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OCCURRENCE AND ECOTOXICOLOGICAL EFFECTS OF MICROPLASTICS IN THE DIEP RIVER, MILNERTON, CAPE TOWN

by

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Declaration

I, Asmat Begum Khan, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

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Abstract

Rivers play an important role in the water cycle and serve as habitats for various species in aquatic ecosystems. They serve as a source of microplastic litter in the ocean. Microplastics are ubiquitous, with the potential for accumulation in the environment. Improperly disposed of plastics often end up in 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, the microplastic burden of the Diep River and some physicochemical parameters of the river water were assessed. Water and sediment samples were collected from five sites on the Diep River and analysed for microplastics. On the field, a 100 L sample was filtered through a 250 µm mesh and 20 L was 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 Fourier-transform infrared spectroscopy (FTIR). Surface water samples were evaluated to determine the ecological risk, effects of microplastic standards on the river, potential climate change effects of microplastics using three bioassays and potential for genetic toxicity. Three test organisms, each representing a trophic level, were exposed to the river water samples, river water samples with microplastics, and distilled water with microplastic at variable temperatures. The organisms used were *Raphidocelis subcapitata* (microalgae), *Daphnia magna* (crustacean), and *Tetrahymena thermophila* (protozoan). The AMES test was used to test for potential mutagenicity. There were significant relationships between microplastics and physicochemical parameters. Fibres and polyethylene were the most predominant microplastics particles identified in water and sediment samples (under microscopy and FTIR respectively). Tourist and recreational areas had higher microplastics burden relative to non-tourist areas. There were significant differences shown in spatial and temporal microplastic distribution based on the proximity to urban/industrial areas and wastewater treatment plants. Different toxicity levels were shown over the four seasons in environmental water, and growth inhibition occurred in environmental samples with microplastics. The climate change effect studies revealed that microalgal and crustacean growth were enhanced in response to temperature rise in the presence of microplastics. A mutagenic response was observed in the Diep River water samples investigated. This study provided information for management strategies in policy development and implementation, protection, and other mitigation strategies about the microplastic burden of the Diep River. 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.

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Dedication

This thesis is dedicated to my dear and beloved mother,

the late Shumsunesia Mowzer Khan, and my dear aunt, the late Zainab Khan Amod.

I will love you forever.

Glossary

ABS	Acrylonitrile Butadiene Styrene
AS	Acrylonitrile Styrene
ATR	Attenuated Total Reflection
BOD	Biochemical Oxygen Demand
BPA	Bisphenol A
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DWAF	Department of Water Affairs & Forestry
EC	Electrical Conductivity
ECHA	European Chemicals Agency
EPBI	Environmental Bio-detection Products Inc.
FTIR	Fourier Transform Infrared
GDP	Gross Domestic Product
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
GIS	Geographic Information Systems
HDPE	High Density Polyethylene
HOC	Hydrophobic Organic Chemicals
ISO	International Organisation for Standardization
LDPE	Low Density Polyethylene
OECD	Organisation for Economic Cooperation and Development
ORP	Oxidation-Reduction Potential
PAC	Plastic-Associated Chemicals
PAH	Polycyclic Aromatic Hydrocarbon Compound
PC	Polycarbonate
PCB	Polychlorinated Biphenyls
PE	Polyethylene
PET	Polyethylene terephthalate
POP	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PUE	Polyurethane
PVC	Polyvinyl Chloride
TDS	Total Dissolved Solids
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Background

Plastics are one of the most versatile materials that are used and discarded daily; large quantities of these materials are being accumulated in the environment (Clunies-ross, 2019). Due to the varied applications of plastics, there is an increase in the amount of plastic waste in the environment. The rapid increase of plastic wastes in the environment poses threats to freshwater ecosystems. Hence, there is a great concern around plastic pollution due to its persistent nature in the environment as the degradation rates of these materials are extremely slow. Water pollution has become a worldwide problem as not only is it aesthetically degrading the environment but may negatively affect the ecological health of freshwater systems.

Microplastics occur as both primary and secondary types in the environment (Eerkes-medrano *et al*., 2015). Primary microplastics are those that were manufactured in smaller size ranges (less than 5mm) for specific purposes. The secondary microplastics on the other hand, are plastics that have been broken down from larger plastics due to physico-chemical degradation processes. The latter category exists in a variety of sizes (Mrowiec, 2017). The various sizes of plastics range from nano- to macro-scale. Although the plastics are broken down, they remain in the environment as microplastics and ultimately end up in freshwater and marine environments, lasting for hundreds of years due to their persistent nature. Several species confuse microplastics for food and selectively feed on them with consequent adverse effects on organisms of all trophic levels (Mrowiec, 2017). Since microplastics cannot be digested by organisms, mortality may occur, resulting in death due to lack of nutrients, starvation and entanglement.

The Diep River originates in the Riebeek Kasteel Mountains north-east of Malmesbury, and flows for approximately 65 km south-west towards Cape Town. Thereafter, it enters the sea at the Milnerton Lagoon (City of Cape Town, 2016). A reconnaissance survey of the Milnerton Beach revealed huge plastic pollution issue. The beach is a popular one and regularly used by many for recreational purposes. The Diep River is the habitat for many plant and animal species. It is therefore important that the sources and effects of plastics be investigated. The need to find possible solutions to protect

Cape Town's freshwater resources, aquatic biodiversity and promote environmental awareness becomes imperative.

1.2 Research problem

Plastic pollution is a major global issue in recent years. Plastics have been found to accumulate in the aquatic environment (Eltemsah & Bøhn, 2019). Plastics pose serious ecological and health risks to man and the environment. Rivers play an important role in the water cycle, serve as habitat to various species, forms part of the ecosystem and may serve as a source of microplastic litter into the ocean. The Diep River runs through the City of Cape Town via neighbourhoods with different land use types into the ocean. The river is therefore predisposed to pollution problems due to the diverse anthropogenic activities in its vicinity along the river course. Hence, it was necessary that a study on the assessment of microplastic pollution in the Diep River be undertaken in order to understand the possible impacts of microplastics occurrence on ecological and human health. There was also a need for scientific information to develop strategic policy decisions to protect the Diep River from degradation.

1.3 Research Questions

- Is the Diep River contaminated with microplastics?
- Is there a relationship between the physicochemical characteristics of the Diep River water samples and the occurrence of microplastics in the river?
- What are the potential ecological and human health risks of microplastics in the Diep River system?

1.4 Aims and Objectives

The purpose of this study was to evaluate the occurrence of microplastics particles and the potential ecological risk in the Diep River system.

Specific objectives were to:

1. Quantify microplastics burden of the Diep River.
2. Evaluate the spatial and temporal variations of microplastics distribution in the Diep River.
3. Evaluate the physicochemical characteristics of the Diep River water samples relative to microplastics distribution.
4. Assess possible ecological and human health risks of microplastics in the Diep River.

1.5 Delineation of the research

The study focussed on microplastic pollution in the Diep River within the Milnerton area. The study emphasis was only on plastic particles in the size range of ≤ 5 mm. The only form of pollution measured in this study is plastic pollution and some water physico-chemical quality parameters. Tests for indicators like microbial organisms, such as bacteria were not considered.

1.6 Significance of the study

Due to the fragile nature of aquatic ecosystems, any pollution into aquatic waters can cause disturbances in the water with both direct and indirect effects on ecological and human health. Negative effects on organisms could include problems such as ingestion of plastic wastes, leading to immediate death, disruption of feeding and breeding patterns and decline in population densities. Persistent organic pollutants and pathogens could be sorbed on microplastics and bio-accumulate throughout the food web which could expose wildlife and humans to these chemicals. Not only are negative effects exerted on aquatic organisms, it also affects the aesthetic value of the river. The loss of species, biodiversity and the aesthetic value ultimately result in a loss of value. Therefore, it was important that an assessment of microplastic pollution in the Diep River (Milnerton) be conducted to provide information about the microplastic burden of the river and consequently, its health and the need to identify strategies for the river's protection.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Microplastics are synthetic polymers with a size limit of 5 mm and are either intentionally manufactured or are as a result of the breakdown of larger plastics. They have extremely useful properties and have become increasingly ubiquitous. Although they have many useful and desirable properties, they end up in environments where they do not originate. Aquatic organisms may ingest microplastic due to its size, bioaccumulation potential and consequent mimicry for food (Wagner *et al*., 2014). This review therefore sought to provide information on the sources, occurrence, distribution, characterization, and the potential ecological and human health effects of microplastics in freshwater systems.

2.2 Plastics

Plastic is defined as 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. The term 'plastic' is widely used to describe synthetic organic polymers that have been fashioned by polymerisation processes (Oladejo, 2017).

2.2.1 Properties and importance of plastics

According to Andrady (2011), plastics are versatile materials, lightweight, strong and potentially transparent, which makes them suitable for a variety of applications. The author posits that plastics are excellent packaging materials due to their excellent oxygen/moisture barrier properties, bio-inertness, lightweight and low cost. Their suitability for use in various applications make these plastics very sought-after. Bhowmick *et al*. (2021), stated that these desirable properties, including its hypoallergenic and easily sterilisable nature have made them a competent material that are useful in numerous business sectors, such as infrastructure, agriculture and primary manufacture, textile, healthcare, food supply, transportation, personal care products, communications and more. There are 7 main types of plastics namely, Polyethylene terephthalate (PET or PETE); High Density Polyethylene (HDPE); Polyvinyl Chloride (PVC); Low Density Polyethylene (LDPE); Polypropylene (PP);

Polystyrene (PS); and Other (which depend on resin or a combination thereof: Polycarbonate (PC); Acrylonitrile Styrene (AS); and Acrylonitrile Butadiene Styrene (ABS)) (Oladejo, 2017). Although hundreds of commercial plastic supplies are available, very few are polymers, which is composed of more than 80% of the total market demand (Razeghi *et al*., 2021). PP, LDPE and HDPE are frequently used typically in packaging whereas PVC, Polyurethanes (PUE), PET and PS are also widely used due to the varied applications (Razeghi *et al*., 2021).

Synthetic polymers have unique material properties. Klein *et al*. (2015), states that the success of synthetic polymers is due to their lightweight and the ability to resist mechanical, chemical and biological stresses, its cheap production and simple packaging (Klein *et al*., 2015). However, plastics do not always have desired properties. Kärrman *et al*. (2016) mentioned that monomers are polymerized into macromolecules or chains to make plastic polymers. Many chemicals are added to plastic products during their production or usage (The Royal Society, 2019). This is to enhance and improve its strength, flexibility and durability. Additives, such as stabilisers, flame retardants, pigments and fillers are added in the operation procedure (Kärrman *et al*., 2016). To improve plastic properties for its intended use, polymer additives are added to change or improve their properties. For instance, softeners decrease plastic brittleness, stabilisers prohibit oxidation of plastics or additives, and blowing agents used for flame retardants or additional refining. Different polymers will have different toxicities because of the types and quantities of additives used in manufacturing processes. For this reason, different polymers will have different characteristics. These chemical additives vary depending on the type of polymer and play a huge role in the reason why plastics are so prevalent, diverse and versatile in the present-day.

2.3 Microplastics

The greatest size limit of microplastics is 5 mm, though it does not have a fixed lower limit (Li *et al*., 2018). Microplastics in the environment can be categorized into two, namely, primary microplastics and secondary microplastics. Li *et al*. (2018) defines primary microplastics as microplastics that are originally manufactured with a size that is less than 5 mm. These microplastics are intentionally used as resin pellets, which are the raw materials used in the production of plastic products, or as ingredients in personal care products (Wagner *et al*., 2014) and are mostly found in textiles (as

secondary microplastics), medicines and personal care products such as facial and body scrubs (Li *et al*., 2018). The authors defined secondary microplastics as microplastics that are derived from fragmentation of large plastic debris, as a result of processes such as photo-degradation, and interconnection between physical, chemical and biological components. These secondary microplastics originate mainly from fishing nets, industrial resin pellets, domestic goods and plastic debris waste (Li *et al*., 2018) or of garment fragmentation during washing (Strady *et al*., 2021). Microplastics are characterised by a broad range of polymer types, particle sizes and densities and possible exposure can harm aquatic ecosystems and have the potential to adversely impact human health (Razeghi *et al*., 2021).

2.4 Distribution of Microplastics in Water

Plastics have high volume usage and usually end up in the marine environment. Particles with high densities are bound to sink to the bottom and stored in sediment, while low density are likely to float at the ocean surface (Van Cauwenberghe & 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 be submerged into the underlying sediment and low-density particles will remain 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 *et al*., 2020). Plastic debris transported from riverine areas to land systems account for 80% of debris into the marine environment (Sarkar *et al*., 2020).

Van Cauwenberghe & Janssen (2014), stated 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 remain in the 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 persistence. The dispersal, abundance and incidence of microplastics are influenced by environment type, physical characteristics of microplastics, climate, industrialisation, urbanisation, waste management, development and societal standards (Milološa *et al*., 2021). The introduction of plastic fragments into terrestrial and aquatic ecosystems result from anthropogenic interferences such as industrial and

domestic activities in coastal areas. These sources are diverse and usually consist of both primary and secondary plastic classification (Pereao *et al*., 2020b).

2.5 Microplastics in Freshwater

Freshwater resources include streams, rivers, lakes, dams and ponds. It represents the most complex system with regards to microplastic transmission and retention. Freshwater resources receive microplastic from the terrestrial environment. Additionally, they are pathways for microplastics release into the marine environment. Rivers may also serve as reservoirs for the formation of microplastic due to the disintegration of plastics that are in the water body, and as sinks that retain microplastic in sediment (Horton & Dixon, 2018). Martinez-tavera *et al*. (2021) mentioned that the largest proportion of plastic contamination are in marine environments, with approximately 1,15-2,41 million tons per year of plastic waste derived from river systems. This is because most plastics were utilized and discarded inland and into adjoining freshwaters. There is dearth of information on plastic accumulation and effects in freshwater and terrestrial ecosystems relative to marine ecosystems (Eerkes-medrano *et al*., 2015). Freshwater resources may accumulate several microplastic particles and fibres, but fewer studies have been reported relative to marine systems (Horton *et al*., 2017).

However, some studies have been done on microplastic abundance in freshwater matrices. The results revealed that average microplastics abundance counts deviated significantly from hardly any to several million pieces per cubic meter (Li *et al*., 2018). Microplastics can rapidly be transferred to the aquatic environment; though, in remote and isolated freshwaters, microplastics are trapped in water and sediment (Li *et al*., 2018). Additionally, studies were done on microplastic concentrations in marine and freshwater settings in Viet Nam (Strady *et al*., 2021). Results revealed that microplastic concentrations are associated with adjacent human activities using plastic such as aquaculture, fishing, households, landfills, urban pressure and the release of treated and untreated wastewater (Wagner & Lambert, 2018; Strady *et al*., 2021). Rivers are dynamic freshwater bodies. Numerous plastic materials that are introduced into the environment get redistributed within environmental compartments due to mobility; microplastics undergo varied rates of degradation and could be transferred and spread at quicker rates than larger plastics (Wagner & Lambert, 2018).

A substantial amount of microplastic contamination is attributed to the breakdown of non- reusable plastic, industry abrasives and artificial clothing fibres as a result of laundry (Allen *et al*., 2019). The aquatic environment receives primary and secondary microplastics primarily from wastewater effluents that originate from domestic or factory-made sources(Strady *et al*., 2021) which emphasizes that rivers are indeed dynamic freshwater bodies. There are several microplastic sources released into the aquatic environment. These sources can be derived from waste through solid waste collection, handling, landfills or conveyance, from tyres, automobile debris, agriculture, runoff, wind and atmospheric effects (Strady *et al*., 2021). River channels may experience heavy microplastic contamination and a considerable amount of microplastics are transported from riverine systems to the oceans (Woodward *et al*., 2020). Wagner & 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.

In general, river beds are highly oxygenated. It provides shrimps, stoneflies and caddisfly larvae invertebrates with a habitat essential for their survival (Woodward *et al*., 2020). Woodward *et al*. (2020) further discussed that important food resources including algae and decomposed organic matter are present in river bed sediments, ideal for aquatic ecosystems. This zone (river bed) is of particular importance because it is a feeding zone for many aquatic species. Woodward *et al*. (2020) pointed out that river bed sediments are the most suitable sample environment and setting to determine the degree of microplastics and possible threats to the ecosystem. Freshwater and soil environments are susceptible to point and non-point plastic sources, and immense investigations are needed for the understanding of microplastic exposure, transfer, and ecological effects (Horton *et al*., 2017). According to Ghayebzadeh *et al*. (2021), there exist variances in microplastic abundance and spatial distribution in water and sediment which is primarily influenced by environmental and anthropogenic factors.

2.5.1 Effects of Microplastics on Aquatic Life

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. Many organisms including plankton, fish, birds and mammals can ingest microplastics. Microplastic are small in size with a large specific surface area which means that microplastics are available to a variety of biota (Wagner *et al*., 2014) and are

biologically available to aquatic organisms (Kärrman *et al*., 2016; Ghayebzadeh *et al*., 2021). Microplastic ingestion was reported for species such as mussels, crabs, sea birds and fish (Klein *et al*., 2015). It was recorded that low density and buoyant microplastics are mainly linked with low trophic level organisms whereas denser microplastics are related to benthic invertebrates (Sarkar *et al*., 2020). Wagner *et al*. (2014) opined that several organisms can ingest microplastics with the potential for bioaccumulation at all trophic levels. Studies have shown detrimental effects of microplastics on young and fully-grown fish (Klein *et al*., 2015) and some studies observed physical damage of microplastic exposure on organisms (The Royal Society, 2019).

Biofilm occurrence on plastic surfaces has increased the probability of microplastic consumption, which triggers an organism's sensorial reactions (Razeghi *et al*., 2021; 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 may mistake microplastics for food and may directly ingest microplastics, or choose to ingest microplastics instead of prey (Kärrman *et al*., 2016). 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 lower-level organisms such as zooplankton, isopoda, or mysid shrimps, and biomagnification is expected. Biomagnification of ingested or inspirited plastic particles could reportedly occur; for example, planktivorous fishes ingest microplastics and were then preyed on by predators (Kärrman *et al*., 2016). In addition, plastic biomagnification was shown under laboratory conditions, for crabs (*Carcinus maenas*) that were fed mussels (*Mytilus edulis*), that ingested 0,5 µm fluorescent polystyrene microspheres (Kärrman *et al*., 2016). Medaka fish, *Oryzias latipes,* were also dietarily exposed to virgin and marine PE (<0,5 mm) resulting in bioaccumulation, tumor formation and liver stress (Miloloža *et al*., 2021).

Previous studies have suggested that microplastics ingested by many types of fish at larval stages resulted in impediments of the gastrointestinal tract, decrease in feeding activities as a result of false satiation, weakened fish reproduction and diminished larval growth (Campos *et al*., 2021). Larval swimming behaviour, induction in inflammatory and metabolic responses, and microplastic translocation to tissues are

effects that could occur when fish are exposed to smaller microplastics (Campos *et al*., 2021). It was proposed that the ingestion rate of microplastics in aquatic organisms is dependent on the physical properties of microplastics – size, colour, density and organism's feeding behaviour (Razeghi *et al*., 2021). Klein *et al*. (2015), stated that the gastro-intestinal systems of small crustaceans such as isopods will not necessarily block microplastics due to its small size, and hence digested microplastics can be expected. Studies have shown that harmful effects of microplastics were revealed to organisms, especially aquatic organisms owing to the direct interaction to microplastic particles (Miloloža *et al*., 2021). When microplastics are eaten by aquatic animals, it affects animals that eat them and can accumulate in the food chain affecting top predators including humans (Jiang *et al*., 2018). Microplastics were identified as several comestibles including honey, drinking water, beverages, fish, mussels, table salt and sugar (Shopova *et al*., 2020).

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. According to Klein *et al*. (2015), microplastic retention time is dependent on the uptake route and the organism. The retention time might be long enough that microplastic end up in the next trophic level. This is likely the reason why there is a potential for bioaccumulation. Although adverse effects of microplastic to organisms that have ingested and excreted microplastics particles have not shown a clear trend or affected their mortality, studies have shown negative effects of microplastics on young and adult fish (Klein *et al*., 2015). Ingestion and absorption of microplastics may bring about physical damage like blockage in the intestine and internal abrasions (Kabir *et al*., 2021). According to The Royal Society (2019), some studies observed physical damage of microplastic exposure on organisms, which brought about some non-fatal internal damages, particularly in the gut, liver and mouth cavity (The Royal Society, 2019).

Algae are the main producers in aquatic systems, have a low life expectancy, are sensitive to fluxes in the water environment, and the water status can be reflected by anatomy and load of algae in water (Wang *et al*., 2021). According to Wang *et al*. (2021), it was observed that microplastics are capable of being adsorbed onto algal surfaces and the growth of microalgae can be inhibited by polystyrene nanoparticles, which can decrease chlorophyll content and affect photosynthesis. Any negative effect

on algae affects the entire food chain because algae are primary producers and serve as a basis for the aquatic food chain (Milološa *et al*., 2021). Adverse effects of microplastics on algae were reported with the most common effect being on its growth (Milološa *et al*., 2021). Microalgae are affected by microplastics by growth and photosynthetic reduction, which reduces chlorophyll content, which induces oxidative stress, and result in changes to morphological characteristics (Patil *et al*., 2021). Microplastics adsorb on algae surfaces, decreasing photosynthesis efficiency or reducing chlorophyll level in cells, and prevents the exchange of nutrients, gasses and toxic metabolites (Milološa *et al*., 2021).

Eerkes-medrano *et al*. (2015) pointed out that potential effects of microplastics at tissue and cellular levels studies have been shown, such as in *Mytilus edulis*, where microplastic ingestion caused inflammatory responses in tissues and diminished membrane stable cells of the digestive system. Changes in morphological appendages and haemolymph proteome as a result of microplastics were found in blue mussel *Mytilus edulis* in the reef habitat (Sarkar *et al*., 2020). Ingested microplastics in *Daphnia magna* have also been displayed to traverse into cells and be translocated into oil storage droplets (Eerkes-medrano *et al*., 2015). False satiation, weakened motion, and increase in buoyancy are some effects of microplastics on organisms (The Royal Society, 2019). Patil *et al*. (2021) indicated that most animals cannot digest plastic or make use of it as an energy source because they lack enzymatic pathways for the breakdown of plastics, which aids the accumulation of plastic in the digestive tract. Microplastics were found to influence the egestion of *Daphnia magna;* when egestion levels are very low or absent, it leads to a reduction in food intake, resulting to starvation (Milološa *et al*., 2021). Patil *et al*. (2021) suggested that larger particles will excrete, while smaller ones would translocate.

Microplastic impacts on reproductive rates varies in species. For example, reproduction rates declined in freshwater crustaceans whereas the reproductive rates in *Daphnia magna* were unsubstantiated (The Royal Society, 2019). However, effects of phthalates on *Daphnia magna* showed a decline on reproductive rates, size, and lifecycle, and bisphenol A (BPA) effects impaired genetic information that triggered stress reactions and diminished reproductive rates (The Royal Society, 2019). Furthermore, microplastics can act as a vehicle for chemicals, firstly, chemicals that are combined during plastic manufacture to enhance plastic production; and secondly chemicals which are present in the water body may sorb on the microplastic surface

(Pereao *et al*., 2020a). Consequently, unreacted substances or additives from microplastic chemicals can leach into nearby environments (Pereao *et al*., 2020a)

According to Kärrman *et al*. (2016), 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 (Kärrman *et al*., 2016). As a result of sorption to microplastics and desorption of additives from microplastics, exposed organisms are vulnerable to contamination effects. Plastic additives such as metals and phthalates are not chemically bonded to polymers and can therefore be leaked out of the plastic (Cormier *et al*., 2021). Therefore, organisms and the environment are exposed to contamination from chemical sorption and desorption processes.

Leaching from consumer plastics to water was demonstrated to occur and be toxic to freshwater organisms (Kärrman *et al*., 2016). As a result of sorption to microplastics and desorption of additives from microplastics, organisms that come into contact with it are exposed to contamination and thus contaminants can enter the aqueous environment as well as into 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*. (2020a), mentioned that concentrations of organic contaminants are present in plastic debris at approximately submicrograms per gram to nanograms per gram with most contaminants adsorbed from nearby water bodies whereas other contaminants are added during plastic manufacture.

2.5.2 Ecological and Human Health Risks

There is a lack of studies done on population effect and microplastic risk assessment in scientific literature (Kärrman *et al*., 2016). However, studies have shown that microplastics can accumulate in larger animals as they pass up the food chain; with severe microplastic effects shown with an increase in trophic levels (The Royal Society, 2019). Microplastics were recorded to have negative effects on organism's energy, protein content, detoxification systems and behaviour (Razeghi *et al*., 2021). Findings revealed that microplastic exposure was toxic to fish species and resulted in changes in their biochemical and immunological properties, with changes being even greater

when microplastics were combined with Cadmium (Cd), and suggested that Cd and microplastics have synergistic effects (Razeghi *et al*., 2021). Plastic polymers are classified as carcinogenic and mutagenic to humans, extremely noxious to aquatic organisms and have persistent effects, and as a result, emerging microplastic pollution pose serious environmental, human health and environmental protection and sustainability risks worldwide (Kabir *et al*., 2021).

It is possible that micro and nano plastics are introduced into the food web via trophic transfer through the ingestion of seafood (Domenech & Marcos, 2021). Kärrman *et al*. (2016) added that the risk of microplastic transmission from the gastro-intestinal tract to other tissues in humans and other mammals is factual and plastics can intensify effects on ecosystems that are currently under strain. Due to the increased changes and binding abilities of microplastics, oxygen functional groups in microplastics support the dispersal and contact with cell compounds of living organisms (Liu *et al*., 2021). The few studies that have been conducted on soil and freshwater species validated potential microplastic effects to be destructive to species functioning across various niches (Horton *et al*., 2017). Kabir *et al*. (2021) stated that prior studies specified that significant hazards are caused by small microplastics, and there is a higher likelihood that aquatic organisms will ingest and biologically transfer microplastics particles. Aging results in the breakdown of microplastics into smaller particles; this reduced size will then allow microplastic to easily be able to enter the intestinal mucosa and internal biological circulation system via sorption, endocytosis and phagocytosis (Liu *et al*., 2021).

Studies on microplastics in fishes, shrimps and mussels that have commercial importance, exhibit possible microplastic exposure pathways of humans via their diet (Kärrman *et al*., 2016). As mentioned previously, microplastics can undergo further polymerisation as well as leached polymer additives. Sorbed contaminants may undergo adsorption and desorption processes. Additives and low-molecular organic products are able to discharge from older microplastics and induce secondary chemical risks (Liu *et al*., 2021). Consequently, changes in properties of microplastics have the possibility to influence microplastic interaction with environmental pollutants, which could affect and change ingestion behaviour and microplastic risk by organisms (Liu *et al*., 2021). Klein *et al*. (2015), mentioned that persistent organic pollutants ((polycyclic aromatic hydrocarbon compounds (PAH's) and polychlorinated biphenyls (PCB's)) were discovered in microplastics found in sediments around the globe.

Aqueous concentrations of pollutants can be increased through desorption of highly contaminated microplastics when entering a less polluted aquatic system (Klein *et al*., 2015). Plastic-associated chemicals (PAC's) (bisphenol A and phthalates) are well known endocrine disrupting chemicals that impede the hormonal system, play a role in atherosclerosis, and are associated with cardio-vascular diseases (Kärrman *et al*., 2016).

Toxicological effects of microplastics on freshwater (micro) organisms are scanty, as most studies were done on marine (micro) organisms (Miloloža *et al*., 2021). Organisms (*Vibrio fischeri, Algae, Daphnia magna and Fishes)* were exposed to a wide range of microplastic types and concentration ranges represented in Figure 1, which show ecotoxicological concentrations that were recognized for distinct organism level studies (Miloloža *et al*., 2021). This showed that organisms at different trophic levels are responsive and vulnerable to a broad range of microplastic concentrations.

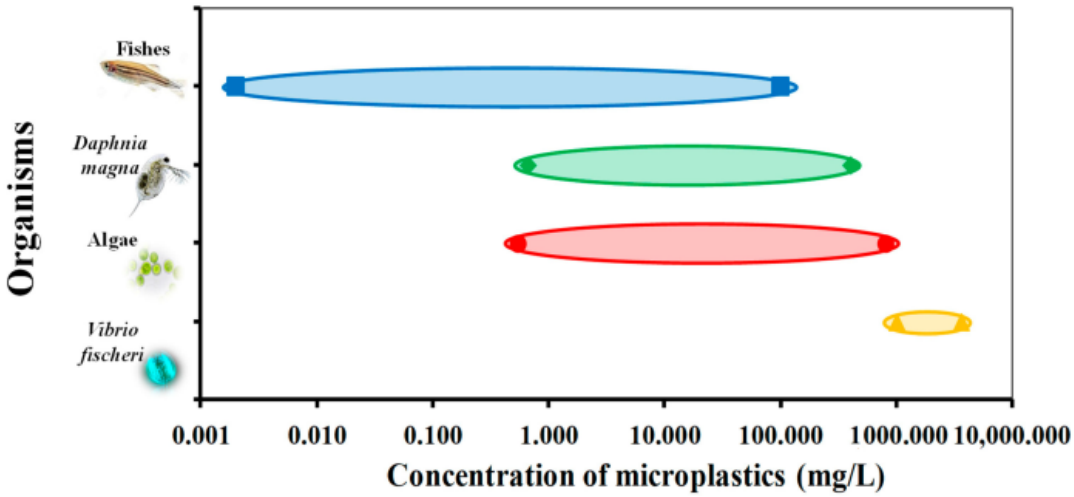

Figure 2. 1. The range of ecotoxicological concentrations for different trophic levels (Miloloža et al., 2021).

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 & 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 *et al*., 2020). A study done on the exposure of Polystyrene (PS) particles in the 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 included oxidative stress and morphological changes on freshwater algae *Chlorella pyrenoidosa* cell (Milološa *et al*., 2021).

There are various plastic types that exist with significant differences in the ecological risks of each plastic type (Jiang *et al*., 2018). There is a possibility that microplastics are able to influence the function of soil and freshwater affecting the growth of plants and structure of soil (The Royal Society, 2019). Results from Wang *et al*. (2021), indicated that there are synergistic effects of microplastics and metals, whereby algal bloom formations are promoted in the presence of both microplastics and Pb^{2+}. Furthermore, biogeochemical cycles could be affected when there is a change in sediment quality or the extent to which light penetrates into water as a result of microplastic aggregation (Eerkes-medrano *et al*., 2015). Given the above, microplastics have the possibility to influence plant growth and soil structure and could restrict pathways for nutrients and essential elements in the environment.

The toxins in repeating monomeric units of polymers such as PVC, PS and PC are linked to cancer in humans, rats and invertebrates, and can cause abnormalities in reproduction (Pereao *et al*., 2020a). Cytotoxicity was shown on human intestine cells with the use of polystyrene microplastics with higher toxic effects of microplastics induced by larger sized polystyrene microplastics (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 (Milološa *et al*., 2021). *Daphnia magna* were able to ingest PE microplastics of 63 -75 µm in size 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). The authors stated that smaller PE microplastics are more toxic for *Daphnia magna* than larger ones. The reason for this finding could be due to smaller microplastics that adhere to inner and outer surfaces, and consequently impair the filtering activity, compromises gut integrity and enters cells and tissues of the organism (Milološa *et al*., 2021).

Campos *et al*. (2021) reported on microplastics and its potential negative effects on finfish hatcheries. The study revealed that microplastics triggered biochemical

responses on early life stages, and consequently, short term effects could include inefficiency in growth and development of finfish and may have long term health and life trait effects to the organism. According to Domenech & Marcos (2021), there were specific short- and long-term effects of microplastics. Studies need to be undertaken on bioaccumulation of micro and nano plastics in each organ; and data suggested that micro and nano plastics are easily internalised by human cell models and cause changes to their functionality (Domenech & Marcos, 2021). However, Shopova *et al*., (2020), stated that it is difficult to compare the condition in humans to studies conducted with invertebrates, mussels or fish.

While some studies showed that possible toxicological effects like inflammatory responses, damage to the immune system and cells, and disturbances of reactive oxygen species levels could occur; other studies showed that no toxicity occurred even at higher concentrations of microplastic particles (Shopova *et al*., 2020). Most studies use in vitro cell models; microplastic toxicity analysis that make use of in vitro human cells are described to have triggered oxidative stress (Patil *et al*., 2021). The effects of polypropylene microplastics were investigated on human cell lines and it was proposed that they were toxic to cells in both size and concentration; and impacts were wielded by cytokines production from immune cells after cells and polypropylene microplastics were directly in contact (Patil *et al*., 2021).

Microplastics have strong hydrophobicity and easy adhesion of pollutants (Wang *et al*., 2021). Microplastics can serve as a mechanism that transport harmful hydrophobic organic chemicals (HOC), persistent organic pollutants (POP's), additives, plasticizers and heavy metals (Razeghi *et al*., 2021). Razeghi *et al*. (2021) also stated that toxins in the environment can use microplastics as routes to enter the food chain which may result in the accumulation of toxins in the human body. This suggests that microplastic impacts are likely to spread across trophic levels. Hydrophobic organic chemicals which include polychlorinated biphenyls (PCB's) polycyclic aromatic hydrocarbons (PAH's), organochlorines and trace metals are typically sorbed onto plastic materials (Cormier *et al*., 2021). These microplastic particles, through diet, are possible transport mechanisms of heavy metals to the human body and have a great potential to cause adverse effects (Martinez-tavera *et al*., 2021).

Furthermore, Martinez-tavera *et al*.(2021), indicated that microplastics less than 20 µm may possibly enter all mammalian organs whereas microplastics smaller than 0,1 µm can enter all cell membranes. Microplastic presence in freshwaters can also affect the

edibility of many organisms that form part of the human diet. Additionally, humans are exposed to microplastics through ingestion of crops that have been watered with contaminated water (Domenech & Marcos, 2021). The occurrence of microplastics in the environment 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. This ultimately exposes humans to contamination and may cause potential health risks.

2.6 Global Concern around Microplastics

Plastics have been manufactured since the early 1940's, and there has been a rapid surge in the number of plastics being produced ever since, which lead to a widespread usage of plastics in various applications (Pereao *et al*., 2020a). Worldwide plastic production and end user trends, improper plastic waste disposal and demographics suggest that there will be an increase in plastic production in the future. Demands for plastics are increasing rapidly and production patterns are expected to have quadrupled by 2050 (Karbalaei *et al*., 2018). With increase in plastic production, plastic wastes in the environment and in particular microplastics, will increase immensely. There are still much needed data required for the development of microplastics standards. The European Chemicals Agency (ECHA) view present microplastic data as inadequate to obtain a threshold value of microplastic concentration that may prove that risks are controlled, which means that it has to be considered a risk when any microplastic is released into the environment (Milošža *et al*., 2021).

The high demand and inadequate management of plastic waste caused an upsurge in plastic residues in different environments, namely oceans, freshwaters, food, and human beings (Martinez-tavera *et al*., 2021). Plastic fragmentation research indicated that microplastics are comprehensive and were detected on mountain tops, deep seas and in Arctic ice, which creates pollution all over the world (Bhowmick *et al*., 2021). Microplastics were detected in an extensive range of shapes, sizes, polymers and concentrations in marine, freshwater and atmospheric environments, agroecosystems, food, biota, potable water, and isolated locations (Campanale *et al*., 2020). This confirms 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 quantity of plastics contributed to municipal waste and its proportion to global produced waste (Pereao *et al*., 2020a). Available information on microplastic occurrence and effects affirms classification as an environmental pollutant. Globally, much research is still required to fully understand microplastic exposure and effects in the environment; this makes it a cause for concern.

2.6.1 Microplastics in South Africa

Plastics are utilized in all sectors within South Africa's economy, with the plastic manufacturing industry contributing 1,6 % and 14,2 % to the Gross Domestic Product (GDP) and manufacturing sector in 2014 (Verster *et al*., 2017). 1, 1 million tonnes of plastic waste were produced in 2017, which is equivalent to 19 kg of plastic per capita per year and 53 g per person per day (Verster & Bouwman, 2020). The plastic industry is identified by the South African government as a primary sector for economic growth and encourages export, trade policy measures, innovation, and recycling (Verster *et al*., 2017). Verster & Bouwman (2020), mentioned that microplastic in aquatic ecosystems come from Wastewater Treatment Plant effluents, sewer overflows, discharge, and sludge runoff from agriculture and industries and other possible sources of microplastics in aquatic systems in South Africa come from urban runoff and informal settlements that result from littering and poor waste management.

Although microbeads, which are primary microplastics, have been banned in countries such as Canada, United States of America, United Kingdom, France, Sweden, Taiwan, South Korea and New Zealand, it has not been banned in South Africa. However, Verster & Bouwman (2020), further mentioned that some initiatives were implemented by the South African cosmetics industry to replace microbeads with other materials. Research on microplastics in South Africa is scanty with most published work on particles that exceed 5 mm (Verster *et al*., 2017). Not many studies looked at microplastics in freshwater systems in South Africa (Verster & Bouwman, 2020), and although there is scarcity of microplastic data for the South African aquatic environment, there is progress in its development (Pereao *et al*., 2020a). Verster & Bouwman (2020), pointed out that the concentration of microplastic fragments were excessively found in segments of the Vaal River, microplastic levels in the Crocodile and Klip Rivers reached 4, 5 particles per litre, and the microplastic load in the

Bloukrans River sediments fluctuated between 6 and 160 particles per kg of dry sediment during summer and winter, (high and low flow), respectively.

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*., 2020a). Verster *et al*. (2017), opined that South Africa is faced with two issues. First, subsistence farming takes place in many rural areas. Water, food security, and well-being of the population may be negatively affected due to water and soil contamination with microplastics if used for drinking and crop production. Second, South Africa has a rich natural biodiversity and microplastic pollution is a potential threat to biota. Currently, studies on microplastic impact on biota are being conducted, which are yet to be fully understood (Verster *et al*., 2017), and research on potential risks of microplastics in South Africa is ongoing in order to make better decisions and protect environmental and human health

2.6.2 Microplastics in the Diep River

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 & Dudley, 1988). There have been numerous studies done on South African coasts which have confirmed high microplastic and microfibre concentrations; with evidence that showed important correlations between microfiber pollution hotspots alongside South African coasts and land pollution sources which included wastewater treatment plants and rivers (De Villiers, 2019). The Diep River, which is the largest river in the City of Cape Town municipality, had the most abundant sediment microfibre levels in the Western Cape (De Villiers, 2019).

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. The experiments were divided into four main sections. Section 3.3 describes the sampling of physico-chemical properties that were taken from the Diep River water samples. Section 3.4 describes the microplastic sampling in freshwater and sediment samples. Section 3.5 and 3.6 describes the ecological and human health risk assessment studies, respectively.

3.2 Study Area

Sampling points were identified along the Diep River based on land-use practices and site accessibility (Table 3.1). Samples were collected to assess the physico-chemical characteristics of water and the microplastics pollution burden of the river. A map of the study area was created using Geographic Information Systems (GIS). The locations of the sampling sites were selected and identified using Google Earth as shown in Figure 2.

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

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 - canal 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 (along a recreational area and 1km from the Milnerton Lagoon)	33 52' 53,6" S 18 29' 22,2" E	DR-5

Figure 3.1. Sampling sites on the Diep River

Samples were collected at 5 points along the Diep River (Milnerton) twice per season to evaluate possible spatial and seasonal variations in microplastic pollution and physico-chemical properties of the river.

Site 1 was near farming practices and opposite an informal settlement (Dunoon); Site 2 was along the Table Bay Nature Reserve and in a residential area; Site 3 was at a canal along the recreational/commercial/industrial area which connects to the river; Site 4 was along a recreational and residential area near the river and in close proximity to a wastewater treatment plant; and Site 5 was 1 km from the Milnerton Lagoon where recreational activities take place.

All instruments that were used were calibrated prior to use. Distilled water was used to rinse all equipment (glass beakers, sieve, metal spoon, buckets, falcon tubes, zip lock bags) before use to avoid cross contamination.

3.3 Physico-Chemical Properties

Spatial and seasonal variations in physico-chemical characteristics of the river were observed. Assessments were made twice per season. Both in-situ and ex-situ measurements were done. For the *in-situ* analyses of water samples were collected and filled into a glass beaker with a volume of 500 ml. Samples were analysed for pH, dissolved oxygen, conductivity, temperature, redox potential, and total dissolved solids (TDS) were conducted on the respective sampling site, using the Lovibond® Water Testing MultiMeter Instrument. The *ex-situ* analyses of water samples involved

transportation of samples on ice-chests to the laboratory for the assessment of chemical oxygen demand (COD) and biochemical oxygen demand (BOD).

3.4 Sampling procedures for microplastics analyses in water and sediment samples of the Diep River

Two environmental matrices (water and sediment samples) were collected and analysed in this study. All equipment and storage containers were cleaned and rinsed with distilled water. Samples were collected in the opposite direction (against) of the wind to prevent cross contamination. Cotton clothing were worn to limit microplastic contamination.

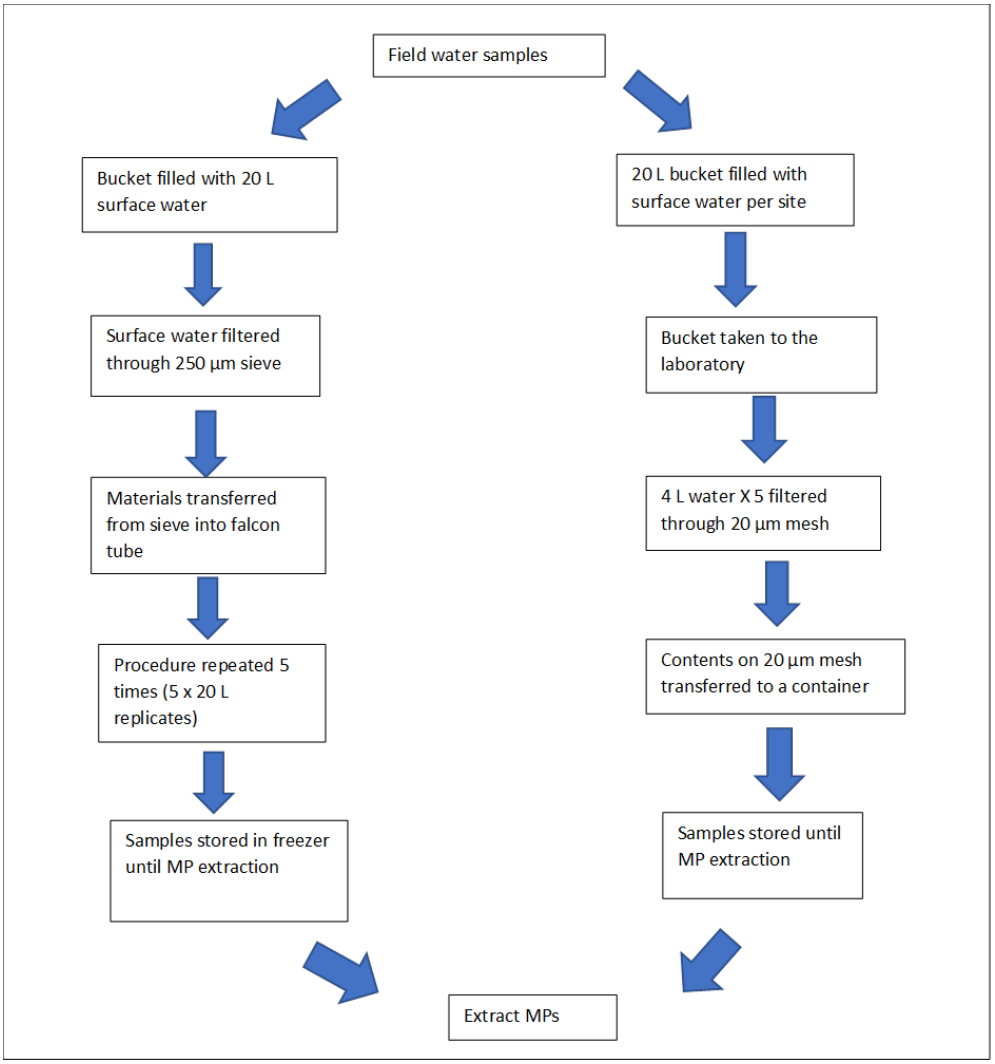

Figure 3.2. Stepwise sampling protocol for microplastic analyses in water

For water samples, there were two procedures used for collection and preparation for each site. The two procedures are represented in Figure 3.2. The first procedure was to collect 5 replicates of 20 L on site. This amounts to a total of 100 L of water that was processed at each site. The samples were processed in the field by filtration through a

250 µm mesh sieve. The particles that were trapped on the sieve was transferred into falcon tubes and labelled. The falcon tubes were thereafter taken to the laboratory for analysis. Another 20 L surface water sample was collected at each site and taken to the laboratory for filtration through a 20 µm mesh sieve.

Sediment samples were collected with a metal spoon. At each site, five samples were collected at about the same point for water sampling. The metal spoon was used to scoop a depth of 5 cm of sediment, transferred into a zip lock plastic bag and labelled. The bag was then taken to the laboratory for analysis.

3.4.1 Extraction and analyses of microplastics in water and sediment samples

3.4.1.1 Control Experiments

A clean petri dish was always left opened with a clean 20 µm mesh inside to serve as a control sample for possible laboratory contamination. Microplastics found on the control petri dishes were analysed and accounted for in the laboratory procedures and reporting. One each of clean 20 L plastic bucket, plastic zip lock bag and falcon tube were used as controls for each sampling event.

3.4.1.2 Extraction of microplastics from water samples:

10% Potassium Hydroxide (10% KOH) was used for digestion. The 100 L water samples filtered on site were digested prior to filtration using the 10% KOH stock solution. The 10% KOH solution was added in the ratio 2:1 (10% KOH: sample) to ensure that all the organic material was removed from the sample. The sample was then placed in the oven at 50 ºC for 24 h.

Thereafter, a hypersaline solution was added to the digested sample in the ratio of 3:1 (Hypersaline solution: Sample). The mixture was then stirred vigorously for 2 min and was thereafter allowed to settle for 15 min until a supernatant was formed. The supernatant was then filtered through a 20 µm mesh through a vacuum pipe system. This was repeated thrice. The mesh containing the microplastics was transferred onto a petri dish and labelled. This process was repeated for each sample and analysed under the microscope once dried.

The 20 L bucket water sample was divided into 5 x 4 L and filtered through a 20 µm mesh. The mesh was rinsed with 10 µm distilled water into a glass jar to be digested. A hypersaline solution was thereafter added to the samples as explained above. The supernatant was then filtered through a 20 µm mesh, the remaining filtered water in the flask was added into the jar, stirred to separate any microplastics in the sample

and left to settle for 15 min before filtering the supernatant again. This was repeated thrice to extract any remaining microplastics. The mesh containing the microplastics were transferred into a petri dish which was then labelled. This process was repeated for each sample and analysed under a microscope once dried.

3.4.1.3 Extraction of microplastics from sediment samples

The sediment samples were weighed into an aluminium foil container, covered with aluminium foil, placed into the oven, and dried for 48 h. Thereafter, 5 x 20 g of the dried sample was weighed into a glass jar. The sample was digested in 10% KOH solution to ensure that all the organic material is removed from the sample. It was then placed in the oven at 50 ºC for 24 h. After digestion, the hypersaline solution was added to the sample in the ratio of 3:1 (Hypersaline: Sample). A supernatant was formed and filtered through a 20 µm mesh through a vacuum pipe system, the remaining filtered water in the flask was added into the jar, stirred to separate any microplastics in the sample and left to settle for 15 min before filtering the supernatant again. This was repeated thrice to extract any remaining microplastics. The mesh containing the microplastics was transferred into a petri dish and labelled. This process was repeated for each sample and analysed using stereomicroscopy once dried.

3.4.1.4 Microscopic Analysis

The were 6 different types of microplastics analysed and classification were based on different shapes - fibre, fragment, pellet, film and foam adopted from the method by GESAMP, (2019). The stereomicroscope (BS-3060CT, BestScope, China) was used to examine the physical properties of the particles. Microplastic particles were counted, measured, and classified in terms of its type, colour and size. The criteria used to classify microplastic type is presented in Table 3.2.

Table 3 2: Criteria used for microplastic types

Fibres	Thin elongated pieces with one dimension significantly greater than the other two
Fragments	Thick pieces with three size dimensions comparable
Films	Pieces with their thickness significantly lower than other two dimensions
Spheres	Pieces with a homogeneous sphere
Pellets	Pieces with homogenous flat sphere
Foam	Lightweight pieces

3.4.1.5 Fourier Transform Infrared (FTIR) Analysis

FTIR was used to determine the chemical properties of microplastics. A PerkinElmer FT-IR Spectrometer Spectrum Two system was used to identify suspected microplastics particles polymer types using the Attenuated Total Reflection (ATR) mode. Microplastics greater than 1000 µm were selected and analysed under FTIR. The FTIR spectrometer was wiped with alcohol and the background on the monitor was scanned. The microplastic was carefully placed onto the crystal centre of the spectrometer with a fine tweezer. It was then previewed on the system before being scanned. The peaks produced on the monitor were compared with spectral libraries for polymer identification.

3.5 Ecological Risk Assessment of microplastics

3.5.1 Microplastic 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 (Cannif and Hoang, 2018) which represent a similar size range to food ingested by crustaceans. Transparent polystyrene granular plastics purchased from Sigma-Aldrich were grinded and used as models for microplastics. Their size range were between 200 µm-1000 µm with a density of 1.04 g/ml. 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 for a week at room temperature. The stock solution was diluted with distilled water to the final concentrations of 400 mg/L, and ultrasound bathed for 15 min at 50 W for dispersion prior to the toxicity assay.

3.5.2 Ecotoxicological Studies

Three experiments were conducted for each bioassay:

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.

3.5.2.1 *Raphidocelis subcapitata* toxicity tests

The Algaltoxkit F^{TM} supplied by MicroBiotests Inc. (Belgium), were used to carry out the tests. The test species was *Raphidocelis subcapitata*, and the OECD Guidelines 201 was used. Algal beads were de-immobilised. An algal density of 1 x 10^6 cell/mL was prepared from the concentrated algal inoculum, by measuring the optical density of the inoculum on a spectrophotometer (Jenway 6300) at a wavelength of 670 nm.

Dilution series of the samples were prepared, and each flask was inoculated with 1 x 10^4 cell/mL as the test start concentration. There were 6 treatments including a control, and each treatment had 3 replicates. The inoculated samples were incubated at 23 ºC, with a sideway illumination of 1000 Lux for 72 h. Optical density measurement of test cells was made at 24 h intervals over a period of 72 h. The data was used to determine the yield and growth inhibition of *Raphidocelis subcapitata* after exposure to the water samples.

3.5.2.2 *Daphnia magna* acute toxicity testing

An aquatic crustacean, the water flea (*Daphnia magna)*, were exposed to the water samples, using the ISO 6341 method. Instructions from (Daphtoxkit F Magna^{TM}, Microbiotests Inc., Belgium), were followed to assess the risk of microplastics exposure on *Daphnia magna*. The young daphnids were pre-fed 2 h before the commencement of the experiment to prevent starvation to death.

The hatching petri dish were placed on a light table and five actively swimming neonates were transferred into each of the test wells. The multiwall plate was covered and incubated in darkness at 20ºC. After the incubation period of 48 h, the test plate was scored to determine the amount of immobilised (regardless of movement of the antennae) or dead daphnids.

3.5.2.3 Short-term assessment of chronic toxicity using *Tetrahymena thermophila*

The freshwater ciliate protozoan, *Tetrahymena thermophila*, procured from Protoxkit F^{TM} (Microbiotests Inc., Belgium), were exposed to the water samples. The Protoxkit assay is a multi-generation growth test that included 5-6 generations and is completed over a 24 h period. Disposable 1 cm path polystyrol spectrophotometric cells were used as test containers. The turnover of the substrate into ciliate biomass is what the test depends on. Inhibited growth cell culture would remain turbid whereas normal multiplying cell cultures would clear the substrate suspension.

The optical density measurement of the turbidity with the use of a spectrophotometer (Jenway 6300) at a wavelength of 440 nm provided information on the degree of inhibition. There were 6 treatments, including the control, with each treatment having 2 replicates. Holding trays of experimental cells were incubated in darkness at 30 ºC over a 24 h period. Optical densities were measured at the start and end of the experiment.

3.5.2.4 Evaluation of toxicity

The classification system was based on a ranking in 5 acute hazard classes (Persoone *et al*., 2003). The percentage effect (PE) for each microbiotest 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

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):

a) Calculation of weight scores

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

battery No “significant” toxic effect PE < 20% - score 0

• Significant toxic effect 20% ≤ PE < 50% - score 1

• Toxic effect 50% ≤ PE < 100% - score 2

• PE = 100% - score 3

b) Calculation of the class weight score

Class weight score = (∑ all test scores)/n where n = number of tests performed.

c) Calculation of the class weight score as a percentage

Class weight score in % = (class score)/ (maximum class weight score) × 100

3.6 Genetic Toxicity Testing

3.6.1 Ames Mutagenicity Test

The Ames test is an approach used to test for mutagenic materials in water, sediment, air and chemicals (Ames *et al*., 1975). The Ames test was done with the use of the EPBI Muta-ChromoPlate™ test and performed entirely in liquid culture. A 96-well microplate type of the *Salmonella typhimurium* Ames Test was used. The *Salmonella typhimurium* strain, TA100, was used for sample screening with and without metabolic bioactivation. The standard mutagens used in this experiment were Sodium azide (NaN3) used with strain TA 100, and 2-Aminoanthracene (2-AA) used exclusively with the metabolic bioactivation S9 experiment. Plates were scored visually; yellow and partial yellow wells were scored as positive and purple wells as negative. 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.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter reports the physicochemical properties of the Diep River water samples, microplastics occurrence in the river and the ecological risks associated with microplastic pollution of the Diep River. Recovery studies of microplastics standards in water were carried out and the method was 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 the Diep River was evaluated. Two different environmental matrices (water and sediment) were collected from the river and analysed. 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.

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. This chapter also aims to report the ecological risk assessment of microplastics on the Diep River. The potential climate change effects of microplastics on three aquatic organisms and genetic toxicity. Results obtained from the bioassay experiments using environmental water samples and microplastic standards, climatic effects and the genetic toxicity potential are presented in this chapter.

4.2 Microplastic recovery studies

The polymer recovery rates and potential effects of sieving on polymers of different types and sizes were investigated using two mesh sizes (250 and 20 µm). None of the treatments led to significant polymer loss and no visual changes were detected in the polymers after the sieving process. The virgin polymers were exposed to digestion methods to detect potential effects of the reagents on the polymers. After the microscope viewing and FTIR analysis used to complement the information, no visual changes were detected. 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 the 20 µm mesh size was more efficient to extract micro and nano plastic with high microplastic recovering rates (95 ± 4%).

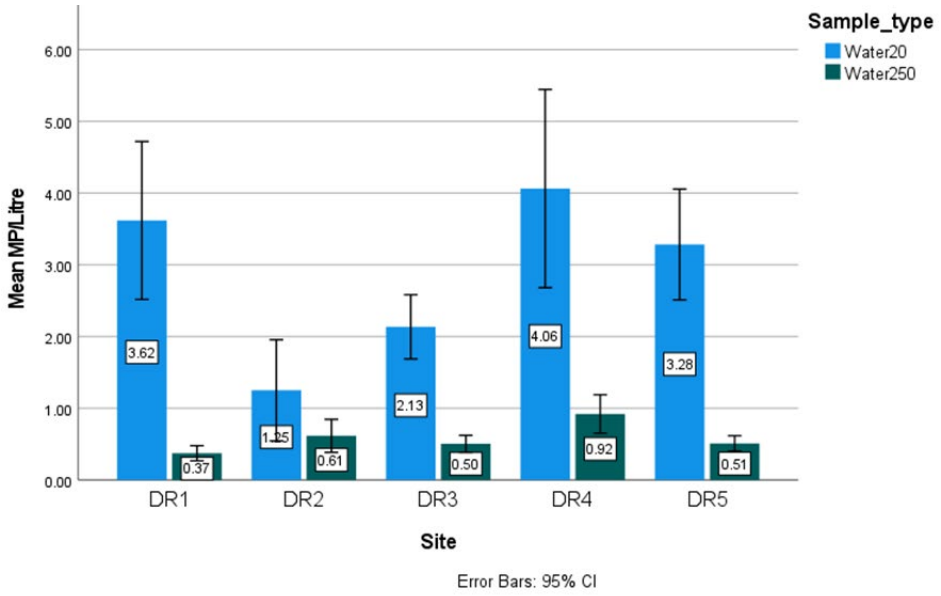

Figure 4.1. Microplastics recoveries from the Diep River water samples using two different mesh sizes

Figure 4.1 shows microplastic abundance in water samples using two different mesh sizes for filtration. Water20 represents samples that were filtered through the 20 µm mesh size and the Water250 denotes samples that were strained through a 250 µm sieve. Multiple orders of magnitude of microplastics were recovered using the 20 µm mesh sieve relative to the samples prepared using the 250 µm sieve. The results obtained revealed that there was a significant loss (more than 50%) of microplastics with the use of the 250 µm compared to the 20 µm mesh in all sites.

4.3 Spatial and temporal distribution of microplastics in water samples of the Diep River

Mean microplastics for all sites combined are presented in Figure 4.2 and results obtained from the two extraction processes in the Diep River water samples over four seasons are presented in Table 4.1.

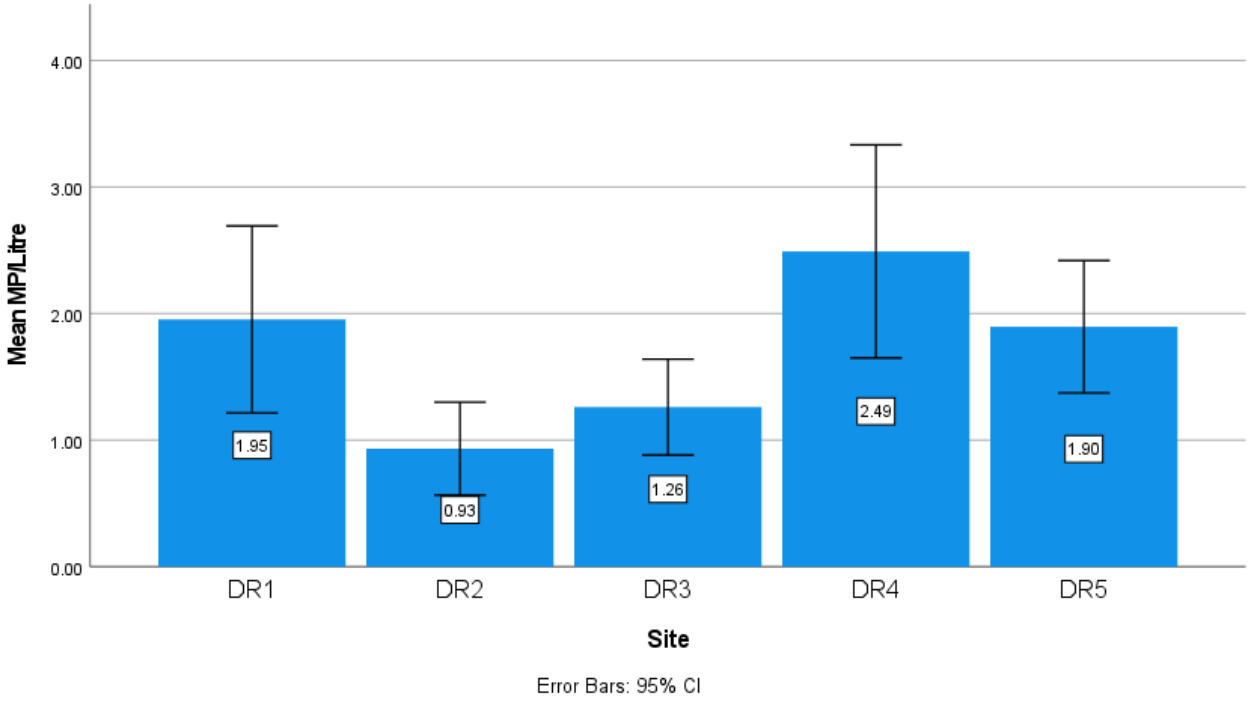

Figure 4.2. Microplastic abundance in the Diep River water samples

There were 187 water samples collected. Microplastics were detected in all except for one sample from DR-2 (the site in the vicinity of Table Bay Nature Reserve). The results show that DR-4 (2.49), a site in close proximity to a wastewater treatment plant, had the highest mean microplastic load, followed by DR-1 (1.96), which is near farming practices and opposite an informal settlement. DR-2 (0.93) recorded the lowest mean microplastic per litre in water.

Table 4.1. Seasonal 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
DR-1	1,6±0,29	0,23±0,11	5,1±2,20	0,54±0,19	-	-	2,44±0,83	0,19±0,10
DR-2	-	-	1,25±0,99	0,61±0,32	-	-	-	-
DR-3	2,5±0,59	0,52±0,28	1,42±0,52	0,53±0,22	-	-	2,2±0,78	0,46±0,17
DR-4	3,3±0,96	0,44±0,22	4,88±3,99	1,16±0,66	-	-	3,2±1,16	0,92±0,31
DR-5	2,55±1,11	0,59±0,24	5,25±2,27	0,68±0,31	1,95±0,74	0,24±0,08	2,15±1,10	0,28±0,12

Increased mean microplastics per litre were observed in Water20 and Water250 respectively for DR-1 (5.1 and 0.54), DR-4 (4.88 and 1.16) and DR-5 (5.25 and 0.68) in spring. The 20 µm size mesh extracted more microplastics, in multiple orders of magnitude and the observation demonstrates the loss of microplastics during onsite filtration using a larger mesh size. 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 and the proximity to a wastewater treatment plant. No samples were taken at DR-1, DR-2, DR-3 and DR-4 during the summer season due to excessive vegetation growth and the surface water having dried up, and at DR-2 during the autumn and winter seasons due to excessive vegetation growth. Apart from DR-3, the results were consistent with those of Schell *et al*. (2021), who reported the highest occurrence of microplastics in spring.

4.4 Spatial and temporal distribution of microplastics in sediment samples of the Diep River

There were 85 sediment samples collected. Microplastics were detected in all sediment samples, except for one sample from DR-2 (the site in the vicinity of Table Bay Nature Reserve). Figure 4.3 shows the results obtained in sediment samples for all sites combined. Data for sediment samples were extrapolated to present microplastics per kg of sediment. The distribution of microplastics in the sediment samples varied significantly *($p \leq 0.05$)* across all sites.The abundance and distrubution of microplastics in sediment samples during autumn, spring, summer and winter are shown in Figure 4.4.

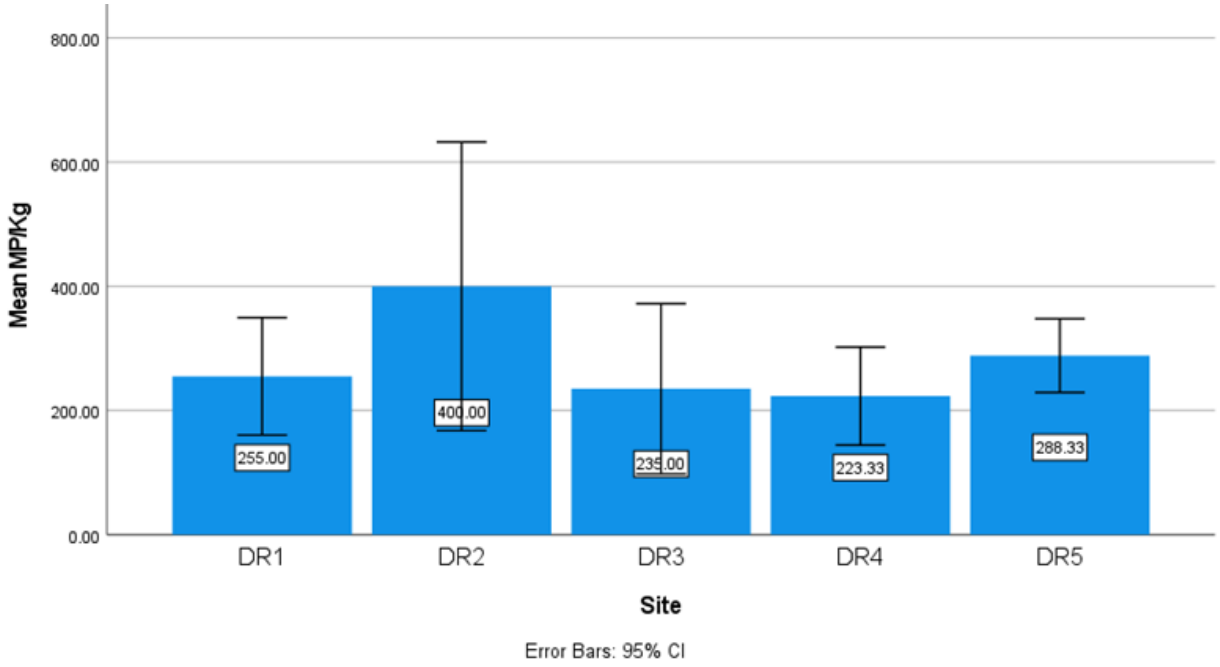

Figure 4.3. Spatial distribution of microplastics in the Diep River sediment (mean microplastic per kg)

In contrast to microplastic abundance in water samples, the results revealed that DR-2 (400.00) which is the site along the Table Bay Nature Reserve, had the highest mean microplastic per kilogram of sediment, and the lowest in water samples, whereas DR-4 (223.33) recorded the lowest mean microplastic per kilogram of sediment, but had the highest in water samples. The water at DR-2 was slow moving (as expected from a nature reserve) while DR-4 (near WWTP) had faster moving water. It can be expected that slow moving water will have less MP in surface water. As a result, most MP would settle in sediment. Figure 4.4 represents the abundance and distribution of microplastics per kilogram of sediment over 4 seasons. The results revealed that sediment samples had between 110 and 450 mean microplastic per kilogram of sediment.

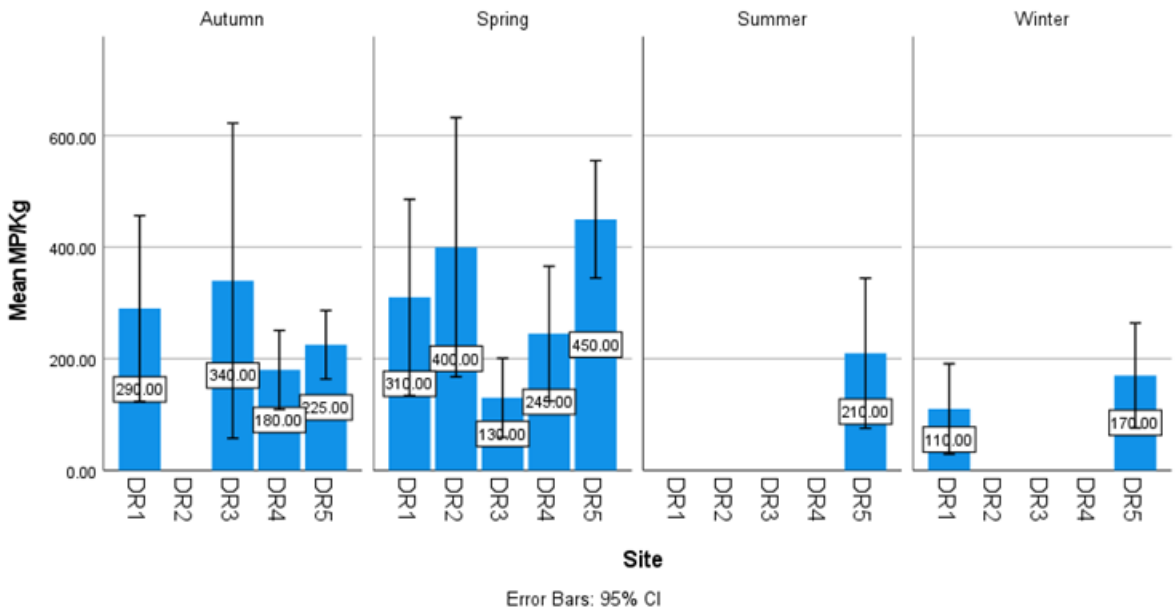

Figure 4.4. Seasonal distribution of microplastics in sediment samples of the Diep River over 4 seasons

Microplastic abundance values ranged between 180 and 340 microplastics per kilogram during autumn (March and May); 130 and 450 microplastics per kilogram in spring (September and November); 210 in summer (February), and 110 to 170 microplastics per kilogram in winter. During the spring season, DR-1 (310.00), DR-4 (245.00,) DR-5 (450.00) recorded the highest microplastic concentration in all seasons except for DR-3 (130.00), which was found to be higher in the autumn season with a mean of 340.00 microplaatic per kg. It must be noted that DR-3 is a canal that connects to the Diep River. The reason for the high microplastic load found at DR-3 in autumn may be attributed to seasonal influences. In addition, the autumn season follows the dry summer season. Low rainfall may have resulted in the settling and accumulation of microplastics in sediment at this site.

4.5 Morphological distribution of microplastics 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 such as 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. The MPs recovered from the Diep River were characterized based on shape, color and size.

4.5.1 Microplastics classification using microscopy – shape distribution

The Diep River receives microplastics from various sources that include a wastewater treatment plant, informal and human settlements, and industrial and recreational activities. The types of microplastics analysed were classified based on different shapes - fibre, fragment, pellet, film and film adopted from the method by GESAMP, (2019). The stereomicroscope (BS-3060CT, BestScope, China) was used to examine particles based on physical appearance. Figure 4.5 shows the percentage distribution of microplastic shape in water and sediment samples for each site. The most categories of microplastic types were found at DR-3, the site near recreational, commercial and industrical activities along with DR-4, which is in close proximity to a wastewater treatment plant. Figure 4.5 showed that DR-3 (16.25%) had the most fragments compared to all the other sites and this suggests that the combination of recreational, commercial and industrial practices nearby is a causal factor. The most predominant shape found at all sites were fibres which amounted to 95.80% in DR-1, 88.19% in DR-2, 74.99% in DR-3, 71.98% in DR-4 and 93.06% in DR-5. There were four sites, DR-1 (3.19%), DR-2 (6.64%), DR-3 (16.25%) and DR-5 (3.57%), that recorded fragments as the second most abundant shape types, whilst only one site, DR-4 (13.20%), recorded foam as the second most abundant microplastic type followed by fragments.

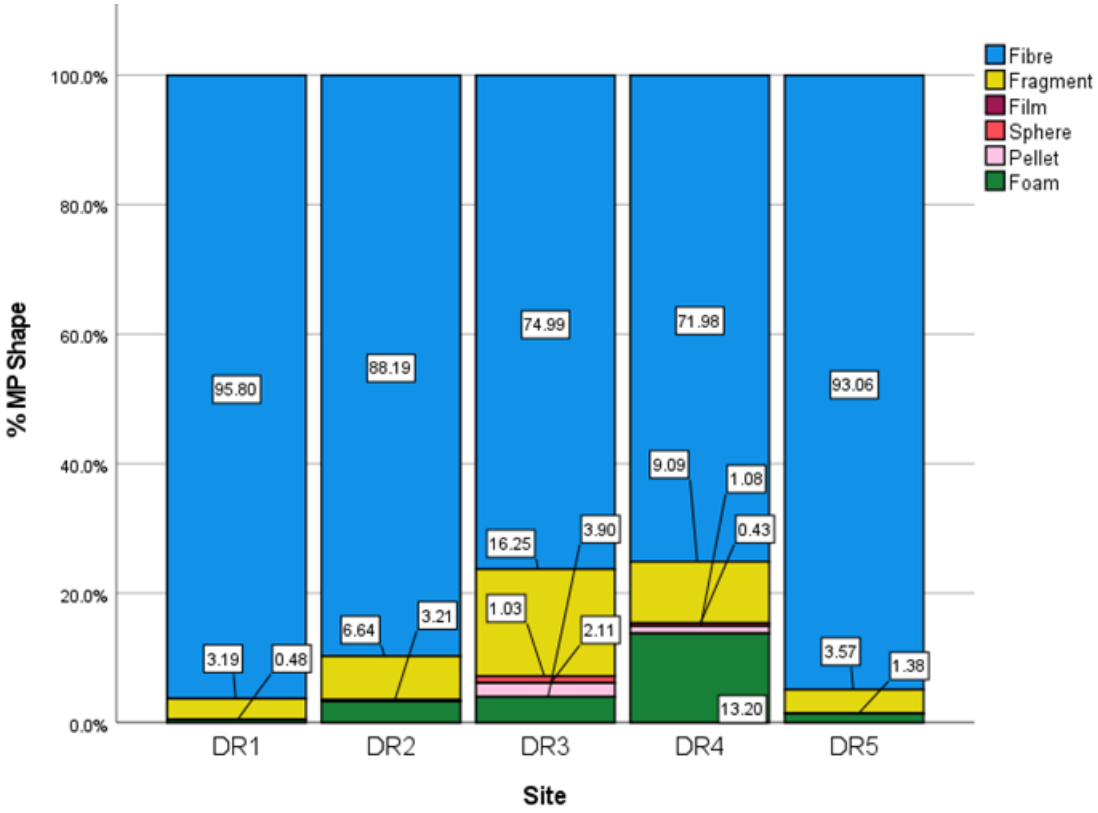

Figure 4.5. Percentage distribution of microplastic (shapes) in water and sediment samples for different sites along the Diep River

Among all study sites, DR-4, which was near the wastewater treatment plant and along an industrial and recreational area, recorded the highest percentage of foam (13.20%) and could be as a result of industrial waste or littering. For the different sampling sites, DR-1 was near the industrial and farming practices and opposite an informal settlement (Dunoon); DR-2 was along a Nature Reserve and near a residential area; DR-3 was on a canal along recreational /commercial/industrial activities which connects to the river; DR-4 was along a recreational and industrial area near the river and in close proximity to a waste water treatment plant; and DR-5 was 1 km from the Milnerton Lagoon, Woodbridge Island, where recreational activities take place. These variations in MP occurrence may have been caused by seasons and anthropogenic activities. Microplastic abundance and percentage distribution of microplastic shape in all sites combined is presented in Figure 4.6.

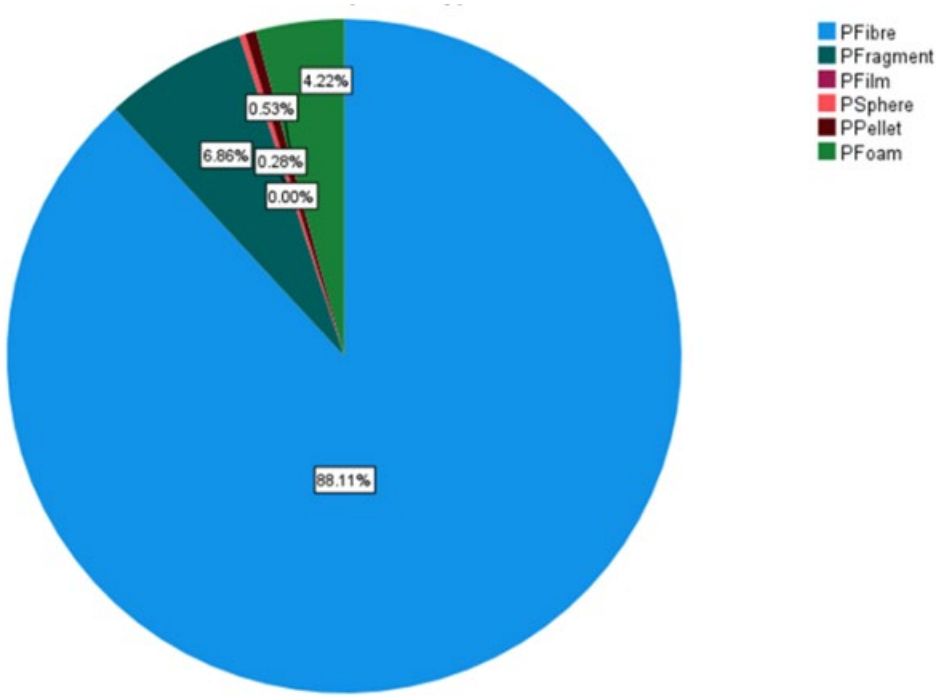

Figure 4.6. Abundance and percentage distribution of microplastic shape in the Diep River water and sediment samples

The most dominant microplastic type in water and sediment samples were fibres (88.11%), followed by fragments (6.86%), foam (4.22%), pellets (0.53%) and sphere (0.28%). This study agrees with a previous work that reported that fibre is the most dominant microplastic particle type in freshwater ecosystems (Wang *et al*., 2017; Jiang *et al*., 2018; Jiang *et al*., 2019; Ghayebzadeh *et al*., 2021; Strady *et al*., 2021). The percentages of film in water and sediment samples were almost undetected and insignificant with a contribution of less than 0.01%. Figure 4.7 shows selected images of microplastics found in various study sites such as (a) pellet, (b) foam, (c) fragment, (d) fragment, (e) fibre and (f) fibre which were photographed using the BestScope BHC3E-1080P HDMI Digital Camera (China) that was connected to the microscope. Microplastics found in this study are a combination of both primary and secondary microplasics. Pellets are primary microplastics that were initially produced as micro sized beads for industrial , personal-care, and medical purposes (Horton *et al*., 2017; Zhang *et al*., 2017; Tien *et al*., 2020) and are often entered into wastewater treatment plants or discharged into the aquatic environment (Tien *et al*., 2020). Secondary microplastics, such as foam, fragment and fibre, originate from the breakdown of bigger plastics into smaller pieces due to photo-oxidative and mechanical degradation (Zhang *et al*., 2017; Lambert *et al*., 2017; Pereao *et al*., 2020a).

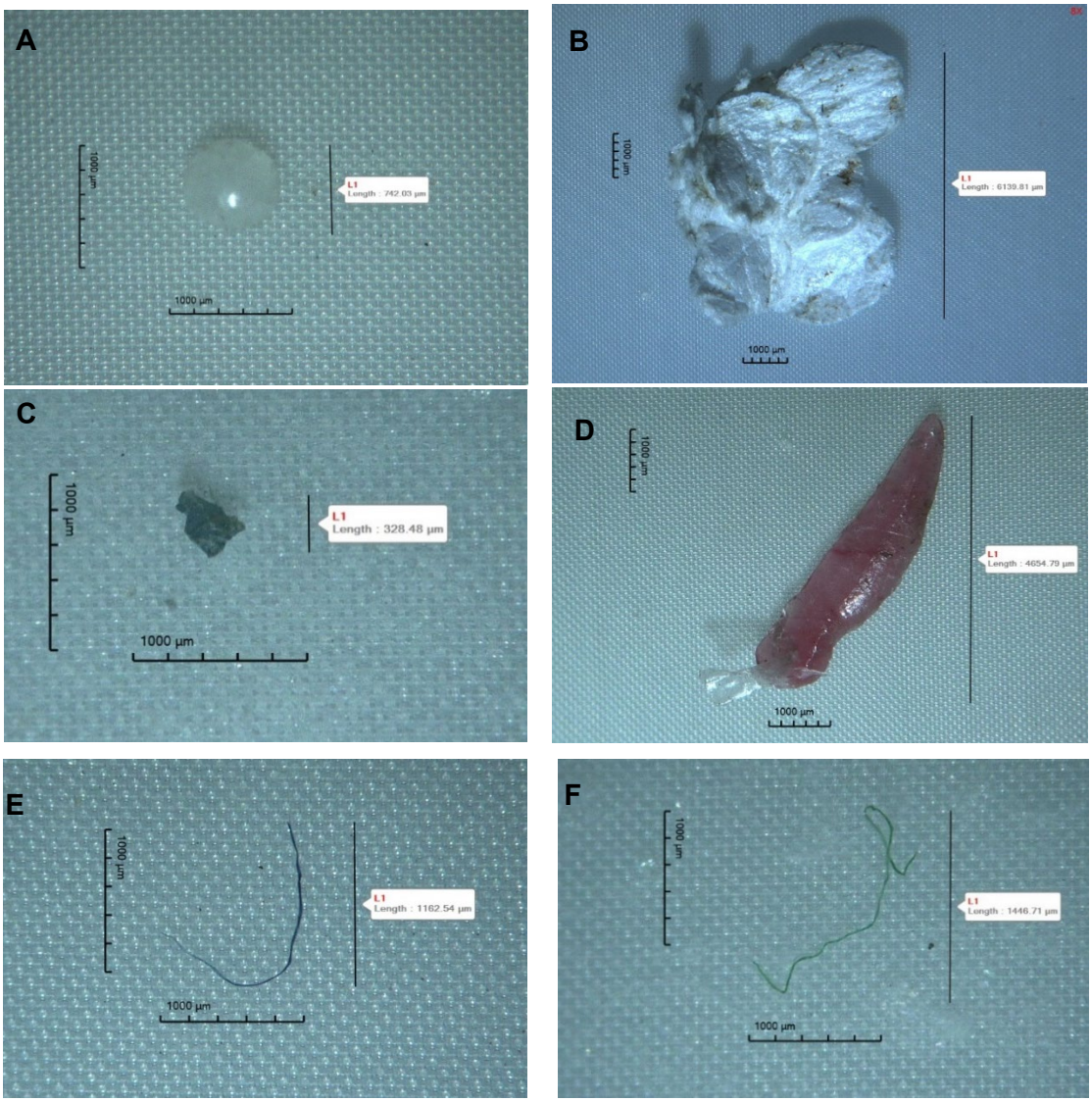

Figure 4.7. Microplastic particles types found in the Diep River (a) pellet, (b) foam, (c) fragment, (d) fragment, (e) fibre and (f) fibre.

4.5.2 Microplastics classification using microscopy- colour distribution

Figure 4.8 presents the percentage distribution of microplastic colours recovered from different sites along the Diep River. Microplastic colour were divided into 6 categories (white, transparent, yellow/brown, red/pink, blue/green and black/grey). The black/grey category in DR-1 (55.94%), DR-2 (51.45%), DR-3 (44.20%), DR-4 (40.10%) and DR-5 (46.01%) were found to be the most abundant.

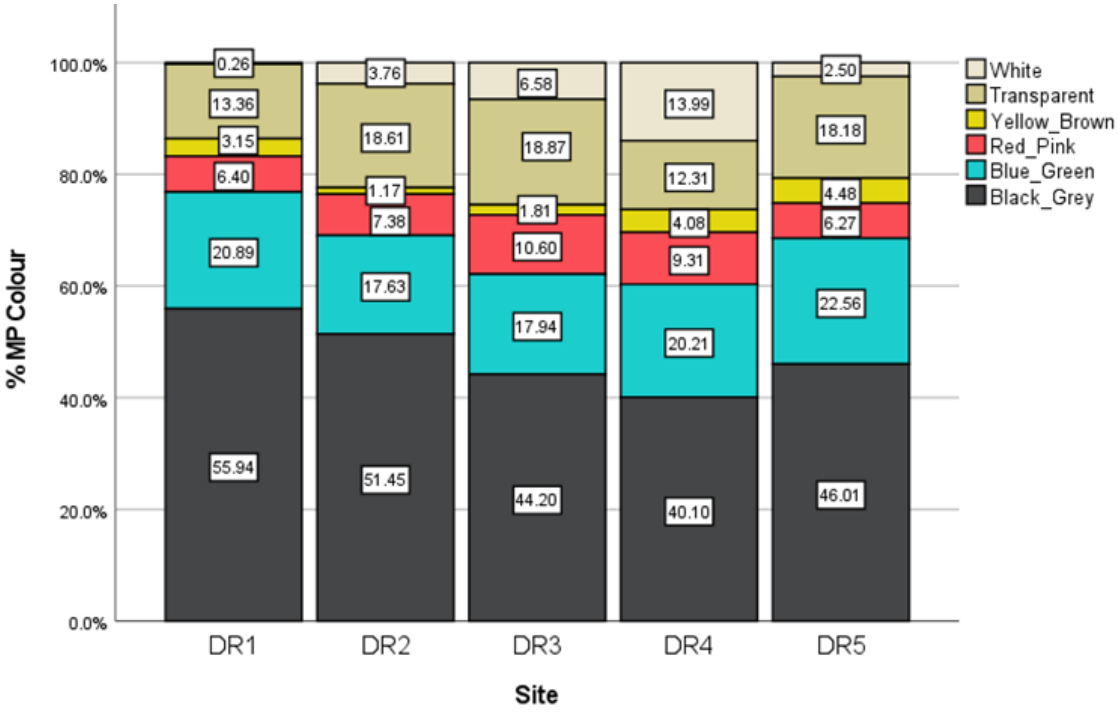

Figure 4.8. Percentage distribution of microplastic colours in water and sediment samples on different sites along the Diep River

For DR-1 (20.89%), DR-4 (20.21%) and DR-5 (22.56%), the blue/green category were the second most abundant colour shown, whereas the second most abundant colour found in DR-2 and DR-3 (18.61% and 18.87%, respectively) were transparent microplastics. White microplastic occurred the least in DR-1 (0.26%) and DR-5 (2.50%) while yellow/brown microplastic occurred the least in DR-2 (1.17%), DR-3 (1.81%) and DR-4 (4.08%). Samples showed no clear trends in colour analysis for different sites and all categories were found at all sites. Not only was the study area near to a wastewater treatment plant, a nature reserve, as well as an informal settlement, it was also surrounded by residential, recreational, commercial, industrial, and agricultural zones. The different variety of colours from the study may be attributed to a combination of land-use practices and anthropogenic activities in the vicinity of the river. In addition, a greater diversity in microplastic colours is an outcome of anthropogenic activity (Edo *et al*., 2020). Figure 4.9 characterized the microplastics colour for all sites combined. The percentage distribution of colours black/grey (47.29%) and yellow/brown (3.39%) respectively, occurred the most and the least in this study.

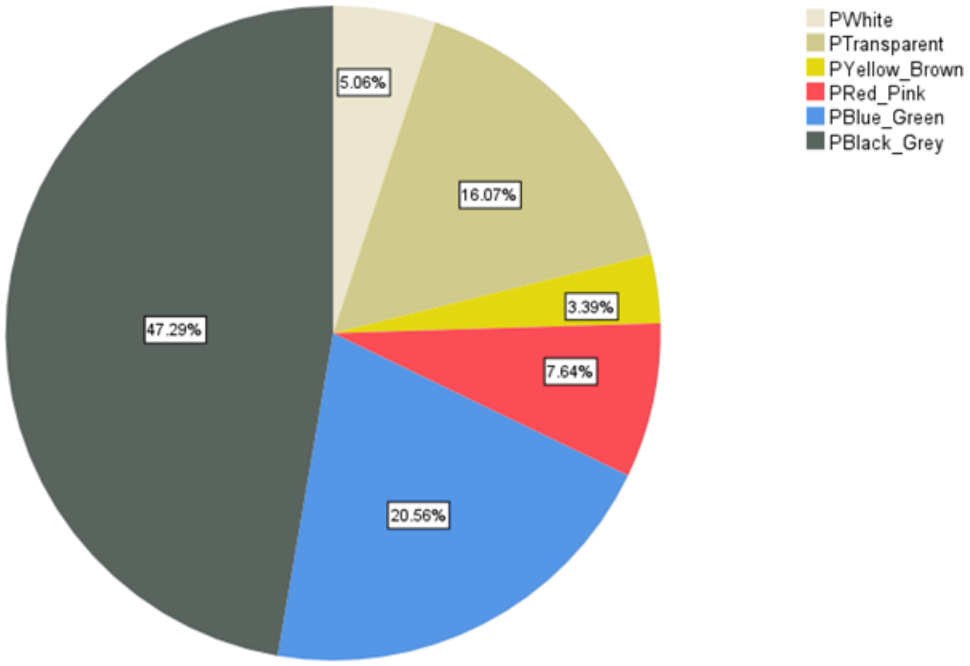

Figure 4.9. Abundance and percentage distribution of microplastic colour in the Diep River water and sediment samples over four seasons

Dark colours were the most common categories present with black/grey (47,29%), and blue/green (20,58%) as the two most abundant colours. Transparent colours contributed to slightly less that the blue/green category with 16.07%, whereas the percentages of white and yellow/brown accounted for only 5.06% and 3.39%.

4.5.3 Microplastics classification using microscopy – size distribution

The percentage distribution of microplastics sizes in water and sediment samples are represented in Figure 4.10. Microplastic sizes showed a trend between different sites in terms of the most and least abundant sizes. The sizes of microplastics were divided into 4 categories according to their size: <500 µm, 500-2000 µm, 2000-5000 µm and > 5000 µm. Microplastics occurred the most and least in the size categories of 500-2000 µm and ≥5000 µm, respectively: DR-1 (68.92% and 2.87%), DR-2 (59.62% and 4.58%), DR-3 (62.75% and 3.71%), DR-4 (68.80% and 3.50%) and DR-5 (65.55% and 4.55%).

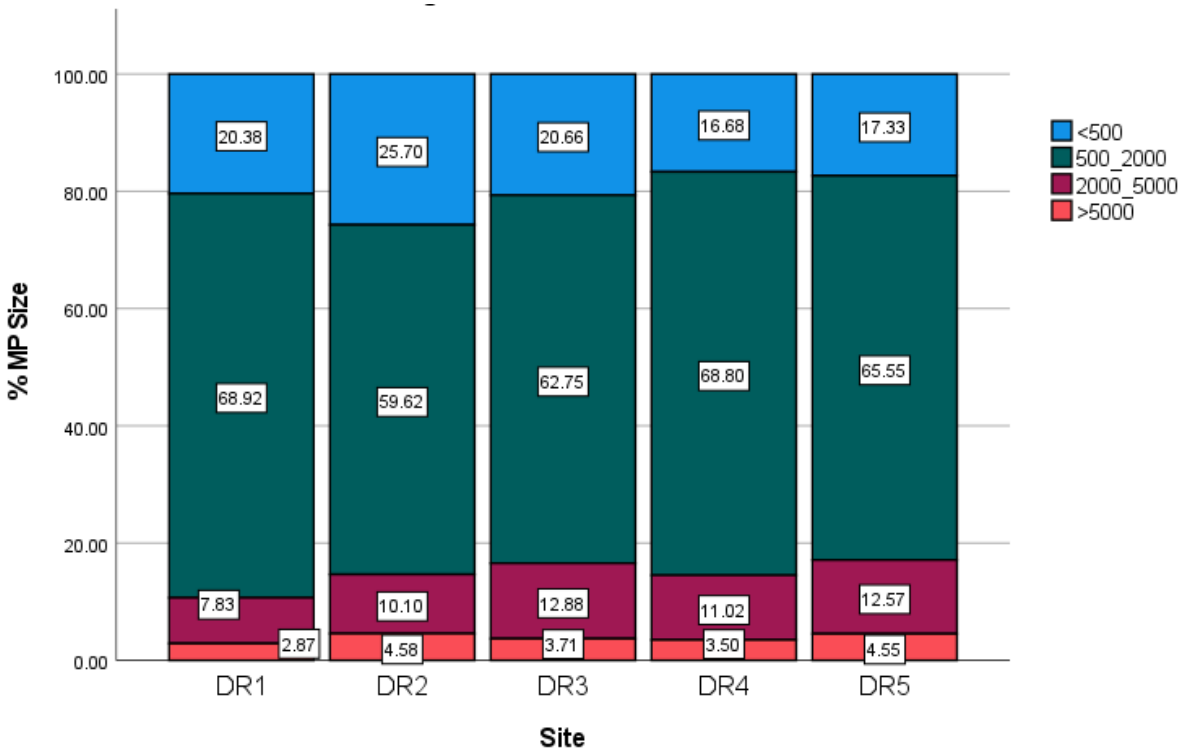

Figure 4.10. Percentage distribution of microplastics size in water and sediment samples

Microplastics in the size range of ≤500 µm occurred the second most, while microplastics in the size range of 2000-5000 µm occurred the second least. This study showed a high proportion of small sized particles. This is likely the result of degradation of larger plastic debris into numerous smaller particles (Zhang *et al*., 2015; Wang *et al*., 2017). Microplastic capacity to adsorb or leach contaminants and additives is influenced by the size of the particle. The potential for surface chemical interactions and binding with hydrophobic chemicals is amplified with a higher surface area per unit of mass and decrease in microplastic size (Horton *et al*., 2017). Figure 4.11 shows the percentage distribution of microplastic size for all sites combined.

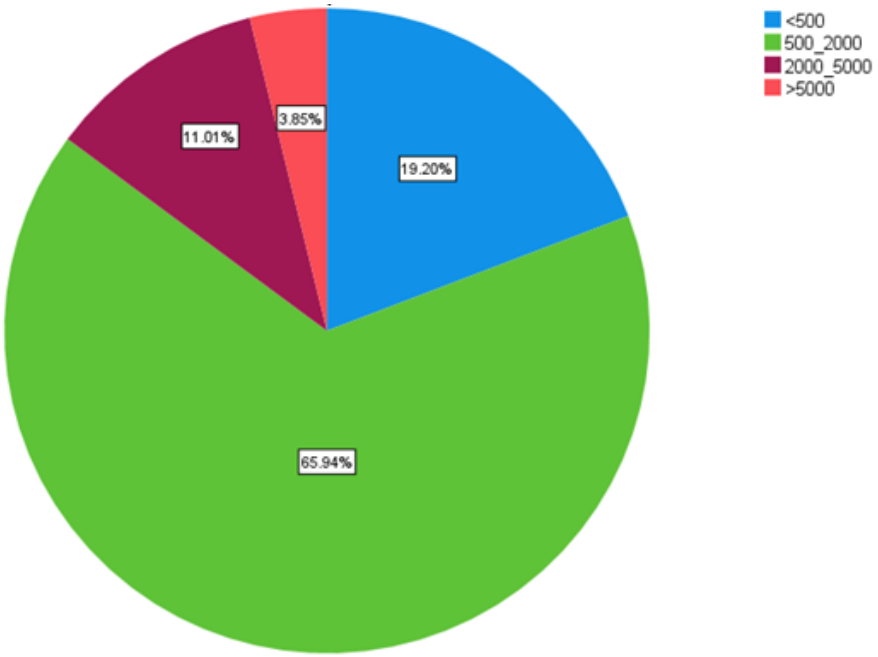

Figure 4.11. Abundance and percentage distribution of microplastic size in the Diep River water and sediment samples over four seasons

The percentage of microplastics with the size of 500-2000 µm occurred most and microplastics with the size of > 5000 µm occurred the least. Majority of microplastics were in the size category of between 500-2000 µm (65.94%), followed by <500 µm (19.20%), 2000-5000 µm (11.01%) and lastly, > 5000 µm (3.85%).

4.6 Microplastics occurrence in Woodbridge Island Beach water and sediment samples

The Woodbridge Island Beach was sampled during spring and winter to investigate microplastic occurrence in water and sediment. The same protocol of the Diep River water samples was followed for the beach water samples. For beach sediment samples, five samples were collected 5 m apart along the strandline at a depth of 5 cm each. Figure 4.12 represents mean microplastic abundance per litre in water samples during the spring and winter seasons. Figure 4.13 represents mean microplastic abundance per kg in sediment samples during the spring and winter seasons. The percentage distribution of microplastics type, colour and size in water and sediment samples of the beach are presented in Figure 4.14.

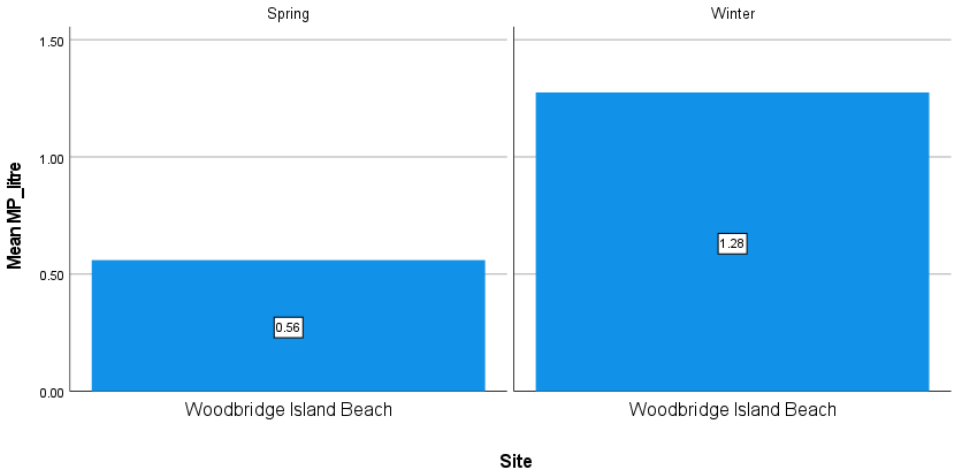

Figure 4.12. Microplastics abundance per litre of Woodbridge Island Beach water samples during the spring and winter seasons

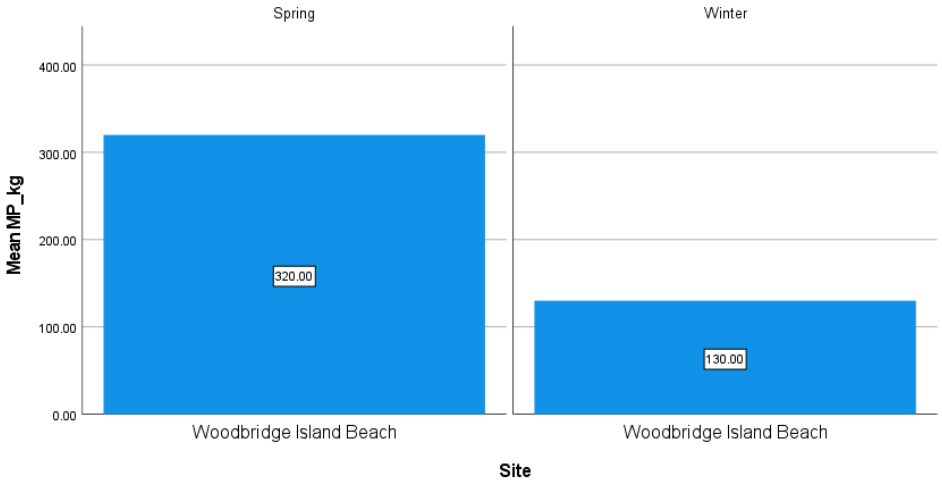

Figure 4.13. Microplastics abundance per kg in Woodbridge Island Beach sediment samples during the spring and winter seasons

Figure 4.12 show the abundance of microplastics per litre in beach water samples during the spring and winter seasons. There was a higher abundance of microplastics in samples during the winter season. The results revealed that 0.56 mean microplastics per litre were recorded in spring compared to 1.28 mean microplastics per litre during winter. Figure 4.13 presents the abundance of microplastics per kg in beach sediment samples during the spring and winter seasons. Microplastics per kg of sediment samples were the highest during the spring season (320.00) compared to microplastics in sediment samples during winter (130.00). It can be assumed that there is a greater influx of microplastics in water during the winter season and a higher retention of microplastics in sediment during spring.

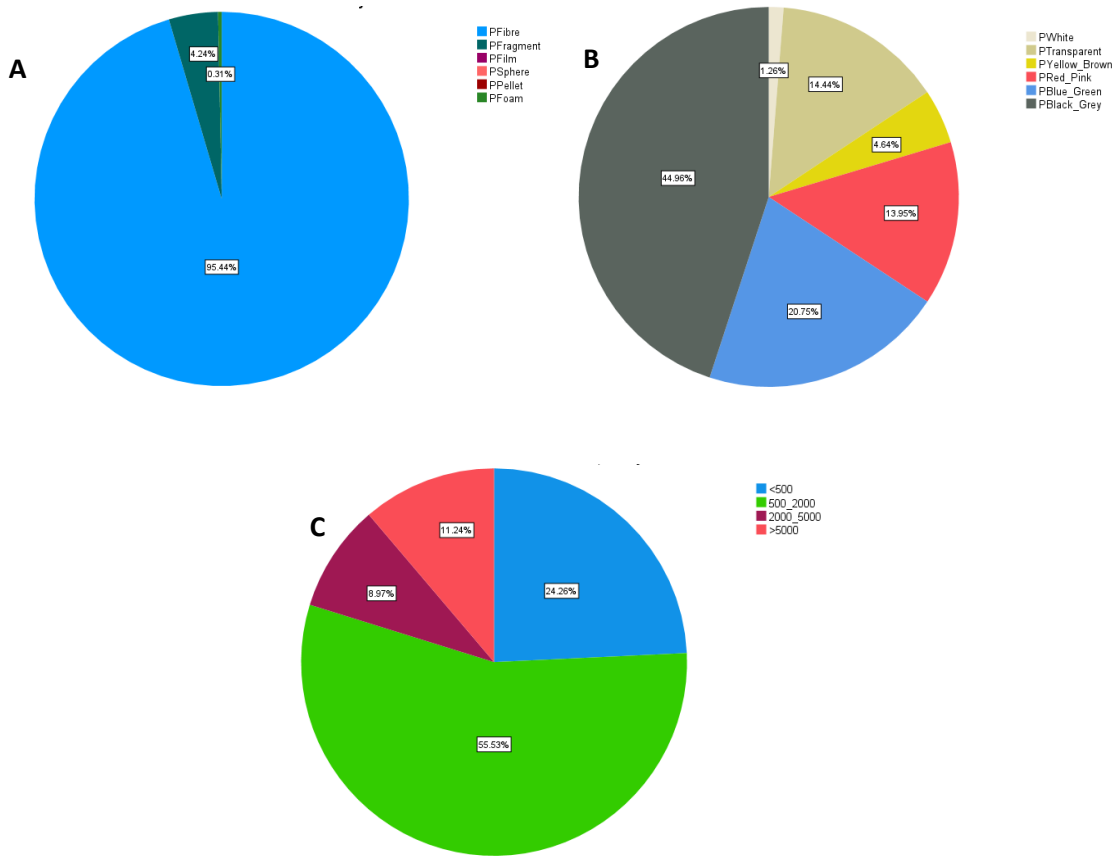

Figure 4.14. Abundance of microplastics types shape (A) colour (B) and size (C) in beach samples

The morphological characteristics were recorded for microplastics in beach samples. Morphological characteristics are presented in Figure 4.14 in terms of microplastic shape (A), colour (B) and size (C). The three common types of microplastics found were fibres, fragments, and foam. Fibres (95.44%) were the most dominant microplastics found in beach samples followed by fragments (4.24%). Microplastic colours detected were mostly black/grey (44.96%) followed by blue/green (20.75%). Other microplastic colours in this study were recorded as transparent (14.44%), red/pink (13.95%), yellow/brown (4.64%), and white (1.26%). There were four size categories of microplastics in this study. The most dominant size category in which microplastics occurred in was 500 – 2000 µm (55.53%), <500 µm (24.26%), >5000 µm (11.24%) and occurred the least in the 2000 – 5000 µm (8.97%) size category.

4.7 Chemical properties of microplastics

Plastic is an artificial, macromolecular compound that is polymerized by adding or condensing a monomeric raw material and its properties are enhanced by additives, which generates different polymers with various characteristics (Jiang *et al.*, 2018). The nine categories identified were polyvinyl chloride, fibre, polyisobutene, polyether

urethane, polystyrene, cotton, polypropylene, polyethylene and other (calcium stearate and calcium hydroxystearate). The percentage distribution of the nine polymer types detected in the Diep River water samples per site are presented in Figure 4.15. The samples analysed under FTIR in DR-1 were all polyethylene polymers. Samples detected in DR-2 were all polystyrene polymers. The samples analysed under FTIR for DR-3 reported polyethylene (42.71%) as the most abundant polymer followed by polystyrene (30.00%), polypropylene (19.38%), cotton (3.13%), and other (4.79%).

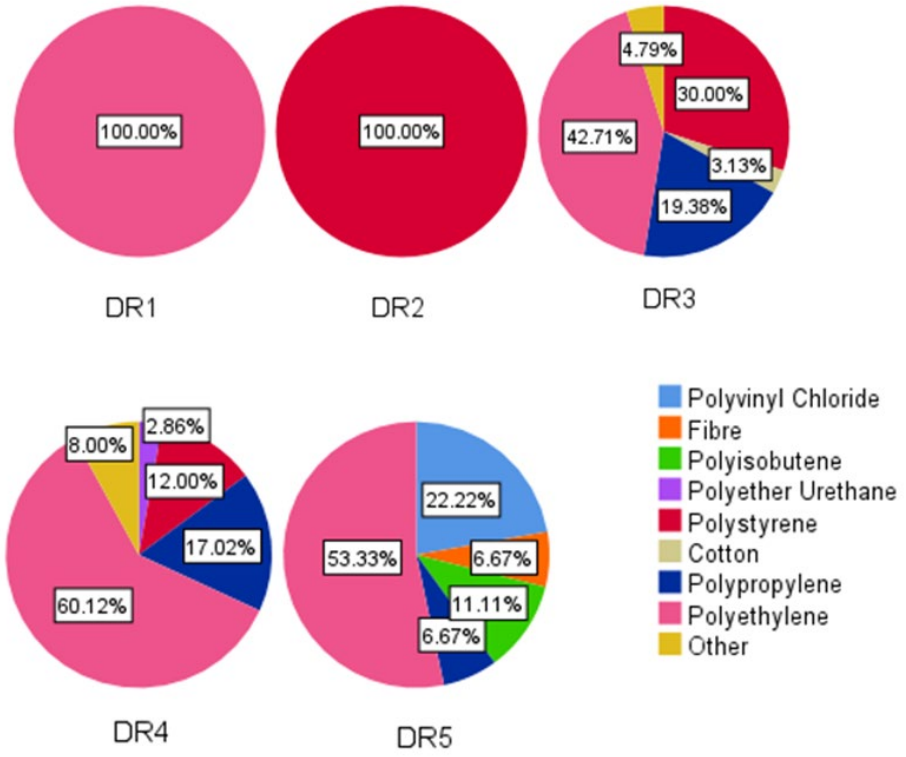

Figure 4.15. Percentage distribution of polymer types in water samples per site

In DR-4, the most abundant polymer found was polyethylene (60.12%), polypropylene (17.02%), polystyrene (12.00%), polyether urethane (2.86%) and other (8.00%). The five polymers reported at DR-5 were polyethylene (53.33%), polyvinyl chloride (22.22%), polyisobutene (11.11%), fibre (6.67%), and polypropylene (6.67%). These polymers are likely contributions from anthropogenic activities. The surrounding areas include a wastewater treatment plant, industrial and recreational area. DR-3, which is a canal that connects to the river is probably a contributing factor of the types of polymers found at DR-4, apart from the surrounding activities in this area. Polyethylene was the main type of polymer found at DR-1 (100.00%), DR-3 (42.71%), DR-4 (53.61%) and DR-5 (53.33%). Polyethylene is present in plastic bags and storage containers, and are known to float (GESAMP, 2019) in water. Anthropogenic activity

is likely the main contributor of this polymer at these sites as plastic litter was present nearby (Figure 4.16).

Figure 4.16. Accumulation of plastic litter at DR-5

Figure 4.17 represents the percentage distribution of polymer types found in all the river water samples for all sites combined. The most abundant polymer present was polyethylene (52.24%), followed by polystyrene (20.00%), polypropylene (13.04%), polyvinyl chloride (4.76%), polyisobutene (2.38%), fibre (1.43%), polyether urethane (1.02%), cotton (0.89%), and other (4.23%).

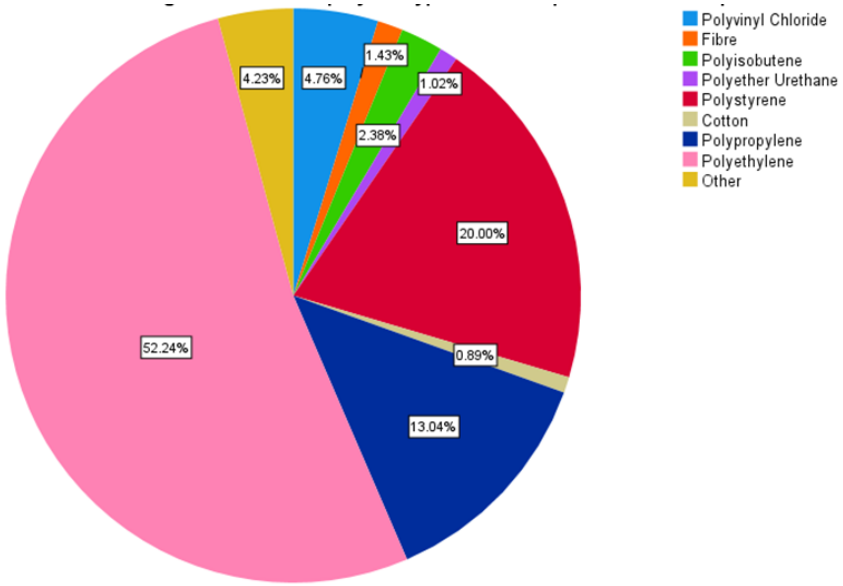

Figure 4.17. Percentage distribution of polymer types in the Diep River

Polyethylene and polypropylene have a density of slightly less or equal to water, and is assumed to float and are not easily retained in water (Besseling *et al*., 2017). The observed polymer distribution is consistent to other studies that assessed microplastic polymers in rivers, which also reported primarily polyethylene, polystyrene and polypropylene (Gasperi *et al*., 2014; Mao *et al*., 2020). The FTIR spectra for the main polymers found at different sites in the Diep River are presented in Figure 4.18.

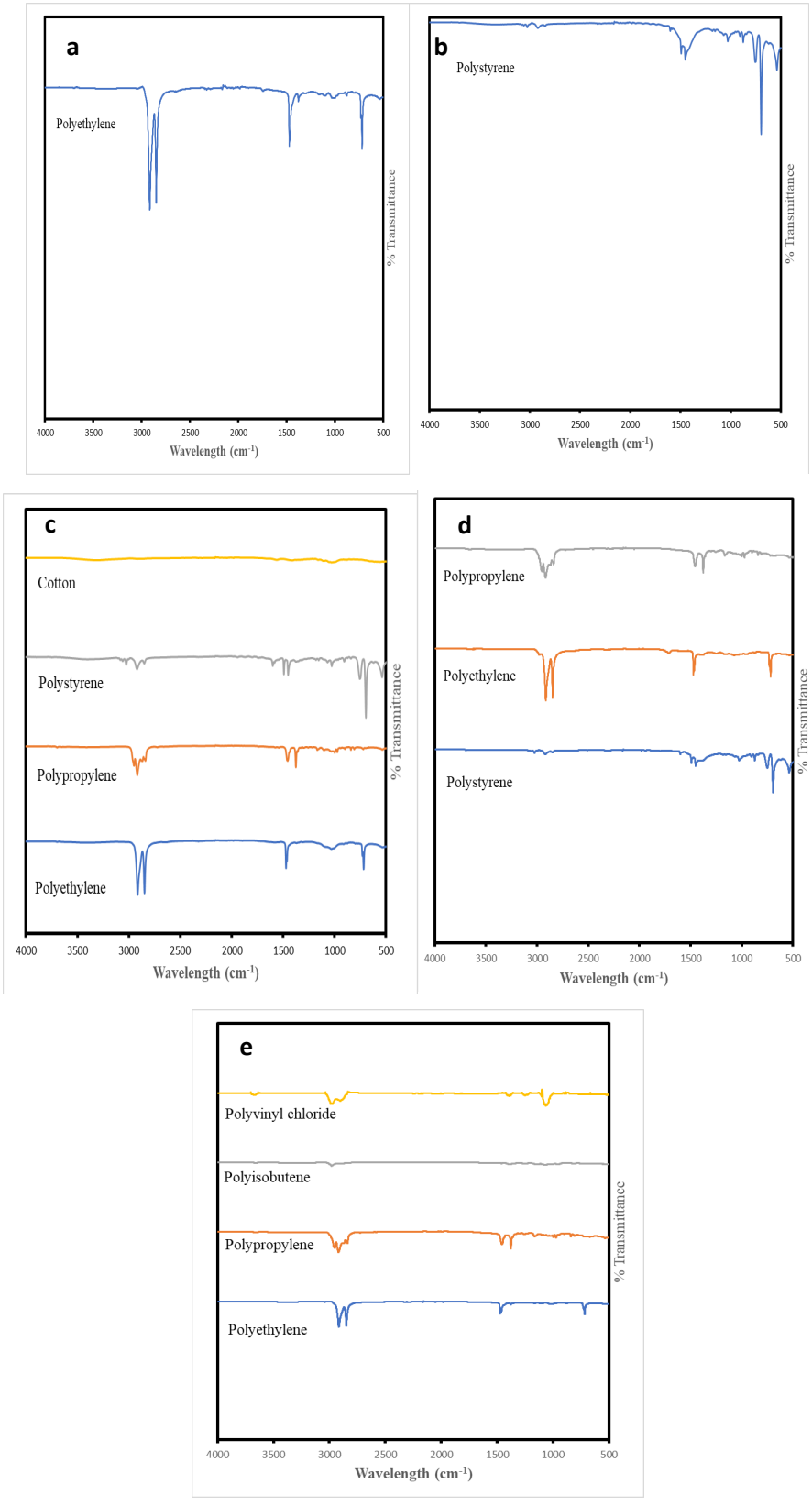

Figure 4.18. FTIR spectra of the main polymers found at a) DR-1, b) DR-2, c) DR-3, d) DR-4 and e) DR-5 in the Diep River

4.8 Physico-chemical properties of the Diep River water samples

The physicochemical parameters of water are used to describe the 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 obtained results in Table 4.1 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 4.2. Seasonal variation of the physicochemical properties of water samples of the Diep River (mean±SD)

Parameter	Season	DR-1	DR-2	DR-3	DR-4	DR-5	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	-	-	-	-	7,7		
	Autumn	9,6*	-	2,4*	3,3*	3,3 ± 1,27		
	Winter	8,3*	-	6,1*	5,6*	4,6*		
EC (mS/m)	Spring	3580,0 ± 777,82	3380,0 ± 1484,92	676,0*	2575 ± 176,78	5740 ± 3945,66	≤2500	1500
	Summer	-	-	-	-	34900*		
	Autumn	1990,0*	-	2940,0*	2310,0*	33500,0 ± 1979,90		
	Winter	1960,0*	-	2160,0*	1783,0*	20000,0*		
Temperature (°C)	Spring	20,85 ±4,60	19,4 ± 5,09	16,6*	20,3 ± 3,82	18,75 ± 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 ± 39,60	119,0*	94,0 ± 4,24	77,5 ± 27,58	N/A	700
	Summer	-	-	-	-	41,0*		
	Autumn	6,4*	-	27,0*	-9,0*	-125,5 ± 187,38		
	Winter	59,0*	-	65,0*	52,0*	47,0*		

TDS (ppm)	Spring	239,0 ± 52,33	225,0 ± 97,58	454,0*	172,0 ± 11,31	384,5 ± 265,17	0-450	1000
	Summer	-	-	-	-	233,0*		
	Autumn	133,0*	-	196,0*	154,0*	223,0 ± 15,56		
	Winter	1319,0*	-	143,0*	1180,0*	134,0*		
COD (mg/L)	Spring	69,8 ± 5,42	71,59 ± 0,59	39,3*	66,17 ± 12,02	77,3 ± 7,78	≤30	≤100
	Summer	-	-	-	-	226,2*		
	Autumn	102,7*	-	54,83*	55,3*	880,17 ± 20,02		
	Winter	81,7*	-	59,3*	75,0*	95,3*		
BOD (mg/L)	Spring	115,8 ± 0,66	152,5 ± 45,66	260,6*	209,7 ± 74,94	212,64 ± 68,5	10	N/A
	Summer					375.15*		
	Autumn	284,9*		63,6*	220,3*	272,8 ± 214,6		
	Winter	102,54*		204,4*	189,0*	191,2*		

*Sampled once

The pH values of the Diep River water samples were slightly higher than the recommended limits at DR-2 (9.16) and DR-4 (9.13) during spring, DR-5 (9.48) during summer, DR-1 (9.65) and DR-3 (9.38) during autumn, and DR-1 (9.74), DR-3 (9.28), DR-4 (9.6) and DR-5 (9.83) in the winter season. Generally, most of the pH measurements obtained were higher than the WHO maximum standards of 8.5 and results showed that the pH was close to neutral and alkaline for all the seasons investigated. Biological activity in eutrophic systems can cause high pH values which may fluctuate widely from >6-10< over a 24 h period as a result of changing rates of photosynthesis and respiration (DWAF, 1996).

The 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 DO results during spring for DR-1 and DR-5 (11.8 and 11.3 mg/L, respectively) showed more oxygen availability in the waterbody whereas DO values were very low at DR-2 (4.9), DR-3(6.4) and DR-4 (4.1). DO values had slightly less oxygen at DR-1 (7.7) during summer and were very low at DR-3 (2.4), DR-4 (3.3) and DR-5 (3.3) in autumn. The winter season also recorded low DO values at DR-3 (6.1), DR-4 (5.6) and DR-5 (4.6). Fertilizer, plastics, and solid waste that enter the river may impact DO values and low DO values may have threatening implications on ecosystems (Gqomfa *et al*., 2022).

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 mS/m, respectively. The influence of anthropogenic and wastewater discharges into aquatic systems can increase electrical conductivity (Gqomfa *et al*., 2022). In a study conducted on microplastics in sludge solutions, electrical conductivity was higher than the control with no microplastics present, which can be due to metal additives leaching from the microplastics during hydrothermal treatment (Li *et al*., 2022).

The measured temperature values were within the permissible limit of WHO and DWAF standards and there was 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 TDS values were observed in DR-1 and DR-4 during the winter seasons. The high TDS values might be due to the natural and anthropogenic factors from the domestic and industrial activities in the area.

The COD values for all seasons and sites were within the WHO standards, except for DR-5 during summer and autumn. While DR-1 demonstrated slightly higher COD values than the permissible WHO limit during the autumn season, DR-5 exceeded the WHO standards during the summer and autumn seasons. COD and BOD are vital for controlling and management of water pollution (Prambudy *et al*., 2019). Good water quality is indicated by having low COD levels but high levels specify pollution which may be detrimental to aquatic organisms (Gqomfa *et al*., 2022). High BOD values in a river indicates the presence of organic matter in rivers (Sharma *et al*., 2019) and is indicative of the amount of DO consumed by aerobic organisms, that oxidizes organic matter in the water (Najafzadeh & Ghaemi, 2019). Results of COD and BOD from this study indicate that the river water is rich in organic matter which may be attributed to runoff from upstream agricultural practices, discharges from the wastewater treatment plant nearby and industrial discharges into the river. The Spearman correlation analysis coefficients for the physico-chemical properties of water samples analysed and microplastics occurrences in the Diep River during this study is presented in Table 4.3 below.

Table 4.3. Spearman's correlation coefficient for the physicochemical parameters and microplastics occurrences in the Diep River

	pH	DO (mg/L)	EC (mS/m)	Temp (°C)	ORP (mV)	TDS (ppm)	COD	BOD	Water250	Water20	Sediment
pH	--										
DO (mg/L)	0.032	--									
EC (mS/m)	-0.011	-0.158	--								
Temp (°C)	-0.19	0.303	0.337	--							
ORP (mV)	-0.381	0.243	-0.221	0.266	--						
TDS (ppm)	-0.096	0.325	0.045	0.298	0.278	--					
COD (mg/L)	0.411	0.054	0.478*	-0.174	-0.348	0.044	--				
BOD (mg/L)	0.047	-0.097	0.042	-0.231	-0.075	-0.277	0.388	--			
Water250	-0.374	-0.327	0.018	0.092	0.488*	0.207	-0.182	0.046	--		
Water20	-0.181	0.042	0.092	0.022	0.129	0.06	-0.012	-0.056	0.356	--	
Sediment	-0.037	-0.028	0.389	0.406	0.126	0.103	0.118	0.219	0.356	0.274	--

* Correlation is significant at p≤0.05 level (2-tailed)

There was a negative correlation between pH and EC, temperature, ORP, TDS, microplastics in 250 µm; 20 µm and sediment samples, while a positive correlation existed between pH and DO, COD and BOD. This suggests that microplastics in both water samples (250 and 20 µm) and sediment samples will likely be present in low pH waters. There was a significant (r=0.478) positive correlation between EC and COD, which indicates that COD will increase in waters with high EC values. An increase in EC will increase the need for oxygen, which may be detrimental for organisms in the water. A significant positive correlation (r=0.488) was confirmed between ORP and 250 µm mesh samples. This indicates that larger microplastics are likely to be found in high ORP waters. A higher ORP indicates that substances in the water could oxidize constituents and therefore, decompose wastes in the water. Therefore, it can be assumed that larger sized microplastics presence support this process. The only

parameter which had a negative relationship with EC was ORP, whereas BOD was the only parameter which had a negative relationship with TDS. 20 µm mesh microplastics demonstrated a negative correlation with BOD, whereas 250 µm mesh and sediment samples showed a positive correlation. This suggests that smaller sized microplastics presence will be more prevalent in waters with low organic matter, whereas larger sized microplastics and microplastics in sediment are associated with waters that contain high organic matter content. Seven parameters, DO, EC, temperature, ORP, TDS, 250 µm mesh and sediment samples, correlated positively with 20 µm mesh samples while three parameters, pH, COD and BOD, showed a negative relationship. Hence, the dissimilar relationship between microplastics in 20 µm mesh samples with the three parameters (pH, COD, and BOD), specify that a rise in pH, COD and BOD will limit smaller sized microplastic particle accumulation and abundance.

4.9 Ecological Risk Assessment

Ecological risk assessment is a process used to evaluate the possibility of stressors (Emmanuel *et al*., 2005) and address possible negative effects on organisms from its surroundings. Organisms used for the risk assessment studies included are algae, *Raphidocelis subcapitata* (the primary producer), a crustacean, *Daphnia magna* (a consumer that feed on green algae; a useful ecotoxicity model with high sensitivity to environmental toxicants (Canniff & Hoang, 2018; Aljaibachi *et al*., 2020)), and ciliate protozoan, *Tetrahymena thermophila*, a eukaryotic organism and decomposer.

4.9.1 Ecotoxicological Studies

A battery of biotests were used (Microbiotests Inc., Belgium) and consisted of 3 freshwater organisms for three trophic levels: producers –*Raphidocelis subcapitata*, (72 h growth inhibition test), consumers – *Daphnia magna*, 48 h acute mobility inhibition test) and decomposers - *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.

The classification system was based on a ranking in 5 acute hazard classes (Persoone *et al*., 2003). The percentage effect (PE) for each microbiotest 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

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)

a) Calculation of weight scores

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

battery No “significant” toxic effect PE < 20% - score 0

• Significant toxic effect 20% ≤ PE < 50% - score 1

• Toxic effect 50% ≤ PE < 100% - score 2

• PE = 100% - score 3

b) Calculation of the class weight score

Class weight score = (∑ all test scores)/n where n = number of tests performed.

c) Calculation of the class weight score as a percentage Class weight score in % = (class score)/ (maximum class weight score) × 100.

4.9.1.1 Microplastic 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 (Canniff & Hoang, 2018) which represent a similar size range to food ingested by crustaceans. Transparent polystyrene granular plastics were grinded and used as models for primary microplastics. Their size range were between 200 µm-1000 µm and had a density of

1.04 g/ml. A concentration of 1000 mg/L stock solution of microplastics was prepared with dry particles and distilled water through shaking and sonicating, and were kept in storage for a week at room temperature. The stock solution was diluted with distilled water to the final concentrations of 400 mg/L, and ultrasound bathed for 15 min at 50 W for dispersion prior to the toxicity assay.

4.9.1.2 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 at least, 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 4.19, 4.21 and 4.23 represent the percentage inhibition of microalgae, crustacean and protozoa, respectively, at different sites along the Diep River in autumn, spring, summer and winter. Figures 4.20, 4.22 and 4.24, demonstrate the percentage inhibition of microalgae, crustacean and protozoa, respectively, at different sites of the Diep River during the spring season which were exposed to microplastic standards.

4.9.1.2.1 *Raphidocelis subcapitata* 72 h growth inhibition test

Growth inhibition was shown at all sites for microalgae, *R. subcapitata*. The mean growth inhibition of *R. subcapitata* exposed to environmental samples in different sites along the Diep River presented in Figure 4.19 ranged from 8.3% to 34.6%. The samples from DR-1 had the highest growth inhibition of microalgae (19.8%) in the spring season compared to other seasons 17.5% in autumn, and 13.0% in winter. The corresponding values for DR-3 are 10.0% in spring, 12.3% in autumn, and 20.6% in winter. Comparable values at DR-4 were 34.6%, 14.9%, and 14.5%, respectively . Similarly, values at DR-5 were 13.2%, 13.2%, 8.3%, respectively; an additional sample obtained in summer had 13.8% inhibition.

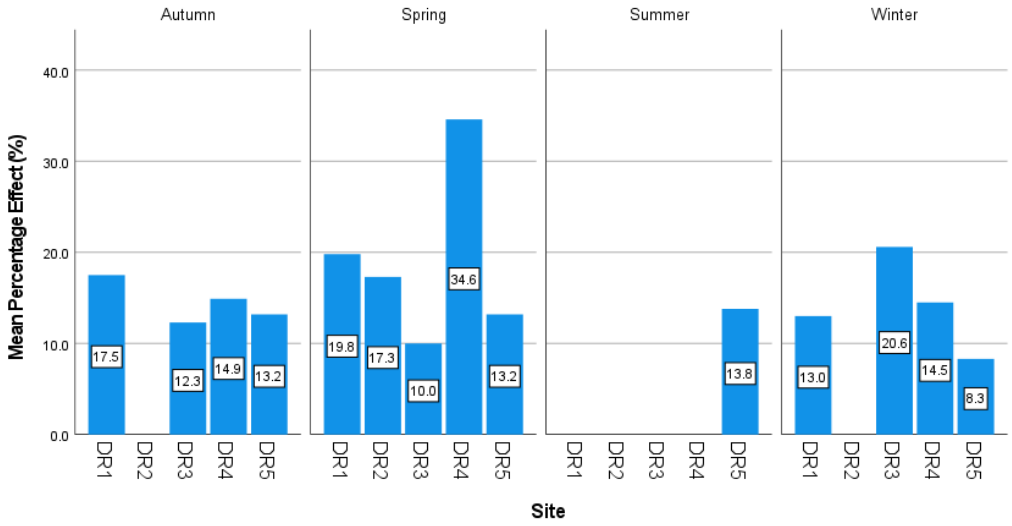

Figure 4.19. Percentage inhibition of microalgae in different sites along the Diep River over 4 seasons

In general, environmental samples demonstrated no significant toxic effect for *R. subcapitata* (Figure 4.19). The mean growth inhibition was below 20.6% for all samples, with the exception of the spring DR-4 sample, with a mean PE of 34.6%. Results of *R. subcapitata* exposure to environmental samples of the Diep River over the four seasons might be due high nutrient waters as these are associated with algae growth and therefore experience a lower growth inhibition (Morrison et al., 2001). River algal blooms as a result of nutrient load and water pollution are increased by industrial and domestic discharges and typically occur during late winters and early springs with low temperatures (Xia et al., 2019). The lowest PE occurred at DR-5 during winter (8.3%). Algal bloom at DR 5 during winter might be due to a combination of the low temperatures in winter as well as a combination of discharges contributing to the river site downstream.

In environmental samples with microplastics, *R. subcapitata* 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 4.20) was demonstrated at DR-1(15.0%), DR-2(25.0%), DR-3(13.0%), DR-4(22.4%) and DR-5(21.0%). In Figure 4.20, three sites; DR-2(25.0%), DR-3(13.0%) and DR-5(21.0%) showed a greater growth inhibition compared to the spring samples in Figure 4.19 where DR-2(17.3%), DR-3(10.0%) and DR-5(13.2%) had no microplastics standards.

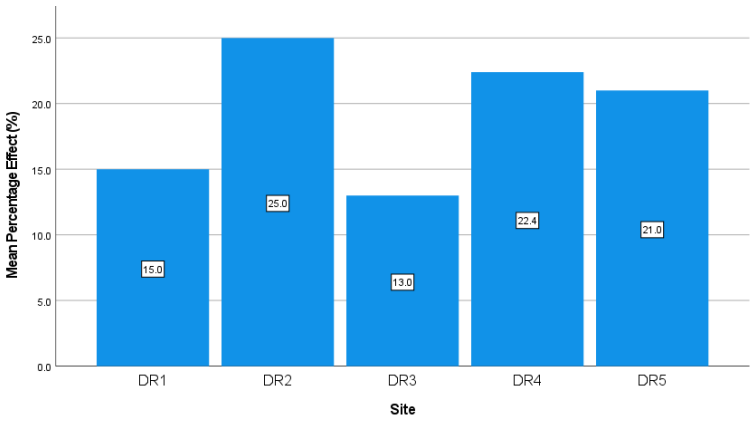

Figure 4.20. Percentage inhibition of microalgae in spring environmental samples with microplastic standards in different sites along the Diep River

The results of *R. subcapitata* exposed to environmental samples with microplastics demonstrated a negative relationship, as a higher PE is a consequence of the inhibition of algal growth. Other workers reported that nanoparticles can reduce and negatively affect chlorophyll and photosynthesis, and cause growth inhibition of microalgae (Wang et al., 2021). The results obtained are consistent with various studies that have reported a negative influence on algal growth in the presence of microplastics (Miloloža et al., 2021).

4.9.1.2.2 *Daphnia magna* 48h acute mobility test

The mortality of *D.magna* exposed to environmental samples of the Diep River over four seasons, is presented in Figure 4.21. The percentage mortality values ranged from 0% to 100%. Mortality for *D.magna* was observed at DR-4(5%) and DR-5(100%) during autumn, respectively, DR-2(15%) and DR-4(5%) during spring, respectively, DR-5(100%) in summer, and DR-3(5%) in winter. Alternatively, no mortality was recorded at DR-1 and DR-3 (autumn), DR-1, DR-3 and DR-5 (spring), and DR-1, DR-4 and DR-5 (winter). The maximum mortality of *D.magna was* demonstrated at DR-5(100%) in both autumn and summer seasons. DR-5 is a downstream site and in close proximity to the lagoon and beach. It must be noted that *D.magna* is a freshwater species and survival may have been influenced by a combination of factors such as the buffering of ocean water, and contaminant transfer downstream. Only one site, DR-3(5%), showed mortality of *D.magna* during the winter season.

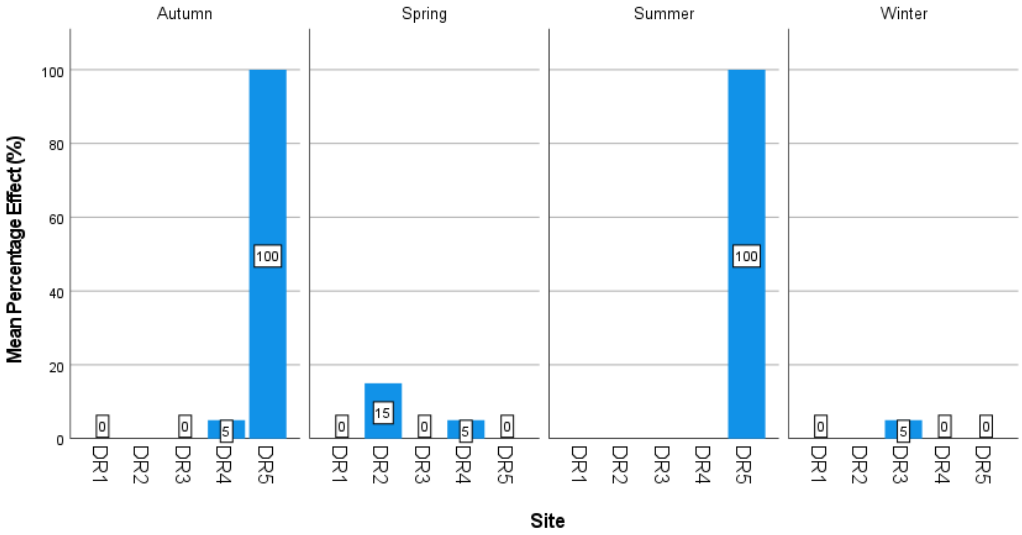

Figure 4.21. Percentage mortality of *D. magna* across sites along the Diep River over 4 seasons

In environmental samples with microplastics, shown in Figure 4.22, results demonstrated that *D.magna* were highly sensitive to microplastics in environmental samples. Mortality occurred at all sites with the addition of microplastic standards. All 5 sites in the presence of microplastics; DR 1 (25%), DR 2 (30%), DR 3 (30%), DR 4 (25%) and DR 5 (25%), showed *D. magna* PE increase of ≥100% compared to samples with no microplastics standards (DR 1(0%), DR 2(15%), DR 3(0%), DR 4(5%) and DR 5(0%), respectively (Figure 4.21).

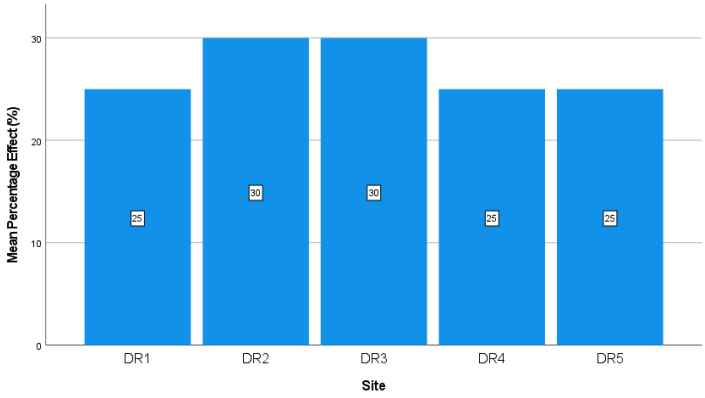

Figure 4.22. Percentage mortality of D. magna in spring environmental samples with microplastic standards in different sites along the Diep River

Size differences of microplastics may have direct adverse effects, with the larger size of PS microplastics possibly associated with a higher toxicity (Schür et al., 2020). The results of *D. magna* exposed to environmental samples with microplastics demonstrated an adverse effect on mortality. These findings revealed that *D. magna* survival displayed sensitivity in the presence of microplastics and are in accordance

with a study conducted by Eltemsah & Bøhn, (2019), which revealed some sensitivity to acute toxicity of microplastics.

4.9.1.2.3 *Tetrahymena thermophila* 24 h chronic growth inhibition test

The results of growth inhibition test using the protozoan, *Tetrahymena thermophila,* are shown in Figure 4.23. Growth inhibition of *T. thermophila* exposed to environmental samples of the Diep River over four seasons ranged from -100% to 100%. In autumn, DR-1(100.00%) and DR-5 (100.00%), respectively, recorded the highest mean growth inhibition compared to growth inhibition at all sites in other seasons. Growth inhibition of protozoa at DR-3 was found to be -30.88% in autumn, 16.89% in spring and 98.43% in winter. At DR-4, growth inhibition of *T. thermophila* was 69.03% in autumn, -60.87% in spring and -100.00% in winter. Growth inhibition was shown at DR-5 in autumn (100.00%), spring (68.63%), summer (-100.00%) and winter (10.87%), respectively. Negative values are indicative of cell proliferation which might be due to influences of water pollution and an increase in nutrients.

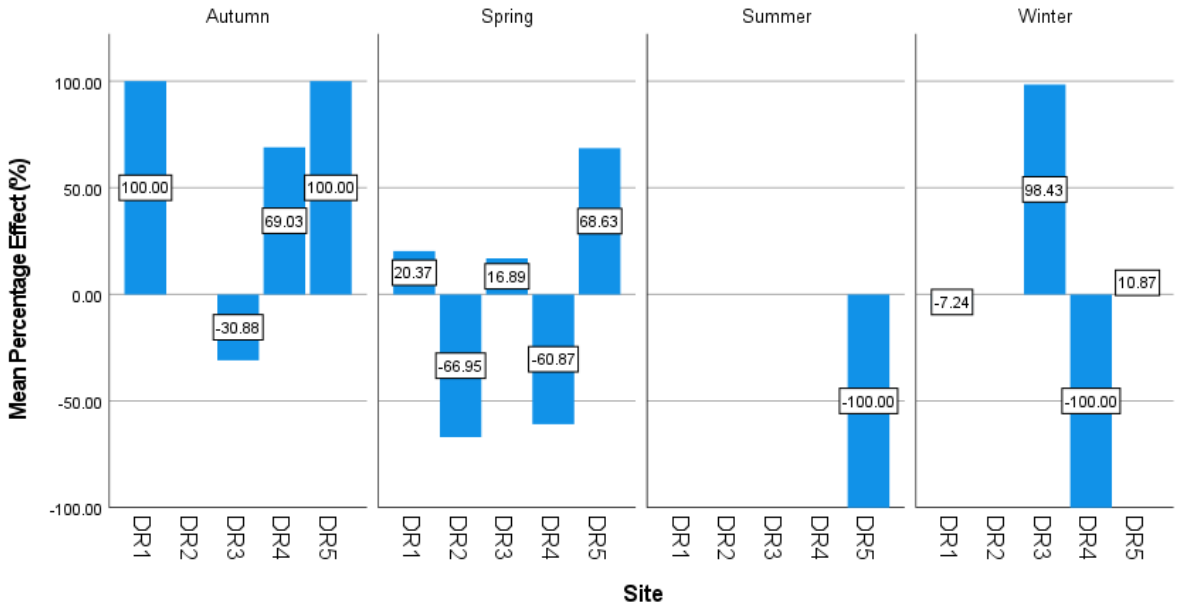

Figure 4.23. Percentage inhibition of protozoa in different sites along the Diep River

Growth inhibition was pronounced in protozoa that were exposed to environmental samples that contained virgin microplastics (Figure 4.24). Figure 4.24 shows growth proliferation in DR-1(-45.14%), and growth inhibition in DR-2 (78.74%), DR-3(100.00%), DR-4(91.27%) and DR-5(100.00%), respectively, in contrast to spring samples that contained no microplastics in Figure 4.23 (DR-1(20.37%), DR-2(-66.95%), DR-3(16.89%), DR-4(-60,87%) and DR-5(68.63%), respectively. Cell

proliferation results in the overgrowth of protozoan which may significantly exert pressure on the oxygen demand of the river.

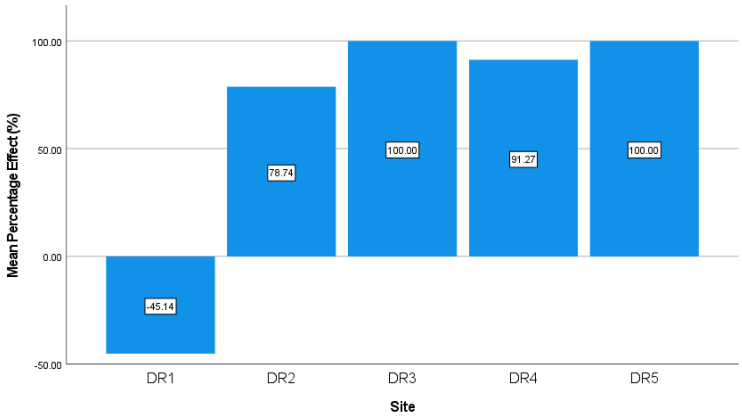

Figure 4.24. Percentage inhibition of protozoa in spring environmental samples with microplastic standards in different sites along the Diep River

T. thermophila have an important role in aquatic food chain; they serve as food sources for zooplankton and bacteria cultivators, and their occurrence or shortage can provide an indication of pollution (Pereao et al., 2021). The results in this study were in line with a study conducted by Sun et al.,(2021), which established that protozoan cell densities exposed to a high microplastic concentration caused growth inhibition and mortality, and a reduction in protozoan photosynthesis (Sun et al., 2021).

4.9.1.2.4 Acute Hazard Classification of the Diep River water samples

The hazard classification classes for environmental samples and environmental samples spiked with virgin microplastics were determined. This experiments were used to assess possible adverse effects of microplstics on aquatic ecosystems. The rationale behind this classification system focussed on the biological analyses that were based on the sensitivity of organisms to the Diep River samples. Figure 4.25 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 had 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 the spring and winter seasons. In Figure 4.26, the hazard classification classes at different sites along the Diep River spring samples with microplastics are shown. Figure 4.26 shows that DR-1 was attributed to Class 4 as opposed to Class 2 in the absence of microplastics (Figure 4.25). On the other hand, DR-2 and DR-4, belonged to Class 3 in Figure 4.26 as opposed to Class 4 in the absence of microplastics (Figure 4.25). DR-3, in Figure 4.26, was categorized as Class 4 in contrast to Class 1 in the absence of microplastics (Figure 4.25). Lastly, DR-5 belonged to Class 4 (Figure 4.26) as opposed to Class 3 (Figure 4.25) without microplastics standards.

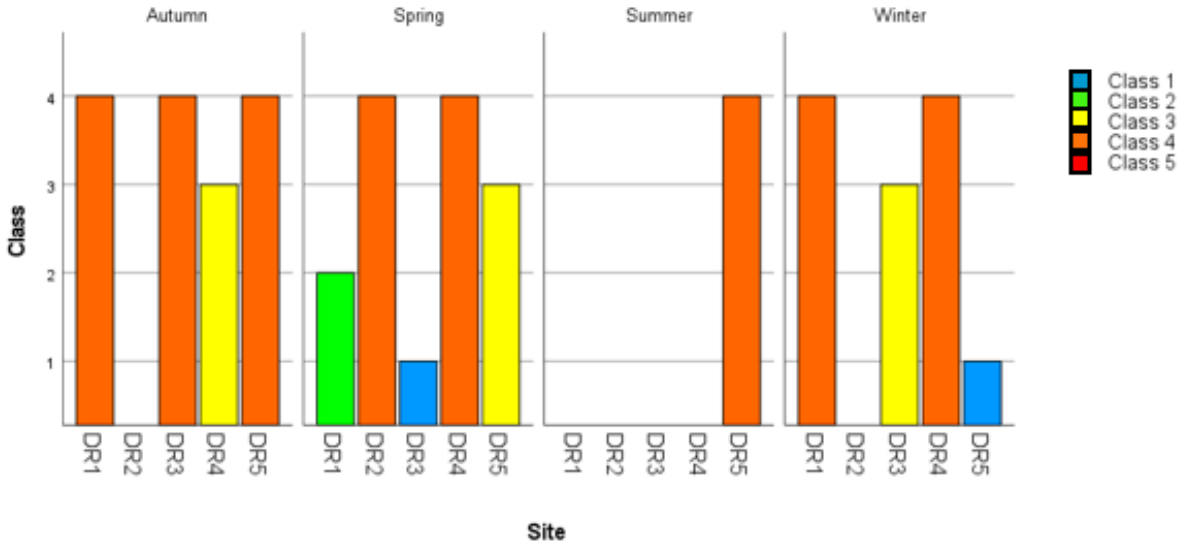

Figure 4.25. Hazard classification classes of different sites along the Diep River over 4 seasons

The weight score reflects the magnitude of the toxicity in each class and is directly proportional to the toxicity of water in that class. The class weight scores for all sites over the four seasons are presented in Table 4.4. In autumn, DR-1 and DR-3 belonged to Class 4 with a class weight score of 33,33% whereas DR-5 belonged to the same class with a higher weight score of 66,67%. 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 33,50% 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 33.00% and 33.33%. DR-4 and DR-5 belonged to Class 3 and Class 2, respectively, with weight scores of 44.33% and 33.50%. 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 perceived twice during the winter season which was at DR 1 and DR 4, with weight scores of 33.33%. DR-3 belonged to Class 3 during winter. However, the weight score was 50.00%, and higher than all sites during this season. Therefore, it can be concluded that this site contained toxic chemicals during the winter season.

Table 4.4. 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	DR-1	1	33,33
Autumn	DR-3	1	33,33
Autumn	DR-4	0,67	33,50
Autumn	DR-5	2	66,67
Spring	DR-1	0,33	33,00
Spring	DR-2	1	33,33
Spring	DR-3	0	0,00
Spring	DR-4	1,33	44,33
Spring	DR-5	0,67	33,50
Summer	DR-5	2	66,67
Winter	DR-1	1	33,33
Winter	DR-3	1	50,00
Winter	DR-4	1	33,33
Winter	DR-5	0	0,00

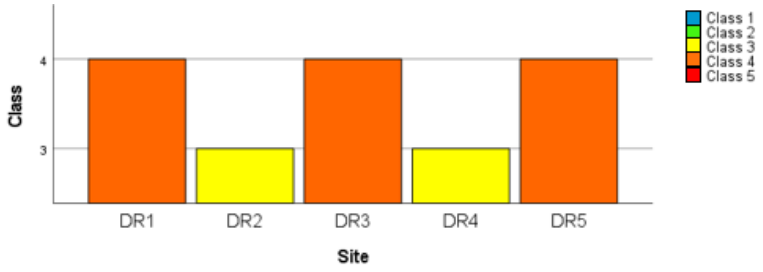

Figure 4.26. Hazard classification classes of different sites along the Diep River spring samples with microplastics

Table 4.5 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 a weight score of 44,33%. DR-5, which also belonged to Class 4, had a high weight score of 55,67%. On the other hand, DR-2 and DR-4 belonged to Class 3, with the highest

weight score of 66,50%. It can be assumed that a combination of the environmental samples with microplastics resulted in a higher toxic response at these sites.

Table 4.5. 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	44,33
DR2	1,33	66,5
DR3	1,33	44,33
DR4	1,33	66,5
DR5	1,67	55,67

4.9.1.3 Microplastic and climatic effect studies

4.9.1.3.1 *Raphidocelis subcapitata* growth inhibition study

In Figure 4.27, growth inhibition of *R. subcapitata* was found to be the greatest at a 1.0 ºC temperature increase (19.40%) compared to a 0.5 ºC (16.20%) temperature increase. Growth inhibition was found to be the least at a temperature increase of 1.5 ºC (11.60%). This suggests that *R. subcapitata* growth was enhanced and thrived at a 1.5 ºC temperature increase in the presence of microplastics. Therefore, it can be concluded that a high temperature in the presence of microplastics may support algal growth and result in formation of algal blooms and eutrophication.

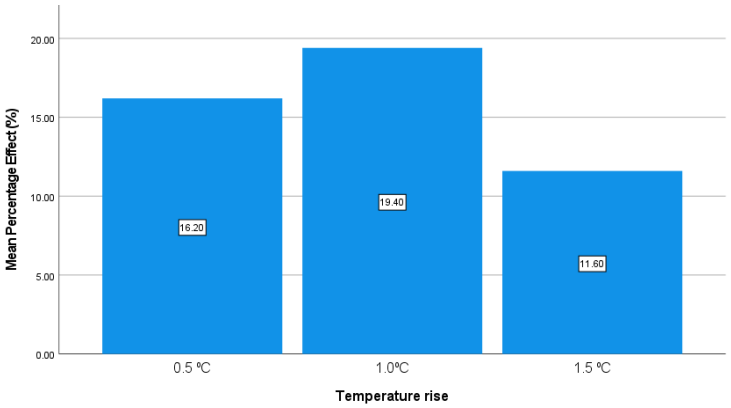

Figure 4.27. Percentage inhibition of R. subcapitata in distilled water with microplastics at three different temperatures

4.9.1.3.2 *Daphnia magna* immobility study

Figure 4.28 demonstrates the effect of temperature rise on *D.magna* in the presence of microplastic. Mortality of *D.magna* was shown to be 40% at a 0.5 ºC temperature increase, 25% at a 1.0 ºC temperature increase and the highest mortality recorded at a 1.5 ºC temperature increase (55%). The results demonstrated that *D.magna* were

the least and most sensitive at a 1.0 ºC and 1.5 ºC temperature increase, correspondingly. Hence, it can be assumed that a high temperature in the presence of microplastics may be detrimental to *D.magna* survival.

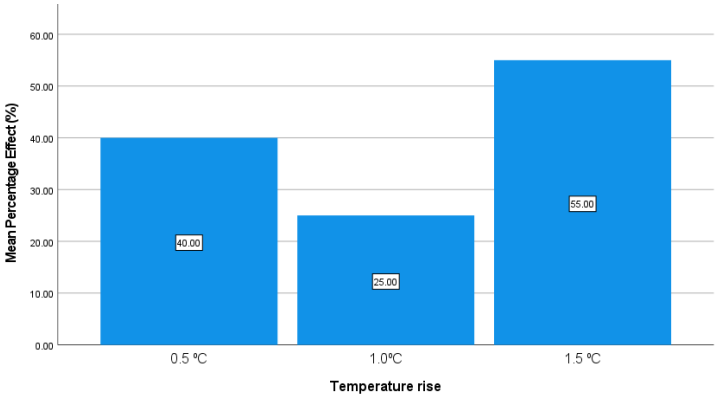

Figure 4.28. Percentage inhibition of crustacean in distilled water with microplastics at three different temperatures

4.9.1.3.3 *Tetrahymena thermophila* growth inhibition study

In Figure 4.29, growth inhibition was observed for *T. thermophila* at the three different temperature changes with virgin microplastics occurrence. Growth inhibition of *T. thermophila* was 66.73%, -17.90%, and 95.56% at a 0.5 ºC, 1.0 ºC and 1.5 ºC temperature increase, respectively. Both cell inhibition and cell proliferation were demonstrated. The results revealed that a high temperature in the presence of microplastic greatly hindered protozan growth.

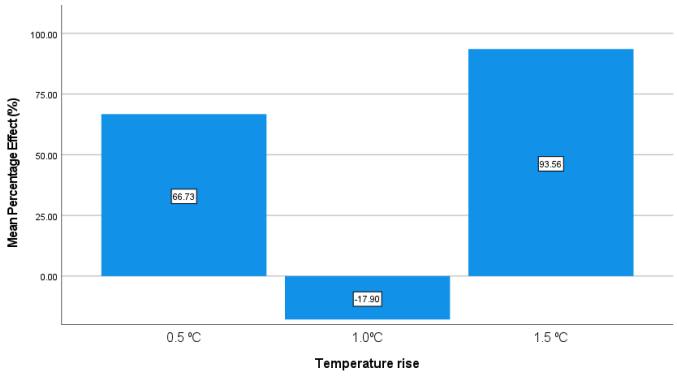

Figure 4.29. Percentage inhibition of protozoa in distilled water with microplastics at three different temperatures

4.9.1.3.4 Acute Hazard Classification of Distilled water with virgin PS-MP

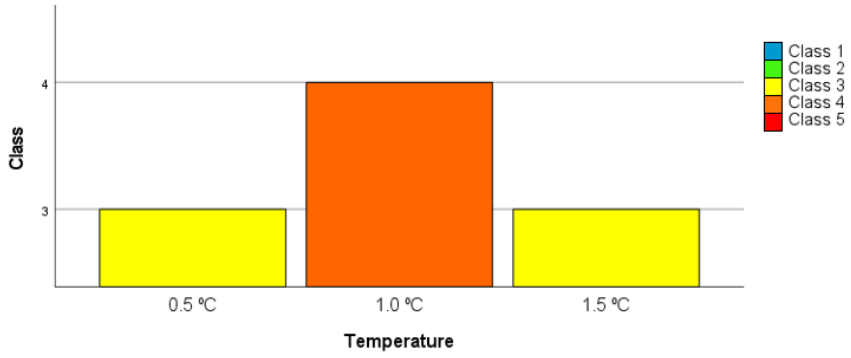

Figure 4.30. Toxicity classification for distilled water with virgin PS-MP

The hazard classification classes in Figure 4.30 shows the effect of the 3 different temperature rise of distilled water with microplastics. Temperature rise of 0.5 ºC and 1.5 ºC belonged to Class 3 whereas a temperature rise of 1.0 ºC was attributed to Class 4 (High acute hazard class).

Table 4.6. 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 %
0,5 ºC	1	50
1 ºC	1,33	44,33
1,5 ºC	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 0.5 ºC, which belonged to Class 3, the weighted score was 50%, whereas with a temperature rise of 1 ºC, the weighted score was 44,33%. Although a temperature rises of 1.5 ºC belonged to Class 3, it had the highest weight score of 66.50%, indicating that an increase in temperature by 1.5 can enhance the toxic effect of microplastics on organisms.

4.10 Genetic Toxicity Testing

4.10.1 Ames Mutagenicity Test 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 (Fowler *et al.*, 2018; Zeiger, 2019). The

investigation of the ability for the Diep River to elicit a mutagenic response by the Ames test was carried out in a 96 well microplate. DR4, the site with the highest load of microplastic pollution, was selected for this experiment. The contents of each plate and number of positive wells with and without S9 are presented in Table 4.7.

Table 4.7. The plate contents and number of positive wells observed in the absence and presence of S9

Plate	Concentration	Bacteria	Day 2	Day 3	Day 4	Day 5	Day 6
Background	-	+	5	9	10	10	14
S9-							
Positive Control	-	+	18	94	96	-	-
Blank (DR4)	100,00%	-	0	5	74	94	94
DR4 a	100%	+	3	41	67	94	95
DR4 b	57.14%	+	8	32	56	85	91
DR4 c	28.57%	+	5	20	60	89	89
S9+							
Positive Control	-	+	52	72	81	93	94
Blank (DR4)	100,00%	-	55	95	96	-	-
DR4 a	100%	+	66	96	-	-	-
DR4 b	51.61%	+	96	-	-	-	-
DR4 c	19.35%	+	96	-	-	-	-

It must be noted that the blank samples are filtered environmental water samples. The results show that constituents in the water itself are mutagenic. The TA 100 *S. typhimurium* strain was effective in producing a mutagenic response. Testing the environmental water samples with bioactivation S9 expanded the detection capability of the assay. Based on the results of this experiment, mutagenic responses were detected with and without bioactivation S9. Therefore, chemicals in the environmental water may be considered a potential mutagen.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter presents the conclusion and recommendations for the research study. This research was set out to evaluate the occurrence of microplastic particles and the potential ecological risks in the Diep River system. It is imperative to assess if the research has achieved the aims and objectives set out in the introductory chapter.

The four objectives were to:

- Quantify microplastics burden of the Diep River.
- Evaluate the spatial and temporal variations of microplastics distribution in Diep River.
- Evaluate the physicochemical characteristics of the Diep River water samples relative to microplastics distribution.
- Assess possible ecological and human health risks of microplastics in the Diep River.

The following conclusions were drawn based on the results obtained from this study.

5.2 Conclusion

This research was set out to evaluate the occurrence of microplastic particles and the potential ecological risks in the Diep River system. The microplastics load and some physicochemical characteristics of the river were assessed to determine water quality and extent of microplastics in water and sediment samples from this study. 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. 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 and pH. Higher values of and 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. The suitability of the waterbody for 3 aquatic organisms at different sites along the Diep River over four seasons was evaluated. 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 enhanced microalgal and crustacean growth recorded at 1.5ºC and 1ºC temperature rise, respectively, 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.

5.3 Recommendations

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.

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. Studies on plastic degradation and coexistence with other pollutants are important to fully understand effects dynamics on exposed biota.

This study also recommends further investigations on the exposure of bioassays to different types and sizes of polymers to better understand potential stressors and associated ecological risks.

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Appendices

Appendix 1. Site 1 (DR-1), opposite an informal settlement and adjacent to farming practices

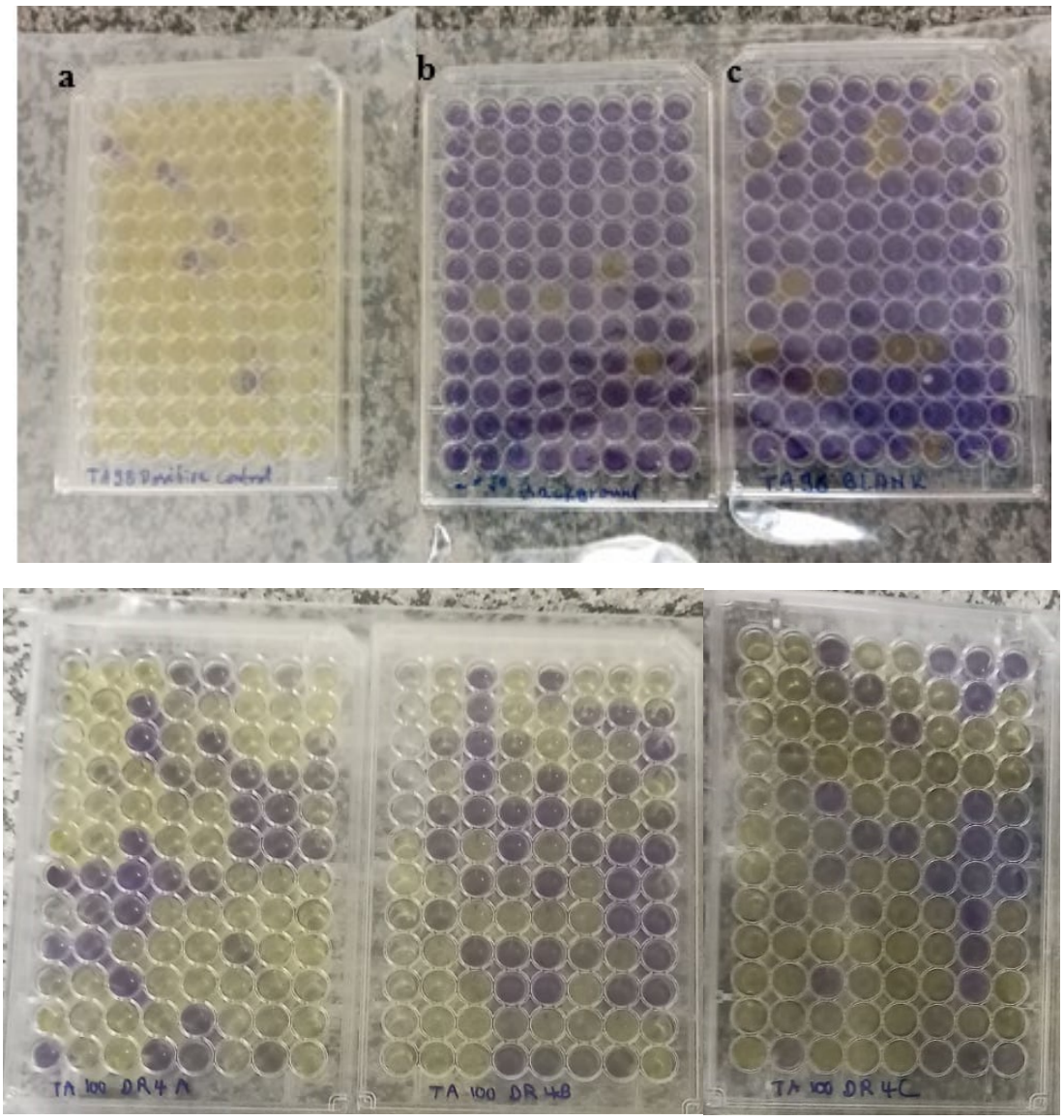
Appendix 2. A site on the Diep River (DR-1) with no water during the summer drought

Appendix 3. Plant cover on the Diep River due to eutrophication at DR- 2 (the site near a nature reserve and residential area)

Appendix 4. Vegetation on the Diep River at DR-4 (the site near a recreational area and in close proximity to a wastewater treatment plant)

Appendix 5. Vegetation removal from DR-4

Appendix 6. Percentage inhibition of algae, *Raphidocelis subcapitata*



Appendix 7. Some AMES mutagenicity test plates showing response after incubation