshwater organisms have been demonstrated (Pittura et al., 2018). As a result of sorption to microplastics and desorption of additives from microplastics, exposed organisms are vulnerable to contamination effects. Leachate analyses have highlighted the presence of phthalates and metals used as plasticizers, stabilisers or colouring agents in plastics, and it must be noted that plastic additives including metal stabilisers and heavy-metal pigments are not chemically bound to the polymers and can therefore easily be leaked out of the plastic (Cormier et al., 2021). Leaching from consumer plastics to water was demonstrated to occur and be toxic to freshwater organisms (Pittura et al., 2018). As a result of sorption to microplastics and desorption of additives from microplastics, organisms that come into contact with MP are exposed to contamination and thus contaminants can enter the aqueous environment including organisms. Aquatic organisms are also exposed to contaminants such as pesticides, insecticides, pharmaceuticals or other pollutants that may sorb on the microplastic particles (Klein et al., 2015). Pereao et al., (2020), mentioned that organic contaminants are present in plastic debris at an approximate concentration of sub micrograms per gram to nanograms per gram and most contaminants adsorb from the surrounding water whereas others are added during the plastic manufacturing process.

2.8 ANALYSIS AND CHARACTERIZATION OF MICROPLASTICS

Microplastics bioavailability, ubiquity in the aquatic environment and capacity for transporting toxic chemicals is becoming a global and major ecological and environmental problem lately and has increased the importance of the microplastic analysis in environmental and experimental samples. Microplastics monitoring in several biotic and abiotic environmental conditions can provide the required scientific evidence needed to determine microplastic historical trends, concentration hot spots, pollution status and the exposure and fate of organisms. The microplastics monitoring studies require comparable and reliable sampling and analytical procedures since microplastic analytical study methods is an emerging new field still at the developmental stage. Moreover, the varied assortment of microplastics sizes and the complex constituent of the polymer types, colours and shapes prevented scientists from developing a reliable microplastic characterisation and classification data thereby making data assessment and comparison additionally problematic and challenging. Currently, microplastics identification can be based on the combinations of chemical and physical characterisation of sample particles isolated from the combinations of organic and inorganic residue constituents after the clean-up and extraction process (Shim et al., 2017), but there is still the need to develop and improve novel procedures to decrease the identification effort and time for detecting sub-micron plastics in ecological samples. The analytical methods used in an environmental microplastics sample consist of the extraction, separation (or isolation), identification, and classification (or quantification). Despite the lack of standardized methods to assess number and amounts of plastics in the environment, microplastics is a growing potential threat. Results from different studies indicate that the amount of microplastics are increasing in the aquatic environment but there are also inconclusive data pointing on more stable abundance of plastic accumulation (Barnes et al., 2009; Goldstein et al., 2012). At the same time, the micrometre fraction of pollutants seems to be increasing as well as the geographical distribution. This study presents the review of microplastics sampling techniques in water and sediment as well as identification methods for analyses. The information was organised into the following sampling stages: the collection of bulk samples, separation of sample, processing and digestion, and identification based on their relevance in the result validation process.

2.8.1 Sample collection

The sediment and water collection of environmental samples is an initial basic phase in microplastics sampling practises. The widespread dispersal of microplastics particle samples in the water column is based on several properties which include environmental properties like wind, water density, waves and currents and on adsorption of chemicals, density, size, shape and biofouling. The quality and quantity of recovered microplastics are hugely and extremely dependent on the sample depth and location as well as the characteristics of the environment such as the density and the hydrodynamic profiles. However, the choice of the medium sampled depends on both the obtainable equipment and the objective or purpose of the research study. The collected water samples are stored in glass amber bottles for further processing in the laboratory while higher water volumes collection can be essential towards obtaining a representative sampling which is often further reduced in situ by using sieves, nets or pumps. Collected environmental sampled size reduction can similarly be realised via pre-treatment employing steel meshes with decreasing sizes in the lab (Ziajahromi et al., 2017b). The microplastics distribution in sediments is typically irregular and uneven but mostly influenced by the environmental factors and properties such as currents and winds. The accurate estimate of the

concentration of microplastics in the sediment samples will involve a description of the sampling depth because the topmost 1-5 cm depth always have concentrations which are higher than the soil top 10 cm depth as well as the number of replicates (Besley et al., 2017). Results obtained for sediment samples will depend mostly on the sampled area (like intertidal areas, transects, high tide line) plus the depth since some areas may have higher microplastics concentrations.

2.8.2 Separation of microplastics sample

Microplastics need to be separated from collected sediment and water samples before it can be characterized and quantified. These examining samples may then be subjected to two separate stages: (1) a sample reduction step which allowed a reduction in the volume sampled, for example, bulk collection and sieving or the use of nets for collection and (2) separation phase typically by density separation and/or filtration using NaCl as recommended by both NOAA (Masura et al., 2015) and MSFD (MSFD, 2013).

2.8.2.1 Sieving or filtration

Sieving or filtration is the most common technique of separating microplastics from collected environmental water samples and used for any plastic containing supernatant obtained from sediment samples after density separation. The sieve's mesh or filter's pore size can vary greatly and thus control the detection of lower sized microplastics. Though mesh sizes or small pore could be affected by a rapid blockage by mineral and organic matter and for sediments samples, they may first be pre-treated using larger sieves, before the use of density separation and finally by supernatant filtration using sieves or filters (Prata et al., 2019).

2.8.2.2 Density separation

Solutions of density >1.4 g cm^{-3} is recognised as a requirement for microplastics separation from sediment because plastic density depends on additive concentration, polymer type and by even the organisms and substances adsorbed (Quinn et al., 2017). This density differences can, therefore, be employed to separate plastics with 0.8-1.6 g cm^{-3} from environmental sample sediment having the density with 2.7 g cm^{-3} and by the careful mixture of the collected sediment samples by saturated salt solutions before further re-collecting the microplastics supernatant for further filtration (Rocha-Santos and Duarte, 2015). NaCl is eco-friendly, cheap and highly available and commonly used salt in density separation of solutions (Nuelle et al., 2014). NaCl of reagent grade is often recommended since the extraction efficiency is higher especially for slightly heavier plastics like high-density polyethylene (HDPE) and can also achieve a slightly higher density (Sánchez-Nieva et al., 2017) but NaCl (1.2 g cm^{-3}) and NaBr (1.4 g cm^{-3}) was found to have larger error bars and low recovery rates (<90%) (Quinn et al., 2017) but $ZnBr_2$ (1.7 g cm^{-3}) and also NaI (1.6 g cm^{-3}) was found to easily separate other heavier plastics with tight error bars and good recovery rates (99%).

2.8.3 Removal of organic matter and sample processing

The environmental samples collected often contain biological material which is confused frequently with microplastics constituents (like dark algae fragments) which may lead to the plastic environmental sample

concentrations being overestimated and consequently increase the number of particles considered for further investigation. Hence, there is the requirement to create a simple digestion process which can reduce the organic materials without affecting the polymer chemical and structural integrity (Felsing et al., 2018). However, the need for digestion will vary and depends on the amount of organic matter in the samples.

2.8.3.1 Acid digestion

A method able to degrade organic material with some caution is acid digestion because it could lead to a microplastics underestimation in the environmental samples. There is also an optimum temperature and acid concentration required to effectively remove biological matter in any reasonable time. However, some polymers like polyethylene terephthalate (PET), nylon, may also be degraded due to their low resistance to acids especially in high temperatures and high concentration conditions (Qiu et al., 2016). Solutions of hydrochloric acid (HCl) appears to have the lowest treatment efficiency impact in biological material treatment with very large quantities (Zhao et al., 2017) while nitric acid has been used extensively in acid digestion but it might also leave tissue debris or oily residues, cause melting of PS (polystyrene) and loss of nylon or yellowing of polymers.

2.8.3.2 Alkali digestion

Another acid digestion substitute having good potential is the alkali digestion but it can still discolour or damage polymers, re-deposit tissue remains on surfaces of plastic, and or leave oily residues thereby obscuring characterization from vibrational spectroscopy (Wagner et al., 2017). KOH can digest organic material and recover plastics very well but may nevertheless cause degradation of polyester, PE, nylon, polycarbonate (PC), PVC, cellulose acetate (CA), LDPE and PET, discolouration of unplasticized PVC (uPVC), PE and nylon (Maes et al., 2017). NaOH similarly can cause degradation of polymer and colour change in PET and PVC (Dehaut et al., 2016). However, alkali and acid digestion can be sequentially used such as HNO_3 and NaOH with good recovery rates and digestion of biological matter (Roch and Brinker, 2017).

2.8.3.3 Oxidizing agents

An oxidizing agent like the hydrogen peroxide (H_2O_2, 30-35%) are capable of digesting organic substances more efficiently and effectively than acid and alkali digestion using HCl and NaOH with none or little polymer degradation (Nuelle et al., 2014; Zhao et al., 2017). However, the incubation temperature appears to be the determining factor with regards to H_2O_2 digestion efficiency but overall, H_2O_2 actions are possibly able to eliminate all organic material efficiently with only very slight consequence on the microplastic integrity.

2.8.3.4 Enzymatic digestion

Digestion by an enzyme is another alternative being used in digestion technique. Enzyme digestion is less probable to induce microplastics damage and much safer because it may be used without a fume hood (Maes et al., 2017). Nevertheless, the enzyme effectiveness varies with the type of organic matter present with the environmental samples. The enzyme procedures include pre-digesting the sediments sample at 45°C for 60

min using a 2.5% industrial enzyme blend which is followed by H_2O_2 (30%) debris removal (Crichton et al., 2017).

2.8.4 Identification methods

It is problematic to classify microplastics of different polymer types, sizes and shapes fully and reliably from complex environmental matrices with the use of a sole analytical technique. Hence, the use and combination of additional or more analytical methods were widely suggested. Generally, microplastic analysis now involves two major phases: chemical characterization (like spectroscopy) for confirmation of plastics and physical characterization of possible plastics by microscopy as shown in Figure 2.3.

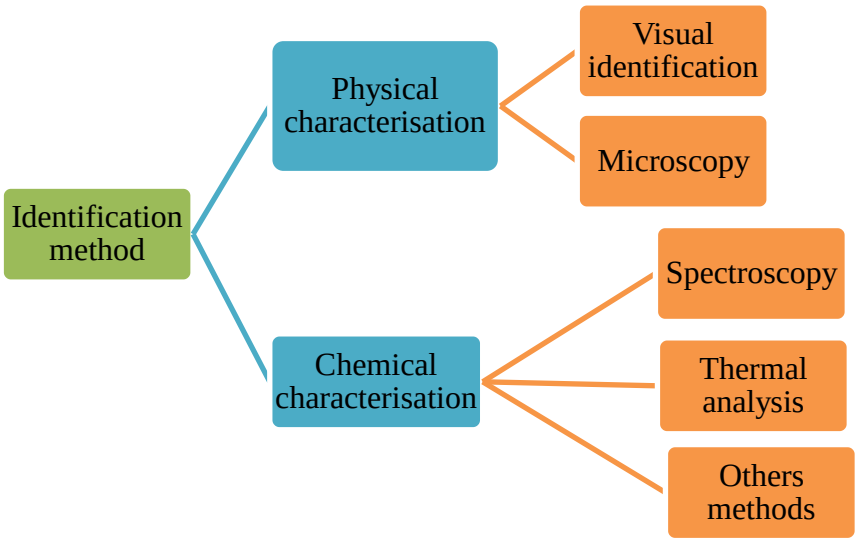

Figure 2.4. Overview of characterization methods used for microplastic analysis

Microplastic characterisation, quantification and identification were investigated respectively for effluent, sewage, marine sewage disposal, washing machine effluent, seawater, vertebrate and invertebrate ingestion, muddy and sandy sediments, facial cleansers and plankton samples (Browne et al., 2011; Ivar do Sul and Costa, 2014) and evidence signify that microplastics physicochemical characteristics like chemical composition, density, shape, size and colour significantly influenced the microplastic transport and bioavailability in the river environments (Rocha-Santos and Duarte, 2015). The major techniques include the following:

2.8.4.1 Visual identification

Visual identification and sorting of bigger sized microplastics provide a fast, easy and simple process for both trained specialists and the inexperienced volunteers with little or no training (Hidalgo-Ruz and Thiel, 2013). Identification and sorting are generally simultaneously performed using the naked eye in a tray with forceps because of the comparatively large microplastics size ranges. Some pre-production resin pellets and colourful plastic fragments and small plastics which has the range of 2-5 mm size may be easily identified using this visual method (Heo et al., 2013). However, the outcomes on separation and identification of microplastics samples often depend on the characteristics like structures and forms, brightness, shininess, hardness

determined by applying tweezers, colours and the specific elastic consistency of sample particles (Fries et al., 2013).

2.8.4.2 Microscopy

Dissecting or stereo microscopy is a generally acknowledged and useful technique for identifying microplastics in the size range of hundreds of micron like neuston net samples (Desforges et al., 2014). Images magnified by microscopy provide detailed structural information and surface texture of the sample that is adequately crucial for the identification of plastic-like ambiguous particles. Previous research studies which were confirmed by subsequent spectroscopic analysis showed that identified false plastic-like particles were about 20% and approximately 70% for transparent particles with the use of microscopy (Song et al., 2015). The scanning electron microscopy (SEM) also can offer high-magnification and very clear plastic-like particles images. High-resolution surface texture images of the particles enable the discernment of microplastics from organic matter (Cooper and Corcoran, 2010). Additional analysis using energy-dispersive X-ray spectroscopy (EDS) can also provide the elemental composition of the sampled materials (Vianello et al., 2013) and are very useful in the identification of carbon dominant plastics matter from inorganic particles. The SEM coupled with EDS is expensive and involve some considerable effort and time for sample preparation and examination which can decrease the number of samples that can be handled at a certain period. In SEM, the colours of the plastic cannot be used as identifiers but SEM-EDS micro-analyser has been used to obtain material information with regards to chemical composition and morphology of microplastics which require prior mounting and pre-selection of the analyzed particles (Fries et al., 2013). Some other innovative microscopy methods that were applied to identify microplastic materials in other specific instances include polarised optical microscopy which was used successfully for the identification of polyethylene (PE) particles in toxicity experiments and laboratory examination (Von Moos et al., 2012).

2.8.4.3 Fourier Transform Infrared spectroscopy

Fourier Transform Infrared spectroscopy (FTIR) spectroscopy gives information on the exact functional chemical bonds of microplastic particles. Identification using FTIR analysis can show the microplastic abundance and polymer composition which can provide sufficient proof or clues about the source, input pathway or origin of the polymer samples (Shim et al., 2017). There are unique spectra that are produced from different bond compositions which differentiate plastics from any other inorganic and organic particles and carbon-based polymers are easily identified with the use of this method. The well-established polymer spectrum library allows the confirmation of plastics and the identification of specific polymer types. The IR spectrum helps to avoid false-positive quantification associated with non-plastic particles using the fingerprinting of each plastic-like particle and decreases the probability of missing microplastic particles with no texture or colour. The attenuated total reflectance (ATR), reflectance and transmission modes are offered in FTIR analysis for polymers and microplastics. The FTIR-ATR measurement is a surface contact analysis method and the pressure from the ATR probe can damage fragile and extremely weathered microplastics or tiny plastic particles may be pulled by adhesion from the electrostatic interaction with the probe tip from the filter paper. Smaller microplastics need the application of micro-FTIR (μ-FTIR) which is used on a single platform but switched between the IR probe and object lens to perform microscopic observation of plastic-like

micro-sized particles before spectroscopic confirmation. Currently, μ-ATR-FTIR can be an advantageous method for identifying microplastics in environmental samples the identification of plastic-like particles by microscopy followed by chemical confirmation by spectroscopy. Nevertheless, microplastics having a < 50 μm maximum length require several trials because it may be problematic to obtain clear spectra that permit accurate and distinct identification.

2.8.4.4 Raman spectroscopy

An essential tool for the analysis and identification of very small microplastics <20 μm particles is Raman microscopy (Araujo et al., 2018) but the use is not widespread partly due to some of the drawbacks such as proneness to spectral distortion induced by fluorescence and long measurement time. Raman analysis identifies plastics particles and provides the polymer composition profiles of each sample just like FTIR. Raman spectroscopy is very similar to the FTIR technique with regards to the combination of microscopy with non-destructive chemical analysis including the requirement for expensive instrumentation. The different spectra and responses from microplastic in Raman spectroscopy and FTIR are that they can compromise each other in complex microplastic identification. The Raman spectroscopy laser beam which falls on the microplastic object results in an unlike frequency of several back-scattered lights which now depends on the atoms and molecular structure present which then produce the unique spectrum for each polymer.

2.8.4.5 Thermal analysis

The thermal analytical method measures the variations in the chemical and physical properties of polymer dependency on their thermal stability and was tried lately for microplastic identification (Majewsky et al., 2016). The thermal analysis provided the alternate method to the spectroscopy of chemical identification for certain types of polymeric materials, but the analysis is a destructive method, which prevents subsequent and further analysis of microplastic samples. Differential scanning calorimetry (DSC) is one suitable technique for the thermal properties study of polymeric particles. This technique needs a reference material for the identification of plastic types since each polymer product has varying characteristics in DSC. The DSC is useful for identifying specific primary microplastics like polyethylene microbeads which have available reference materials (Castañeda et al., 2014) and the examination is relatively fast and simple but has the limitations of identifying microplastics from several polymer products in environmental samples. Gas chromatography-mass spectrometry (GC-MS) combined with thermal analysis may be used to simultaneously analyse additive chemicals materials in microplastics (Fries et al., 2013). Pyrolysis gas chromatography-mass spectrometry (Pyro-GC-MS) is another technique that analysed thermally decomposed gas obtained from microplastics and the generated pyrograms after the analysis is compared to known polymer samples with reference pyrograms.

2.8.4.6 Other methods

Other identification methods include atomic force microscopy (AFM) which can combine with either Raman or IR spectroscopy and has the potential for nano plastic analysis. The AFM may provide nanometre resolutions images and the probes may be operated with the objects in both contact and noncontact modes. AFM combined with Raman or IR spectroscopy may also be used to analyse the chemical composition of the polymer materials. AFM-IR is the actual merging of dual instruments into one while Raman spectroscopic

analysis is the simple combination of two instruments with the independent or simultaneous scanning of the same sample material (Dazzi et al., 2015). The X-ray fluorescence (XRF) portable spectrometer can also be used to evaluate the polymer elemental composition via the reflection and diffraction of radiation and this has more potential use in the detection of some adsorbed metals and polymer additives (Turner, 2017).

2.9 ECOLOGICAL AND HUMAN HEALTH RISKS

Microplastics can accumulate in larger animals as they pass up the food chain and the effects of microplastics appear to become more severe as the trophic level increases (Guo et al., 2020). Adverse effects of microplastics on organisms have been reported in their total energy, protein content, detoxification systems as well as behaviour (Razeghi et al., 2021). Plastic polymers are classified as carcinogenic and mutagenic to humans, highly toxic to aquatic life and have long lasting effects, and as a result, emerging microplastic pollution pose serious environmental, human health and global environmental protection and sustainability risks (Kabir et al., 2021). It is thus possible that micro and nano plastics enter the food web via trophic transfer through seafood (Domenech and Marcos, 2021). Due to the increased changes and binding abilities of microplastics, oxygen functional groups in microplastics support the dispersion and interaction with cell compounds of living organisms (Liu et al., 2021). The few studies that have been conducted on soil and freshwater species generally confirm the potential for microplastics to have detrimental effects on the physiology of species across many ecological niches (Horton et al., 2017b). Kabir et al., (2021) showed that small microplastic particles cause great hazards and created a high probability of both ingestion and biological transportation by aquatic organisms.

Aging leads to the breakdown of microplastics into smaller-sized particles which will allow microplastics to enter into the intestinal mucosa and the internal biological circulation system more easily through sorption, endocytosis and phagocytosis than bigger-sized particles (Liu et al., 2021). Studies already demonstrated the presence of microplastics in commercially important fishes, shrimps and mussels, as a possible microplastic exposure pathway of humans via their diet (Ziccardi et al., 2016). Sorbed contaminants may undergo adsorption and desorption processes while additives and low-molecular organic products are able to be released from older microplastics to induce secondary chemical risks (Liu et al., 2021). (Klein et al., (2015), showed that persistent organic pollutants ((polycyclic aromatic hydrocarbon compounds (PAHs) and polychlorinated biphenyls (PCBs)) were detected in microplastics from sediments around the world. Toxicological effects of microplastics on freshwater aquatic (micro) organisms are still scarce, since most studies were done on marine (micro) organisms (Miloloža et al., 2021). Figure 2.5 showed several organisms such as *Vibrio fischeri, Algae, Daphnia magna and Fishes* were exposed to a wide range of microplastic types and concentrations with results indicating that organisms at different trophic levels are responsive and vulnerable to a broad range of microplastic concentrations.

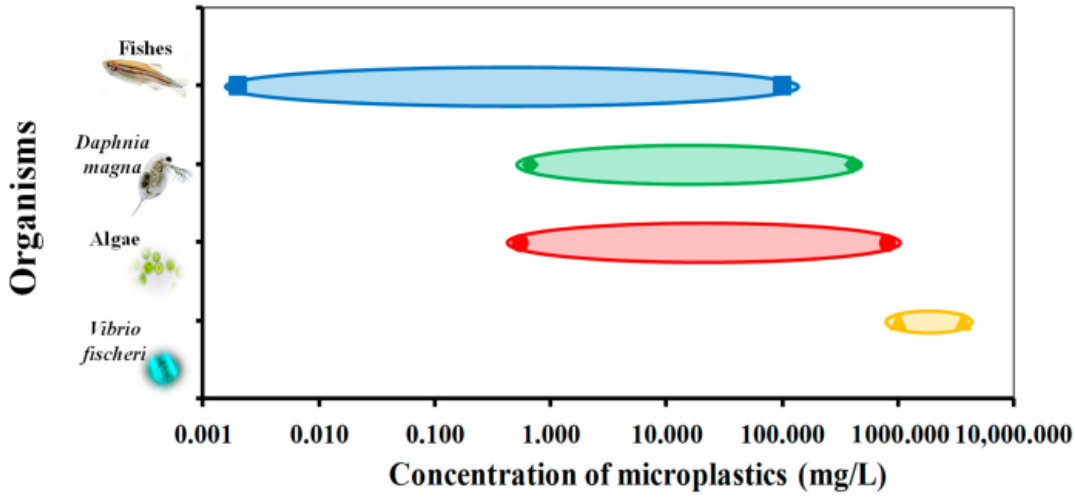

Figure 2.5. The range of ecotoxicological concentrations for different organism levels (Miloloža et al., 2021)**.**

There are numerous ecotoxicological effects of microplastic polymers such as mortality, growth reduction, productivity, population sizes, gene expression and oxidative stress that have been shown under laboratory studies (Kabir et al., 2021). Additionally, microbiota can be disturbed, which will affect homeostasis and stimulate toxicological effects in metabolic processes in mammalian models (Domenech and Marcos, 2021). Examples of toxicological implications of microplastic exposure include neurotoxic disorders in fishes, decreased growth and reproduction competency in freshwater crustacean amphipod *Hyalella azteca*, oxidative stress and genotoxicity in marine mussel *Mytilus galloprovincialis* when exposed to polyethylene (PE) microplastics (Sarkar and Kumar Manna, 2020). A study done on the exposure of Polystyrene (PS) particles in size ranges of between 0,1 µm and 1,0 µm, and at concentrations of 10, 50 and 100 mg/L induced the algal growth inhibition with a higher effect with smaller particles; effects include oxidative stress and morphological changes on freshwater algae *Chlorella pyrenoidosa* cell (Miloloža et al., 2021).

Many types of plastics exist and ecological risks of various types of plastics differ significantly (Jiang et al., 2018). There is a possibility that microplastics have the potential to affect soil structure, plant growth, and soil or freshwater ecosystem functioning. Results from Wang et al., (2021), indicated that there are synergistic effects of microplastics and metals, and that the synergistic effects of microplastics and Pb^{2+} may promote the formation of algal blooms in freshwater environments. Eerkes-Medrano et al., (2015) suggested that microplastic accumulation in pelagic and benthic habitats might alter light penetration into the water column or change sediment characteristics, and these changes could affect biogeochemical cycles. Pereao et al., (2020), claimed that polymers like polyvinylchloride (PVC), polystyrene and polycarbonate are made of monomeric repeating units which can induce reproductive abnormities and release toxic monomers associated with cancer in humans, invertebrates and rats. Reduced cell viability, increased intracellular ROS levels, changes in membrane integrity and function, and inhibition in mitochondrial membrane potential are indicators of cytotoxicity in a study which was done on size-dependant adverse impact of polystyrene microplastics of 0,1 µm and 5 µm size on human intestine cells (Patil et al., 2021).

Daphnia, which is a genus of planktonic freshwater crustaceans are commonly used in ecotoxicological laboratory tests. Studies have shown that ingestion are dependent on size, shape, type and concentration of microplastics (Miloložа et al., 2021): *Daphnia magna* are able to ingest PE particles in the size range of 63-75 µm and PET long fibres <1400 µm while elevated PE particles concentrations decreased mobility of *Daphnia magna* and they were more severely affected by irregular shaped beads (10-75 µm) compared to regular shaped beads (10-106 µm). Microplastics have strong hydrophobicity and easy adhesion of pollutants (Wang et al., 2021). Microplastics can act as vectors for the transportation of hazardous hydrophobic organic chemicals (HOC), persistent organic pollutants (POP's, additives, plasticizers and heavy metals (Razeghi et al., 2021). Razeghi et al., (2021) also showed that microplastics can act as pathways of toxic chemicals in the environment, and work their way up in the food chain, which may lead to the concentration of toxins in humans which suggests that microplastic impacts are likely to spread across trophic levels. Chemicals sorbed on plastic materials are typically hydrophobic organic compounds and includes polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbon compounds (PAH) and organochlorine pesticides, and also trace metals (Cormier et al., 2021).

The effects of polypropylene microplastics were investigated on human derived cell line and it was proposed that they were cytotoxic in size- and concentration-dependant manner; and impacts were wielded by cytokines production from immune cells after direct contact of cells and polypropylene microplastics (Patil et al., 2021). Additionally, humans are exposed to microplastics through ingestion of crops that have been watered with contaminated water (Domenech and Marcos, 2021) used for crop production, and as a result, microplastics end up in soils where they undergo degradation processes by microorganisms. The presence of microplastics in the environment, specifically in freshwaters, have a possibility of bio-accumulating in the food chain. It can negatively affect many kinds of species and can transfer chemicals into freshwater and organisms that form part of the ecosystem. These microplastics can be highly toxic to organisms, pose a great risk on their health, and can lead to decline in their population which ultimately exposes humans to contamination and puts their health at risk.

2.10 GLOBAL CONCERN AROUND MICROPLASTICS

Plastics have been manufactured since the early 1940's, and there has been a rapid increase in the number of plastics being produced ever since, which lead to a widespread usage of plastics in various applications (Pereao et al., 2020b). Global plastic production trends, consumer use patterns, inappropriate plastic waste disposal and demographics suggest that there will be an increase in plastic production in the future. Demands for plastics are growing exponentially and trends of production are expected to quadruple by 2050 (Karbalaei et al., 2018). With increase in plastic production, plastic wastes in the environment and microplastics, will increase immensely. The European Chemicals Agency (ECHA) considers that current information on microplastics is insufficient to derive a robust predicted non-effective microplastics concentration, i.e. a threshold value that could be used as proof that risks are adequately controlled. This means that any microplastic released in the environment should be considered as risky (Miloložа et al., 2021). The high consumer demand and insufficient plastic waste management have resulted in the accumulation of plastic remains in different environments, namely oceans, freshwater lakes and rivers, food, and even humans (Martinez-Tavera et al., 2021). Microplastics are spread all over the world and have been detected in beaches,

seabed sediments, wastewater effluents, surface waters, freshwater systems and in sea ice in the Arctic (De Bhowmick et al., 2021), states that research on plastic fragmentation indicated that microplastics are widely found in detectable range from mountain tops to the deepest part of the oceans and even Arctic ice, which creates pollution all over the world. Microplastics particles have been detected in a broad range of shapes, sizes, polymers and concentrations in the marine and freshwater ecosystems, agroecosystems, atmosphere, food and potable water, biota and other remote locations (Campanale et al., 2020). This shows that microplastic particles in the environment are extensive and wide-ranging in a ubiquitous fashion. The concern is that microplastics are foreign particles that are detected in natural systems and organisms, from which they do not originate. Although plastics provide extensive benefits, it also became a growing concern of ecological anxiety due to the number of plastic contributions to municipal waste and the proportion to global generated waste (Pereao et al., 2020b). The available information on the occurrence and effects of microplastics affirms classification as an environmental pollutant. Globally, much research is still required to fully understand exposure and effects of microplastic in the environment, and that makes it a cause for concern.

2.11 MICROPLASTICS IN SOUTH AFRICA

Plastics are used in every sector in the South African economy, because the plastic manufacturing industry contributed 1,6% to the Gross Domestic Product (GDP) and 14,2% to the manufacturing sector in 2014 (Verster et al., 2017). South Africa generated 1,1 million tonnes of plastic waste in 2017, which was equivalent to 19 kg of plastic per capita per year and 53 kg per person per day while the plastic industry was identified by the South African government as a priority sector for economic growth which encouraged export, trade policy measures, innovation, and recycling (Verster et al., 2017). Microplastic in aquatic ecosystems in South Africa come from Wastewater Treatment Plant effluents, sewer overflows, discharge, and runoff from sludge from agriculture and industries, urban runoff and informal settlements that result from littering and poor waste management (Verster and Bouwman, 2020). Although microbeads, which are primary microplastics, have been banned in several countries such as Canada, United States of America, United Kingdom, France, Sweden, Taiwan, South Korea and New Zealand but it has not been banned in South Africa but there are some initiatives that have been implemented by South African cosmetics industry to replace microbeads with other materials (Verster and Bouwman, 2020).

Research on microplastics in South Africa are still scarce and scanty but developing (Pereao et al., 2020b) with most published work on particles greater than 5 mm (Verster et al., 2017) and only few of these studies considered microplastics in freshwater systems (Verster and Bouwman, 2020). High concentrations of microplastic fragments were found in some sections in the Vaal River, the Crocodile and Klip Rivers with microplastic levels of up to 4, 5 particles per litre, and the microplastic load in the sediments of the Bloukrans River ranged between 6 and 160 particles per kg of dry sediment in summer (high flow) and winter (low flow), respectively (Verster and Bouwman, 2020). Microplastic effects on South African aquatic ecosystems are still mostly speculative due to limited reports in toxicological risks that microplastics have on aquatic organisms (Pereao et al., 2020b). South Africa is faced with two issues on microplastic pollution in the aquatic ecosystems. First, subsistence farming takes place in many rural areas where water, food security, and well-being of the population may be negatively affected due to water and soil contamination with microplastics when used for drinking and crop production (Verster et al., 2017) and secondly, South Africa has a rich natural

biodiversity and microplastic pollution is a potential threat to biota. Currently, studies on the impact of microplastics on biota are being conducted, which are yet to be fully understood (Verster et al., 2017).

2.11.1 Diep River

The Diep River rises from the Riebeek-Kasteel Mountains and flows south-westerly through Malmesbury, Table View and Milnerton, to drain into Milnerton Lagoon. The river is about 65 km long and forms an extensive vlei (wetland) at the Table Bay coastline, known as Rietvlei, which is largely utilised for recreational activities. Hence, the tidal inlet at the Milnerton lagoon and the wetland system (Rietvlei) constitute the important features of the Diep River (Jackson et al., 2009; Mafejane et al., 2002). The Diep River catchment has industrial areas, agricultural areas, formal and informal settlements, and wastewater treatment works with a total area of 1495 km^2. The catchment is low and flat (making it ideal for crop cultivation) but has isolated mountains (the Perdeberg, Kasteelberg and Paarlberg mountains) on its eastern boundary. The Diep River catchment is bound by the following towns: Riebeek-West (to the north), Paarl (to the east), Atlantis (to the west) and Milnerton (to the south) (Mafejane et al., 2002). The Messelbark River is the major tributary of the Diep River, while the Riebeek River, Klein River, Swart River, Platklip River and the Sout River are the others. The Diep River and its tributaries experience high water level in winter due to rainfall, but low water level and even dries up at certain locations in summer due to high evaporation regimes (Mafejane et al., 2002). Also, due to extensive siltation over the years as a result of catchment erosion, the Diep Rietvlei system serves as a storage area of sediment-rich water during river floods and after the flood, and there is a reduction in water level (Paulse et al., 2009). The Diep River is rich in biota-flora, that include phytoplankton/diatoms, algae, aquatic and semi-aquatic vegetation, terrestrial vegetation; and fauna such as zooplankton, aquatic invertebrates and fish (Grindley and Dudley, 1988). De Villiers also reported microfibre pollution in the South African coastal environment (de Villiers, 2018; De Villiers, 2019). There is evidence that showed a significant association between microfiber pollution hotspots along South Africa's coastal environment and land-based pollution sources such as wastewater treatment works, and in some cases, rivers (De Villiers, 2019). The Diep River, which is the largest river in the Cape Town metropolitan area, had the most abundant sediment microfibre levels in the Western Cape (De Villiers, 2019).

2.11.2 Plankenburg River

The Plankenburg River rises from the mountains of the Boland region, Western Cape, South Africa. It is about 10 km long and flows through Stellenbosch (known for winery) and Kayamandi township (informal residence) (Jackson et al., 2009). The Plankenburg River is the major tributary of the Eerste River in the Stellenbosch area, with the Kromme and the Jonkershoek Rivers being the other tributaries of the Eerste River. The Plankenburg River flows south-easterly and joins the Eerste River at the Adam Tas bridge which ultimately opens into the ocean at Macassar beach. The Plankenburg River services various industrial and agricultural activities, which includes irrigation of edible crops and some of the establishments on the Plankenburg River catchment includes clothing factory, cheese factory, spray painting, mechanical workshops, wineries and dairy factories (Nleya, 2005; Paulse et al., 2009). Most environmental risk assessment research on microplastics

was conducted on the marine environment (Wagner et al., 2014) but the microplastics reports on freshwater is growing (Adam et al., 2019).

2.11.3 Research gaps in microplastics pollution

In a systematic review of global scientific literature on aquatic microplastics, most research on microplastics pollution focussed on toxicology and environmental chemistry. Studies that focused on exposure and effects assessment of microplastics in the environment are therefore necessary because ecological studies on microplastics pollution are developing (Pauna et al., 2019). There is also insufficient data on the partitioning of organic contaminants in aquatic systems in combination with microplastics. Monitoring of spatio-temporal occurrence of microplastics in the environment, transportation and fate as well as ecological and human health implications of microplastics pollution; singly and in combination with other contaminants is thus important (Zhou et al., 2015). Organic hydrophobic contaminants on microplastics may be distributed between the plastic surface and thus act as sorbents for organic contaminants (Ziccardi et al., 2016). Urban centres were identified as a major source of plastics pollution in South Africa (Ryan et al., 2019) where illegal waste dumping and microplastics emissions from wastewater treatment plants contribute to microplastics burden of aquatic systems (Ryan, 2020). Though, inland plastic monitoring before entry into the sea has been suggested (Ryan et al., 2020), because aquatic organisms may ingest microplastics with possibilities of sub-lethal effects and mortality (Naidoo and Rajkaran, 2020). Ecological and human health risk of microplastics in aquatic ecosystems was also identified as a research gap (Ziccardi et al., 2016) and currently, because studies on MPs pollution is very scanty in South Africa, there is the need for this project study. This study also emphasized the need for ecological and human health risk assessment of microplastics pollution in South Africa because microplastics are pollutants of emerging concern and have been classified as possessing in endocrine-disrupting properties.

2.12 CONCLUSIONS

Plastics are ubiquitous in the environment due to their widespread uses. They are relatively cheap and durable making them preferred materials for use in industrial, household, and personal care products. The benefits, uses, occurrence and detrimental effects on man and environment have been described. Some analytical methods for microplastics analysis in environmental samples were presented. Two freshwater systems – the Diep and Plankenburg Rivers are being assessed for microplastics pollution because they are possible sources of exposure of microplastics to aquatic communities and humans. The increasing water scarcity necessitates investigation into environmental burden of microplastics and the different risk factors that may negatively affect water resources. Microplastics monitoring in the environment therefore becomes imperative. The increased requirement for environmental monitoring of microplastic pollution in water and sediment at global and national levels require the improvement in current approaches and procedures and the advancement of novel techniques to decrease identification effort and time. We have assessed the microplastic distribution in the Diep and Plankenburg Rivers over four seasons. Sampling plan was twice per season but only two seasons could be sampled twice due to the COVID-19 pandemic national restrictions in 2020 and 2021. A combination of analytical approaches and techniques assisted in the identification of microplastics in environmental matrices. Methodologies used, sampling and results obtained for microplastics analyses are presented in subsequent chapters.

CHAPTER 3: RESEARCH DESIGN AND METHODS

3.1 INTRODUCTION

This chapter presents the experimental details of this study and is subdivided in alignment with the objectives of this work. Each of the seven sections addresses an important aspect of the microplastic analyses. Section 3.2 describes the microplastic sampling in freshwater and sediments. Section 3.3 describes the microplastic extraction from environmental matrices and Section 3.4 describes the microplastics morphological and chemical characterization. The microplastic quality assurance and quality control, and data analysis are described in Section 3.5 and 3.6. The research study areas are described in Section 3.7. Ecological and human health risk assessment studies will be presented in the next deliverable.

The workflow designed was adapted to the suggestions of Cowger et al., (2020) as presented in Figure 3.1.

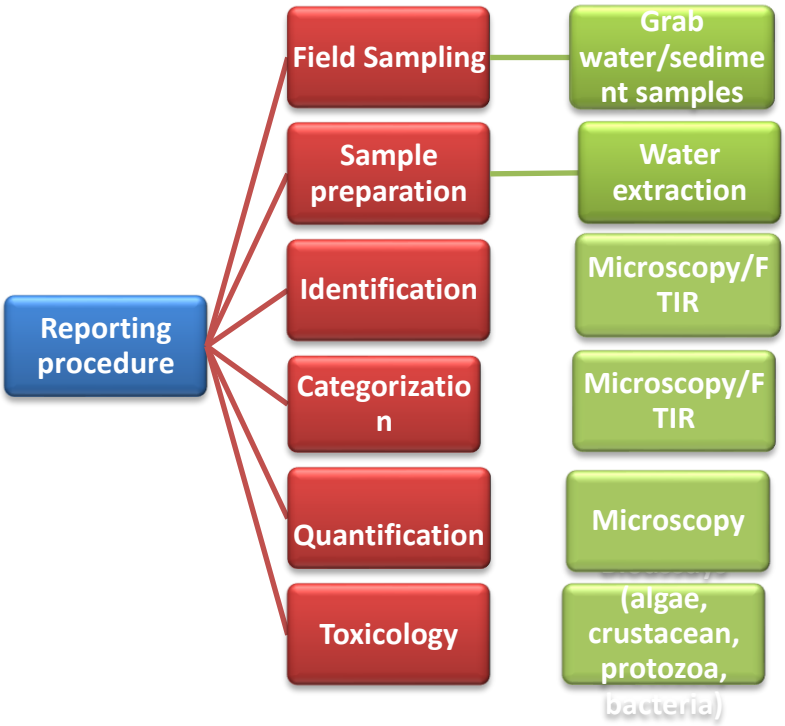

Figure 3.1. Workflow of microplastics analyses in the Diep and Plankenburg Rivers (Adapted from Cowger et al., 2020)

3.2 MICROPLASTICS ANALYSES METHODOLOGY

3.3 METHOD DEVELOPMENT AND RECOVERY STUDIES

The methods development for this study was aimed at water volume reduction and organic matter removal in freshwater samples while maintaining microplastics integrity and avoiding contamination. For the water volume reduction approach, samples were sieved through two mesh sizes (250, 20 um). The 20 um was efficient to extract micro and nano plastic with high microplastic recovering rates (95 ± 4%). Virgin polymers (polystyrene, nylon-6, and polyethylene terephthalate) obtained from Sigma-Aldrich were characterised using Fourier transform infrared spectrophotometry.

Microplastic recovery

To investigate the recovery efficiency and the potential effects of the methodology on microplastics, virgin polymers of different sizes (1000-2000 um) were used. Effects of digestion reagents on polymers, and extraction method efficiency were studied. For polymer recovery, the water samples were spiked with 50 fragments of each polymer type (PS, Nylon, and PET) in three replicates per polymer size. After the application of the selected methodology, the filter was observed under the microscope and photographed. Microplastics particles were confirmed using FTIR-ATR. Recovery rates were estimated as:

$$= \frac{Final\ number\ of\ particles}{Initial\ number\ of\ partcles}\ X\ 100 \quad \text{.....equation 3.1}$$

Results indicated no significant change in polymer structural integrity.

3.4 QUALITY ASSURANCE AND QUALITY CONTROL

Preventive measures were undertaken to minimise potential contamination of microplastics particles during sampling and laboratory processing according to standard used by Wang et al., (2018). Microplastics equipment were rinsed thoroughly with distilled water before use. When possible, plastic equipment was replaced by non-plastics, and when not, it was rinsed adequately with distilled water and blank experiments conducted to account for possible contributions from materials used. Cotton laboratory coats were always worn with nitrile gloves during the whole process and filters and solutions were covered by aluminium foil during the process. Reverse Osmosis (RO) water, Saturated NaCl solution and KOH were filtered through 10 µm size mesh under vacuum. Three blank experiments were conducted to handle potential contamination in the laboratory by running the same process with samples in parallel. If microplastics was detected on the mesh, then the amount was deducted from the final values.

3.5 FRESHWATER SAMPLING

The sampling points were selected based on possible routes of microplastic entry, including human and industrials activities. Site selection was informed by the different land use practices in the vicinity of the rivers. and sampling was undertaken over four seasons for the Diep and Plankenburg Rivers, – summer, spring, autumn, and winter. Onsite, a 30 L water sample was filtered in three replicates of 10 L per site. A 10 L metal

bucket was used to collect water while standing opposite to the windward side of the sampling point to prevent any contamination of the samples from laboratories clothes. The bucket was immersed 20 cm deep below the surface water opposite to the water flow direction. A total of 30 L of water was filtered in batches through 250 µm stainless steel sieve (three replicates of ten litres per sampling site); and the remaining particles on the sieve was transferred and stored in a small glass jar at 4ºC and taken to the laboratory for further analysis. At each sampling interval, the metal bucket and stainless sieve were cleaned carefully with deionized water to reduce cross-contamination. Offsite, a 12 L sample of the surface water was collected at each sampling site and taken to laboratory for further analysis. The 12 L brought into the laboratory was filtered through 20 µm mesh in three replicates of 4 L per site. Five sampling points were selected for the Diep River and four sampling points were selected for the Plankenburg River based on the land use practices to determine the occurrence of plastic items at the different sites. Temperature (T°C), pH, total dissolved solids (TDS), redox potentials (ORP), dissolved oxygen (DO), and electrical conductivity (EC) were measured in situ at each sampling location using a multi-parameter equipment (SensoDirect 150, Lovibond and Tintomete, Germany). Other physicochemical parameters like biological oxygen demand (BOD), chemical oxygen demand (COD), total phosphorus and total nitrogen were also measured in the laboratory.

3.6 SEDIMENT SAMPLING

Sediment samples were collected with a metal scoop to approximately 5 cm depth at each sampling site to achieve three replicate samples per site. Sediment samples were transferred into aluminium foil, stored in sealed plastic bags, stored in ice-chests, and transported to the Microplastic Laboratory, Cape Peninsula University of Technology. The samples were air dried in white dissecting trays prior to analysis and covered to avoid contamination in the laboratory.

3.7 MICROPLASTIC EXTRACTION BY DENSITY SEPARATION

The collected samples were processed to isolate the microplastics. MPs was extracted from the 12 L offsite sample taken to the laboratory by filtration through a vacuum pump system with 20 µm mesh. Alkali digestion was used to degrade organic matter prior to the isolation process (Maes et al., 2017). Residues of samples processed onsite was already stored in glass jars and labelled appropriately. The residues was transferred into 500 ml glass beaker with 10% Potassium hydroxide (KOH) in a ratio of 2:3 (w/v) and covered with aluminium foil for 24 h in the oven at a temperature of 50°C. Hypersaline solutions (NaCl 360 $g \cdot \ell^{-1}$) was added to the digested sample, stirred vigorously for 2 min, and was allowed to settle for 15 min and then filtered through a vacuum pump system with 20 µm mesh. This extraction process was repeated three times for each sample. The filters were then placed in clean petri dishes for further analysis. The extraction of microplastics from sediments samples was carried out with density fractionation method as described by Sparks, (2020); Sparks et al., (2021) with minor modification. Sediment samples were oven-dried at 50°C for 48-72 h to constant weight. A 20 g dry sediment from each replicate was transferred into glass beaker with 10% Potassium hydroxide (KOH) in a ratio of 2:3 (w/v) and placed in oven at 50°C for 24 h. Saturated NaCl solution (360 $g \cdot \ell^{-1}$) in a ratio of 1:2 (V/V) was added to the digested samples which was then stirred with a clean stainless spoon for 2 min and allowed to settle for 30 min. The dry samples in beakers were always covered with aluminium foil to avoid air-bone microplastics contamination. The supernatant of the solution containing

floating particles was vacuum filtered through a 20 µm nylon mesh pre-washed with a 10 µm filtered RO water, which was repeated three times for each sample. The meshes were then placed in clean petri dishes for further analysis.

3.8 MICROPLASTICS MORPHOLOGICAL AND CHEMICAL CHARACTERIZATION

3.8.1 Microscopy

The dried meshes placed in the pre-cleaned petri dishes were examined for microplastics based on physical appearance under a Stereo microscope (BS-3060CT, Bestscope, China). Particles was categorised based on their morphological characteristics (size, shape, and colour) as described by Hidalgo-Ruz and Thiel, (2013), and Masura et al., (2015). Microplastics was classified and measured by their sizes into six categories: C6 (<63 µm), C5 (63-500 µm), C4 (500-1000 µm), C3 (1000-2000 µm), C2 (2000-5000 µm) and C1 (>5000 µm). Particles were also classified based on their shapes (fibre, fragment, pellet and film) according to the method by Zhang et al., (2017). All suspected microplastic particles were photographed using a BestScope BHC3E-1080P HDMI Digital Camera (China) connected to the microscope. Microplastics abundance and mass was recorded in terms of Items/L and items/g dry sediment. Selected microplastics particles larger than 500 µm were analysed using Fourier transform infrared (FTIR). The following criteria was used to differentiate the particles types that were collected 1) thick pieces with three size dimensions comparable (fragments); 2) pieces with a homogeneous sphere/spherule (pellets); 3) pieces with their thickness significantly lower than other two dimensions (films); 3) lightweight pieces (foam) and 4) thin elongated pieces with one dimension significantly greater than the other two (fibres). Particles of uncertain nature were not accounted for in the final estimation (M. O. Rodrigues et al., 2018; Zobkov and Esiukova, 2017).

3.8.2 Fourier transform infrared spectroscopy

A Perkin Elmer FTIR system with Thermos Fisher Nicolet™ iN™10 and Bruker LUMOS was used to identify suspected microplastics particles polymer types. Transmittance mode was chosen for Nicolet™ iN™10 and spectra was acquired using LUMOS by choosing the Attenuated Total Reflection (ATR) mode, and the chemical composition were compared with other spectral libraries (Peng et al., 2018).

3.9 DATA ANALYSIS

Statistical analyses were conducted with IBM SPSS Statistics version 25. Prior statistical analysis data was tested using the Kolmogorov-Smirnov test for normality and the Levene's test for variance. Non-parametric analyses were conducted on the data using the Kruskal-Wallis test for analysis between microplastic abundances in surface water and sediment, with the significance of differences between the groups was determined by the Mann-Whitney U test with a <0.05 significance level. Post hoc analyses for significant differences between sites were conducted using pairwise comparisons of the Kruskal-Wallis analysis. The physicochemical parameters were assessed with the least significant difference test.

3.10 STUDY AREA

Sampling points were identified on the Diep and Plankenburg Rivers to assess microplastics pollution of both rivers.

3.10.1 Diep River

The GIS coordinates and description of the sampling sites on the Diep river are presented in Table 3.1. The map of the sampling sites on Google Earth is presented as Figure 3.2.

Table 3.1. Description and GIS Coordinates of sampling sites on the Diep River

S/N	Description	GIS Coordinates	Site code
1	Farming practices and opposite an informal settlement (Dunoon)	33 48' 03,9" S 18 32' 09,2" E	DR-1
2	Residential/Table Bay Nature Reserve	33 50' 14,3" S 18 31' 10,1" E	DR-2
3	Theo Marais – channel along the recreational /commercial/industrial activities	33 84' 6, 372" S 18 51' 6,750" E	DR-3
4	Theo Marais – wastewater treatment plant	33 84' 6,626" S 18 51' 5,461" E	DR-4
5	Woodbridge Lagoon (recreational activities a	33 52' 53,6" S 18 29' 22,2" E	DR-5

Figure 3.2: Sampling sites on the Diep river

Samples were collected at 5 points along the Diep River (Milnerton) twice per season to evaluate the possible spatial and seasonal variations in microplastic pollution and the physico-chemical properties of the river. Site 1 (DR-1) was near farming practices and opposite an informal settlement (Dunoon); Site 2 (DR-2) was along the Table Bay Nature Reserve; Site 3 (DR-3) was on a channel along the recreational area which connect to the river; Site 4 (DR-4) was along a recreational and residential area near the river and in close proximity to a wastewater treatment plant; and Site 5 (DR-5) was 1km from Woodbridge Lagoon where recreational activities take place.

3.10.2 Plankenburg River

The Plankenburg River is approximately 10 km long and flows through residential, agricultural and industrial sectors (clothing factory, cheese factory, wineries and dairy factories) and agricultural activities (irrigation of crops) of the area (Paulse et al., 2009). The river runs through Stellenbosch (Western Cape Province), popular for its wine estates and runs adjacent to the Kayamandi Informal Settlement that lacks proper sanitation. Storm water and sewage pipes from the settlement drains into the river and reduces the water volume and quality rendering it unsafe for domestic, agricultural, and recreational purposes. The four sampling sites description and GIS Coordinates are described in Table 3.2 and Figure 3.3.

Table 3.2. GIS Coordinates of the Plankenburg River sampling sites

S/N	Description	GIS Coordinates	Site code
1	Krom discharge point into Plankenburg River	33°55'51,597"S 18°51'7,253"E	PR-1
2	Confluence of the Krom Plankenburg Rivers	33°55'52,51"S 18°51'6,52"E	PR-2
3	Industrial and recreational park	33°55'51,496"S 18°51'6,157"E	PR-3
4	Kayamandi Informal Settlement/Commercial activities	33°54'10,870"S 18°50'30,724"E	PR-4

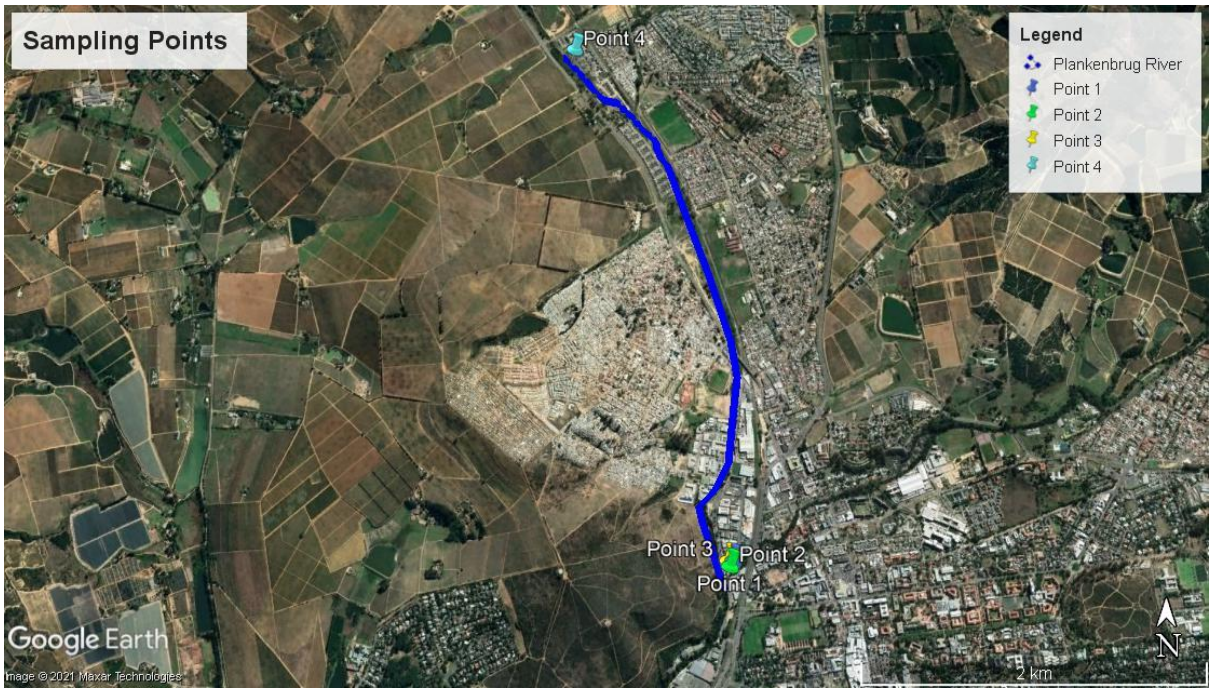

Figure 3.3: Sampling points on the Plankenburg River

3.11 ECOLOGICAL RISK ASSESSMENT

3.11.1 Microplastics stock suspension preparation

Studies have shown that *Daphnia magna* are able to ingest microplastics in the size range of 1400 µm in length and 528 µm in width which represent a similar size range to food ingested by the crustaceans. For the Diep River water samples exposure studies, transparent polystyrene granular plastics with size range between 200-1000 µm were grinded and used as primary microplastics. Polyethylene microspheres of size range 40-48 µm with a density of 0.94 g/mL purchased from Sigma-Aldrich were however used for the Plankenburg River samples. A concentration of 1000 mg/L stock solution of microplastics were prepared with dry particles and distilled water through shaking and sonicating and were kept in storage at room temperature before use. The stock solution was agitated in a shaker (NUVE BM 30, Turkey) for 2 h at 150 rpm and kept at room temperature. The stock solution was diluted with distilled water to the final concentrations of 400, 200, 100 and 20 mg/L, and ultrasound bathed for 15 min at 50 W for dispersion prior to the toxicity assay.

3.11.2 Ecotoxicological studies using environmental water samples and microplastics microspheres under climate-changing conditions

A battery of biotests were used to assess the ecological toxicity of the river water samples. Three freshwater organisms, each representing a trophic level (producer – *Raphidocelis subcapitata* 72h growth inhibition test, consumer – *Daphnia magna* 48h mobility test and decomposer – *Tetrahymena thermophila* 24h chronic growth inhibition test).

1. The model organisms were exposed to environmental water.
2. The model organisms were exposed to environmental water containing microplastic standards.

3. The model organisms were exposed to distilled water with microplastic standards at 3 different temperatures to assess the effects of temperature rise by 0.5°C, 1°C and 1.5°C and to understand potential responses of the model organisms to climate change effects.

3.11.2.1 Raphidocelis subcapitata 72 h growth inhibition test

Raphidocelis subcapitata toxicity tests were carried out using Algaltoxkit FTM supplied by MicroBiotests Inc. (Belgium). The OECD Guideline 201 (OECD 2002) method was used. The algal beads were de-immobilized according to the manufacturer's instructions. An algal density of 1×106 cells/mL was prepared from the concentrated algal inoculum by measurement of the optical density of the inoculum on a spectrophotometer (Jenway 6300) at a wavelength of 670 nm. The dilution series of the samples were prepared, and each flask was inoculated with 1×104 cells/mL as the test start concentration. The control was one of six treatments, and each treatment was in triplicates. The inoculated samples were incubated at 23 ℃ with a sideway illumination of 10000 Lux for 72 h. Experiments with temperature increases of 0.5^{o}C,1^{o}C and 1.5^{o}C were also conducted to assess climate change variabilities. Optical density measurements of the test cells were made at 24 h intervals for 72 h. Data were used to determine growth inhibition of *R. subcapitata* after exposure to water samples and microplastics contaminated water. Data analysis was performed using ToxRat® Professional software to determine toxicity endpoints.

3.11.2.2 Daphnia magna 48 h acute immobility test

Daphnia magna was exposed to water samples and microplastics-contaminated water using the ISO 6341 method. Hatching of the ephippia was achieved according to the supplier's (Daphtoxkit F Magna™, Microbiotests Inc., Belgium) instructions. The young daphnids were pre-fed 2 h before the commencement of experiments to prevent "starvation to death". The dilution series of the samples were prepared according to standard procedure OECD Guideline 201 (OECD 2002). The control was one of six treatments, and each treatment contained four duplicates. Five neonates that were actively swimming were put into each of the test wells. The multiwall plate was covered and incubated in darkness at 20 ℃. Experiments with temperature increases of 0.5^{o}C,1^{o}C and 1.5^{o}C were also conducted to assess climate change variabilities. After 24 h and 48 h incubation, the test plate was scored to determine the number of dead. Experimental data were analysed using ToxRAT Professional 3.2® to determine mortality, statistical significance, and critical concentrations.

3.11.2.3 Tetrahymena thermophila 24 h chronic toxicity test

A short-term assessment of chronic toxicity was conducted using *T. thermophila* – a freshwater ciliate protozoa obtained as Protoxkit ^{FTM} (Microbiotest Inc., Belgium). The Protoxkit assay is a 24 h multi-generation growth test that covers 5-6 generations. The experiment depends on the conversion of substrate into ciliate biomass. The proliferating cell cultures cleared the substrate suspension while the growth inhibited culture remained turbid. The optical density measurement of the turbidity using a spectrophotometer (Jenway 6300) at a wavelength of 440 nm provided information on the degree of inhibition. Sample dilution series were prepared according to standard procedures. There were six treatments, control and each treatment had two replicates. Holding trays of experimental cells were incubated in darkness at 30℃ for 24 h. Experiments with temperature

increases of 0.5^{o}C,1^{o}C and 1.5^{o}C were also conducted to assess climate change variabilities. Optical densities were measured at the beginning and at the end of the experiment.

3.11.3 Toxicity evaluation of water samples and microplastics standards suspension

Toxicity and lethal concentration/effect concentration (LC/EC) values are inversely related, and the percentage effects (PE) are used to describe concentration-based toxicity measures. According to Kaza et al., (2007), the data for toxicity of non-diluted river water samples have been expressed as PE of mortality or inhibition of growth and reproduction, depending on the effect criterion of the respective test procedure scoring system. Acute hazard is used to express acute hazard of concentration-based toxicities. According to Persoone et al. (2003), the Acute hazard classification system includes no acute hazard (class I) when PE ≤20%; class II when slight acute hazard 20% ≤ PE < 50%; class III when acute hazard 50% ≤ PE < 100%; class IV when high acute hazard in at least one test PE = 100% and class V PE = 100% very high acute hazard in all tests.

The classification system was based on a ranking in 5 acute hazard classes (Persoone et al., 2003). The percentage effect (PE) for each microbiotests was obtained and the samples were ranked into each of the 5 classes based on the highest toxic response shown in at least one of the biotests used.

Toxicity classes were determined as follows:

Class 1: No acute hazard – PE < 20% in all used biotests

Class 2: Slight acute hazard – 20% ≤ PE < 50% in at least one biotest

Class 3: Acute hazard – 50% ≤ PE < 100% at least one biotest

Class 4: High acute hazard – at least one biotest PE = 100%

Class 5: Very high acute hazard – PE = 100% in all biotests

Class weight scores were evaluated by the allocation of a test score for the effective results of each test of the battery according to equations 3.2 and 3.3 (Kaza et al., 2007; Szklarek et al., 2021).

Class weight score = $\sum all\ test\ scores/n$ ……...equation 3.2

n = is the number of tests performed

a) Calculation of the class weight score as a percentage

% Class weight score = Class score/maximum class weight score x 100 ……...equation 3.3.

A weight score was calculated for each hazard class to indicate the quantitative importance (weight) of the toxicity in that class according to Persoone et al., (2003).

• Allocation of a test score for the results of each biotest in the battery

Score 0 = No significant toxic effect, PE ≤ 20%

Score 1 = Significant toxic effect, 20% ≤ PE < 50%

Score 2 = Toxic effect, 50% ≤ PE < 100%

Score 3 = 100% = PE

3.12 MICROPLASTICS CONTAMINATION FACTOR AND POLLUTION LOAD INDEX

The microplastics contamination factors (MPCfs) and pollution load index (MPPLI) in the rivers were estimated as described in previous studies (Kabir et al., 2021). The MPCf refers to the contamination of MPs in the studied river compared to the background values. The MPCf is known as a standardised monitoring and assessment approach for determining the level of contamination between different sites. MPPLI is the riverine MPs pollution load index, which is the nth root of the total MPs pollution load indices multiplied together. The MPCfs were categorised into risk category according to Verla et al, 2019 and Kabir et al 2021 (Kabir et al., 2021; Verla et al., 2019) for values with MPCf<1 are low contamination, 1≤ MPCf<3 are moderately contaminated, 3≤ MPCf ≤6 are considerably contaminated and MPCf ≥6 very highly contaminated. The MPCf and MPPLI were mathematically computed using equations 3.4 and 3.5. Where MPi is the quantity of MPs in site i while MPb is the minimum baseline concentration taken from the lowest MPs abundance recorded in the literature (Mason et al., 2018) which shares similar environments and analytical context as this study. However, due to lack of available background data in similar environments and the analytical context of this study, the lowest MPs abundance obtained in this study was taken as the baseline concentration.

MPCf = MPi / MPb ……... equation 3.4

$$MPLI_{RIVER} = \sqrt[n]{MPLI_1 \times MPLI_2 \times \ldots . MPLI_n}$$ …….equation 3.5

3.13 HUMAN HEALTH RISK ASSESSMENT

3.13.1 Ames Mutagenicity Test

The Ames test is an approach used to test for mutagenic materials in water, sediment, air and chemicals (Ubomba-Jaswa et al. (2010)). The Ames test was done with the use of the EPBI Muta-ChromoPlate™ test (EBPI Inc., Mississauga, Ontario, Canada). and performed entirely in liquid culture. A 96-well microplate type of the *Salmonella typhimurium* Ames Test was used. The *Salmonella typhimurium* strains, TA100 and TA98, were used for sample screening with and without metabolic bioactivation. Positive controls used included 2-Sodium azide (TA100), 2-Nitrofluorene (TA98), and 2-Aminoanthracene with metabolic bioactivation S9. Negative controls were included in the test and the extent of the background reverse mutation rates were compared to the rates after contact with the samples. If a compound had twice the number of revertant colonies than the background rate of *Salmonella typhimurium*, it was considered a mutagen. The tester *S. typhimurium* strain TA98 +S9 and strain –S9 mix were conducted. Lyophilised bacteria were transferred into the nutrient broth and grown overnight for 16 to 18h.

The liquid reaction medium consisted of Davis-Mingioli salts, D-glucose, D-biotin, L-histidine and bromocresol purple, sterile distilled water and *S. typhimurium* TA 98. The S9 mix consisted of MgCl2 + KCL solution, Glucose-6-phosphate, NADP, Phosphate buffer, sterile water and S9 fraction (hydrate with 2.1 ml of sterile H2O). Water samples were added to the reaction medium and to the S9 mix (strain TA98 +S9). Strain TA98 –S9, were prepared with the samples and the reaction medium. A 96-well microplate with 200 µl in each well

was then filled with the suspension for each test. To prevent evaporation, plates were incubated at 37°C for 6 days. All yellow, partially yellow, or turbid wells were considered positive, and all purple wells were recorded as negative. All yellow, partially yellow, or turbid wells were considered positive, and all purple wells were recorded as negative. For each experiment, a blank, positive control and background (negative control) were run. The blank was used to ensure the sterility of the experiment; aII wells in the blank were expected to be purple. The standard mutagen sodium azide (0.5 µg/100 µl) was used for the positive control, and all wells were expected to be yellow.

CHAPTER 4: RESULTS AND DISCUSSION – PART I (DIEP RIVER)

4.1 INTRODUCTION

The aim of the next two chapters is to report the analyses of microplastics standards and their occurrence in the Diep and Plankenburg River. Recovery studies of microplastics standards were carried out and the method was then used for the extraction of microplastics in environmental samples. Results of the experiments of microplastics recovery from water are presented in this chapter. The spatial and temporal variations of microplastics distribution in both the Diep and Plankenburg Rivers were evaluated. Two different environmental matrices (water and sediment) were collected from both rivers and analysed. This chapter presents the results of the occurrence of microplastics particles in the Diep River. The results obtained for the Plankenburg River will be presented in the next chapter. Five sampling sites were selected for investigations on the Diep River; site selection was determined by a combination of land-use activities in the vicinity of the river and accessibility for sampling.

4.2 MICROPLASTIC RECOVERY STUDIES

The polymer recovery rates and potential effects of sieving on polymers of different types and sizes were investigated considering varying mesh sizes (250 and 20 um). None of the treatments led to significant polymer loss and no visual changes were detected in the polymers after the sieving process. Based on the results from this experiment, the mesh size of 20 um was chosen as the most appropriate protocol for water volume reduction. The virgin polymers exposed to the digestion methods to detect potential effects of the reagents on the polymers showed no visual changes were detected in polymers after the exposure to the digestion reagents after the microscope viewing and in the FTIR analysis used to complement the information based on the visual assessments. All the polymers retained their characteristic peaks after exposure to digestion reagents. This was also corroborated by previous studies that found no changes in the surface structure after digestion (Monteiro et al., 2022). For the water recovery studies, sieving freshwater samples using 20 um mesh size was efficient to extract micro and nano plastic with high microplastic recovering rates (95.0 ± 4%).

4.3 PHYSICO-CHEMICAL PROPERTIES OF THE DIEP RIVER WATER SAMPLES

The physicochemical parameters are used to described the water integrity, fitness and protection of the health of aquatic ecosystems in specific rivers and for some range of activities or uses (Edokpayi et al., 2017). The physico-chemical properties of the Diep River water samples were measured using a SensoDirect 150 multi-parameter equipment (Lovibond® Germany). The parameters measured include the pH, dissolved oxygen (DO), electrical conductivity (EC), temperature (°C), redox potential (ORP), total dissolved solids (TDS) and Chemical Oxygen Demand (COD) (Table 4.1). The obtained results were compared with the South African national standard and water quality for inland waters by Department of Water Affairs & Forestry, (DWAF, 1996) and World Health Organization (WHO, 2017) Guidelines for water quality.

Table 4.1: Physico-chemical properties of water samples from the Diep River over four seasons (mean±SD)

Parameter	Season	DR1	DR2	DR3	DR4	DR5	DWAF	WHO
pH	Spring	8,6 ±1,03	9,16 ± 0,14	7,73*	9,13 ± 0,15	8,4 ± 0,43	6.5-9.0	6.5-8.5
	Summer	-	-	-	-	9,48*		
	Autumn	9,65*	-	9,38*	7.97*	8,75 ± 1,07		
	Winter	9,74*	-	9,28*	9,6	9,83*		
DO (mg/L)	Spring	11,8 ± 5,09	4,90 ± 0,71	6,4*	4,1 ± 1,70	11,3	08-10	N/A
	Summer	-	-	-	-	7,70		
	Autumn	9,60*	-	2,40*	3,30*	3,30 ± 1,27		
	Winter	8,30*	-	6,10*	5,60*	4,60*		
EC (µS/m)	Spring	3580 ± 777	3380 ± 1484	676*	2575 ± 176	5740 ± 3945	≤2500	1500
	Summer	-	-	-	-	34900*		
	Autumn	1990*	-	2940*	2310*	33500 ± 1979		
	Winter	1960*	-	2160*	1783*	20000*		
Temperature (°C)	Spring	20.9 ±4,60	19,4 ± 5,09	16,6*	20,3 ± 3,82	18,8 ± 3,46	≤25	≤ 37
	Summer	-	-	-	-	23,2*		
	Autumn	16,8*	-	18,6*	16,4*	17,85 ± 2,62		
	Winter	16,5*	-	16,8*	16,4*	15,8*		
Redox potential (mV)	Spring	83,0 ± 7,07	65,0 ± 39,6	119,0*	94,0 ± 4,24	77,5 ± 27.6	N/A	700
	Summer	-	-	-	-	41,0*		
	Autumn	6,4*	-	27,0*	-9,0*	-125 ± 187		
	Winter	59,0*	-	65,0*	52,0*	47,0*		
TDS (ppm)	Spring	239 ± 52,3	225 ± 97.6	454,0*	172 ± 11,3	384 ± 265	0-450	1000
	Summer	-	-	-	-	233*		
	Autumn	133*	-	196,0*	154*	223 ± 15,6		
	Winter	1319*	-	143*	1180*	134*		
COD	Spring	69,8 ± 5,42	71,6 ± 0,59	39,3*	66,2 ± 12,0	77,3 ± 7,78		
	Summer	-	-	-	-	226*		
	Autumn	103*	-	54,8*	55,3*	880 ± 20,0		
	Winter	81,7*	-	59,3*	75,0*	95,3*		

*Sampled once

The pH values of the Diep River water samples were within the acceptable limits of DWAF except DR-1 in Autumn and winter seasons. Sites DR-4 and DR-5 also exceeded the DWAR regulatory limit in winter seasons. Generally, most of the pH measurements obtained were higher than the WHO maximum standards of 8.5. The results showed that the pH was close to neutral and alkaline for all the seasons investigated. The winter season pH values recorded 9.7, 9.3, 9.6 and 9.8 for DR-1, DR-3, Dr-4 and DR-5 respectively were higher than those of other seasons. Dissolved oxygen (DO) in water is a key water quality indicator required for the oxidation of organic matter and aquatic life (Barakat et al., 2018) and the obtained DO values varied from 2.4-11.8 mg/L. The results were within the permissible limits of DWAF standards for all the seasons: values observed for DR-1 and DR-5 (11.8 and 11.3 mg/L respectively) during spring showed more oxygen availability in the waterbody. The electrical conductivity (EC) values were generally above the permissible limits of WHO and DWAF standards. The EC values for DR-5 were excessively high for summer, autumn and winter seasons with 34900, 33800 and 20000 respectively.

The measured temperature values were within the permissible limit of WHO and DWAF standards. The results showed no significant difference in all the sampling points and seasons. Redox potential or oxidation-reduction potential (ORP) is a measurement of the chemical species propensity to acquire electrons and thus be reduced in the process (Gao et al., 2003). The total dissolved solids (TDS) values were mostly within the permissible limits of WHO and DWAF standards., Higher values were observed in winter seasons for DR-1 and DR-4. The high TDS values might be due to the natural and anthropogenic factors from the domestic and industrial activities in the area.

Table 4.2: Pearson's correlation coefficient for the physicochemical parameters and microplastics occurrences in the Diep River

	pH	**DO (mg/L)**	**EC (µS/m)**	**Temp (°C)**	**ORP (mV)**	**TDS (ppm)**	**COD**	**Water 250**	**Water 20**	**Sediment**
pH	--									
DO (mg/L)	-0.147	--								
EC (uS/m)	0.091	-0.128	--							
Temp (°C)	0.066	0.206	0.179	--						
ORP (mV)	-0.227	0.246	-.561*	0.279	--					
TDS (ppm)	0.222	0.223	-0.194	-0.123	0.118	--				
COD	-0.061	-0.245	.809**	-0.039	-.748**	-0.126	--			
Water 250	-0.114	-0.224	-0.243	0.221	0.118	-0.041	-0.043	--		
Water 20	-0.295	0.449	-0.178	-0.06	0.103	-0.013	-0.12	0.312	--	
Sediment	0.01	0.035	-0.152	0.448	0.194	-0.153	-0.125	0.197	0.127	--

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The Table 4.2 above presents the correlation analysis coefficients for the physico-chemical properties of water samples analysed during this study. There was negative correlation between pH and DO, ORP, COD, 250 and 20 µm mesh microplastics. Positive correlations existed between pH and EC, Temperature, TDS, and sediment. Significant (r=-0,561) negative correlation existed between EC and ORP. There was a significant (r=0,809) positive correlation between EC and COD; COD had a significant (r=-0,748) negative relationship with ORP and was the only parameter which had a negative relationship with ORP. Temperature and COD were the only 2 parameters which correlated positively with EC. Two parameters, temperature and ORP, correlated positively with microplastics sampled through 250 µm mesh, and four parameters (DO, ORP, Water 250 and sediment) correlated positively with microplastics sampled through 20 µm mesh. Three parameters (EC, TDS, and COD) had negative correlations between microplastics in sediment samples.

4.4 SPATIAL AND TEMPORAL DISTRIBUTION OF MICROPLASTICS IN WATER SAMPLES OF THE DIEP RIVER

Samples were planned to be collected twice per season, but due to COVID 19 restrictions, sampling could only take place once for summer and winter seasons. Samples were collected in September and November 2020 (Spring), February 2021 (summer), March and May 2021 (autumn) and August 2021 (winter). During the summer season, samples were only taken at DR5 because the surface water dried up at other sampling points. The occurrence of microplastics in samples revealed different results and patterns. Two sampling and extraction procedures were used for water samples. For the first procedure, 5 replicates of 20 L water samples were collected (a total of 100 L) and processed onsite. Microplastic extraction was achieved by filtration through a 250 µm mesh sieve. Trapped particles on the sieve were transferred into falcon tubes and transported to the laboratory. The samples were further processed in the laboratory by filtration through a 20-um mesh sieve. The second sample batch (20 L) collected at each site were transported to the laboratory and filtered through a 20-um mesh sieve. The results obtained from the two extraction processes in the Diep River water samples over four seasons are presented in Table 4.3. The smaller and larger mesh size samples are presented as Water20 and Water250 respectively.

Table 4.3: Abundance and distribution of microplastic particles in the Diep River water samples (mean microplastic per litre ± SD)

	Season							
	Autumn		Spring		Summer		Winter	
Site (MP per litre)	Water20	Water250	Water20	Water250	Water20	Water250	Water20	Water250
DR1	1.60±0,29	0.230±0,11	5.10±2,20	0.540±0,19	-	-	2.44±0,83	0.190±0,10
DR2	-	-	1.25±0,99	0.610±0,32	-	-	-	-
DR3	2.50±0,59	0.520±0,28	1.42±0,52	0.530±0,22	-	-	2.20±0,78	0.460±0,17
DR4	3.30±0,96	0.440±0,22	4.88±3,99	1.16±0,66	-	-	3.20±1,16	0.920±0,31
DR5	2.55±1,11	0.590±0,24	5.25±2,27	0.68±0,31	1.95±0,74	0.240±0,08	2.15±1,10	0.280±0,12

Results obtained from the small size mesh extracted more microplastics, in multiple orders of magnitude. The observation demonstrates the loss of microplastics during onsite filtration using a larger mesh size. Occurrence was also different for the different sampling sites as well as seasons. Anthropogenic activities in the vicinity of the sites as well as seasonal conditions such as rainfall and wind conditions contributed to microplastics distribution in the aquatic ecosystem. The site is in close proximity to a wastewater treatment plant. The aquatic environment received primary and secondary microplastics primarily from wastewater effluents that originate from domestic or factory-made sources (Strady et al., 2021). Increased mean microplastics per litre were observed for DR-1, DR-4 and DR-5 in spring. A total of 187 water samples were collected from the Diep River and processed. MPs were detected in all but one water samples. A sample collected froom DR-2 (the site in the vicinity of Table Bay Nature Reserve had no microplastic particle.

4.5 SPATIAL AND TEMPORAL DISTRIBUTION OF MICROPLASTIC IN SEDIMENT SAMPLES OF THE DIEP RIVER

Sediments were collected with a metal scoop at a depth of 5 cm of the flowing river. Five replicates of 20 g dried sediment samples were processed and analysed in the laboratory. Results obtained are presented in Figure 4.1.

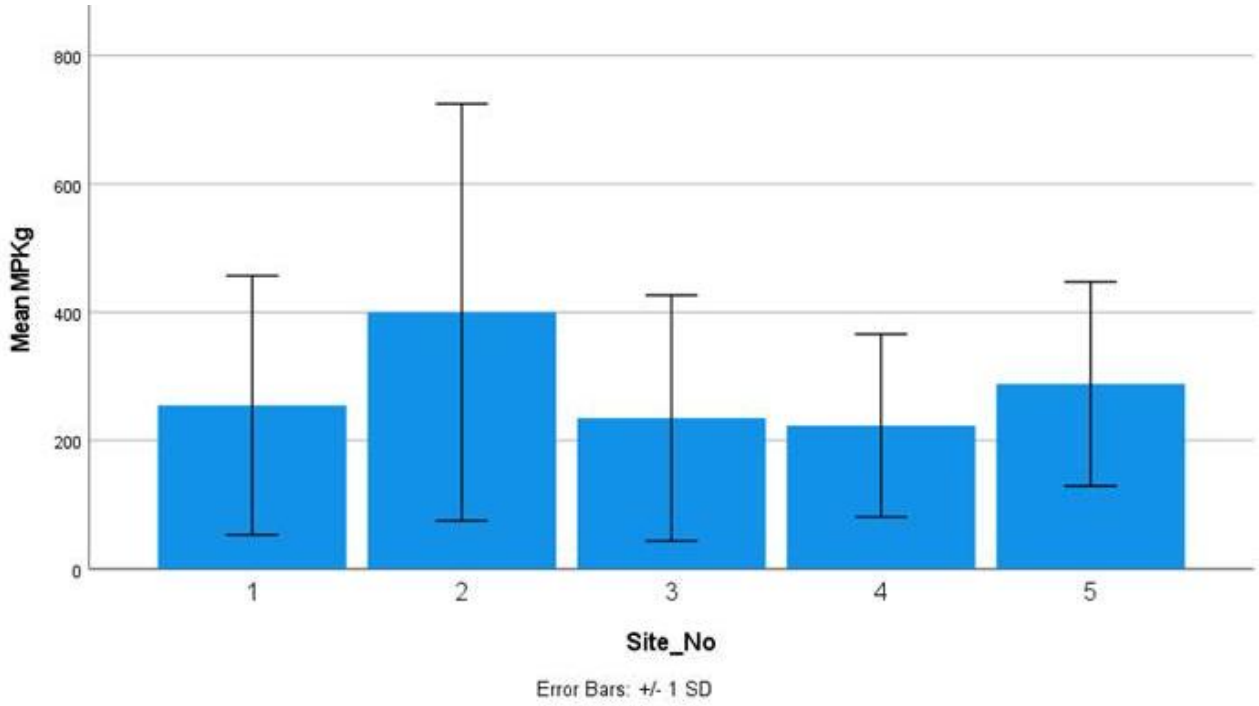

Figure 4.1: Spatial distribution of microplastics in the Diep River sediment (mean microplastic per kg)

Table 4.4: Abundance and distribution of microplastic particles in the Diep River sediment samples (mean ± SD)

	Seasons			
Site (MP per gram)	**Autumn**	**Spring**	**Summer**	**Winter**
DR1	14.5±0,13	15.5±0,25	-	5.50±0,07
DR2	-	20.0±0,32	-	-
DR3	17.0±0,23	14.5±0,06	-	-
DR4	9.0±0,06	12.0±0,17	-	-
DR5	11.5±0,09	22.5±0,15	10.5±0,11	8.50±0,08

The results revealed that sediment samples had between 115 and 450 mean microplastic per gram. DR3 and DR5 were found to have the lowest and highest mean microplastic per gram respectively during the spring season. There was also a significant difference in mean microplastic per gram in the sediment samples for all the seasons and this was attributed to a high volume of beachgoers and activities during spring. The highest microplastics burden in sediment samples in spring was probably influenced by wind transportation and an increase in anthropogenic activities after the cold wet winter season with consequent generation of more plastic wastes that got into waterbodies. Warmer temperatures, lower river volume and weaker flows are possible reasons for higher microplastics occurrence in autumn and spring. A total of 85 sediment samples with replicates were collected in the Diep River. The results revealed that sediment samples had between 5.50 and 22.5 mean microplastic per kilogramme of sediment. MPs were detected in 84 of the 85 sediment samples. MP abundances ranged from 12.0 to 22.5 MP per kilogramme during spring (September and November); corresponding values were "Not available to 10.5 for summer (February), 9 to 17 in autumn (March and May) and 5.50 to 8.50 during winter.

4.6 MORPHOLOGICAL DISTRIBUTION OF MICROPLASTIC IN THE DIEP RIVER

The transport and distribution of plastic debris to the marine, terrestrial and aerial environments are influenced by the environmental conditions and physical properties of plastics (Patil et al., 2021). The ingestion rate of microplastics in aquatic organisms are partly dependent on the physical properties of microplastics size, colour, density and organism's feeding behaviour (Razeghi et al., 2021). Hence, the need to understand the types of microplastics in the river system based on physical attributes. Microplastics recovered from the Diep River were characterized based on shape, color and size. Figure 4.2 represents the 6 different types of microplastics analysed and is presented as percentage of fibres, fragments, films, spheres, pellets, and foam. The percentages of film in water and sediment samples were almost undetected and insignificant, with a contribution of less than 0.01%. The most dominant microplastic type in water and sediment samples were fibres (88.1%), followed by fragments (6.86%), foam (4.22%), pellets (0.53%) and sphere (0.28%).

There were 6 different shape types of microplastics analysed in this study as shown in Figure 4.2 (a). Fibre was the most abundant (93.7%) microplastic particles identified followed by foam (3.88%) and fragment (2.43%). Our study agrees with a previous work that reported that fibre is the most dominant microplastic particle type freshwater ecosystems (Jemec et al., 2016).

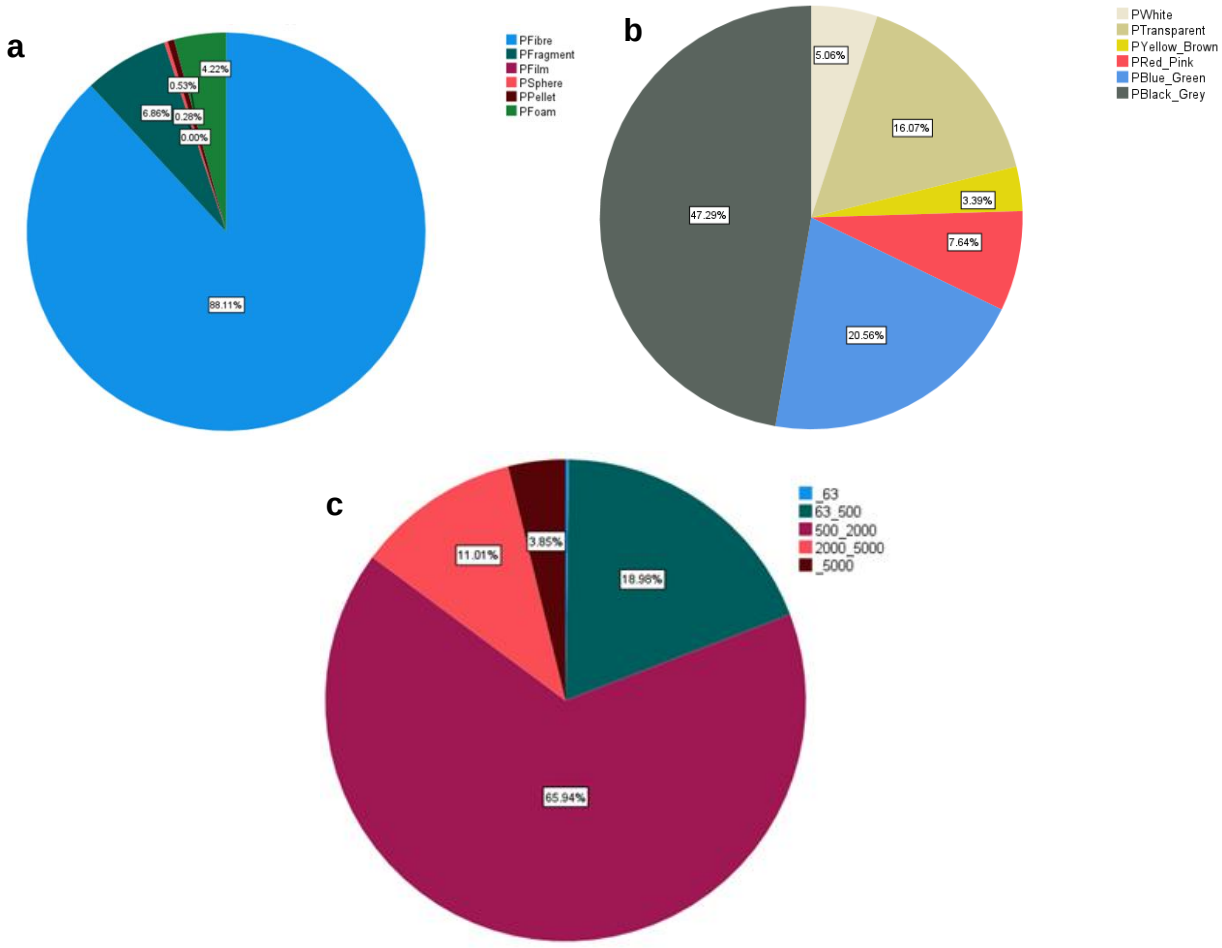

Figure 4.2: Abundance and percentage distribution of microplastics in the Diep River water and sediment samples over fours seasons (a) shapes (b) colour (c) sizes

Figure 4.2 (a) and 4.3 showed that fibres was the predominant shape found in all the sites. DR1, DR2, and DR3 recorded fragments as the second most abundant shape types. Fibres and fragments are both secondary sources of microplastics as the product of the breakdown of larger plastics (Eerkes-Medrano et al., 2015). There was no clear shape trend in the microplastic type at all the different sites because various sites contributed to microplastic pollution in the river. However, all categories of microplastic type were found at DR3 and DR4. The Diep River receives microplastics from various sources, such as from a wastewater treatment plant, informal and human settlements, and industrial and recreational activities. DR3 had the most fragments compared to all the other sites; and this suggests anthropogenic actiivities as a causal factor because of the site'sproximity to a recreational facility. DR4, which was near the wastewater treatment plant and along an industrial and recreational area, recorded the highest percentage of foam and could be as a result of industrial waste or littering. For the different sampling sites, DR1 was near the industrial and farming practices and opposite an informal settlement (Dunoon); DR2 was along a Nature Reserve and near a residential area; DR3 was on a channel along the recreational area which connects to the river; DR4 was along a recreational and

industrial area near the river and in close proximity to a waste water treatment plant; and DR5 was 1 km from the Milnerton Lagoon, Woodbridge where recreational activities take place. These variations in MP occurrence may have been caused by seasons and anthropogenic activities

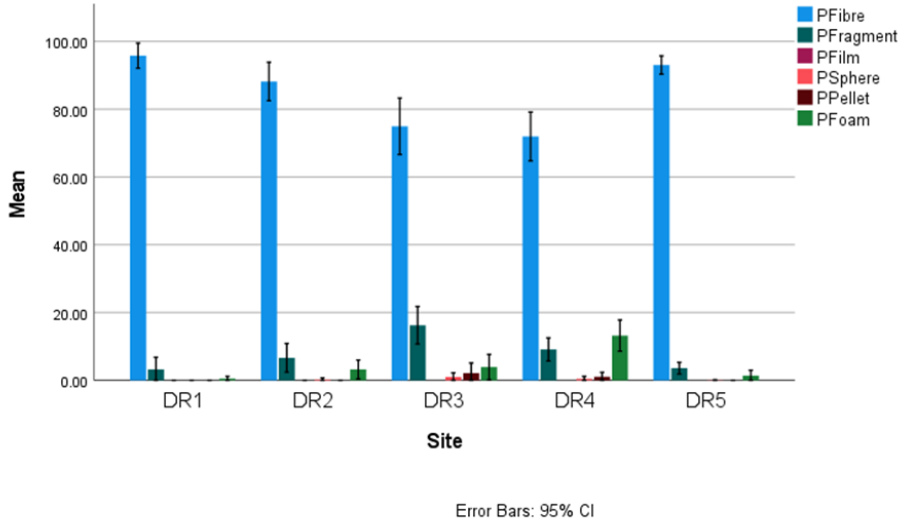

Figure 4.3: Mean percentage distribution of microplastic shapes in water and sediment samples

The microplastics were characterized based on colour; the percentage distribution of colours is presented in Figure 4.2 (b). Microplastics colours that were observed in this study were white, transparent, yellow/ brown, red/pink, blue/green, and black and grey. Black/grey (47.3%) and yellow/brown (3.39%) respectively, were the most and least prevalent of microplastics. Dark colours were the most common categories present with black and grey (47.3%), and blue and green (20.6%) being the two most abundant types. Transparent contributed to slightly less that the blue/green category with 16.1%, whereas the percentages of white and yellow/brown accounted for only 5.06% and 3.39%.

The sizes of microplastics were divided into 5 categories according to their size: <0,63 um, 0,63-500 um, 500-2000 um, 2000-5000 um and > 5000 um. Figure 4.2 (c) shows percentage distribution of each microplastic size found in this study. Majority of microplastics were in the size category of between 500-2000 um, followed by 0,63-500 um. The percentage of microplastics with the size of 500-2000 um occurred most and microplastics with the size of <0,63 um was the least. Majority of microplastics were in the size category of between 500-2000 um (65.9%) followed by 0,63-500 um (18.9%) and 2000-5000 um (11.0%). The smallest (<0,63 um) and largest (> 5000 um) size categories accounted for the least microplastic percentage with < 0% and 3.85%, respectively. Selected images of microplastics found in various study sites are presented in Figure 4.4.

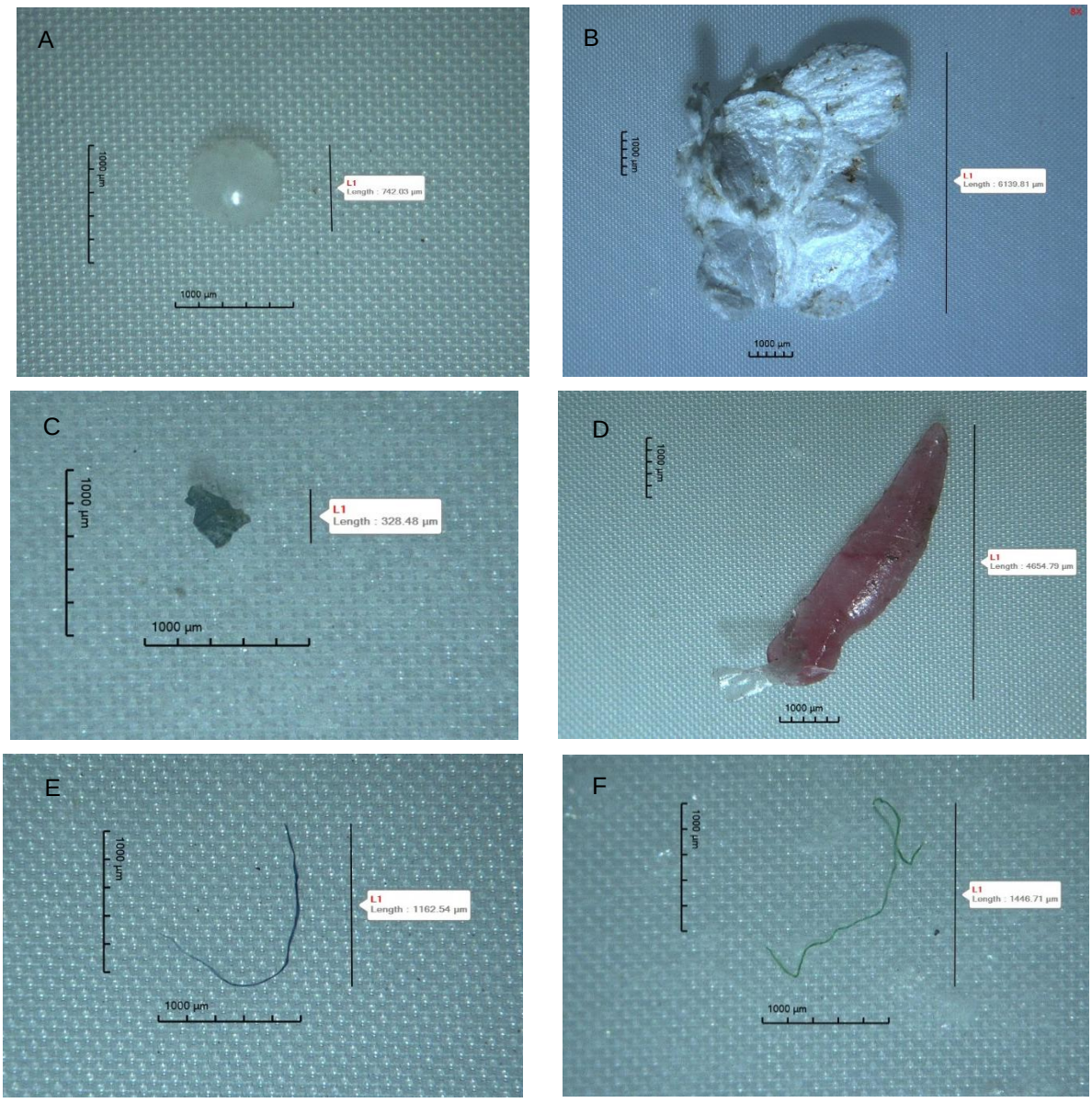

Figure 4.4: Microplastic particles types found in the Diep River (a) pellet, (b) foam, (c) fragment, (d) fragment and (e) fibre.

Size, shape, and abundance of the polymer can influence uptake and effects. Fragmentation rate does not only depend on the access to UV-radiation and other environmental factors, but also the polymer type and presence of additives. Antioxidants and UV light absorbers are added to polymers, especially for outdoor applications, in order to slow down discoloration and impairment from heat and UV-exposure (Lithner et al., 2011). Additives, residual monomers, by-products, and solvents are all low molecular chemicals that can leach out from the polymer to air, water, and organisms. The surface area available for biofouling and sorption of pollutants differs between polymer types and effects from sorbed chemicals have been demonstrated in fish and worms thus proving the capability of microplastics acting as vector for pollutants (Rochman et al., 2013; Teuten et al., 2007).

4.7 CHEMICAL PROPERTIES OF MICROPLASTICS

Plastic is a macromolecular compound that is polymerized by adding or condensing a monomeric raw material (Jiang et al., 2018). It is an artificial product that has undergone polymerisation of monomers. Polymer additives are added to change or improve plastic properties and different polymers will have different characteristics. To determine the type of polymers found in this study, the chemical composition was analysed using Fourier Transform Infrared spectroscopy (FTIR). FTIR data for the spring season are presented in Figure 4.5 (a-d) which represented the percentage distributions of the polymers scanned for the different sites. DR 1 had mostly fibres, and no data was presented 1 as fibres were unable to be scanned as it was either too fine or were lost while being placed onto the crystal.

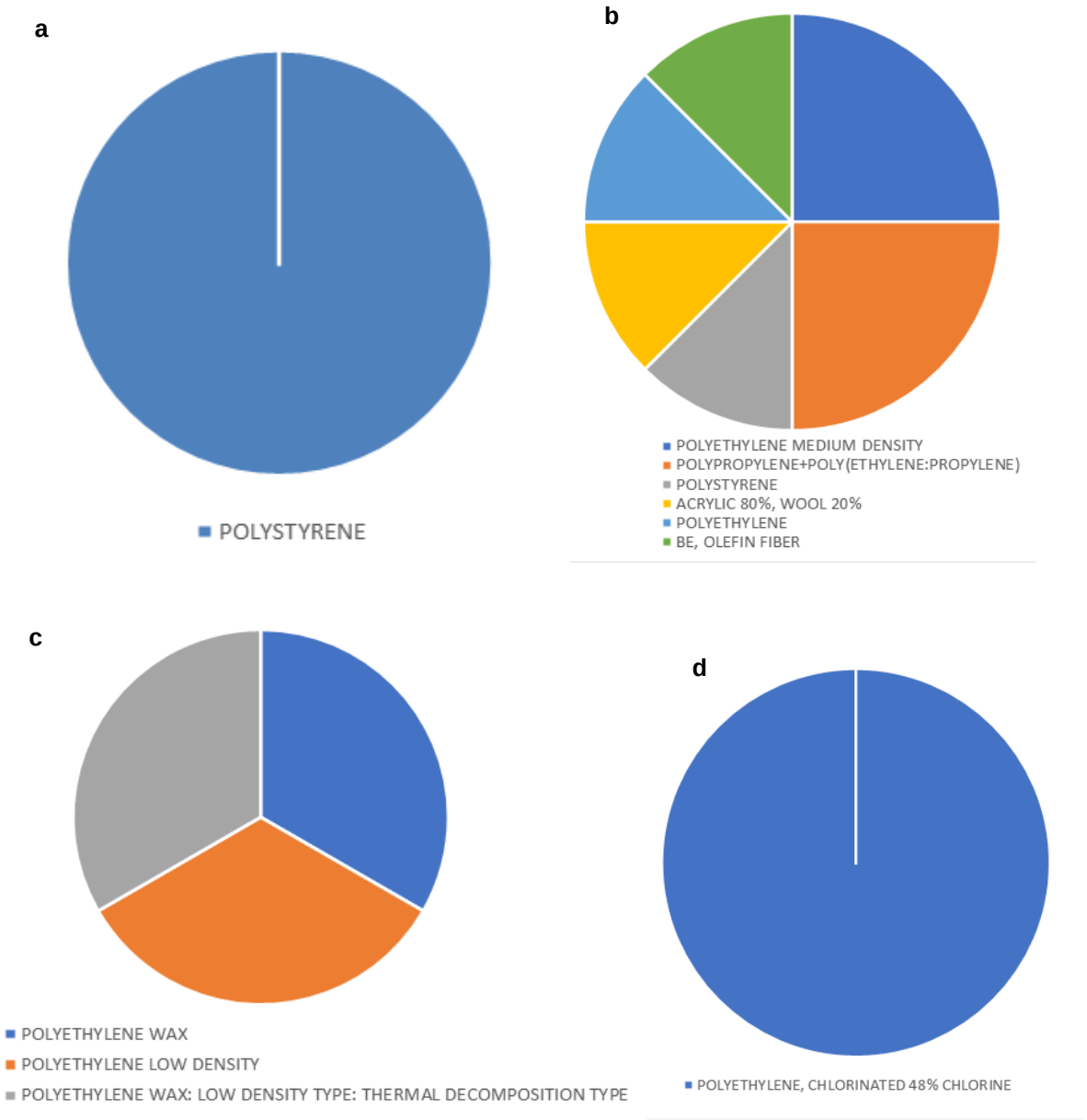

Figure 4.5: Occurrence of polymer types in the Diep River per site (a) DR2 (b) DR3 (c) DR4 (d) DR5 for the spring season

The DR3 sampling sites had most diverse polymers including some of the most common and uncommon types relative to the other locations sampled along the Diep River. Polystyrene was the main polymer found in samples obtained from DR2. Fibres were also present, however, there were complications attaching fibres to the crystal on the FTIR due to its fine size. There were a variety of polymer types found in DR3, which is near a recreational and industrial area. Polymers found at this site are polyethylene medium density, polypropylene + poly (ethylene: propylene), polystyrene, acrylic 80%, wool 20%, polyethylene and olefin fibre. These polymers are likely contributions from both industrial and anthropogenic activities. Polyethylene were the main polymer found in DR4. The different types of polyethylene found at this site were polyethylene wax, polyethylene low density and polyethylene wax: low density type: thermal decomposition type. The surrounding areas include a wastewater treatment plant, industrial and recreational area. DR3, which is a channel that feeds this site is probably a contributing factor of the types of polymers found at DR4, apart from the surrounding activities in this area. The main type of polymer found at DR5 was polyethylene. This polymer consists of a wide variety of plastics that are used by human activity. As this site is a recreational area, anthropogenic activity is likely the main contributor of this polymer in DR5.

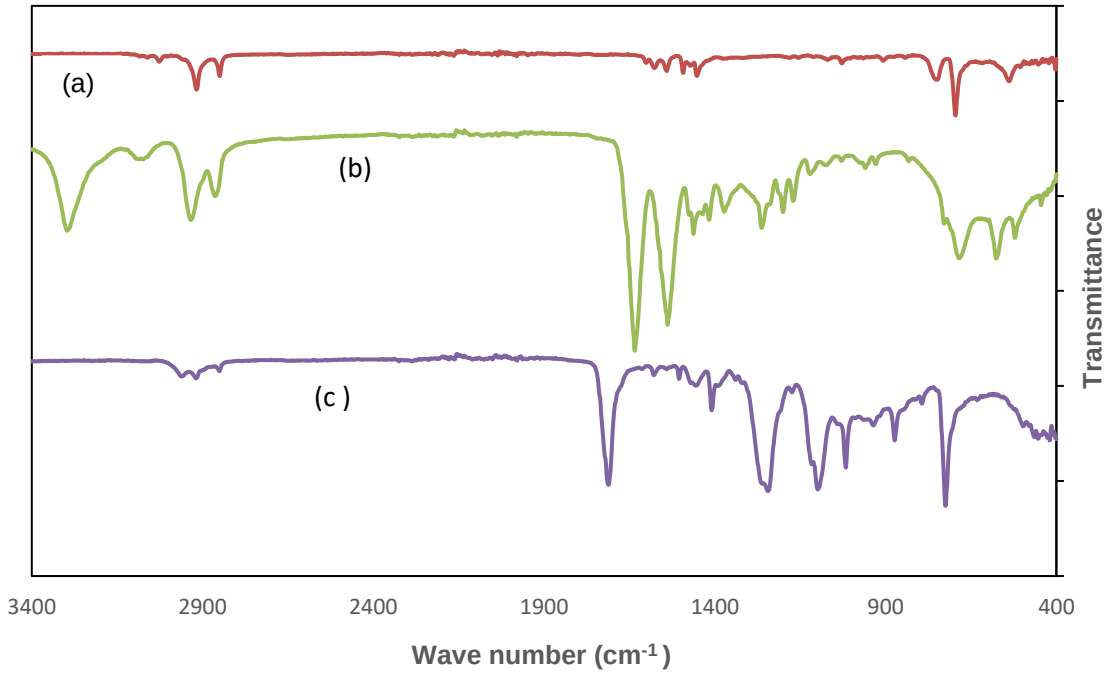

Figure 4.6: FTIR spectra for microplastics standards (a) Polystyrene (b) PA-6 (Nylon) (c) Polyethylene terephtalate

The FTIR spectrum for microplastics standards in Figure 4.6 (a) confirmed the presence of the main absorption characteristic peaks of PS. The following were identified; aromatic C-H bond stretching vibration; (1601; 1493; 1453 cm^{-1}), deformation CH_2 and C=C of the aromatic ring; (1026 $cm^{-1;}$ 756 cm^{-l}). Moreover, the peaks at 1601, 1493 and 1453 cm^{-1} all belong to the characteristic peaks of benzene ring of the polystyrene backbone and the spectrum of PS is in agreement with previously published spectra and bands of the IR spectrum for polystyrene (Pereao et al., 2020a). Chemical studies have also indicated that the monomeric units in polystyrene occur predominantly in a head-to-tail arrangement, i.e. the phenyl groups are attached to alternate carbon atoms (Su, 2013). Figure 4.6 (b) presents the ATR-FTIR spectra of PA-6 (Nylon) and the spectrum

shows the main characteristic vibrations for NH, CH_2 and C=O. The peaks at 3284 cm^{-1} and 3062 cm^{-1} are assigned to N-H stretching, while the peaks at 2925 cm^{-1} and 2854 cm^{-1} are assigned to CH_2. The sharp C=O peak appeared at 1617 cm^{-1} while the peak at 1537 cm^{-1} is assigned to N-H bending (Guerrinia et al., 2009). Other peaks that appeared at 1458 cm^{-1}, 1361 cm^{-1}, 1258 cm^{-1} and 680 cm^{-1} are assigned to C-H bend, C-N stretch, HN-C=O and N-H bending, respectively. The peak assignments for microplastics standards shown in Figure 4.6 (c) depicts the FTIR of PET with bands at 1712 cm^{-1} attributed to the ester carbonyl bond stretching, and the band at 1245 cm^{-1} to the ester group stretching. The band at 1092 cm^{-1} is assigned to the methylene group. The band at 1452 cm^{-1} is assigned to a CH_2 bending mode while the band near 1409 cm^{-1} is assigned to a CH_2 wagging mode of the ethylene glycol segment. The band near 1016 cm^{-1} is assigned to an O-CH_2 stretching mode and the band near 827 cm^{-1} has been assigned to the C=O out-of-plane bending mode coupled with a ring CH out-of-plane bending mode. Other sharp bands at 1400-1600 cm^{-1} and 950-1250 cm^{-1} are assigned to C-C stretching and C-H in plane bending of benzene rings (Liu et al., 2013). The assignments for PET are virtually identical to those reported previously (Chen et al., 2012; Djebara et al., 2012) and the absorption bands are assigned according to the groups in the monomeric unit of PET.

Figure 4.7 presents four polymers that were identified with their match degrees: Four kinds of microplastics, including polypropylene (94.6%), polyethylene (98.4%), polystyrene (93.8%) and cotton 90% (70.2%) were detected in the Diep River.

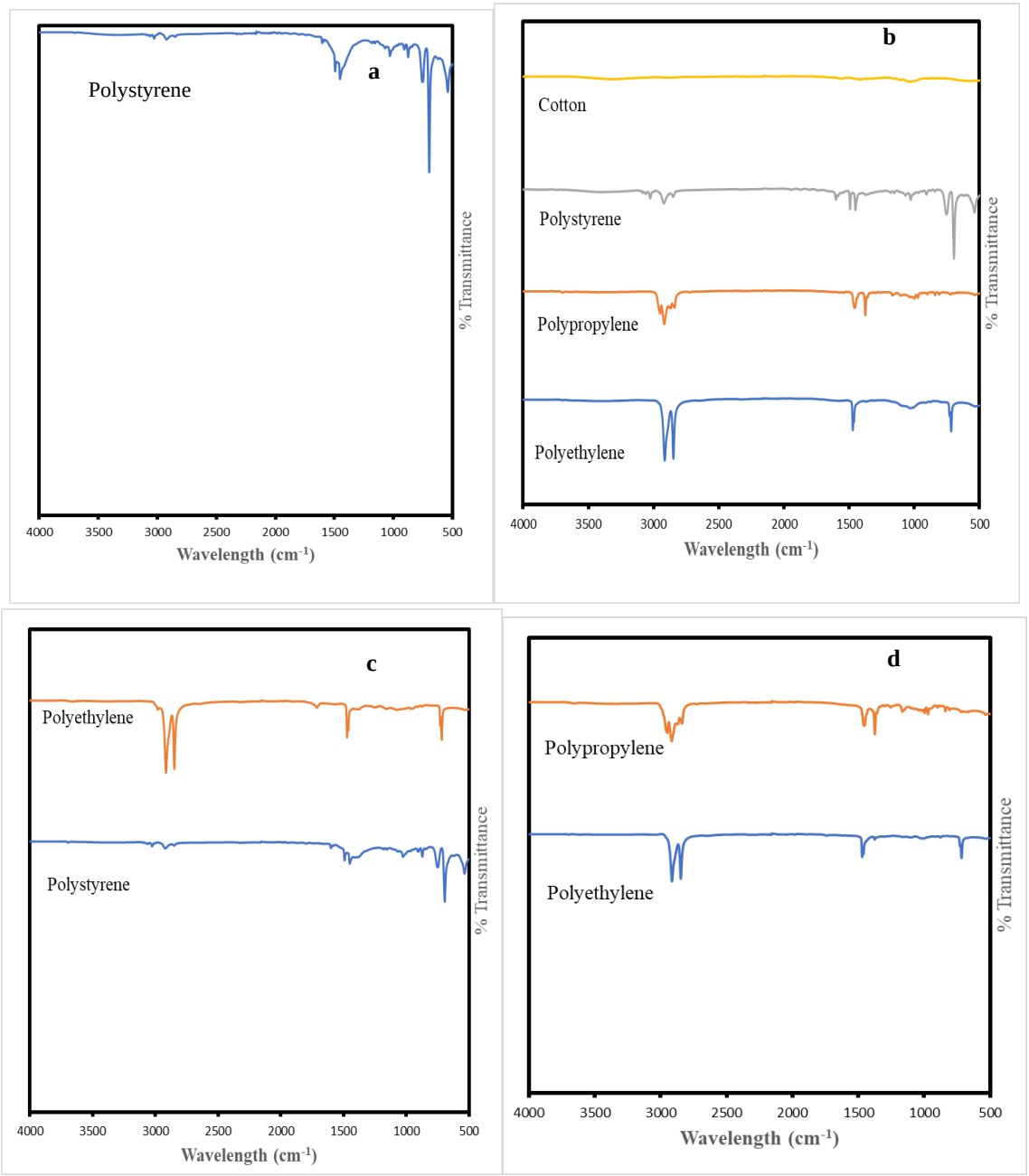

Figure 4.7: FTIR spectra of the microplastics found at a) DR2, b) DR3, c) DR4 and d) DR5 in different sites along the Diep River

The largest number of microplastic types found in this study are polypropylene and polystyrene. In DR2, polystyrene characteristic spectra display peaks at around 3030, 2915, 1601, 1494, 1448, 1028, 877, 757, 716, 695 and 538 cm^{-1}. In DR3, Cotton produced characteristic spectra peaks at 1543 and 989 cm^{-1}; PS characteristic spectra display peaks at 3028, 2914, 2848, 1601, 1494, 1453, 759, 721, 698 and 539 cm^{-1}; while PP display characteristic spectra peaks at 2958, 2920, 2840, 1454 and 1377 cm^{-1}; and PE show characteristic spectra peaks at 2914, 2864, 2847, 1470 and 717 cm^{-1}. It can be seen that the PE sample in DR4 produced characteristic peaks at 2915, 2868, 2849, 1713, 1472 and 718 cm^{-1} while PS produces peaks at wavenumber regions 3028, 2920, 1493, 1450, 872, 753, 724, 696 and 539 cm^{-1}. At DR5, the main peaks of PP are at 2954, 2935, 2918, 2841, 1460, 1377, 1164, 842 and 536 cm^{-1} and PE show main peaks at around 2913, 2864, 2849, 1472 and 719 cm^{-1}. Polypropylene, Low-Density Polyethylene, High-Density Polyethylene, and polystyrene

are used daily typically in packaging (Razeghi et al., 2021) and are among the most commonly used and abundant polymers worldwide (W. C. Li et al., 2016).

4.8 SUMMARY

Microplastics are ubiquitous and originate from various anthropogenic sources. The sources of microplastics to the Diep River include formal and informal residential areas, a wastewater treatment plant, recreational, commercial, and industrial processes This chapter provides data on the spatial and temporal distribution of microplastics in water and sediment samples from the Diep River over four seasons. There are no clear trends for MPs distribution in the Diep River and seasonal and temporal variations are likely due to a combination of differences in seasonal conditions and anthropogenic activities from microplastics sources into the Diep River.

CHAPTER 5: RESULTS AND DISCUSSION – PART II (PLANKENBURG RIVER)

5.1 INTRODUCTION

This chapter presents the occurrence of microplastics particles in the Plankenburg River (PR). The microplastics burden of the river was assessed spatially and temporally. Two different environmental matrices (water and sediment) were collected and analysed. Samples were collected six times rather than eight due to the COVID-19 pandemic restrictions nationally and at CPUT. One field sampling regime were carried out for both the summer and winter seasons. Four sampling sites were selected along the river course with diverse anthropogenic activities.

5.2 MICROPLASTIC RECOVERY USING DIFFERENT MESH SIZES

The comparison of the recovery efficiencies during filtration using two mesh sizes showed that the 20 µm mesh has higher extraction efficiency than the 250 µm (Figure 5.1).

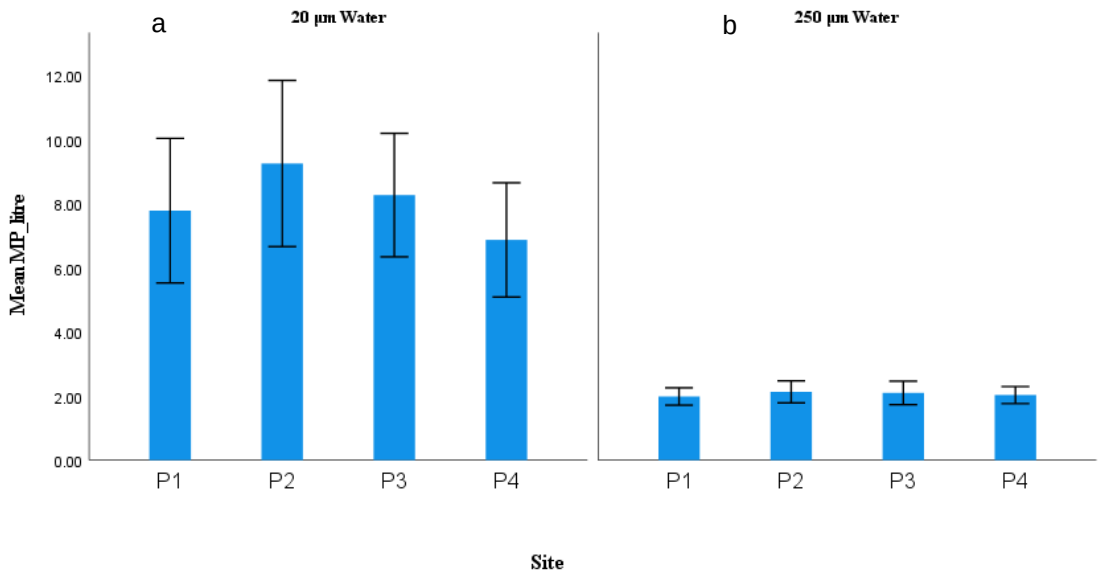

Figure 5.1: Extraction efficiencies of microplastics from water using 20 µm (a) and 250 µm (b) mesh sizes

The extraction efficiencies for the 20 µm mesh sample was in multiple orders of magnitude greater than those observed for the samples processed using the 250 µm.

5.3 PHYSICO-CHEMICAL PROPERTIES OF WATER SAMPLES IN THE PLANKENBURG RIVER

The results of the physicochemical parameters of the Plankenburg River are presented in Table 5.1. The values are compared to the South African national standard and water quality for inland waters by Department

of Water Affairs & Forestry, (DWAF) [235] and World Health Organization (WHO) [236] Guidelines for water quality.

Table 5.1: Physico-chemical properties of the Plankenburg River water samples

Parameter	Season	PR1	PR2	PR3	PR4	DWAF	WHO
pH	Spring	8.75 ±1.17	7.53 ±0.18	8.45 ±1.37	7.89 ±0.81	6.50-9.00	6.00-9.00
	Summer	8.23*	9.90*	8.47*	8.12*		
	Autumn	9.82 ±0.04	9.37 ± 0.76	8.44 ± 0.88	9.70 ±0.11		
	Winter	8.58*	9.39*	8.49*	8.82*		
DO (mg/L)	Spring	4.75 ± 0.49	4.20 ±1.41	4.40 ± 1.13	3.25 ±0.78	8-10	N/A
	Summer	17.7*	7.70*	4.80*	3.80*		
	Autumn	10.4 ±4.03	5.90 ± 1.70	5.90 ± 3.68	6.40 ±4.10		
	Winter	7*	5.30*	4.80*	3.80*		
EC (µS/cm)	Spring	418 ±22.63	678 ±38.2	857 ±100.41	817 ±67.9	≤2500	N/A
	Summer	150*	100*	890*	840*		
	Autumn	300.5 ±187.38	478 ± 147	779 ± 65.8	753 ±75.7		
	Winter	390*	590*	760*	740*		
T°C	Spring	18.9 ± 0.78	19.5 ±2.26	19.3 ±2.05	19.8 ±1.63	≤25	≤ 37
	Summer	19.4*	22.1*	20.7*	21.4*		
	Autumn	16.3 ±2.76	16.2 ±2.97	16.7 ± 2.62	16.8 ±2.47		
	Winter	14.3*	13.9*	13.9*	14.2*		
ORP (mV)	Spring	51 ±31.1	42.5 ±17.7	-34 ±31.1	-500 ±696	N/A	700
	Summer	115*	91*	92 *	73 *		
	Autumn	44.5 ±21.9	48 ± 9.90	-25.5 ±53.0	-110 ±0.71		
	Winter	106 *	92 *	84 *	*		
TDS (ppm)	Spring	287± 13.4	444 ± 28.3	565 ±70.7	551 ±43.8	0-450	1000
	Summer	95*	69.8*	606*	569*		

Parameter	Season	PR1	PR2	PR3	PR4	DWAF	WHO
COD (mg/L)	Autumn	201 ±124.45	314±94.1	516 ±43.1	513 ±39.6	≤30	≤100
	Winter	258*	396*	504*	493*		
	Spring	28.8 ±1.13	30.3 ± 7.07	47.3 ±24.0	62.5 ±45.0		
	Summer	20*	24.5*	49.3*	48.50*		
	Autumn	19.2 ±4.79	20.4 ±14.0	46.7 ±6.72	56 ±12.9		
	Winter	24.3*	24*	39.3*	42*		

*Samples were collected due to Covid-19 regulation

The measured DO, pH, EC, TDS, T°C, COD and ORP levels were in the range of 3.25-17.7 mg/L, 7.53-9.90, 1-8.9 µS/cm, 95-606 ppm, 13.9-22.1°C, 19.2-62.5 mg/L, -500-115 mV, respectively. Dissolved oxygen (DO) in freshwater is an indicator of water quality for aquatic organisms and organic matter and the recorded value ranged from 3.25 to 17.7 mg/L which did fall in the target water quality range of DWAF in summer (17.7 mg/L). The pH value for Plankenburg River was within the water quality limit of DWAF and WHO for spring across the four sites with values ranging from 7.53 to 8.75. In Autumn, pH was within the permissible limits of DWAF and WHO at PR3 with 8.44 and in Summer and Winter pH were in the general limit of DWAF and WHO ranging from 6.5 to 9 and 6.00-9.00, respectively at PR1, PR3, PR4. The electrical conductivity (EC) of surface water is a valuable indicator of salinity with total salt content (Pereao et al., 2021). The obtained EC values were substantially higher than the permissible DWAF standard limit for freshwater system all over the four seasons except in summer at PR1 (150 µS/cm) and PR2 (100 µS/cm) where lower values than ≤250 µS/cm were recorded.

According to Agoro et al., (2018), EC is mainly attributed to the dissolved ions from the decomposed plant matter. It can be suggested that the EC values obtained can be due to the higher amount of dissolved inorganic substances in ionized form. The results of EC all over the four seasons were similar to Edokpayi et al., (2015) study on the Mvudi River, South Africa. The total dissolved solids (TDS) were not in the target water quality range of DWAF at sites PR3 and PR4 for the four seasons on one side, while on the other side all recorded value were in the general limit of WHO which is 1000 mg/L. The highest values obtained at PR3 and PR4 might be due to the anthropogenic activity from the informal settlement and of the industrial area. These results are similar to the one reported by Nephale, (2021) during the study of urban rivers in Limpopo, South Africa.

Temperature levels obtained in this study ranging from 13.9 to 22.1°C were within DWAF and WHO water quality limit for freshwater. This results are similar to the one reported by Nephale, (2021). The measured chemical oxygen demand (COD) values were not in the target water quality range of DWAF at sites PR3 and PR4 for the four seasons on one side, while on the other side all recorded value were in the general limit of WHO which is ≤100 mg/L. The elevated amount at PR3 and PR4 can increase the quantity of biologically active substances such as bacteria in the Plankenburg river. This can be due to organic matter that is available for oxidation in this part of the river that is polluted by human activities. Redox potential or oxidation reduction potential (ORP) is a measure of the ability of a river to break down contaminants and dead plants and animals.

The ORP values of the Plankenburg river were within the permissible limit of WHO ≤700. The very low values observed across the four seasons was due to a lower presence of oxygen in the river system. This mean the bacteria could not efficiently decompose contaminant. The use of different water quality parameters with the different type of MPs necessitates the correlation analysis (r) to discern possible relationships. Table 5.2 shows the correlation coefficients of physicochemical water quality parameters. There was a significant positive correlation between EC and each of TDS (r=0.999; p<0.01), COD (r=0.698; p<0.01) and Sediment (r=0.438; p<0.05). Table 5.2 reveals that significant (p<0.01) positive correlations existed between TDS and COD (r=0.710) and Sediment (r=0.436; p<0.05). According to Chigor et al. (2013) (Chigor et al., 2013), high levels of TDS in water systems have been shown to increase the chemical oxygen demand. COD also correlated positively with Sediment (r=0.581; p<0.05).

Table 5.2: Pearson's correlation matrix for water physicochemical parameters and microplastics occurrences

	pH	DO mg/L	EC µS/cm	T °C	TDS ppm	ORP mV	COD	250 µm	20 µm	Sediment
pH	--									
DO mg/L	0.352	--								
EC µS/cm	**-0.417***	**-0.606****	--							
T °C	**-0.450***	-0.248	0.024	--						
TDS ppm	**-0.409***	**-0.605****	**0.999****	0.031	--					
ORP mV	0.283	.246	-0.340	-0.222	-0.345	--				
COD	-0.377	-0.334	**0.698****	0.198	**0.710****	**-0.753****	--			
250 µm	-0.151	0.123	0.016	0.285	-0.002	0.072	0.058	--		
20 µm	-0.338	**-0.416***	0.140	0.356	0.137	0.170	-0.098	0.133	--	
Sediment	**-0.435***	**-0.512***	**0.438***	0.120	**0.436***	**-0.684****	**0.581****	0.121	0.282	--

*. Correlation is significant at the 0.05 level.

**. Correlation is significant at the 0.01 level.

The correlation analysis (r) for the physicochemical properties was evaluated and the result is presented in Table 5.2. The results showed that most of the tested variable parameters have an erratic correlation pattern and the substantial positive correlations only demonstrated that there was a close relationship of the factors with one another. Significant (p<0.05) inverse relationships existed between pH and each of EC (r=−0.417), Temperature (r=−0.450), TDS (r=−0.409) and Sediment (r=−0.435). The significant positive correlation between electrical conductivity and TDS suggests that these two parameters could very well represent one another in analysis of water quality (Chigor et al., 2013). There is a strong inverse correlation between dissolved oxygen and each of EC (r=−0.606; p<0.01), TDS (r=−0.605; p<0.01), 20 µm (r=−0.416; p<0.05) and Sediment (r=−0.512; p<0.05). Redox potential (ORP) has a significant (p<0.01) inverse correlation between COD (r=−0.753) and Sediment (r=−0.684)

5.4 SPATIAL AND TEMPORAL DISTRIBUTION OF MICROPLASTICS IN WATER SAMPLES OF THE PLANKENBURG RIVER

The distribution of microplastic particles in the river provides an understanding and information on MP occurrence in the freshwater ecosystem. Results of microplastics distribution over four seasons in the Plankenburg River is presented in Table 5.3. A total of 141 water samples with replicates were collected in the Plankenburg river. Spatial and temporal distributions show different pattern of abundance of MPs.

Table 5.3: Abundance and distribution of microplastic particles in the Plankenburg River water sample (mean ± SD)

Site	Season							
	Spring		Summer		Autumn		Winter	
	20 µm	250 µm	20 µm	250 µm	20 µm	250 µm	20 µm	250 µm
PR1	13.0 ± 2.65	1.80 ± 0.38	3.92 ± 0.80	2.93 ± 0.40	5.88 ± 4.31	1.92 ± 0.35	4.92 ± 1.13	1.53 ± 0.40
PR2	14.9 ± 4.18	2.13 ± 0.50	8.92 ± 4.19	2.17 ± 0.65	3.67 ± 1.07	2.08 ± 0.85	9.42 ± 1.61	2.17 ± 1.27
PR3	12.4 ± 3.45	2.70 ± 1.04	8.42 ± 0.14	1.63 ± 0.15	4.04 ± 0.73	1.83 ± 0.39	8.25 ± 2.38	1.87 ± 0.57
PR4	8.33 ± 3.74	1.75 ± 0.36	-	2.37 ± 0.45	4.63 ± 1.20	1.95 ± 0.56	8.42 ± 4.45	± 0.87

Statistical analysis was conducted using SPSS (v27). Statistical tests for normality and equal variance were conducted, resulting in neither criterion being met to do parametric analyses. Non-parametric tests were subsequently done using the Kruskal-Wallis for multiple groups. MP abundances were calculated for each site and presented as the number of MPs per litre (N = 72). Results are reported as means (±SD) and significances set at $p < 0.05$. For microplastics processed under 20 µm There were no significant differences in microplastics concentrations between sampling points analysed (Kruskal-Wallis; $p > .05$). The highest average concentrations of microplastics were recorded in spring (PR2; 14.9 ± 4.18 MPs per litre), summer (PR2; 8.92 ± 4.19 MPs per litre), autumn (PR1; 5.88 ± 4.31 MPs per litre), winter (PR2; 9.42 ± 1.61 MPs per litre) and the lowest across the four seasons were recorded in summer (PR2; 3.67 ± 1.07 MPs per litre) (Table 5.3).

For microplastics processed under 250 µm There were no significant differences in microplastics concentrations between sampling points analysed (Kruskal-Wallis; $p > .05$). The highest average concentrations of microplastics were recorded in summer (PR1; 2.93 ± 0.40 MPs per litre), spring (PR3; 2.70 ± 1.04 MPs per litre), autumn (PR2; 2.08 ± 0.85 MPs per litre), winter (PR4; 2.40 ± 0.87 MPs per litre) and the lowest across the four seasons were recorded in winter (PR1; 1.53 ± 0.40 MPs per litre) (Table 5.3). This study is the first to present data on the occurrence of MPs (< 5 mm) in the Plankenburg River water, South Africa. The mean abundance in the 20 µm and 250 µm water samples were respectively 8.09 ± 4.54 MPs/L and 2.06 ± 0.65 MPs/L. These values are lower than MPs recorded by Zhang et al., (2020), who recorded an abundances of 2345 ± 1858 n/m^3 from the Yongjiang River. Laju et al., (2022) reported MP abundances of 24.4 ± 3.22 items/l (water) from the Kodaikanal Lake in Indian. This study result is similar to that recorded by Alam et al., (2019) where the recorded abundances of microplastics were 5.85 ± 3.28 particles/litre.

5.5 SPATIAL AND TEMPORAL DISTRIBUTION OF MICROPLASTICS IN SEDIMENT SAMPLES IN THE PLANKENBURG RIVER

The results of microplastics distribution in sediments samples over four seasons in the Plankenburg River are presented in Table 5.4. A total of 72 sediment samples were collected. Three replicates per site over the four seasons in six sampling events.

Table 5.4: Abundance and distribution of microplastic particles in the Plankenburg River sediment sample (mean ± SD)

Site	Season			
	Spring	Summer	Autumn	Winter
PR1	75.0 ± 0.25	39.0± 0.06	34.5± 0.16	49.0 ± 0.16
PR2	68.0 ± 0.43	47.5± 0.09	43.5 ± 0.34	84.0 ± 0.86
PR3	68.0 ± 0.32	31.0 ± 0.16	62.5 ± 0.37	66.5 ± 0.28
PR4	106 ± 0.90	59.0 ± 0.30	53.5± 0.42	83.5 ± 0.19

From the results, microplastics in sediments samples were recorded in abundance in the spring season (September) and the lowest amount in summer season (February). The results obtained were found to contain microplastics in a range of 31 to 106 microplastics per kilogramme of sediment samples. Microplastics were unevenly distributed in the different seasons and months in sediment sample. The distribution suggests that runoff from winter rains contribute to microplastic load of the river. However, stronger river flow seems to enhance transport in winter resulting in slightly reduced quantities measured in that season. Reduced flow and large volumes of water in spring accounted for the observed results in spring. A non-parametric independent-sample test (Kruskal-Wallis) was used to test for differences in the samples analysed as the data did not meet the criteria for parametric analysis (homogeneity and variance).

Microplastics abundance was calculated for each site and presented as number of microplastics per gram (N = 18). Results are reported as means (±SD) and significances set at $p < 0.05$. There were no significant differences in microplastics occurrence across all sites for the six sampling events (Kruskal-Wallis; $p > 0.05$). The highest average values of MPs were recorded in spring (PR4; 106 ± 0.90 MPs per kilogramme of sediment sample), summer (PR4; 59.0 ± 0.30 MPs per kilogramme of sediment sample), autumn (PR3; 62.5 ± 0.37 per kilogramme of sediment sample), winter (PR2; 84.0 ± 0.86 MPs per kilogramme of sediment sample). The lowest across the four seasons were recorded in Summer (PR3; 31.0 ± 0.16 MPs per kilogramme of sediment sample) (Table 5.4). This is the first study to present data on the occurrence of MPs (< 5 mm) in the Plankenburg River sediment, South Africa. An overview of the monthly distribution of microplastics in Plankenburg River is given in Figure 5.2. The values obtained in sediment samples is lower than those reported by Jiang et al., (2018), who recorded an abundance of 388.57 ± 66.19 items/kg from the West Dongting Lake.

This study result is similar to that recorded by Rodrigues et al. (2018) where the recorded abundances of MP were 2.6-71.4 mg kg^{-1}. From the results, MPs in sediments samples were highest in spring (September) and the lowest in summer (February). The microplastics were unevenly distributed in the different seasons and months in the sediment samples. The distribution suggests that runoff from winter rains contribute to

microplastic load of the river. However, stronger river flow seems to enhance transport in winter resulting in a slightly reduced quantities measured in that season. It might be suggested that the high occurrences of microplastics in site PR4 can be due to the informal settlement. Study conducted by Huang et al., (2021) shows the influence of human activity on the river pollution.

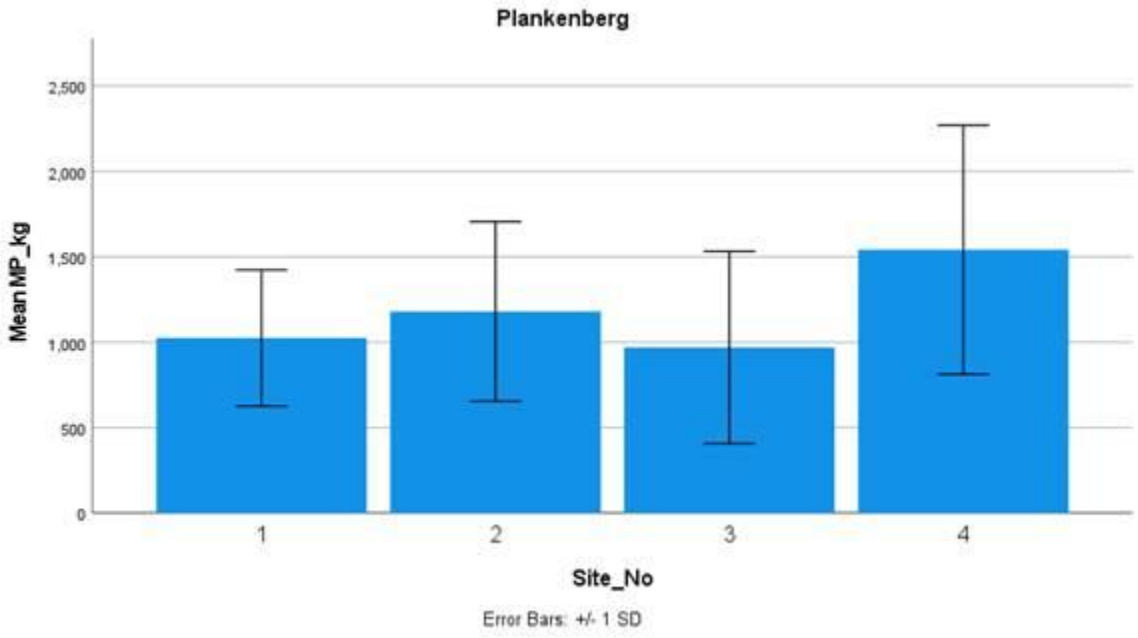

Figure 5.2: Spatial distribution of microplastics in the Plankenburg River sediment (mean microplastic per kg)

5.6 PHYSICAL PROPERTIES OF MICROPLASTIC FOUND IN THE PLANKENBURG RIVER

The types, sizes and colours of MPs filtered from water and sediment are shown in Figure 5.5. Fibres (96.8%) and films (1.79%) were recorded as dominant shapes of plastics particles in the Plankenburg river system under investigation. The MPs shapes was categorized into five: foam, fragment, Sphere, film, and fibre. Each of the site contained varying compositions of shapes, suggesting the diversity of MPs shapes present in the river and fibre was found to be the most prominent reaching 96.8%. Fibre accounted for 95.8%, 98.4%, 95.9% and 97.3% in PR1, PR2, PR3 and PR4, respectively. The MPs shape was regarded as an indicator of its origin and the informal settlement where people are dumping refuse and plastics was along the river flow.

In terms of colours, 78.2% of the MPs were transparent, and 15.9% of blue/green colours. MPs particles colours were identified as shown in Figure 5.3 (b). A total of 1835 MP particles were recorded from the 72 sediment samples. A diverse range of colours which include white, yellow/ brown, black/grey, transparent, blue/green and red/pink MPs were found in the Plankenburg river (Figure 5.3 (b)), and the transparent MPs was the most dominant colour with the most abundant MPs with 80.5% at PR4. Transparent MPs accounted for 78.2% of the total MPs load. Consistent with previous report, transparent and blue/green MPs dominated in the freshwater system (Zhang et al., 2020). The different colours were good for our manual and visual identification of MPs during the sampling processes, even though, they may cause damages to freshwater ecosystems. For example, some freshwater biota might be mistaken by the diverse coloured MPs as their food

and ingest or swallow them, resulting in accumulation and transfer of MPs in the food chain (Wright et al., 2013), which would end up with potential health hazards to the freshwater ecosystem.

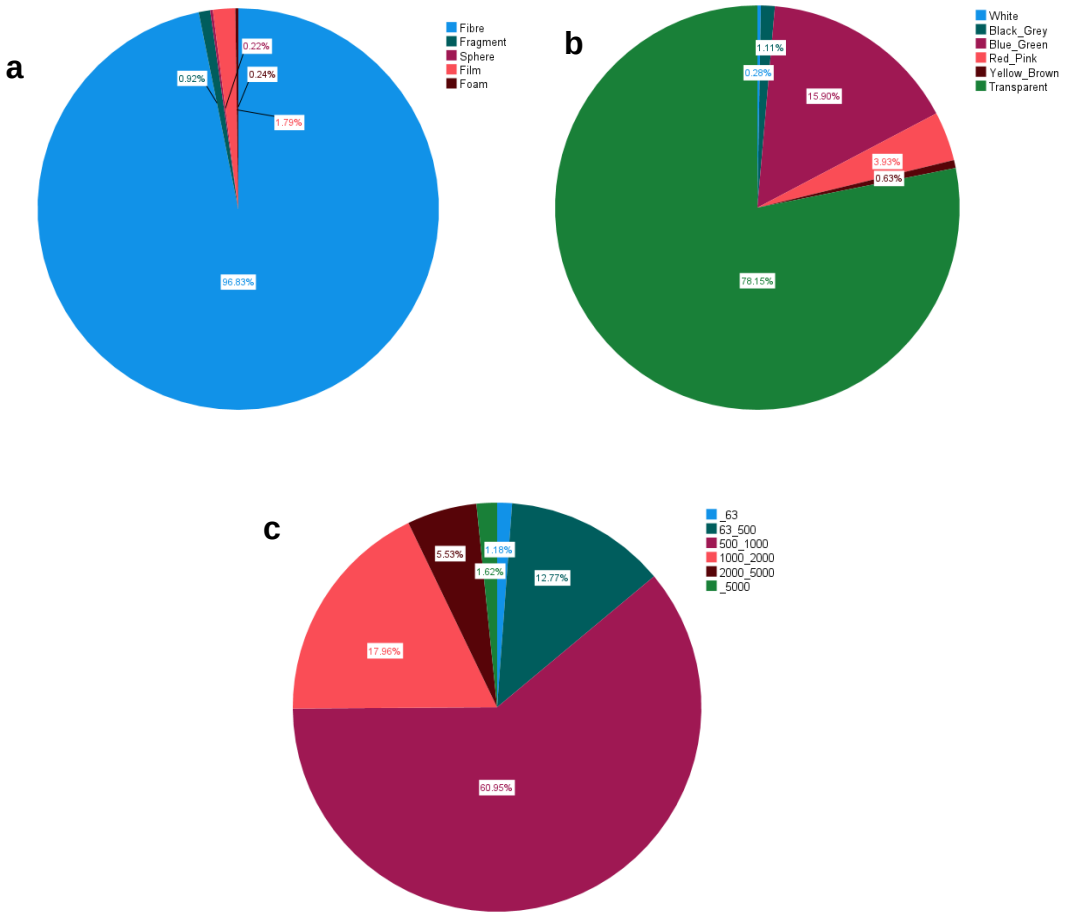

Figure 5.3: Percentage distribution of microplastics in the Plankenburg river (a) shapes (b) colours (c) sizes

The high percentage of plastics particles size were reported for 500-1000 µm (60.9%) which may be contributed from degradation products (waste), and 1000-2000 µm (17.9%) as shown in Figure 5.3 (c). It is well known that pollutants including heavy metals and organics can adhere to the surface of MPs but those with different particle sizes may have varying adsorption capacities for contaminants. Size is an important factor for distribution, dispersal, and biological effects in organisms. Larger particles are capable of being transported in water at longer distances than smaller particles as shown by models of distribution of micro- and macro particles (Lebreton et al., 2012). Smaller particles, especially fibres, can be subjected to air transport and small particles are easier taken up in the food web.

5.7 CHEMICAL ANALYSES OF MICROPLASTICS IN THE PLANKENBURG RIVER

5.7.1 Polymer distribution of microplastics in the Plankenburg River water and sediment samples

Chemical analyses of plastics particles were investigated using FTIR-ATR to determine the different type of polymers recorded in the freshwater system. A total of 249 (13.5%) particles out of the 1835 MPs recorded from water and sediment were analyse for FTIR-ATR polymer type identification. The results obtained are shown in Figure 5.4. FTIR microplastics spectra found at the different sampling were mainly composed of: (a) PR-1 (cotton, polyester and Jute), (b) PR-2 (cotton, polyester, polypropylene, polyacrylamide), (c) PR-3 (cotton, polypropylene, methyl cellulose, polyester) and (d) PR-4 (cotton, polypropylene, methyl cellulose, polyester, polystyrene). Polypropylene characteristic spectra for the four sites display peaks around 1876, 1458, 2917 and 2915 cm^{-1}. Cotton produces peaks around wave number regions 1000 and 3327 cm^{-1}. Polystyrene, peaks were noticed at 995,1227, and 1137 cm^{-1}. The main peaks of polyacrylamide blanks are 1547,1404, 1557 and 3316 cm^{-1}. Polyester [PET] characteristic spectra peaks around 1710,1232, 1089 and 723 cm^{-1}. Methyl Cellulose produces peaks around wave number regions 1085, 2964 cm^{-1}. These results are consistent with those described by other authors (Faure et al., 2015; M. O. Rodrigues et al., 2018; Zbyszewski et al., 2014). The FTIR spectra obtained were compared with reference IR-spectrums and the results are shown in Figure 5.4 for MP found in different sites.

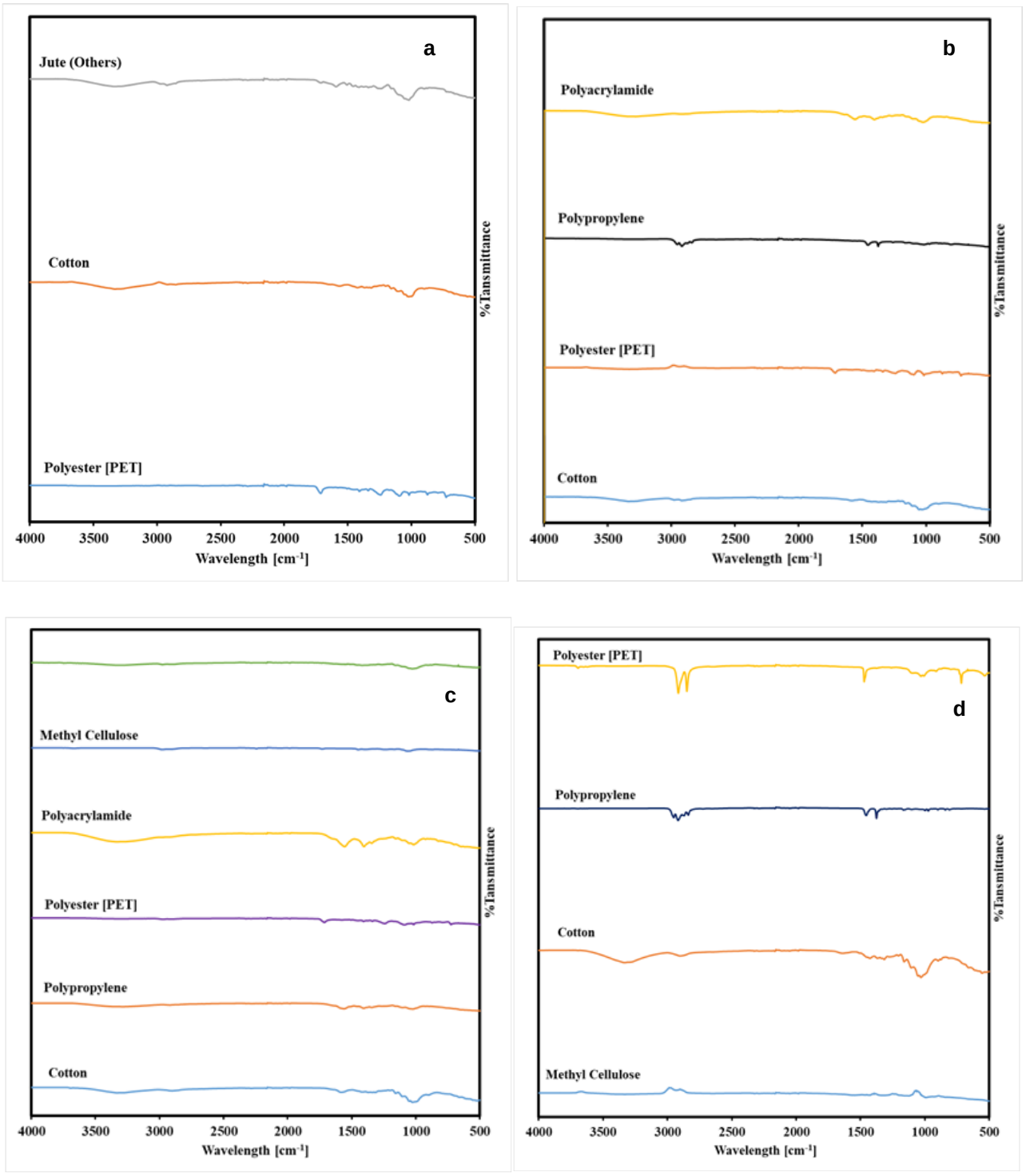

Figure 5.4. FTIR spectra of microplastics found in (a) PR-1 (b) PR-2 (c) PR-3 and (d) PR-4 in the various sites in Plankenburg river

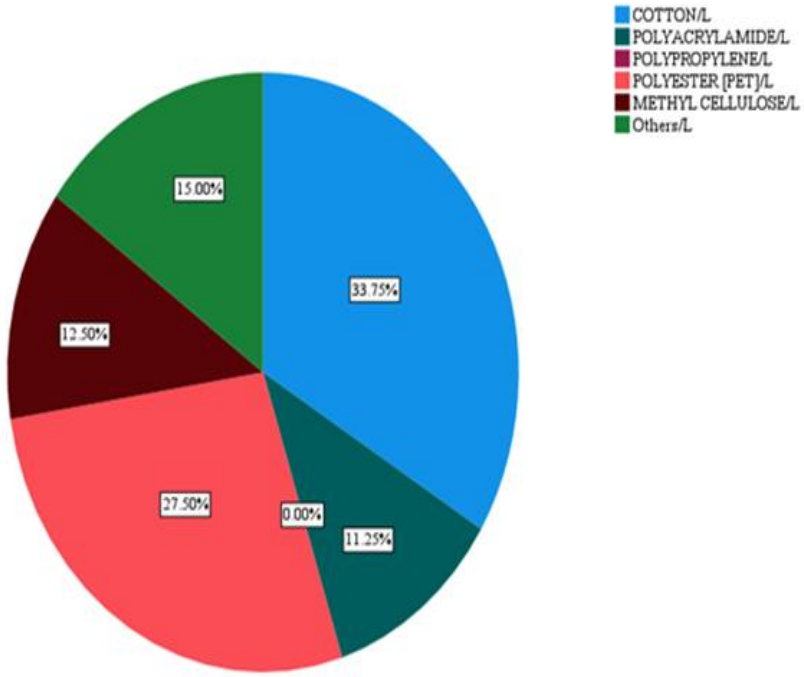

Figure 5.5: Occurrence of polymer types identified In the Plankenburg River water samples (Percentage)

In the Plankenburg River water samples, PET, methyl cellulose and cotton were the most dominant polymers with 50% MP per L, respectively.

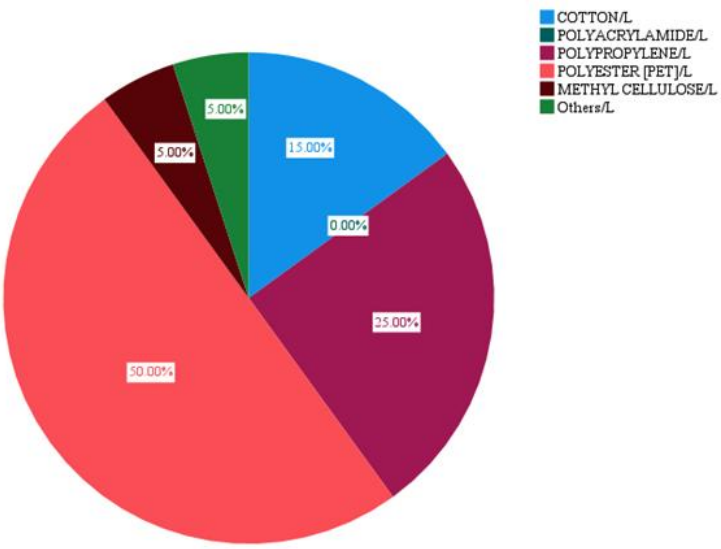

Figure 5.6: Abundance of polymer's type identified In the Plankenburg River sediment samples (Percentage)

The sediment sample results indicate the different percentage of polymer in the sediment per site. In the sediment samples, PET was the most dominant with 80% per gram at PR2 as shown in Figure 5.6.

CHAPTER 6: RESULTS AND DISCUSSON – ECOLOGICAL RISK ASSESSMENT

6.1 INTRODUCTION

The aim of this chapter is to report the ecological risk assessment microplastics occurrence in the Diep and Plankenburg River, potential climate change effects (temperature increases) on ecological systems and human health using three bioassays and the Ames mutagenicity testing. Results of the bioassay experiments using freshwater species as model organisms, exposure to environmental water samples, microplastic standards, temperature changes and the mutagenicity potentials of water samples are presented in this chapter.

6.2 ECOLOGICAL RISK ASSESSMENT

Ecological risk assessment is a process used to evaluate the possibility of stressors. Physical and chemical monitoring are indirect measurements of aquatic health and may vary greatly due to various influences and only provide a brief indication of ecosystem health whereas biological monitoring are direct measurements of biological responses to chemical, physical, and biological influences in their habitat over a longer period of time (Mankiewicz-Boczek et al., 2008). Therefore, biological monitoring is useful in understanding and reflecting water quality.

6.3 BIOASSAY TESTS OF THE DIEP RIVER WATER SAMPLES

The toxicity of the environmental samples, environmental samples with microplastics, and climate change studies were described. The overall ecotoxicity was based on three bioassays and determined for each site. After the PE obtained was determined, the water was ranked into one of five classes based on the highest toxic response showed by atleast one of the biotests applied. The weight score is an additional scoring that was calculated for each hazard class to indicate the quantitative importance (weight) of the toxicity in that class. The higher the weight score, the more the score expresses the toxic hazard of the water in that class (Persoone et al., 2003). Figures 6.1, 6.2 and 6.3, represent the percentage inhibition of microalgae, crustacean and protozoa, respectively, at different sites along the Diep River in Autumn, Spring, Summer and Winter.

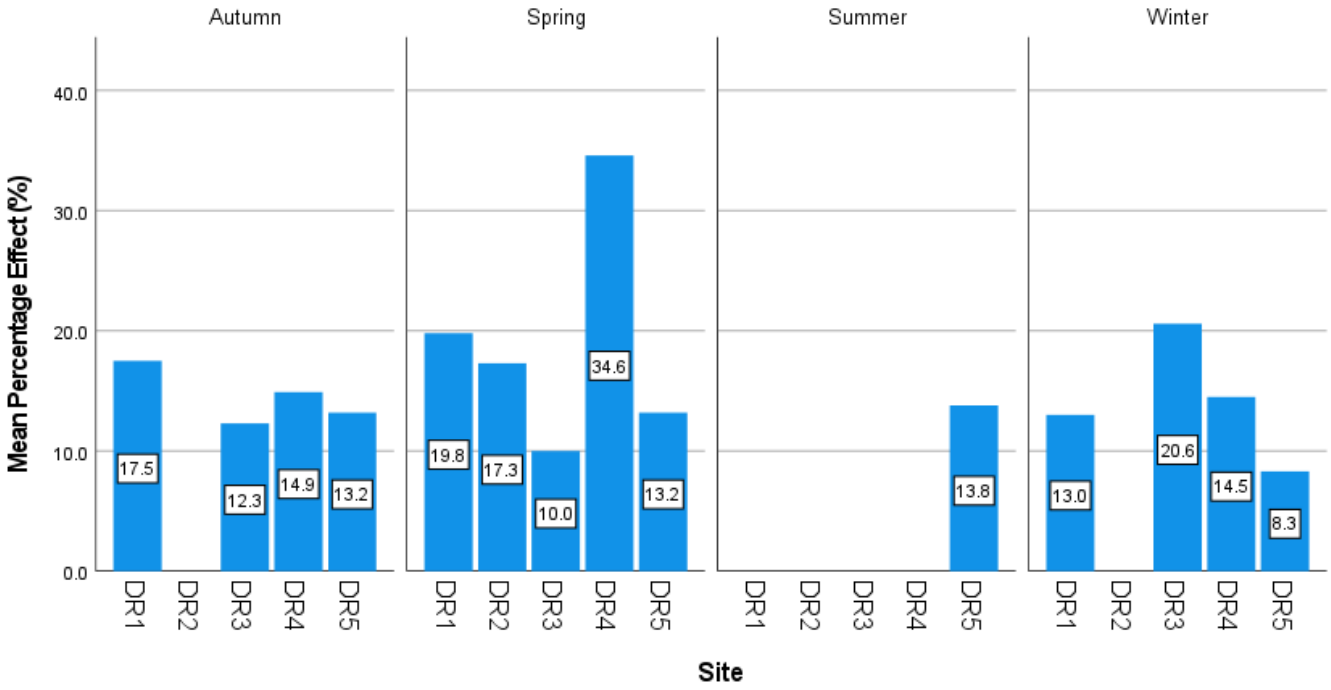

Figure 6.1: Percentage growth inhibition of microalgae in different sites along the Diep River over 4 seasons

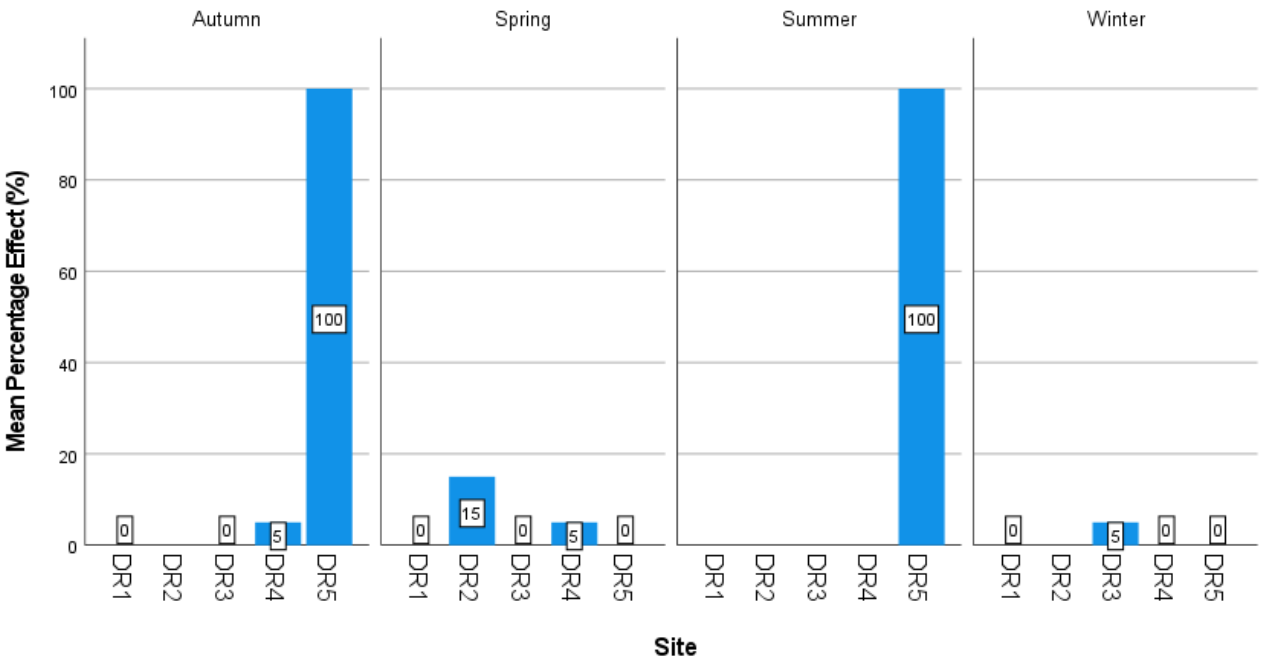

Figure 6.2: Percentage inhibition of crustacean in different sites along the Diep River over 4 seasons

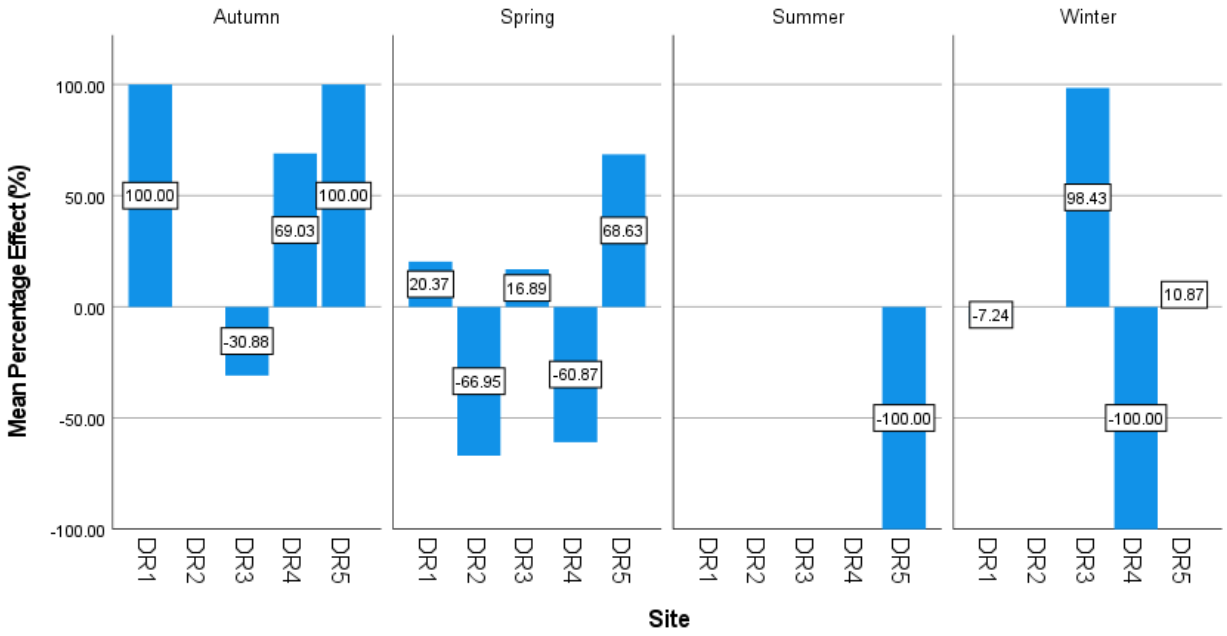

Figure 6.3: Percentage inhibition of protozoa in different sites along the Diep River over 4 seasons

In environmental samples, growth inhibition was shown at all sites for microalgae and protozoa whereas growth inhibition was only observed at DR4 (5) and DR5 (100) during Autumn, DR2 (15) and DR4 (5) during Spring, DR5 (100) in Summer, and DR3 (5) in Winter. In Figure 6.1, DR 1(19.8) recorded the highest growth inhibition of microalgae in the Spring season compared to DR 1(17.5) in Autumn and DR1 (13.0) in Winter. DR 3(20.6) recorded the highest growth inhibition of microalgae in Winter compared to DR3 (12.3) in Autumn and DR 3(10.0) in Spring. Growth inhibition of microalgae during spring was shown at DR4 (34.6) which accounted for more than 50% of growth inhibiton shown at DR 4 in Autumn (14.9) and Winter (14.5). DR 5 had the same growth inhibiton of microalgae in Autumn and Spring (13.2) wiith the least growth inhibition shown during the Winter season (6.3).

Figure 6.2 shows that growth inhibition for crustacean was found to be the highest at DR5 (100) in both Autumn and Summer whereas there was no growth inhibition recorded at DR 5 during the Spring and Winter seasons nor at DR 1 and DR 3 in Autumn, DR1, DR3 and DR5 in Spring, and DR 1, DR 4 and DR 5 in Winter. There was only one site which showed growth inhibition of crustacean during winter, which was at DR 3(5). Protozoan growth inhibition is demonstrated in Figure 6.3. DR1 (100) and DR5 (100) in Autumn recorded the highest growth inhibition compared to growth inhibition at all sites in other seasons. Growth inhibition of protozoa at DR3 was found to be -30.9 in Autumn, 16.89 in Spring and 98.43 in Winter. At DR 4, growth inhibition was 69.0 in Autumn, -60.9 in Spring and -100 in Winter. Growth inhibition was shown at DR5 in Autumn (100), Spring (68.6), Summer (-100) and Winter (10.9). Figures 6.4, 6.5 and 6.6, demonstrate the percentage inhibition of microalgae, crustacean and protozoa, respectively, at different sites of the Diep River in the Spring season which were exposed to microplastic standards.

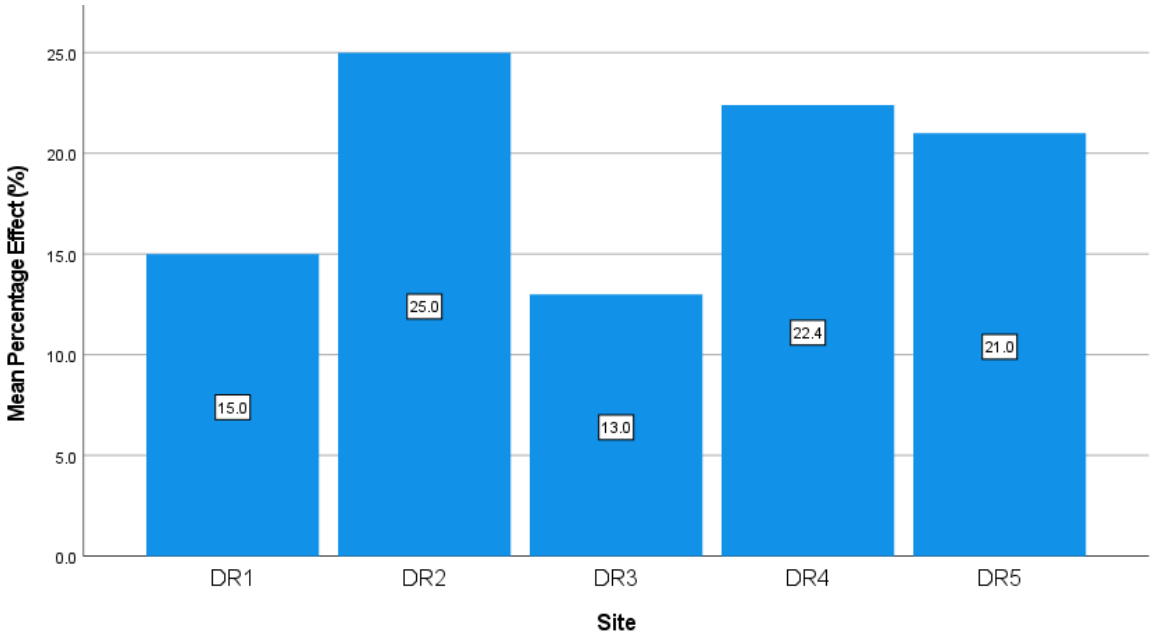

Figure 6.4: Percentage inhibition of microalgae in spring environmental samples with microplastic standards in different sites along the Diep River

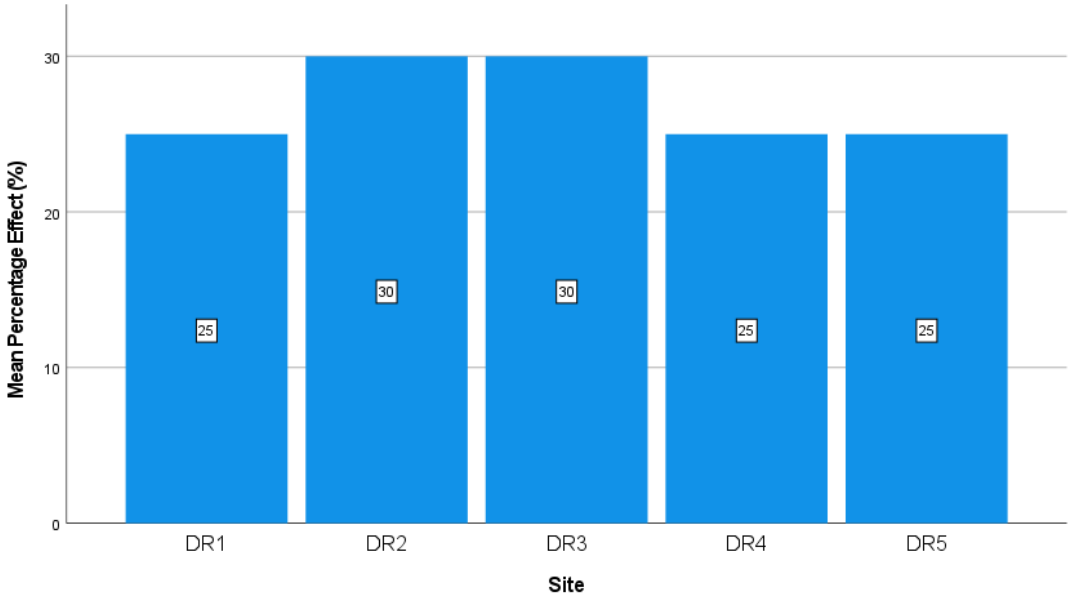

Figure 6.5: Percentage inhibition of crustacean in spring environmental samples with microplastic standards in different sites along the Diep River

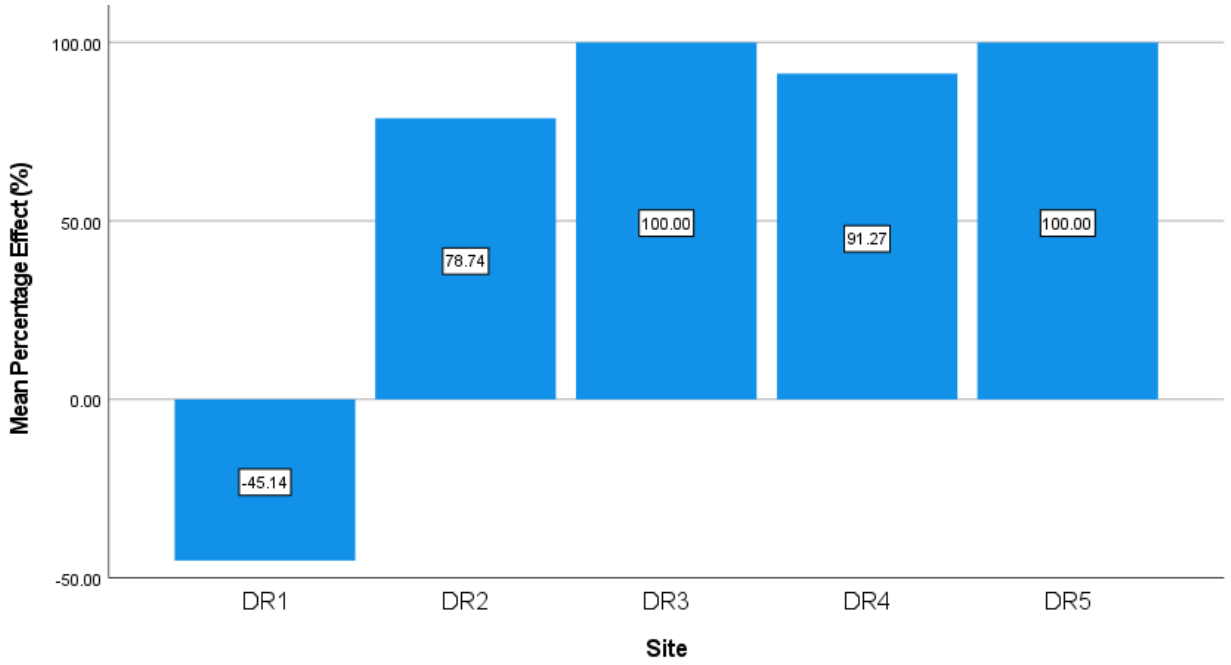

Figure 6.6: Percentage inhibition of protozoa in spring environmental samples with microplastic standards in different sites along the Diep River

In environmental samples with microplastics, bioassays showed inhibiton in growth at all sites. It can be assumed that these values were attributed to the synergistic influence of microplastics and environmental samples. Algal growth inhibition (Figure 6.4) was found at DR1 (15.0), DR2 (25.0), DR3 (13.0), DR4 (22.4) and DR5 (21.0). Three sites, DR2 (25.0), DR3 (13.0) and DR5 (21.0) showed a greater growth inhibition compared to DR2 (17.3), DR3 (10.0) and DR5 (13.2) that had no microplastics as shown in Figure 6.1. Figure 6.5 shows that the crustacean, *Daphnia magna*, were highly sensitive to microplastics in environmental samples.

Growth inhibition was observed at all sites (with the exception of DR1 with the protozoan bioassay that showed cell proliferation) and was increased with the addition of microplastic standards. All 5 sites, DR1 (25), DR2 (30), DR3 (30), DR4 (25) and DR5 (25), showed a greater inhibition of 50% to 200% more growth inhibition compared to samples with no microplastics (DR1 (0), DR2 (15), DR3 (0), DR4 (5) and DR5 (0)) in Figure 6.2. Growth inhibition was enhanced in protozoa that were exposed to environmental samples that contained microplastics (Figure 6.6). Figure 6.6 shows growth inhibition recorded in DR1 (-45.1), DR2 (78.7), DR 3(100), DR 4(91) and DR5 (100) in contrast to samples that contained no microplastics in Figure 6.3 (DR1 (20.37), DR2 (-66.9), DR3 (16.9), DR4 (-60,9) and DR5 (68.6).

6.3.1 Microplastics and temperature variability exposure studies

The effects of temperature increases were evaluated using virgin polystyrene microspheres (PS-MP) in Milli-Q water and the three bioassays previously reported. The percentage inhibition of microalgae, crustacean and protozoa which were exposed to distilled water that contained microplastic standards under 3 different temperatures is presented in Figures 6.7, 6.8 and 6.9, respectively.

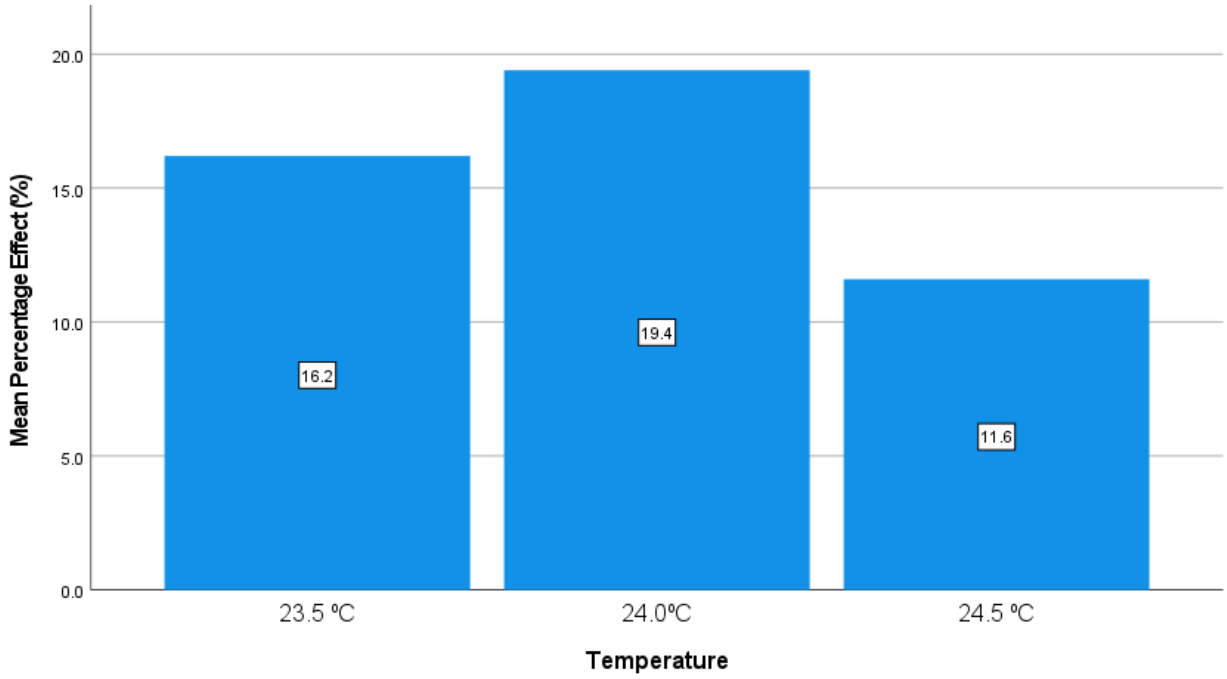

Figure 6.7: Percentage inhibition of microalgae in distilled water with microplastics relative to temperature variation

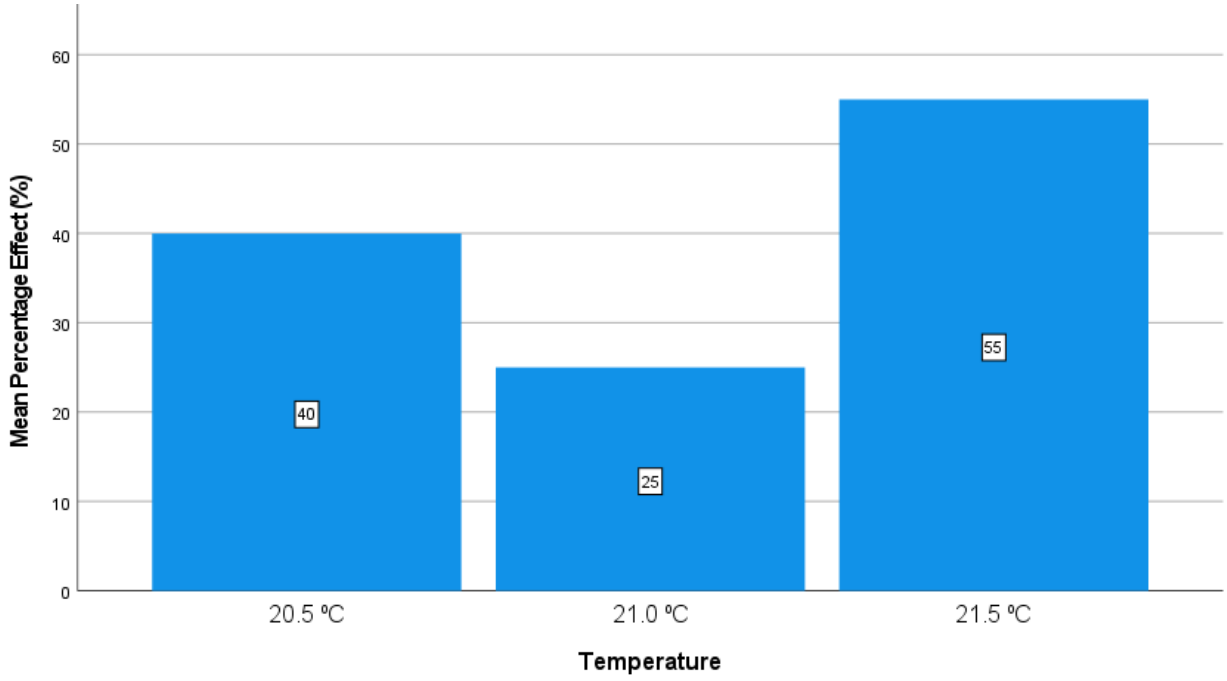

Figure 6.8: Percentage inhibition of crustacean in distilled water with microplastics relative to temperature variation

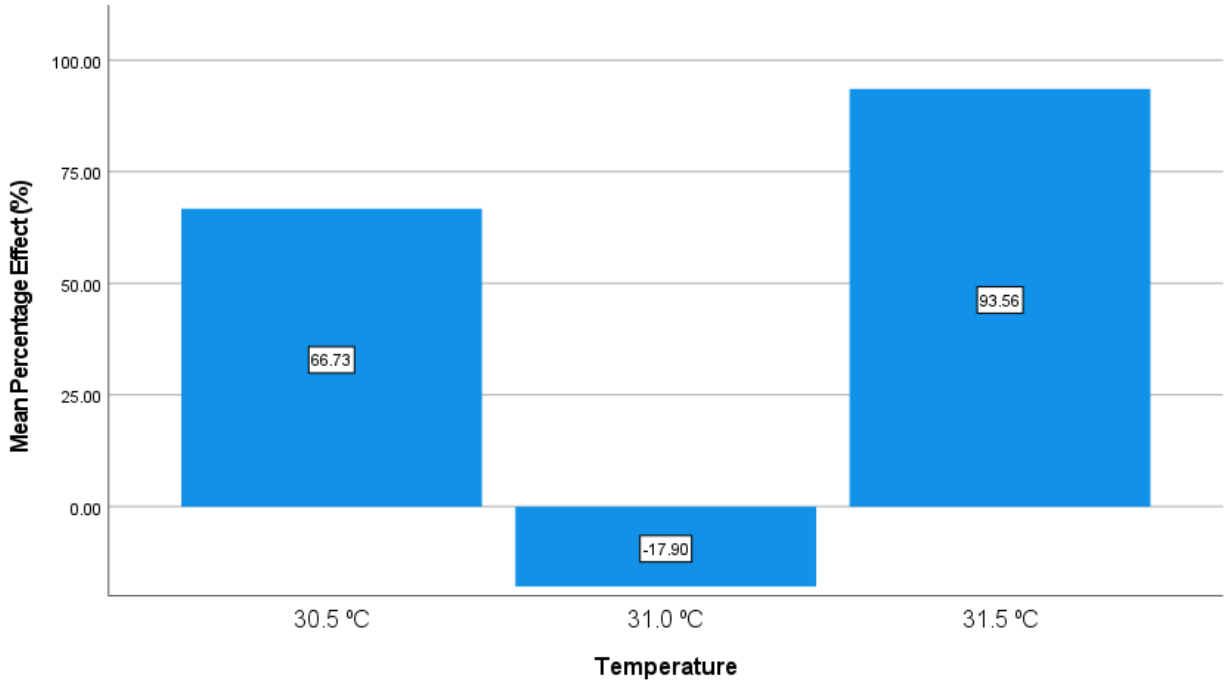

Figure 6.9: Percentage inhibition of protozoa in distilled water with microplastics relative to temperature variation

In Figure 6.7, growth inhibition of microalgae was found to be the greatest at a 24.0ºC temperature increase (19.4) compared to a 23.5ºC (16.2) temperature increase. Growth inhibition was found to be the least at a temperature increase of 24.5ºC (11.6). This suggests that microalgae growth was enhanced and thrived at a 1.5ºC temperature increase in the presence of microplastics. Figure 6.8 demonstrated the effect of temperature rise on crustacean in the presence of microplastic. Growth inhibition of crustacean was shown to be 40.0 at a 20.5ºC temperature increase, 25 at a 21.0ºC temperature increase and the highest growth inhibition recorded at a 21.5ºC temperature increase (55). The results show that crustacean were the least and most sensitive at a 1.00ºC and 1.5ºC temperature increase, correspondingly. In Figure 6.9, growth inhibition was recorded for protozoa at the three different temperature rises with microplastic present. Growth inhibition of protozoa was 66.7, -17.9, and 95.56 at a 30.5ºC, 31.0ºC and 31.5ºC temperature increase.

6.3.2 Acute Hazard Classification of Diep River water samples

The proposed toxicity classification based on a battery of microbiotests are presented below. Biological analyses were based on three bioassays' sensitivity of the surface-water samples from Diep River over the study period. Figure 6.10, Figure 6.11, and Figure 6.12 illustrate the hazard classification classes for environmental samples, environmental samples with microplastics, and climate change effect studies respectively.

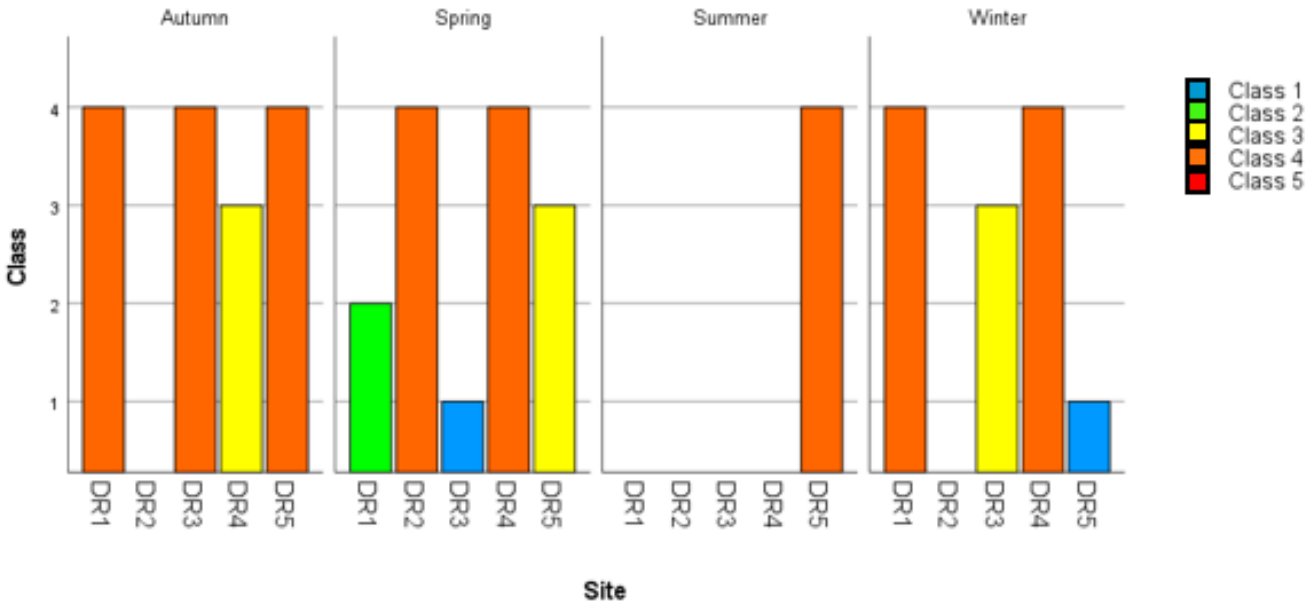

Figure 6.10: Hazard classes of different sites along the Diep River over 4 seasons

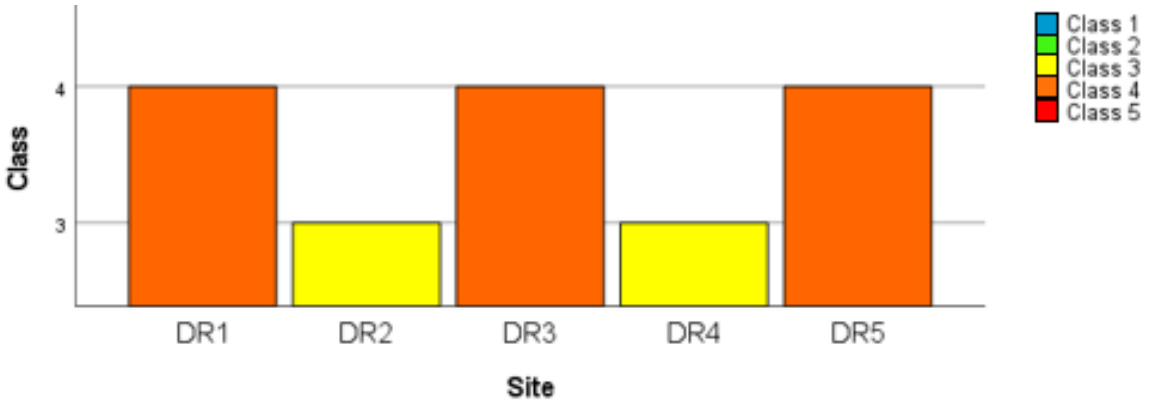

Figure 6.11: Hazard classification of different sites along the Diep River spring samples with microplastics

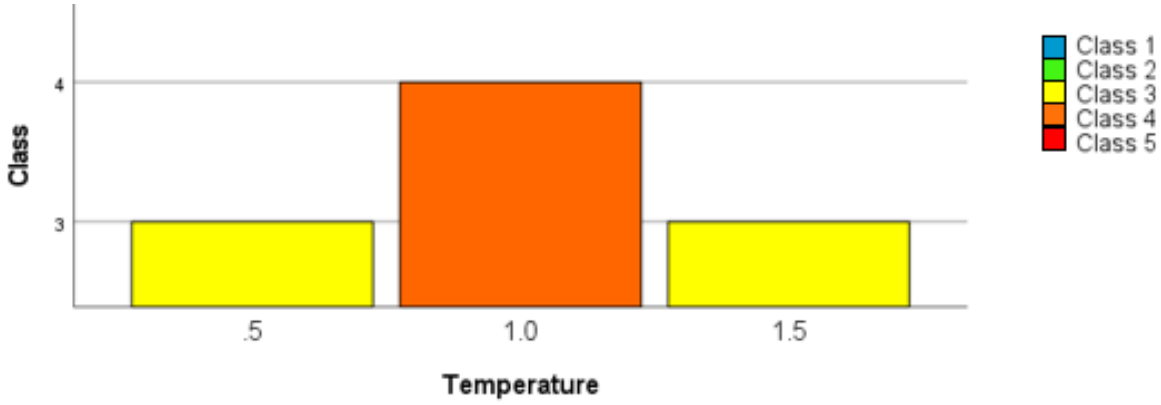

Figure 6.12: Hazard classification classes at 0.5ºC, 1ºC and 1.5ºC temperature increases of distilled water with microplastics

The rationale behind hazard classification classes for environmental samples, environmental samples with microplastics, and climate change effect studies were focussed on the biological analyses that were based on the sensitivity of organisms to the Diep River samples. Figure 6.10 presents the hazard classification classes of the Diep River samples at different sites over 4 seasons. In the Autumn season, DR 1, DR 3 and DR 5 belonged to Class 4 whereas DR 4 belonged to Class 3. This means that at least one biotest reached a PE of 100% in DR 1, DR 3 and DR 5, whereas at DR 4, atleast one biotest reached a PE of 50% or more but the effect level is below 100%. There were more variability in the classes found In the Spring season, such that DR 1 was attributed to Class 2, DR 2 and DR 4 belonged to Class 4, DR 3 was attributed to Class 1 and DR 5 was ascribed to Class 3. The Summer season recorded Class 4 at DR 5, and the Winter season recorded Classes 4, 3, 4, and 1 at DR 1, DR 3, DR 4, and DR 5, respectively. At least one biotest reached a PE of 100% at DR 1, DR 3 and DR 5 during the Autumn season as they all belonged to the high acute hazard class (Class 4) and were found to be higher at these sites compared to the same sites during spring and winter seasons. In Figure 6.11, the hazard classification classes at different sites along the Diep River spring samples with microplastics are shown. Figure 6.11 shows that DR 1 was attributed to Class 4 as opposed to Class 2 in the absence of microplastics in Figure 6.10. On the other hand, DR 2 and DR 4, belonged to Class 3 in Figure 6.11 as opposed to Class 4 in the absence of microplastics in Figure 6.10. DR 3, in Figure 6.11, was categorized as Class 4 in contrast to Class 1 in Figure 6.10 and lastly, DR 5 belonged to Class 4 as opposed to Class 3 in Figure 6.10.The hazard classification classes in Figure 6.12 shows the effect of the 3 different temperature rise of distiiled water with microplastics. Temperature rise of 0.5ºC and 1.5ºC belonged to Class 3 whereas a temperature rise of 1.0ºC was attributed to Class 4 (High acute hazard class). The class weight scores for all sites over the four seasons are presented in Table 6.1.

Table 6.1: Mean class weight score and class weight score as a percentage (%) at different sites along the Diep River over 4 seasons

SEASON	SITE	CLASS WEIGHT SCORE	CLASS WEIGHT SCORE AS A PERCENTAGE (%)
Autumn	DR1	1.00	33.3
Autumn	DR3	1.00	33.3
Autumn	DR4	0.67	33.5
Autumn	DR5	2.00	66.7
Spring	DR1	0.33	33.0
Spring	DR2	1.00	33.3
Spring	DR3	0.00	0.00
Spring	DR4	1.33	44.3
Spring	DR5	0.67	33.5
Summer	DR5	2.00	66.7
Winter	DR1	1.00	33.3
Winter	DR3	1.00	50.0
Winter	DR4	1.00	33.3
Winter	DR5	0.00	0.00

The weight score reflects the magnitude of the toxicity in each class and is directly proportional to the toxicity of water in that class. In Autumn, DR 1 and DR 3 belonged to Class 4 with a class weight score of 33,3% whereas DR 5 belonged to the same class with a higher weight score of 66.7%. It can be concluded that the water at DR 5 contained more toxic chemicals as confirmed by the study. Though DR 4 belonged to Class 3 during Autumn, it had a slightly higher weight score of 33.5% which means that the water was slightly more toxic than water at DR 1 and DR 3. Water belonged to Class 1-4 during the spring season.

DR 1 and DR 2 belonged to Class 2 and Class 4, respectively, with corresponding weight scores of 33.0% and 33.3%. DR 4 and DR 5 belonged to Class 3 and Class 2, respectively, with weight scores of 44.3% and 33.5%. The Summer season belonged to Class 4 with a very high weight score of 66.67% and can be assumed that there was a higher load of toxic chemicals at DR 5 during drier periods (Summer and Autumn). Class 4 was detected twice during the Winter season, which was at DR 1 and DR 4, with weight scores of 33.3%. DR 3 belonged to Class 3 during Winter. However, the weight score was 50.0%, and higher than all sites during this season. Therefore, it can be concluded that this site contained toxic chemicals during the Winter season. The class weight scores for all sites over the four seasons with microplastics are presented in Table 6.2.

Table 6.2: Mean class weight score and class weight score as a percentage (%) at different sites in the Diep River spring samples with microplastics

Site	Class Weight Score	Class Weight Score as a Percentage (%)
DR1	1.33	44.3
DR2	1.33	66.5
DR3	1.33	44.3
DR4	1.33	66.5
DR5	1.67	55.7

Table 6.2 represents the class weight scores for all sites during the spring season which contained microplastics. DR 1 and DR 3 belonged to Class 4 and had weight scores of 44.3%. DR 5, which also belonged to Class 4, had a high weight score of 55.7%. On the other hand, DR 2 and DR 4 belonged to Class 3, with the highest weight score of 66.5%. It can be assumed that a combination of the environmental samples with microplastics resulted in a higher toxic response at these sites. The class weight scores in distilled water with microplastics at 3 different temperatures (ºC) are presented in Table 6.3.

Table 6.3: Mean class weight score and class weight score as a percentage (%) in distilled water with microplastics at 3 different temperatures (ºC)

Temperature rise	Class Weight Score	Class Weight Score as a Percentage (%)
0.50	1.00	50.0
1.00	1.33	44.3
1.50	1.33	66.5

Class weight scores are shown for the three different temperature rises of distilled water with microplastics. the results reveal that at a temperature rise of 0.5ºC, which belonged to Class 3, the weighted score was 50%, whereas with a temperature rise of 1ºC, the weighted score was 44,3%. Although a temperature rises of 1.5ºC belonged to Class 3, it had the highest weight score of 66.5%, indicating that an increase in temperature by 1.5 can enhance the toxic effect of microplastics on organisms.

6.4 BIOASSAY TESTS OF THE PLANKENBURG RIVER WATER SAMPLES

Based on three test models (*R. subcapitata, Daphnia Magna,* and *T. thermophila*) representing the freshwater ecosystem, the toxicity of the river samples was assessed using virgin Polyethylene microplastic (PE-MP). The eco-toxicological approach provides a holistic understanding of the resulting compound mixture present in water samples (de Melo Gurgel et al., 2016).

6.4.1 Raphidocelis subcapitata 72 h growth inhibition test

The mean growth inhibition for *R. subcapitata* exposed to different sampling sites of Plankenburg River samples represented in Figure 6.13 ranged from -1.40% to 22.5%. In spring, no significant difference in mean growth inhibition values was observed between sampling points PR1, PR2 and PR4, except PR3, where a significant decrease in microalgae growth (PE < 0%) was observed. The maximum PE was obtained at PR2 (12.5%), which means that in spring, the river samples had no significant toxic effect on *R. subcapitata* and can be scored 0. In summer, the mean growth inhibition value observed at PR1 (0.80%) was the lowest compared to PR2, PR3 and PR4, with the highest PE obtained at PR4 (12.8%), meaning that in summer, the river samples had no significant toxic effect on *R. subcapitata* and can be score 0 (Figure 6.13).

In autumn, a significant difference in mean growth inhibition was observed between PR1, PR2 and PR3, PR4. A significant increase in microalgae growth was observed at PR1 with 22.5%, meaning that in autumn, the river sample at PR1 had a significant toxic effect (score 1), except the other three sites, which had no significant toxic effect (score 0). In winter, the highest mean growth inhibition was obtained at PR2 (12.5%), and a significant decrease in microalgae growth (PE < 0%) was observed at PR3 (-1.40%). The river water samples in winter at the four sites had no significant toxic effect (score 0).

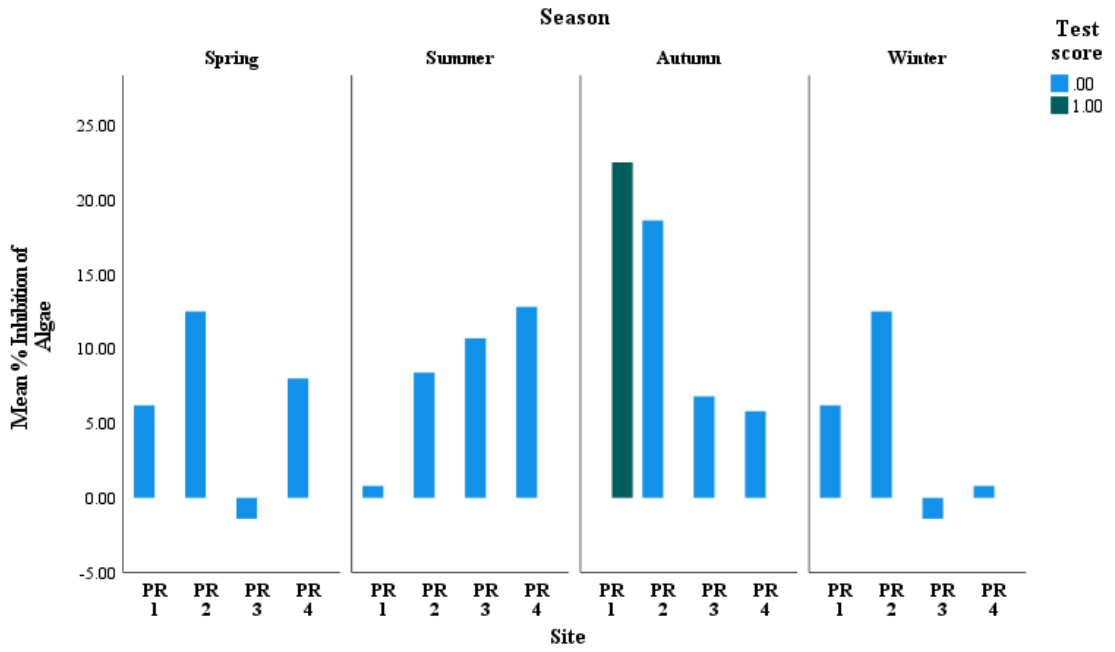

Figure 6.13: Mean toxic response (growth inhibition) of *R. subcapitata* for Plankenburg River sites. PE values < 0% indicate growth stimulation.

In general, the river samples demonstrated no significant toxic effect for *R. subcapitata* (Figure 6.13), where the mean inhibition was below 20.0%, except PR1 in autumn, with a mean inhibition of 22.0%. The results of *R. subcapitata* exposure to the Plankenburg River might be due to the limited amount of nutrients over the four seasons at the different sites. However, the significant toxic effect observed in autumn at PR1 might be due to a consequence of water pollution led by an increase of complex compounds, that provided nutrients for algal proliferation. The algal bloom might also be due to the informal settlement in the vicinity of the sampling site. The obtained results confirmed the value of *R. subcapitata* as a sensitive bio-indicator in ecotoxicological assessment of aquatic systems.

It should be expected that complex compounds enriched in river waters would stimulate *R. subcapitata* growth. The results are similar to Serpa et al. (2014), where *R. subcapitata* exposed to the Cértima river did not cause any algal growth inhibition. The exposure studies of the spring samples with virgin polyethylene microspheres (PE-MP) revealed that there was no significant difference in the mean growth inhibition of *R. subcapitata* at different sampling sites (Figure 6.14a). The percentage growth inhibition for PR2, PR3, PR4 and PR1 were 29.2%, 28.5%, 32.2% and 39.1%, respectively. The percentage effect at PR1 was the highest, and so, it had a significant toxic effect (score 1). This observation is consistent with Li et al. (2020) who reported that exposure to microplastics resulted in the growth inhibition of microalgae.

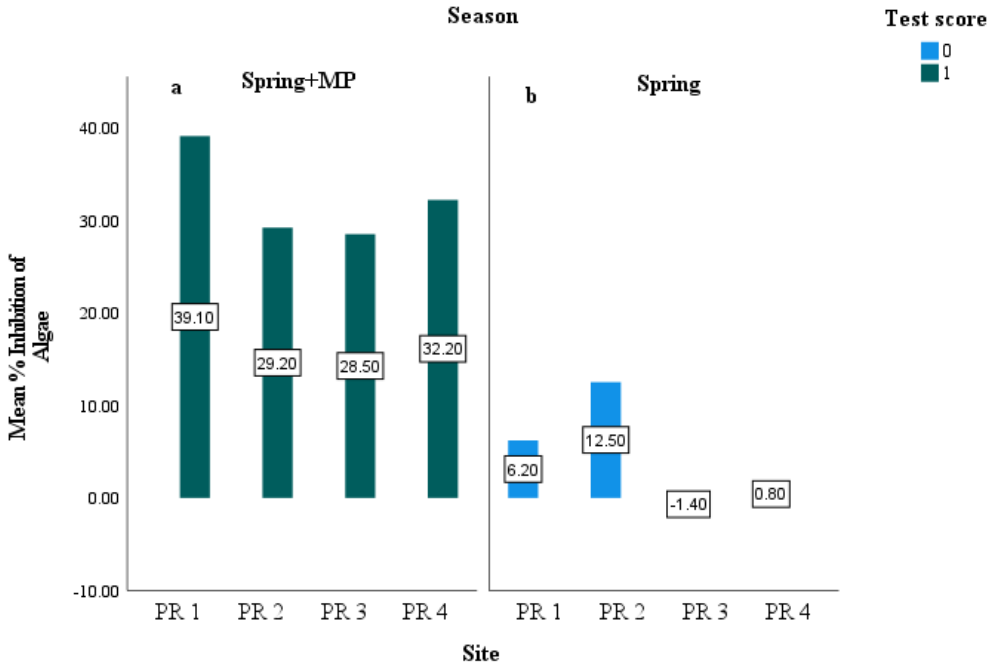

Figure 6.14: Mean toxic response of algae for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b).

The results of the *R. subcapitata* exposed to PE-MP in Figure 6.14a revealed a significant difference in growth inhibition and scoring compared to the results obtained in spring for same samples without MPs (Figure 6.14b). The virgin PE-MP had a negative effect on the microalgae observed as proliferation. This implies the that the mixture had eutrophic potential in the freshwater ecosystems. According to Davarpanah & Guilhermino. (2015), the results of this test might be due to the size of the plastic particles tested with the concentrations tested PE-MP. The results of this study are similar to Casado et al. (2013) results obtained on the eco-toxicological assessment of silica and polystyrene nanoparticles assessed by a multi-trophic test battery.

6.4.2 *Daphnia magna* 48 h acute immobility tests

Daphnia magna acute toxicity test for all samples taken from Plankenburg River is presented in Figure 6.15. In spring, the mean mortality rates of the exposed *D. magna* showed very low toxicity only at PR4 with 5% neonates' mortality, while no neonates mortality occurred at sites PR1 to PR3. According to the later findings, the river samples in the spring exhibited no discernible harmful effects on *D. magna* and can therefore be scored 0. The river samples exposed to *D. magna* in summer and winter presented a 5% mortality rate at PR2 and PR4, respectively and a 10% mortality rate at PR1 in both seasons (Figure 6.15). The PE mortality in both seasons was scored 0 as they presented no significant toxic effect on *D. magna* after 48 h of exposure.

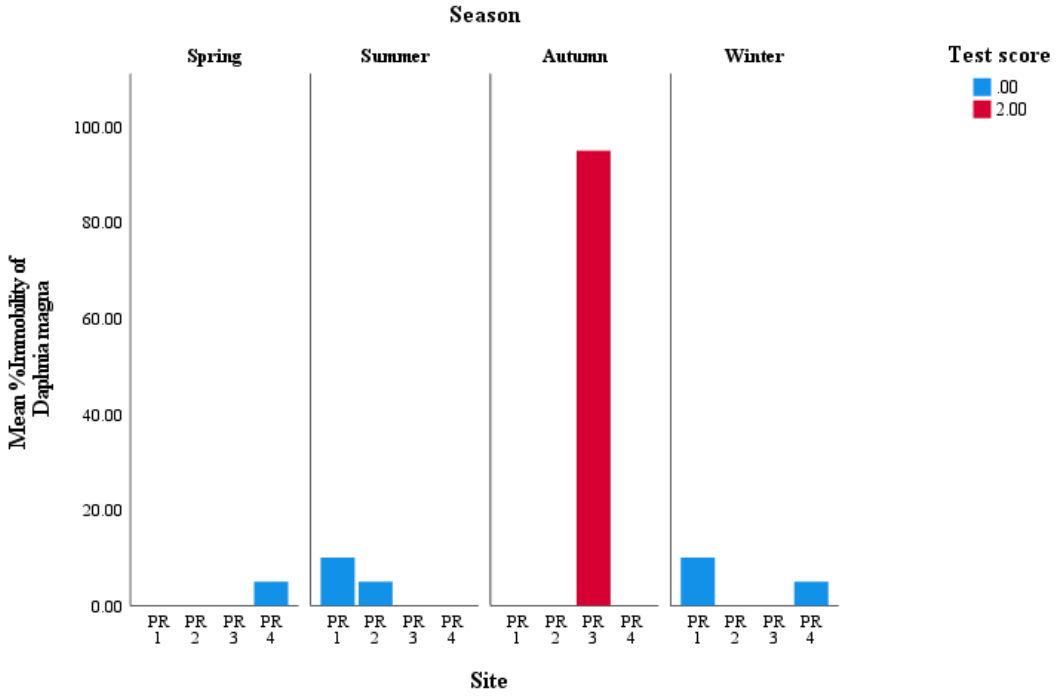

Figure 6.15: Mean toxic response (mortality) of *D. magna* for Plankenburg River sites.

A toxic effect (score 2) to *D. magna* was observed in autumn with a 95% mortality rate at PR3 after 48 h of exposure, while no neonate's mortality was obtained at the other three sites (Figure 6.15) and river water impacted the mortality rate at site PR3 in autumn. The presence of high nutrient availability in the river can favour daphnids mobility which means that the opposite could account for this mortality rate observed at PR3 in autumn. The results obtained are lower compared to the observation by Szklarek et al. (2021) where mortality rate in samples from a river in Poland was assessed over the four seasons.

The toxicity test of *D. magna* exposed to the samples collected in spring with PE-MP is presented in Figure 6.16a. The mean mortality rates of the exposed *D. magna* showed very high toxicity only at PR2 with 20% neonates' mortality, while 5% neonates mortality occurred at sites PR3 and PR4. The latter results mean that the river samples with PE-MP had no significant toxic effect (score 0) on *D. magna*, which is like the test score obtained without PE-MP in Figure 6.16b. The result of this study might be related to the size of the particles used (40-48 mm), and according to Castro et al. (2020), this size class is larger than the size class of PE-MP that had effects on daphnids immobility.

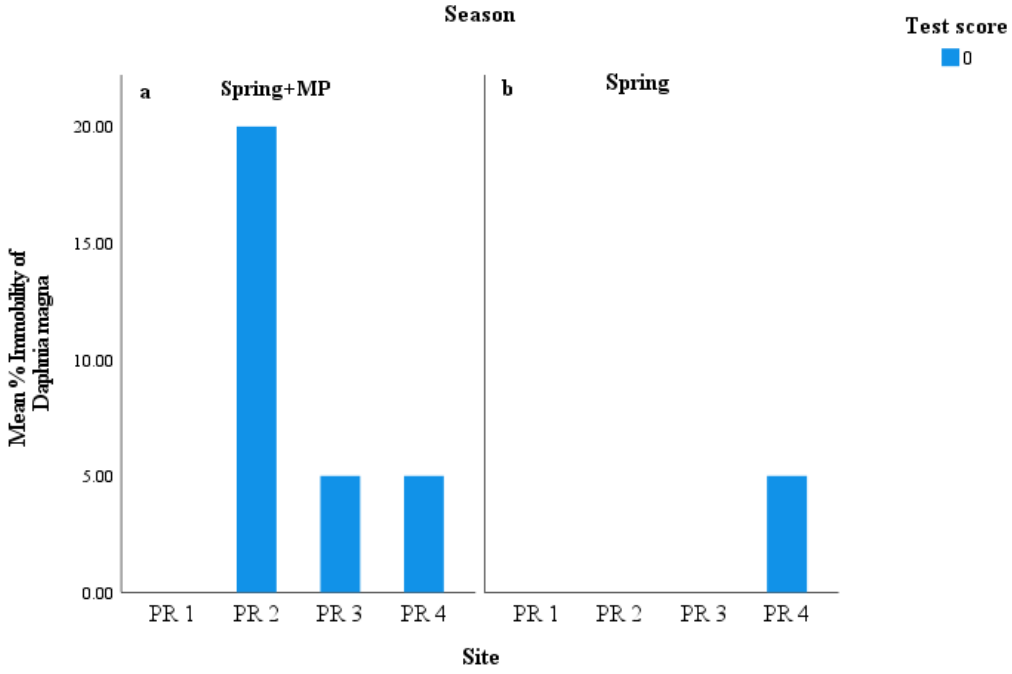

Figure 6.16: Mean toxic response of *D. magna* for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b)

During the exposure period, some *D. magna* were observed swimming in the PE layer on the surface, which puts additional stress on the crustaceans. After a few swimming strokes, the PE-MP attached to the carapax detached again. The results of this study are similar to Rehse et al. (2016) results on short-term exposure of microplastic particles to *D. magna*.

6.4.3 *Tetrahymena thermophila* 24 h chronic growth inhibition test

The mean toxicity of Plankenburg River samples exposed to the ciliate *T. thermophila* presented in Figure 6.17 shows a significant mean percentage growth inhibition over the four seasons. *Tetrahymena thermophila* bioassay showed a high sensitivity response to the river samples with PE < 0% in autumn and winter at PR1 and PR4, respectively. In spring, no significant difference in mean growth inhibition value was observed between sampling points PR1, PR2 and PR3, except PR4, where a small exposure response to *T. thermophila* was observed at 10.9% (Figure 6.17). The river sample at PR4 (spring) had no significant toxic effect (score 0) on *T. thermophila*, while a toxic effect (score 2) was recorded from PR1 to PR3 in the same season with a maximum PE value of 73.5% at PR3. In summer, there was a significant difference in mean growth inhibition value between sampling sites, with a high PE value at PR4 (100%) and a low value at PR1 (28.4%). The river samples had a significant toxic effect (score 1) from PR1 to PR2, a toxic effect (score 2) at PR3 and a PE= 100% (score 3) at PR4.

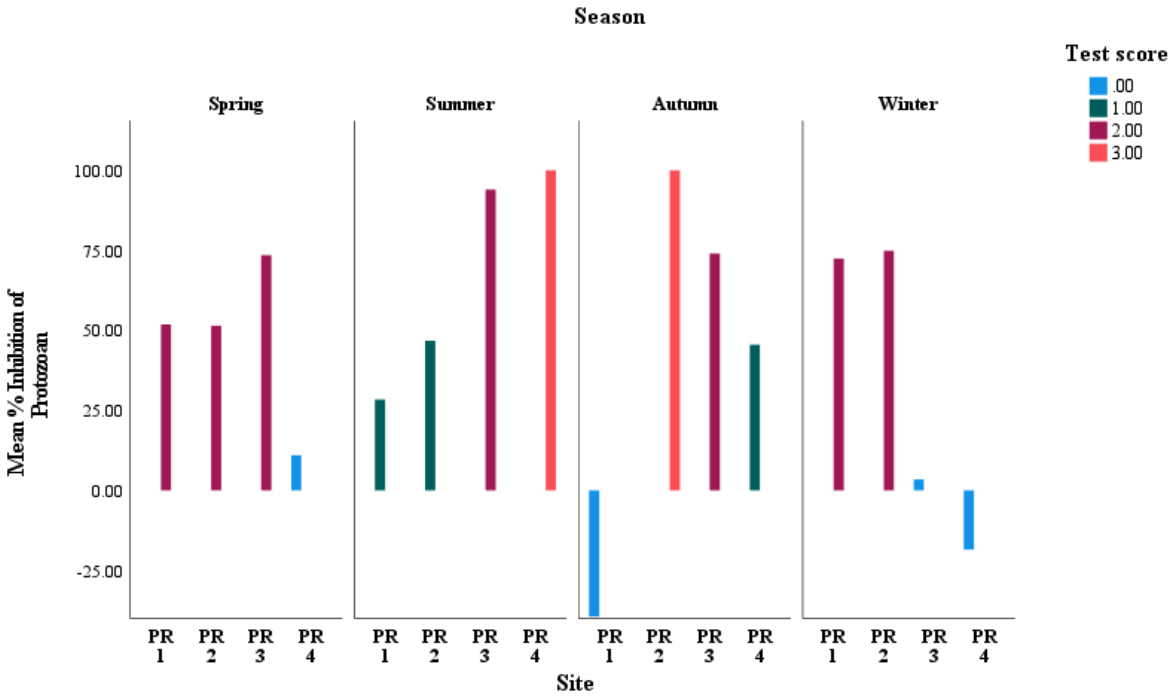

Figure 6.17: Mean toxic response (growth inhibition) of *T. thermophila* for Plankenburg River sites

In autumn, there was a significant difference in mean growth inhibition value between sampling sites, with a high PE value at PR2 (100%) and a low value at PR1 (-39.4%). *T. thermophila* exposure to the river samples presented different responses at each of the four sampling sites, and the highest score (score 3) was obtained at PR2 and the lowest one at PR1 (score 0) (Figure 6.17). In winter, no significant difference in mean growth inhibition value was observed between PR1 and PR2 and presented a toxic effect (score 2) on *T. thermophila* exposure to the river samples at these sites. Very low growth inhibition was observed at PR3 and PR4 with PE < 0%. The river samples at PR3 and PR4 had no significant toxic effect (score 0) on *T. thermophila*. The high effect of *T. thermophila* exposure to the river samples in autumn and winter might be due to the suspended solids or microbial growth in the river water during the two seasons.

According to Pereao et al. (2021) the presence of suspended solids or microbial growth in water can affect cell division and cause a slower growth of *T. thermophila*. Based on the results obtained, the presence or absence of ciliates can be correlated to a specific environmental condition and can be used as a biological indicator of pollution in river water. Thus *T. thermophila* could be an excellent tool to assess toxicity and pollution in aquatic systems. The results obtained in this study are high compared to Szklarek et al. (2021), where *T. thermophila* was exposed to river samples in Poland. The spring mean toxicity of Plankenburg River with PE-MP samples exposed to the ciliate *T. thermophila* presented in Figure 6.18a shows a significant mean percentage growth variation over the four sites. A significant difference in mean growth inhibition value were observed at PR3 (60%), whereas the remaining three sites showed a significant decrease in microalgae growth with PE < 0% (Figure 6.18a). The river sample at PR1, PR2, PR4 had no significant toxic effect (score 0) on *T. thermophila*, while a toxic effect (score 2) was recorded at PR3.

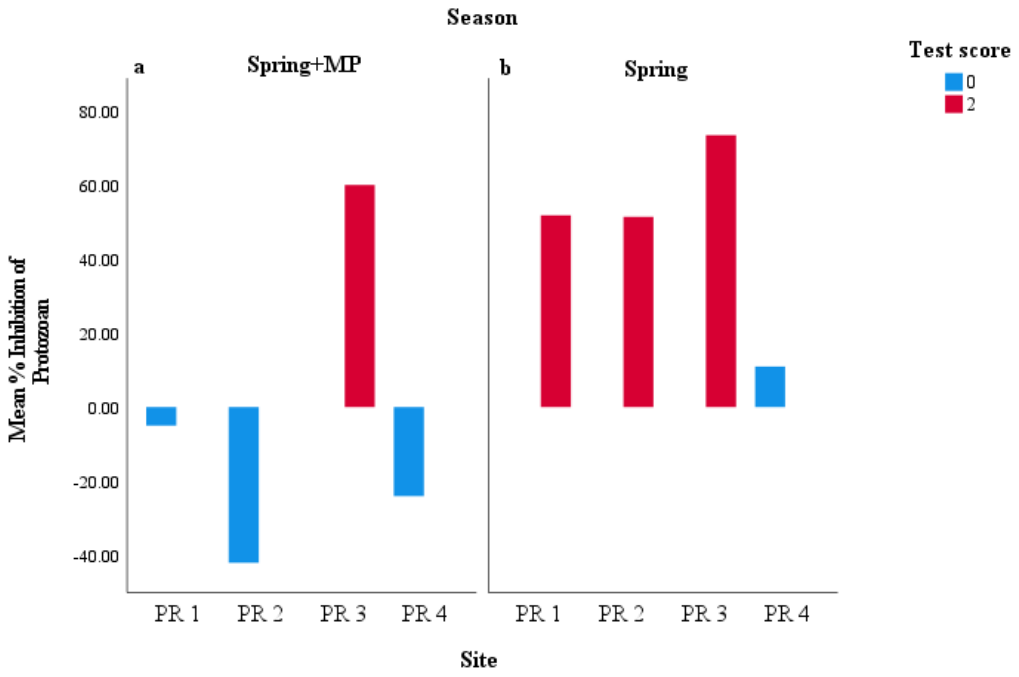

Figure 6.18: Mean toxic response (growth inhibition) of *T. thermophila* for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b).

Results from samples with virgin PE-MP show a significant change in *T. thermophila* growth inhibition after 24 h of exposure compared to samples without PE-MP (Figure 6.18b). In this study, the presence of PE-MP impacted the toxicity effect of *T. thermophila*. The result of this study is similar to Wu et al. (2021) study on the sensitivity of *T. thermophila* when exposed to microplastics. According to Casado et al.(2013), eco-toxicity studies on the sensitivity of trophic level of samples with microplastics when compared to algae and *D. magna*, algae is expected to be the most sensitive trophic level. The results presented in this study have *R. subcapitata* > *Daphnia magna* > *T. thermophila* as sensitive trophic level, which is consistent with Casado et al.(2013) conclusions.

6.4.4 Acute Hazard Classification of Plankenburg River water samples

The proposed toxicity classification based on a battery of microbiotests presented in Figure 6.19 was applied by Mankiewicz-Boczek et al.(2008) and Szklarek et al. (2021), on river samples. Biological analyses were based on three bioassays' sensitivity of the surface-water samples from Plankenburg River over the study period. The river samples that were taken in the spring at the sampling sites PR1, PR2, and PR3 are classified as Class III (Acute hazard) since one of the bioassays examined (*T. thermophila*) yielded a 50% PE 100% result, but no other tests revealed toxic effects. The 20% threshold was not exceeded in any of the three tests, hence the sample for site PR4 falls under the category of no acute hazard (Class I).

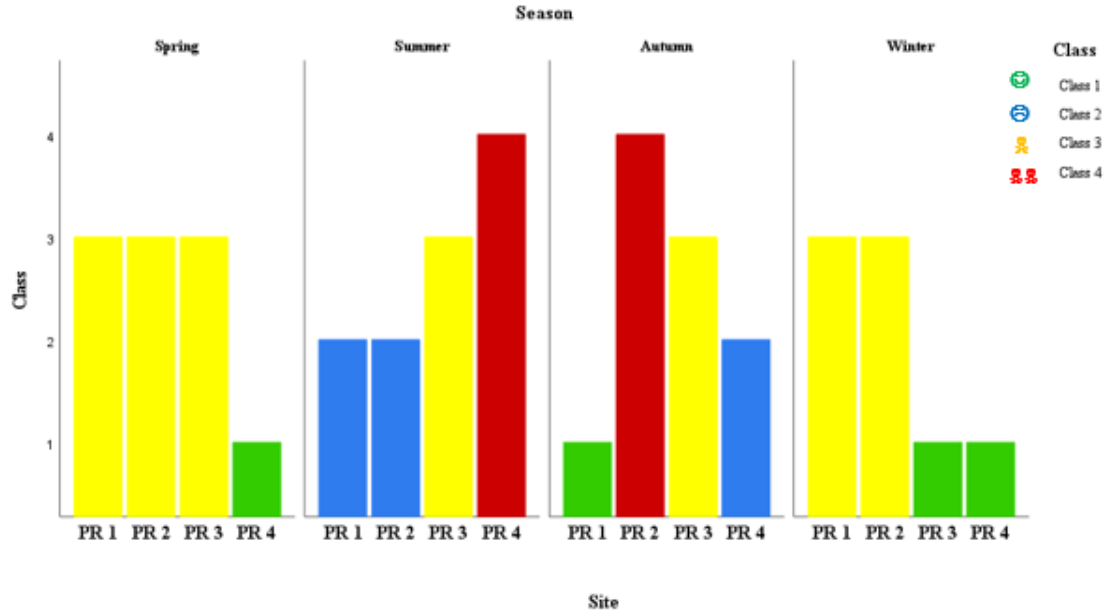

Figure 6.19: Seasonal toxicity classification for Plankenburg River

The percentage of the class weight score in spring presented in Table 6.4 is low at sites PR1, PR2, and PR3 (33.5%) and very low at site PR4 (0%), and it is concluded that Plankenburg River samples contain virtually lower to no toxic chemicals, as against the physicochemical analyses parameters which indicated no pollution.

Table 6.4: Plankenburg River class weight score and percentage

Season	Site	class weight score	class weight score as a percentage
Spring	PR1	0.67	33.5
	PR2	0.67	33.5
	PR3	0.67	33.5
	PR4	0.00	0.00
Summer	PR1	0.33	33.0
	PR2	0.33	33.0
	PR3	0.67	33.5
	PR4	1.00	33.3
Autumn	PR1	0.33	33.0
	PR2	1.00	25.0
	PR3	1.33	66.5
	PR4	0.33	33.0
Winter	PR1	0.67	33.5
	PR2	0.67	33.5
	PR3	0.00	0.00
	PR4	0.00	0.00

The summer samples shown in Figure 6.19 are classified as Class II (Slight Acute Hazard) at sites PR1 and PR2 because only one test (*T. thermophila*) exceeded the 20% threshold while no other tests revealed harmful

effects. Site PR3 samples are classified as Class III (Acute hazard) since only one of the bioassays conducted (*T. thermophila*) exceeded the 50% threshold for toxic effects. The samples from Site PR4 fall under Class IV (High Acute Hazard) since only one of the evaluated bioassays (*T. thermophila*) yielded PE 100%, while no other tests revealed toxic effects. The percentage of the class weight score in summer presented in Table 6.1 is between 33.0% and 33.5%, and it is concluded that Plankenburg River samples contain virtually lower toxic chemicals. The samples shown in Figure 6.19 are from the fall and are considered to pose no acute hazards (Class I) at site PR1 because none of the three tests surpassed the 20% threshold. Site PR2 samples fall under Class IV (High Acute Hazard) since only one of the assessed bioassays (*T. thermophila*) produced PE 100%, while no other tests revealed toxic effects. Site PR3 samples fall into the category of acute hazard (Class III) since two of the examined bioassays (*T. thermophila* and *D. magna*) exceeded the 50% threshold but no other test demonstrated a toxic effect.

The samples collected at site PR4 fall under Class II (Slight Acute Hazard) since only one test (*T. thermophila*) exceeded the 20% threshold while no other tests revealed harmful effects. The percentage of the class weight score in autumn presented in Table 6.4 is 25% (PR2), 33% (PR1, PR4) and 66.5% (PR3), and it is concluded that Plankenburg River at PR1, PR2, and PR4 contains virtually lower toxic chemicals, and at PR3 the river samples can be considered seriously hazardous and acutely toxic to aquatic flora and fauna. The winter samples shown in Figure 6.7 are classified as an acute hazard (Class III) at sites PR1 and PR2 because one of the evaluated bioassays produced a result of 50% PE 100%. (*T. thermophila*). A Low (33.5%) percentage of the class weight score is shown in Table 6.4, indicating a less toxic chemical. The Plankenburg River at the sampling sites PR3 and PR4 are classified as no acute hazard (Class I) because the 20% threshold was not exceeded in all three tests. The very low percentage class weight score at sites PR3 and PR4 in Table 6.4 can be an indication of no toxic chemicals, as against the physicochemical analysis parameters, which indicated no pollution. An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix B-1.

Figure 6.20a presented the toxicity classification of Plankenburg River samples collected in spring with virgin PE-MP. Two types of classes were observed after the exposure of the samples with the three test models bioassays used in this study. Sites PR1, PR2, and PR4 are classified as a slight acute hazard (Class II), as only one test (*R. subcapitata*) are above the 20% threshold for toxic effects. Whereas at site PR3, one of the examined bioassays (*T. thermophila*) exceeded the 50% threshold, and the sample is in the no acute hazard category (Class I). The results of the toxicity classification of Plankenburg River in spring with virgin PE-MP (Figure 6.20a) is completely different from the result obtained

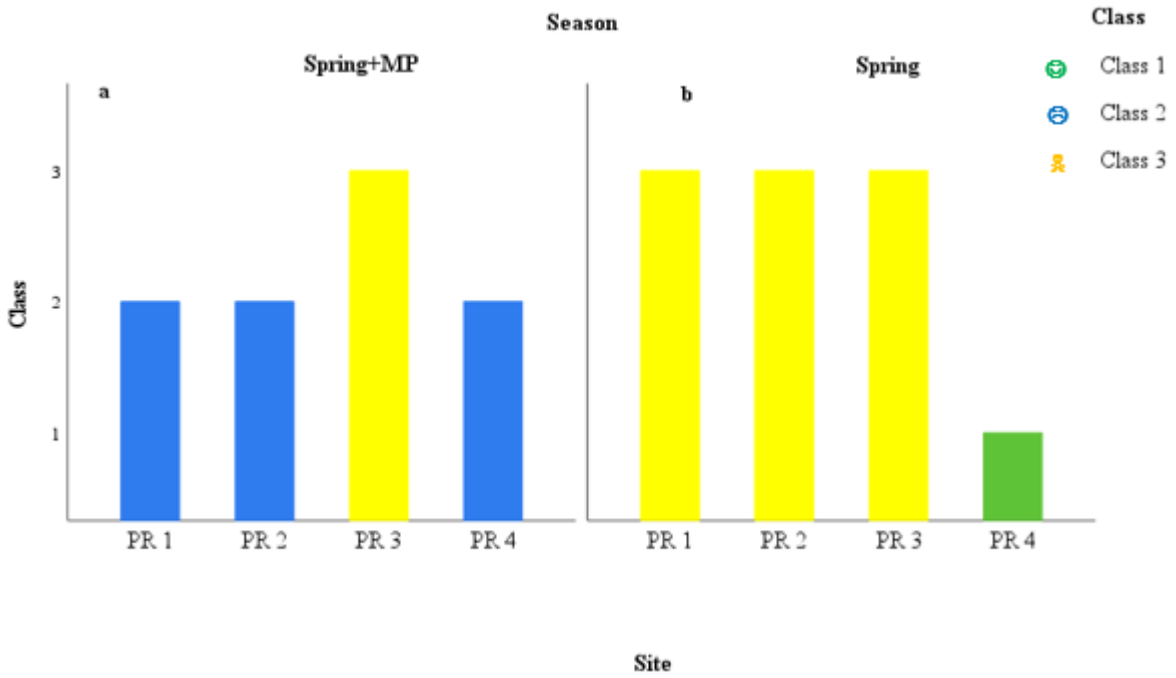

Figure 6.20: Toxicity classification for Plankenburg River with virgin PE-MP (a) and without virgin PE-MP (b).

Figure 6.20b without the virgin PE-MP. A decrease in Classes was observed at PR1 and PR2, with an increase at PR4 and constancy at PR3. The presence of virgin PE-MP impacts the toxicity classification of the freshwater system. Therefore, it will be important to investigate other parameters or chemicals that may influence the impact of virgin PE-MP on freshwater organisms.

Table 6.5: Plankenburg River with virgin PE-MP class weight score and percentage

Season	Site	class weight score	class weight score as a percentage
Spring	PR1	0.33	33.3
	PR2	0.33	33.3
	PR3	1	50
	PR4	0.33	33.3

The percentage of the class weight score of the sample in spring with virgin PE-MP presented in Table 6.5 is low at sites PR1, PR2 and PR4 (33.3%) and relatively high at site PR3 with 50%. It is concluded that Plankenburg River samples in spring with virgin PE-MP contain toxic chemicals at PR3 and lower toxic chemicals at the other three sites. The samples at PR3 can be considered hazardous and acutely toxic to aquatic flora and fauna. An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix B-2.

6.4.5 Microplastics and temperature variability exposure studies

The effects of temperature increases were evaluated using virgin polyethylene microspheres (PE-MP) in Milli-Q water and the three bioassays previously reported.

6.4.6 *Raphidocelis subcapitata* growth inhibition study

The toxicity of the analysed Milli-Q water with virgin PE-MP at three different temperatures (0.5°C, 1°C and 1.5°C) was determined. The mean growth inhibition of *R. subcapitata* exposed to different temperatures shown in Figure 6.21 ranged from 30.1% to 33.2%. No significant difference in mean growth inhibition values was observed between the three temperatures. It can be observed from this study that the increase in temperature might affect aquatic organisms, and an adaptation at high temperature (1.5°C) of *R. subcapitata* can be possible. The results of this study suggest that increased temperature negatively affected aquatic organisms and but *R. subcapitata* has adaptation potentials.

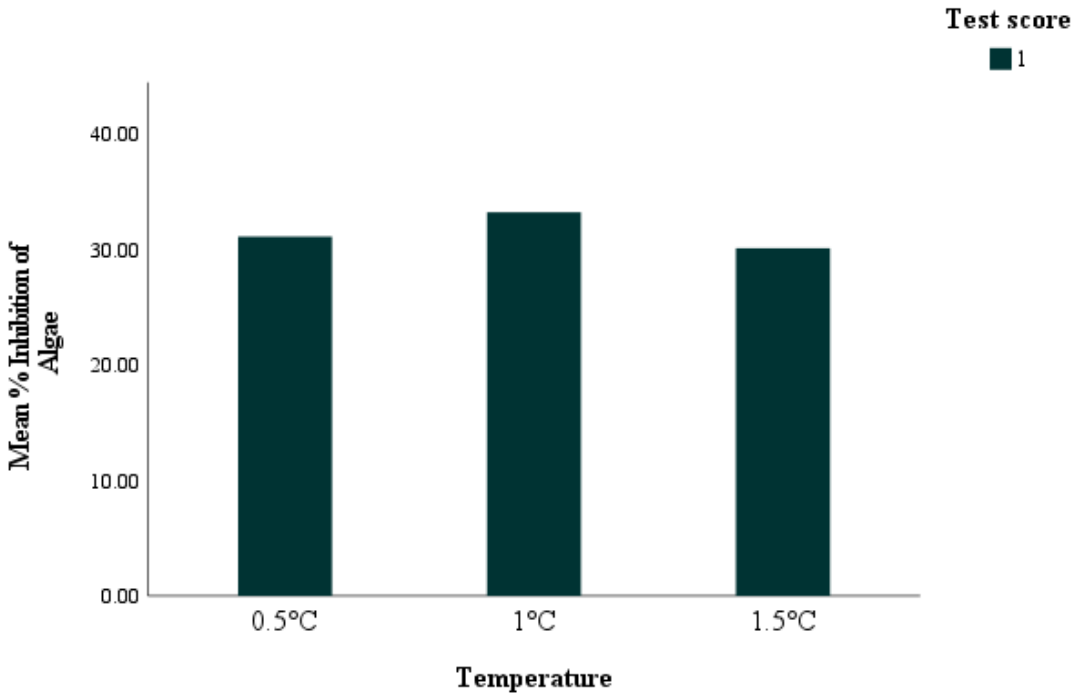

Figure 6.21: Mean toxic response of algae for Milli-Q water with virgin PE-MP

6.4.7 Daphnia magna immobility study

The immobility test for *D. magna* exposed to Milli-Q water with virgin PE-MP at increases of 0.5°C, 1°C and 1.5°C from optimal ambient temperatures are presented in Figure 6.22. The mean mortality rates of the exposed *D. magna* showed high toxicity at the 0.5°C temperature increase with 35% neonates' mortality, while 20% and 25% neonates mortality occurred at 1°C and 1.5°C, respectively. The Milli-Q water with virgin PE-MP was rated 1 with a significant toxic effect on *D. magna*. The result of this study revealed the negative effect of temperature increase on *D. magna*. The implication of this is that temperature increases of 0.5°C in combination with PE-MP could be significantly dangerous to the aquatic fauna.

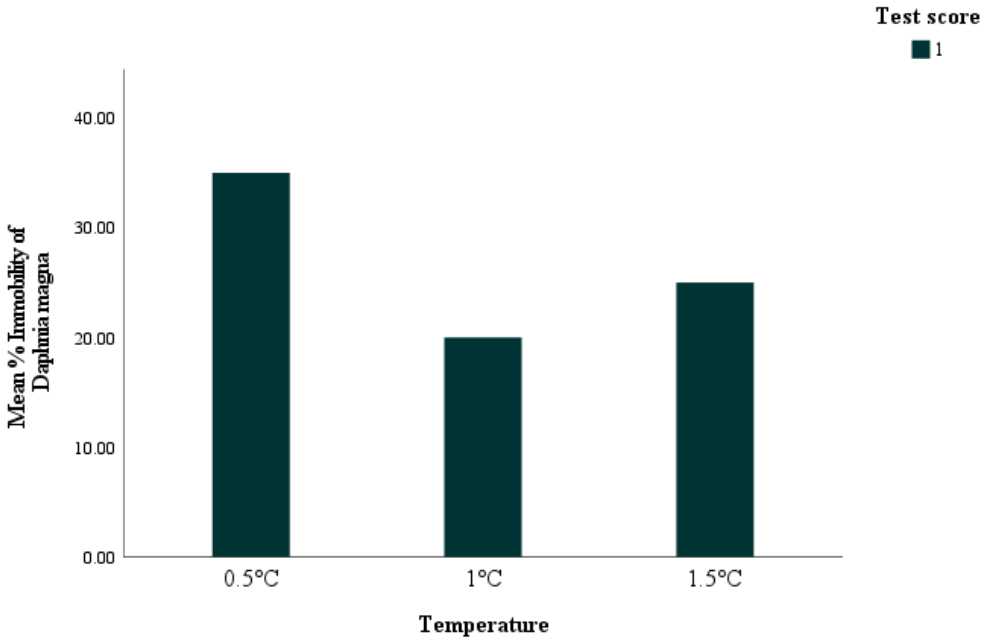

Figure 6.22: Mean toxic response of D. magna for Milli-Q water with virgin PE-MP

6.4.8 *Tetrahymena thermophila* growth inhibition study

The mean percentage inhibition of Milli-Q water with PE-MP exposed to ciliate, *T. thermophila* at three different temperatures (0.5°C, 1°C, 1.5°C) shown in Figure 4.23. There were significant variations in the percentage of growth inhibition over the different temperatures. Cell proliferation was observed at 1°C and 1.5°C with -673.585 and -10.3 percentage of growth, respectively. The Milli-Q water with PE-MP at 0.5°C and 1°C had PE= 100% (score 3) and 1.5°C had a significant toxic effect (score 1) on *T. thermophila*.

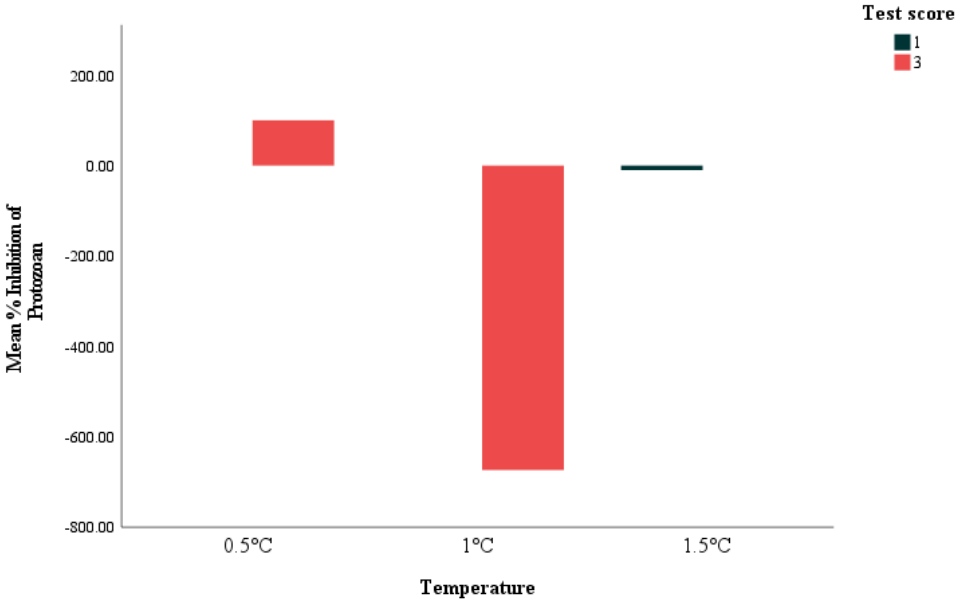

Figure 6.23: Mean toxic response of *T. thermophila* for Milli-Q water with virgin PE-MP

6.4.9 Acute Hazard Classification of Milli-Q water with virgin PE-MP

The proposed toxicity classification using the battery of tests presented in Figure 6.24 was applied on Milli-Q water with virgin PE-MP. Two types of classes were observed after the exposure of the samples with the three test models bioassays used in this study. Water samples with temperatures of 1.50°C increase was categorised as Class II (slight acute hazard) because the 20% threshold was exceeded in two tests (*R. subcapitata* and *D. magna*), but no other test showed a toxic effect. At a temperature of 0.50°C and 1.00°C a PE of 100% was obtained in at least on bioassays tested and classified as high acute risk (class IV).

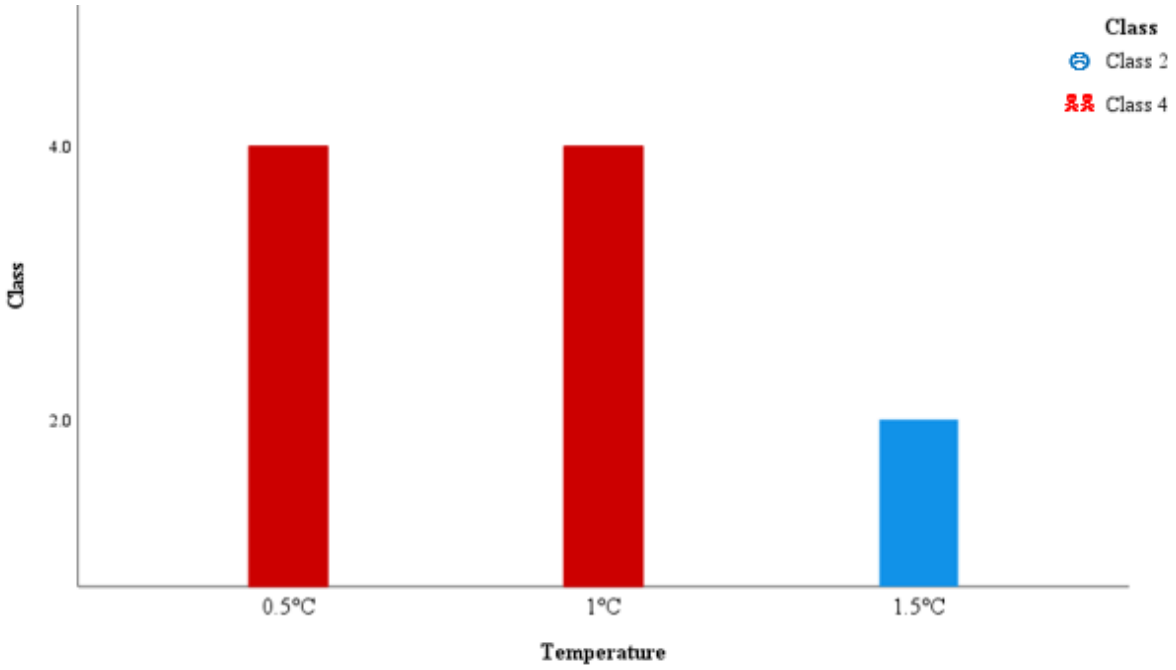

Figure 6.24: Toxicity classification for Milli-Q water with virgin PE-MP

The percentage of the class weight score presented in Table 6.6. is between 100% and 55.67%, and it is concluded that Milli-Q water with virgin PE-MP can be considered hazardous and acutely toxic to aquatic organisms. An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix B-3.

Table 6.6: Milli-Q water with virgin PE-MP class weight score and percentage

Temperature (°C)	Class weight score	Class weight score as a percentage
0.5	1.67	55.67
1	1.67	55.67
1.5	1	100

6.5 MICROPLASTICS CONTAMINATION FACTOR AND POLLUTION LOAD INDEX

The PLI results clearly indicated that all sites on both rivers were polluted with MPs (PLI > 1). Pollution intensity was variable, but some sites had a considerably higher pollution load index value. The findings suggested that

abundance-based higher pollution level (i.e. PLI) yield high pollution risks. We observed that the rivers were polluted all through the four seasons except the site PR4 in summer. The MPPLI value were 2.5 and 1.3 for the Diep and Plankenburg Rivers respectively.

Table 6.7: Microplastics contamination factors and pollution load index for Diep River

	Season								
	Autumn		Spring		Summer		Winter		
Site (MP per litre)	Water20_MPCF	Water250_MPCF	Water20_MPCF	Water250_MPCF	Water20_MPCF	Water250_MPCF	Water20_MPCF	Water250_MPCF	$MPPLI_{250}$
DR1	1.28	1.21	4.08	2.84	0.00	0.00	1.95	1.00	1.90
DR2	0.00	0.0	1.00	3.21	0.00	0.00	0.00	0.00	2.00
DR3	2.0	2.74	1.14	2.79	0.00	0.00	1.76	2.42	2.60
DR4	2.64	2.32	3.90	6.11	0.00	0.00	2.56	4.84	4.10
DR5	2.04	3.11	4.20	3.58	1.56	1.25	1.72	1.47	2.20

As shown in Table 6.7, for Diep River, in Autumn, most sites were moderately contaminated except DR 2 which has no values. However, for spring, most sites were considerably contaminated except DR 2, DR 3 and DR 1, DR 3 which were moderately contaminated using 20 µm and 250 µm respectively. Generally, the Diep River exhibited moderate contamination.

Table 6.8: Microplastics contamination factors and pollution load index for Plankenburg River

	Season								
Site	Spring		Summer		Autumn		Winter		
	MPCF_20 µm	MPCF_250 µm	MPCF_20 µm	MPCF_250 µm	MPCF_20 µm	MPCF_250 µm	MPCF_20 µm	MPCF_250 µm	$MPPLI_{250}$
PR1	3.55	1.18	1.07	1.92	1.60	1.25	1.34	1.00	1.30
PR2	4.07	1.39	2.43	1.42	1.00	1.36	2.57	1.42	1.40
PR3	3.38	1.76	2.29	1.07	1.10	1.20	2.25	1.22	1.30
PR4	2.27	1.14	0.00	1.55	1.26	1.27	2.29	0.00	1.30

For the Plankenburg River (Table 6.8), all the sites in spring season exhibited considerably contaminated risk except PR4 which was moderately contaminated. All sites were moderately contaminated for summer, autumn and winter seasons using both 20 µm and 250 µm filtration. Risk-zones classification and hotspots identification may facilitate further pollution monitoring and management in this environmental context.

6.6 MUTAGENICITY OF THE DIEP AND PLANKENBURG RIVER WATER SAMPLES

Maron &s Ames, (1983) posited that *Salmonella* assay is a generally accepted biotest to detect mutagenicity of individual compounds and environmental samples. Mutagenicity, which alters the DNA structure permanently, has become a growing problem in recent years. According to Liu et al. (2015), an organism's genetic material can modify how it functions in ways that are heritable. The measurement of mutagenic risks has been made possible using short-term bioassays, which can identify a wide range of chemicals that may cause genetic damage. The Ames Salmonella/microsome mutagenicity assay (Salmonella test; Ames test) is

a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage that leads to gene mutations (Ames et al., 1973). The test employs several histidine dependent Salmonella strains each carrying different mutations in various genes in the histidine operon. These mutations act as hot spots for mutagens that cause DNA damage via different mechanisms. When the Salmonella tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence (his+) can form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant. However, when a mutagen is added to the plate, the number of revertant colonies per plate is increased, usually in a dose-related manner. The Ames test is used world-wide as an initial screen to determine the mutagenic potential of new chemicals and drugs. One mutant strain, TA100, allows the detection of base substitution mutation, while another strain, TA98, allows the detection of frameshift mutation. Mutagenic chemicals are also capable of inducing cancer, and this concern has driven most of the mutagenicity testing programs (Mortelmans and Zeiger, 2000).

6.6.1 Mutagenicity testing of the Diep River water samples

The Ames test as is commonly known, is a Salmonella/mammalian microsome mutagenicity test developed from the Bruce Ames' laborartory in California, which tests chemicals for mutagenicity and caters for non mutagenic chemicals through an exogenous metabolic activation system prepared from liver homogenate (S9), that imitate the metabolism of mammals (Mortelmans and Zeiger, 2000). The investigation of the ability for the Diep River to elicit a mutagenic response by the Ames test was carried out. The site with the highest load of microplastic pollution, DR4, was selected for this experiment. The contents of each plate and number of positive wells with and without S9 are presented in Table 6.9.

The blank samples were filtered environmental water samples. The results showed that constituents in the water itself are mutagenic. The TA 100 *S. typhimurium* strain produced stronger mutagenic responses. Testing the environmental water samples with S9 activation enhanced the detection capability of the assay. Based on the results of this experiment, mutagenic responses were detected with and without bioactivation S9. Therefore, the environmental water samples were hazardous to man and the environment.

Table 6.9. Number of positive wells observed in mutagenicity test results with and without S9 activation

Plate	Concentration (%)	Bacteria	Day 2	Day 3	Day 4	Day 5	Day 6
Blank	-	+	5.00	9.00	10.0	10.0	14.0
S9-							
Positive Control	-	+	18.0	94.0	96.0	-	-
Background (DR4)	100	-	0.00	5.00	74.0	94.0	94.0
DR4 a	100	+	3.00	41.0	67.0	94.0	95.0
DR4 b	57.1	+	8.00	32.0	56.0	85.0	91.0
DR4 c	28.6	+	5.00	20.0	60.0	89.0	89.0
S9+							
Positive Control	-	+	52.0	72.0	81.0	93.0	94.0
Background (DR4)	100	-	55.0	95.1	96.0	-	-
DR4 a	100	+	66.0	96.0	-	-	-
DR4 b	51.6	+	96.0	-	-	-	-
DR4 c	19.4	+	96.0	-	-	-	-

6.6.2 Mutagenicity testing of the Plankenburg River water samples

Ames test with TA98 strain was applied for assessing the mutagenicity of the water samples of the Plankenburg River. The Ames test results of the Plankenburg River water samples showing LC10, LC20, and LC50 values are presented. No statistically significant concentration was found (p(F) > 0.05); the selected effective concentrations (LCx) of the reversion *S. typhimurium* rate TA 98 (frameshift mutagen indicator) and the obtained LOEC and NOEC at a 95% confidence limit based on Fieller's theorem (Table 6.10).

Table 6.10: Results of a 48 h of the reverted analysis for Plankenburg River Ames test

Site	Reversion rate	Average LC_{10}	Average LC_{20}	Average LC_{50}	LOEC	NOEC
PR2	TA 98 +S9	n.d.	n.d.	n.d.	≤0.194	< 0.194
	TA 98 –S9	0.406	0.523	0.850	0.571	0.286
PR4	TA 98 +S9	n.d.	n.d.	n.d.	≤0.194	< 0.194
	TA 98 –S9	0.256	0.516	n.d.	≤ 0.286	< 0.286

NOEC: No observed effect concentration; n.d.: not determined due to mathematical reasons; LOEC: Lowest observed effect concentration.

The strain TA98+S9 exposed to the Plankenburg River for 48 hours showed mutagenic activity at LOEC (≤ 0.194) in the Ames test results from sites PR2 and PR4, however, LC50 was not detected at both sampling sites. When the strain TA98-S9 was exposed, a sample obtained at PR2 showed a positive mutagenic response (LOEC of 0.571) with an average LC50 value of 0.850, while at site PR4 mutagenic activity was reported with a LOEC of ≤ 0.286. It is suggested that the Plankenburg River water body's potential for environmental mutagenesis is strongly influenced by the mechanism of reading-frame shifting of genetic macromolecules (Roveri et al., 2021)). This study's findings are comparable to those reported by Roveri et al. (2021), who did find genotoxicity activity in Guarujá surface runoff water. An overview of the number of revertant cells over a period of 6 days was presented in Tables 6.11 and 6.12.

Table 6.11: Results of Plankenburg River exposure to strain TA98+S9 for a period of 6 day

Plate	Concentration (%)	Day 2	Day 3	Day 4	Day 5	Day 6
Blank (sterile water)	-	0	0	0	0	0
Blank PR2	100	73	96	96	96	96
Blank PR4	100	94	96	96	96	96
Background	-	1	2	8	10	12
Positive Control	-	36	96	96	96	96
P2a	100	96	96	96	96	96
P2b	51.6	96	96	96	96	96
P2c	19.4	85	96	96	96	96
P4a	100	96	96	96	96	96
P4b	51.6	96	96	96	96	96
P4c	19.4	96	96	96	96	96

Table 6.12: Results of Plankenburg River exposure to strain TA98 -S9 for a period of 6 day

Plate	Concentration (%)	Day 2	Day 3	Day 4	Day 5	Day 6
Blank (sterile water)	-	0	0	0	0	0
Blank PR2	100	2	2	5	9	13
Blank PR4	100	60	94	94	96	96
Background	-	0	1	2	4	4
Positive Control	-	0	51	90	91	95
P2a	100	3	59	69	81	82
P2b	57.1	2	23	42	72	91
P2c	28.6	1	3	19	32	55
P4a	100	7	31	36	71	94
P4b	57.1	7	23	57	61	78
P4c	28	2	10	14	28	33

The mutagenicity observed was concentration dependent and statistically significant ($p < 0.05$) at all concentrations in the two bacteria strains utilized. However, TA98+S9 was found to be more responsive strain

than TA98-S9 against the test samples in the above, although both showed come similarities in terms of concentration dependence. The result further showed that the mutagenicity is concentration dependent. It is expected than this mixture of compounds previously detected in the urban surface runoff of the western cape effluents can cause potential toxic effects (synergistic, antagonistic, and/or additives) on the aquatic ecosystems because the genotoxicity assays performed with the Ames test with TA98 with and without metabolic activation by S9 showed mutagenic activity for the Plankenburg river samples. The observed mutagenicity of the samples was believed to have been caused by high physicochemical parameters some of which were analyzed in this study (Okunola et al., 2016) but there is also the possibility of other constituents in the sample (though not analyzed) which might be responsible for the observed mutagenicity in this study.

The TA98 and TA100 strains are often used to detect frameshift mutations and base pair substitutions, respectively (Mortelmans and Zeiger, 2000). The presence of mutagenicity in the Diep River may simply indicate the presence of low concentration of a few organic substances (e.g. PCBs and PAH), known to induce damage to genetic material (Khallef et al., 2019). To confirm this hypothesis, further studies are required and should include other strains of bacteria (TA97a, TA102, and TA1535), which are more sensitive to genotoxicity. These physical, chemical, and microbiological results only represent a first snapshot of the water quality from Diep River in Western Cape. These complementary assessment with biological tests (e.g. Ames test and other ecotoxicity tests, with microalgae, crustacean and protozoa, respectively) allowed for an integrated assessment of the water quality.

CHAPTER 7: CONCLUSIONS & RECOMMENDATIONS

7.1 CONCLUSIONS

Microplastics pollution and its effects in aquatic systems have increased but there are still knowledge gaps regarding this issue especially in freshwater systems. This research was set out to evaluate the occurrence of microplastic particles and the potential ecological risks in the Diep and Plankenburg Rivers. The microplastics load and some physicochemical characteristics of the rivers were assessed to determine water quality and extent of microplastics in water and sediment samples. This study provided new insights into MPs abundance and distribution in water and sediments of the Diep and Plankenburg Rivers in the Western Cape province. Spatial and temporal distributions showed substantial differences based on the proximity to urban/industry areas and wastewater treatment plants. Pre-treatment methods used included density separation, filtration, acidic/alkaline digestion to ensure efficient removal of impurities from water and sediment samples. Advanced characterization technologies such as FTIR, were employed to analyse the chemical composition and other properties of MPs. The mesh size of sieves used to extract microplastics in water revealed that the smaller size mesh extracted more microplastics, in multiple orders of magnitude.

7.1.1 The Diep River

The sources of microplastics to the Diep River include formal and informal residential areas, a wastewater treatment plant, recreational, commercial, and industrial processes. Data on the spatial and temporal distribution of microplastics in water and sediment samples from the Diep River over four seasons were provided. There were no clear trends for MPs distribution in the Diep River temporally and spatially. The observations were due to a combination of differences in seasonal conditions, water flow and volumes as well as anthropogenic activities in the vicinities of the sampling sites along the Diep River.

This study demonstrated that the combination of anthropogenic activities including a wastewater treatment plant effluent discharge, greatly influenced microplastic occurrence in the Diep River. Overall, the spring season recorded the most microplastics, which could be influenced by atmospheric effects such as wind transportation and an increase in anthropogenic activity after the cold wet winter season with consequent generation of more plastic wastes that ended up in waterbodies.

There were no clear trends for MPs distribution in the Diep River and seasonal and temporal variations are likely due to a combination of differences in seasonal conditions and anthropogenic activities from microplastics sources into the Diep River There was no clear shape trend in the microplastic type at all the different sites because various sites contributed to microplastic pollution in the river. In surface waters and sediment samples of most of the sites selected on the Diep River, microplastics with particle size of less than 2000 µm were most abundant, fibre was the most common polymer shape and microplastic colour were mostly black/grey. Among all the detected polymer types, PE (Polyethylene) was the predominant type.

The correlation analysis of the physico-chemical properties of water samples and microplastics suggested critical adverse implications with climate change. For example, water acidification will further exacerbate microplastics effects in water bodies considering the negative correlation between microplastics occurrence and pH values. Higher values of temperature may also increase the sediment burden of microplastic pollution with implications for filter feeders and benthic organisms.

Physical and chemical monitoring is useful in the determination of ecosystem health. Additionally, biological monitoring offer more direct measurements of organisms' responses to stress. It provides an enhanced approach to understand river water quality. The Diep River is surrounded by various categories of land use types, which include informal and formal residential areas, a wastewater treatment plant, recreational and industrial areas. This study provided insights into water pollution consequences resulting from land-use practices near river sites. Furthermore, exposure to virgin microplastics with variable temperatures exerted adverse effects on the growth and survival of biota.

The ecotoxicity bioassays exhibited different toxicity levels over the four seasons in environmental water and showed growth inhibition at most sites in environmental samples with microplastics. The bioassay results demonstrated that the Diep River may experience water pollution and have high nutrient load, which may be a consequence of the diversity in land use practices adjacent to the river. Climate change studies demonstrated an aggravated negative effect at rising temperatures in the presence of microplastics. The Ames test results demonstrated that the river water samples produced a mutagenic response, and chemicals in the Diep River sites are potential mutagens and (or) carcinogens.

7.1.2 The Plankenburg River

The occurrence of MPs in both surface water and sediment samples of the Plankenburg Rivers was highest in spring. Infrared spectroscopic analysis of Plankenburg River samples confirmed that the dominant polymer type was polyethylene), shape was fibre and polyethylene was the most abundant polymer type. The size range of 500 to 1000 µm (61.9%) was the dominant MPs at different sites. Spatial and temporal distributions showed differences based on anthropogenic activities, and the COD and BOD values recorded for the river water samples were above the South African water guideline threshold.

The battery of biotests showed variation in the levels of toxicity of river water samples over the four seasons. This affirms the need for routine biotest analyses in addition to the physicochemical assessment of rivers. Such a strategy guarantees the establishment of a complete cause-and-effect study and identifies practical remedies to improve the quality of water resources. The eco-toxicity assessment of the river water samples revealed potential adverse impacts on the ecosystem. Those observations could not be captured with the obtained with physicochemical parameters' values.

Plankenburg River sample without virgin PE-MP showed a very high sensitivity on *T. thermophila* compared to the other bioassays tested. The highest toxicity level was recorded in summer and autumn, with high acute hazard (class IV) at PR4 and PR2, respectively. The exposure of bioassays to the Plankenburg River with

virgin PE-MP presented a totally different sensitivity to biological organisms used in this study. The samples were more sensitive to *R. subcapitata*, with Class II (Slight Acute Hazard) being the lowest and Class III being the highest (Acute hazard). The presence of virgin PE-MP in the river might have contributed to the degradation of river water quality, which might have a toxicological impact on freshwater organisms.

The experiment on the effects of climate change using three different temperatures with an increase of 0.5°C showed similar sensitivity with the spring samples and the virgin PE-MP added to the bioassays. Temperature increases negatively affected test organisms. The genotoxicity results revealed that the Plankenburg River water had mutagenic activity – a concern for human health and the aquatic ecosystem.

7.1.3 General

This study demonstrated that the microplastic pollution occurrence in the investigated freshwater systems was predominantly due to human activities in the vicinity of the sampled sites. This study provides a baseline data for future monitoring and assessment of water and sediment in the South African freshwater systems.

Generally, MPs were also detected in freshwater systems near the wastewater treatment plants as well as freshwater environments in the city with dense population, agricultural practises, and industrial activities. Microplastics with particle size less than 1 mm were most abundant and fibre was a common polymer shape in surface waters and sediment samples of most of the sites selected on both the Diep and Plankenburg Rivers. PMPs have been reported to have ecological impacts on aquatic organisms, soil biota and human health. Inhibition in growth and decrease in assimilation efficiency will be investigated in both aquatic and terrestrial organisms in the next phase of the study. This study demonstrated that the microplastic pollution occurrence in the investigated Plankenburg Rivers freshwater systems was predominantly due to the increased population density within the vicinity of the freshwater resource and the accompanying surrounding intense anthropogenic activities. This study provides an understanding of the microplastics occurrence in the Diep and Plankenburg River system and gives a baseline for future monitoring and assessment of water and sediment in the South African freshwater systems.

The three bioassays used to assess ecotoxicity showed that both rivers were highly polluted. Microplastics contamination was demonstrated as exerting environmental stress on the test models. Temperature increases exacerbated the negative effects of microplastics on aquatic organisms. The tested environmental water samples were also mutagenic. The TA98 was the more responsive strain for mutagenicity testing for both the rivers investigated. The results of this study showed that water samples from both rivers might potentially cause adverse and/or non-adverse mutagenic effects. The presence of mutagenicity suggests the presence of some organic substances (e.g. PCBs and PAH), known to induce damage to genetic material (Khallef et al., 2019). These results represent a first snapshot of the water quality from Diep and Plankenburg Rivers in the Western Cape. The complementary assessment using physico-chemical and biological tests allowed for an integrated assessment of the water quality. The effects observed may be extrapolated to human beings and other species that are part of ecosystems.

The tree major objectives of this project were achieved. The pollution loads and possible ecological and human health effects of microplastics in the Diep and Plankenburg Rivers were assessed. Results have been presented using different fora. Three masters' students were trained to completion and a postdoctoral fellow trained. An emerging researcher, Dr Conrad Sparks also had the opportunity to be part of the project. The involvement of the international collaborators was constrained in part, by the COVID-19 pandemic outbreak, funding availability to mobility and different institutional calendars across the globe.

7.2 RECOMMENDATIONS

The outcomes of our field and community engagement activities on this project provided us with valuable insights into microplastic pollution prevention, mitigation, and control. We therefore recommend the following:

1. The study recommends that a reduction in the environmental sample volume to 20 L per site and filtration through a 20 µm mesh is sufficient for high microplastic recovery rates. This is ideal for microplastic sampling of surface water which is especially useful for developing countries with limited equipment.
2. Further studies on microplastics abundance and its relation to physico-chemical analysis in water is recommended to explore and understand microplastic prevalence in different river sites and the potential implication it may have on aquatic life and human health.
3. Investigations on the exposure of bioassays to different types and sizes of polymers are needed to better understand these potential stressors and associated ecological risks.
4. There is a need to investigate plastic degradation and coexistence with other pollutants are important to fully understand effects dynamics on exposed biota.
5. There is the need for further investigations on the effect of runoff, wind, and season on the occurrence of microplastics in freshwater ecosystems.
6. Further studies are required and should include other strains of bacteria (TA97a, TA102, and TA1535), which are more sensitive to genotoxicity. This study is of public health importance as mutagenicity tests are used for an indication of potential carcinogenicity This is especially desirable in developing countries where indiscriminate disposal of wastes is on the increase.
7. Currently, ecotoxicology testing is not required by the South African DWAF, it is important to regularly include ecotoxicological (acute and chronic) bioassays in national environmental monitoring programmes for water quality management.
8. Regulatory limits for commercial and industrial discharge as well as implementation strategies are imperative for effective control of microplastic pollution.
9. To improve the ecological health of these rivers, there is a need for the provision of basic sanitation and sustainable waste management systems across social divides of the society.
10. Environmental education and awareness in communities across different socio-economic divides of the society.
11. Producers of plastic products should be accountable for the life cycle of their products.
12. Consumers can be provided with incentives for lifestyles that are healthier for man and the environment.

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CHAPTER 8: ENGAGEMENT WITH STAKEHOLDERS

The project proposed to undertake stakeholder engagement activities. Results of the project were presented in SETAC Africa, SETAC Europe and SETAC North America meetings as well as WISA conference. Some of the presentations were virtual platform speeches and others, in-person platform and poster presentations. The presentations include:

SETAC Africa Biennial Meeting (2021)

Water quality monitoring and ecological risk assessment of the Veldwachters River, Cape Town

Authors: Sihle Mlonyeni, Omoniyi Pereao and Beatrice Opeolu

Abstract

Microplastics (MP) occurrence in aquatic ecosystems is a global concern. MPs enter freshwater and marine ecosystems through many sources. Significant contribution is from wastewater treatment plant (WWTP) effluents discharged into receiving waterbodies. This study investigated the physicochemical properties of the WWTP effluent and the occurrence of MPs in the plant's influent and effluent samples. Water and sediment samples were collected upstream, point of discharge and downstream of the receiving Veldwachters River in spring and autumn months. MPs in water and sediment samples were extracted, digested, and identified using microscopy and Fourier-transform infrared spectroscopy (FTIR). The physicochemical characteristics for both seasons were largely within the South African regulatory limits for effluent discharge. The total dissolved solids (TDS) values in spring (470 ppm) were an exception with slightly higher values than the required standard (460 ppm). Particles of MPs in the 2000-1000 µm and 1000-500 µm were the most predominant and fibre/filament were the mostly identified MPs types in the samples. The WWTP processes did not effectively remove MPs from wastewater. Other sources of pollution contributed to the MP burden upstream and downstream of the river. These results will provide insights into the fate and transport of MPs in freshwater; this will be valuable for the holistic assessment of the environmental risk posed by MPs.

Microplastics occurrence and spatial distribution in the Plankenburg River, Stellenbosch, South Africa

Authors**:** Komlan Apetogbor, Omoniyi Pereao, Conrad Sparks and Beatrice Opeolu

Abstract

Several studies on microplastic (MP) pollution in marine systems have been reported in South Africa but there is little information about microplastics contamination in freshwater systems in the country. The abundance and occurrence of microplastics were determined in the Plankenburg River, which flowed through different land-use practices in Stellenbosch. The physico-chemical characteristics of the river's water samples were measured onsite to determine the river quality relative to regulatory limits. Bulk sampling method was used for microplastic sampling using a metal bucket. Onsite, 100 L water samples were filtered per site. An additional 20 L sample was collected and transported to the laboratory for processing five replicates of 4 L per site. Potassium hydroxide (10%) was used for organic matter decomposition. The recovered microplastics will be characterised morphologically using a stereo microscope. Polymer type identification was done by a random selection of microplastics particles larger than 500 µm using Fourier transform infrared (µ-FTIR) analyses. The

results showed an uneven distribution and abundance of microplastics in the freshwater system. Microplastics occurrence was dependent on the anthropogenic activities in the vicinity of the sampling points. More studies on the potential adverse effect(s) posed by microplastics on biota and freshwater environments are on-going. Preliminary results of toxicity to aquatic biota will be presented and discussed.

Assessment of Microplastic Pollution in the Diep River (Milnerton), Western Cape, South Africa.
Authors**:** Asmat Begum Khan, Omoniyi Pereao, Conrad Sparks, Beatrice Opeolu

Abstract

Rivers play an important role in the water cycle and serve as habitat to various species in aquatic ecosystems. They serve as a source of microplastics litter into the ocean. Microplastics are ubiquitous, with the potential for accumulation in the environment. Improperly disposed plastics often end up in the freshwater ecosystems. The Diep River runs through the City of Cape Town via neighbourhoods with different land use types into the ocean. In this study, microplastics particle burden in the Diep River was assessed. Water and sediment samples were collected from five sites on the Diep River and analysed for microplastics. Some physico-chemical parameters of the river water were measured onsite. On the field, 100 L sample was filtered through a 250 µm mesh and 20 L collected for processing in the laboratory. The 20 L sample was filtered through a 20 µm mesh in the laboratory. The microplastics extracted were characterized using microscopy and FTIR. The results revealed that the Diep River physico-chemical parameters such as pH, dissolved oxygen, conductivity, and temperature were within the South African Water Quality standards regulatory limits. Fibres were the most predominant microplastics particles identified in water and sediment samples. Tourist and recreational had higher microplastics burden relative to non-tourist areas. This study will provide information for management strategies in policy development and implementation, protection, and other mitigation strategies about the microplastic burden of the Diep River.

SETAC North America Meeting (2021)

Risk Assessment of Polystyrene Microplastic in the Plankenburg River, Stellenbosch, South Africa
Authors**:** Komlan Apetogbor, Omoniyi Pereao, Conrad Sparks, Beatrice Opeolu

Abstract

Plastics are widely used in industrial, commercial, and domestic processes and activities due to their versatility. This has resulted in their occurrence in environmental compartments, including waterbodies. The concern over the ecological effects of microplastics (MPs) have been reported with evidence of ingestion and trophic chain transfer in freshwater organisms. Preliminary studies revealed the occurrence of microplastics in the Plankenburg River. Anthropogenic activities in the vicinity of the sampling points were major drivers of microplastics in the Plankenburg River. The ecological and human health risks potential of MPs in the Plankenburg River using eco-toxicological bioassays was performed. *Daphnia magna, Raphidocelis subcapitata and Tetrahymena. thermophila* were the three test models used for eco-toxicological risk assessment. Mutagenicity was assessed using Ames test in addition to probabilistic models for human health risk assessment. Dark red polystyrene plastic microspheres (10 µm) were used in the experiment as primary microplastics. Experiments were conducted to assess effects of temperature rise (0.5°C, 1°C and 1.5°C) for each of the three bioassays which provided insights into the potential response(s) of the model organisms to

climate change effects. Results of daphnids mortality, algal growth, and yield inhibition as well as the protozoan growth inhibition will be presented. Potential adverse effect(s) posed by microplastics to humans such as mutagenicity will be presented.

SETAC North America Meeting (2022)

Occurrence and toxicity studies of microplastics in effluent samples of a wastewater treatment plant
Authors: Sihle Mlonyeni, Omoniyi Pereao, and Beatrice Opeolu

Abstract

Wastewater treatment plants (WWTP) are designed to safely dispose wastewater that has been generated during water use, by reducing or removing contaminants that may impact human health and the environment. However, due to population growth, the technologies used are unable to handle the increasing loads received by WWTPs. Emerging contaminants such as microplastics (MPs) further contribute to the deteriorating quality of the WWTPs effluent discharged into rivers. This study assessed the quality of the WWTP's effluent using *Daphnia Magna, Raphidocelis subcapitata* and *Tetrahymena thermophila* as tests species. The occurrence of MPs was determined in the influent and effluent to assess the WWTP's MP removal efficiency. The MPs in water and sediment samples were extracted, digested, and identified using microscopy and Fourier-transform infrared spectroscopy (FTIR). The ecotoxicity classifications showed a very high acute toxicity (class V) for the autumn season, while spring showed an acute toxicity (class III). The most prominent MP forms found in the effluent samples were fibres, with the most common colours being black/grey and 55% of MPs analysed using FTIR-ATR were polyether urethane. The findings of this study showed that dilution of the effluent reduced toxic effects exerted on test organisms. However, design of new technologies that can remove MPs and/or upgrade of WWTPs is now required for water sustainability.

Environmental assessment of the ecological risks in the Diep River (Milnerton), Western Cape, South Africa.
Authors: Asmat Begum Khan, Omoniyi Pereao, Conrad Sparks and Beatrice Olutoyin Opeolu

Abstract

Rivers serve as habitats for several aquatic organisms, and they play important roles in water cycle as well as other ecosystem functions. The presence of chemicals in the environment remains a global challenge that requires monitoring and mitigation. The complementary use of chemical analyses with biotests provides more robust information about ecosystems' response to pollution events. The Diep River runs through the City of Cape Town across neighbourhoods with different land use types into the ocean. In this study, water quality of the Diep River was assessed. Surface water samples were analysed for physico-chemical parameters and bioassays. Three test organisms, each representing a trophic level, were exposed to the river water samples. The organisms used *Raphidocelis subcapitata* (microalgae), *Daphnia magna* (crustacean), and *Tetrahymena thermophila* (protozoan). The pH values ranged between 7,73 and 9,83; dissolved oxygen was between 2,4 and 11,8 mg/L; conductivity -676 and 20000,0 m/s; and temperature values ranged between 15,8 and 23,2ºC. The results revealed that the Diep River water physico-chemical parameters were within the South African Water Quality standards with a few exceptions. Microalgae growth inhibition in all sampling sites showed no

acute hazard (Class I) and the percentage effect (PE) did not exceed < 20%. The ecotoxicological approach used can add value to hazard and risk assessment of the river and contribute to the management of water quality along the Diep River.

Acute and chronic toxicities of polyethylene microspheres on aquatic organisms exposed to Plankenburg River water samples.

Authors: Komlan Apetogbor, Omoniyi Pereao, Conrad Sparks and Beatrice Opeolu

Abstract

Ecological effects of microplastics (MPs) in freshwater systems is a global concern. The ubiquity of microplastics coupled with its adverse impact on the ecological system requires an understanding of the associated risks. The assessment of the ecological risk of microplastics in the Plankenburg River water samples was assessed. Test organisms were exposed to polyethylene in the laboratory and endpoints measured. The three test organisms used were *Daphnia magna, Raphidocelis subcapitata and Tetrahymena. thermophila*. Primary microplastics, polyethylene microspheres (40-48 µm) were used in the experiment The river water samples obtained at different sampling sites (without virgin polyethylene spikes) had different hazard classification due to human activities in the vicinity of the sites. Class III was obtained from site 1 to site 3 (Acute hazard) and the percentage effect (PE) was within 50% and 100% at the different sites in one test (*Tetrahymena. thermophila* growth inhibition assay). However, no other tests showed toxic effects. Anthropogenic activities in the vicinity of the sampling sites were major drivers of hazard toxicity in the Plankenburg River.

Sihle Mlonyeni gave a presentation titled “A wastewater treatment plant discharge into the Veldwachters River, Western Cape – A blessing and/or a curse?” during the World Environment Day symposium organised by The Environment, Climate Change and Sustainability Research Focus Area at CPUT. The event took place on 08 June 2022. The presentation is presented as Appendix C.

An overview of the project was also presented virtually at the WISA conference in 2022. The title of the presentation was “Spatio-temporal distribution of microplastics in the Diep and Plankenburg Rivers, Cape Town, Western Cape” (Appendix D).

The project leader, Prof Opeolu co-delivered a workshop on plastic pollution. The event was convened under the auspices of the Africa Plastic Initiative. The Chief convener of the workshop was Prof Nelson Odume (Rhodes University). A position paper is under preparation now for publication as on output of the initiative. The workshop was themed “African Plastic Initiative. A session titled “Sampling, Quantification, Monitoring of Microplastics in Water and Wastewater Systems: An African Perspective” was delivered by Prof Opeolu.

At CPUT, a training workshop was delivered on 23 March 2023 and a symposium on 24 March 2023. The community engagement programmes description is as follows:

”The analyses of contaminants of emerging concerns (CECs) are a global challenge. Over the past few decades, studies on these group of chemicals are predominantly conducted in global north. Infrastructural

limitations and human resources are major constraints to assessing ecological and human health implications of these pollutants. More recently, data is becoming more available in the southern hemisphere with improvement in infrastructural availability and human capacity development initiatives of funding agencies. At the Cape Peninsula University of Technology (CPUT), the Environmental Toxicology and Chemistry Research Group has been at the forefront of emerging contaminants studies over the past decade. Some of the chemicals that have been studied include phenolic compounds, polycyclic aromatic compounds, perfluorinated compounds, human and veterinary pharmaceuticals, nanomaterials and more recently, microplastics.

The Environmental Toxicology and Chemistry Research Group is inviting postgraduate students and emerging researchers at CPUT and elsewhere to a two-day event to share knowledge and data on some of the group's studies. The first day (23 March 2023) will be a training workshop on sampling, physico-chemical analyses, statistical analyses, and risk assessment of some of these contaminants. A symposium that is focuses on microplastic analyses and risk assessment will take place on the second day (24 March 2023). Both events will be on Microsoft Teams.

At the end of the two days, attendees will be able to conceptualize research in the field of emerging contaminants."

The two events were virtual and there were 30 and15 participants at the respective events. Details of both programmes are presented as Appendix E. Participants wishes to have an in-person follow-up training later in the year. The project group will consider organising in June 2023 before the final reference group meeting in July 2023.

Published Article in peer-reviewed Journal

Komlan Apetogbor, Omoniyi Pereao, Conrad Sparks, Beatrice Opeolu (2023). Spatio-temporal distribution of microplastics in water and sediment samples of the Plankenburg river, Western Cape, South Africa, Environmental Pollution, Volume 323, 2023, 121303, ISSN 0269-7491, https://doi.org/10.1016/j.envpol.2023.121303.

Other manuscripts are under preparation.

APPENDIX A: PROJECT TEAM

Prof Beatrice Opeolu – Project Leader (CPUT)

Dr Omoniyi Pereao – Postdoctoral Fellow (CPUT)

Dr Conrad Sparks – Collaborator (CPUT)

Prof Bryan Brooks –Collaborator (Baylor University, USA)

Dr Dirk Jungmann – Collaborator (Technische Universität Dresden Germany)

Dr Hilmar Boernick – Collaborator (Technische Universität Dresden Germany)

Miss Asmat Khan – Master's Degree student (CPUT)

Miss Sihle Mlonyeni – Master's Degree student

Komlan Apetogbor – Master's Degree student

Note: Dr Nohako was removed from the project team due to unresponsiveness during the field work for this project. She however made some contribution to another project (that focused on wastewater treatment plants) funded by the NRF through her students.