Ecological and human health risk assessment of microplastics in the Plankenburg River, Stellenbosch, Western Cape

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DECLARATION

I, Komlan Mawuko Apetogbor, declare that the contents of this thesis represent my own unaided work and that the 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 23 / 11 / 2022**

ABSTRACT

Microplastics (MPs) pollution has become a subject of environmental concern due to its ubiquity in the environment. Understanding the problems posed by microplastics is necessary due to their prevalence and persistence in samples of water and sediment taken from the Plankenburg River in South Africa's Western Cape. The aim of this study is to investigate and evaluate the occurrence of microplastics particles with a view to determining the potential ecological and human health risk in Plankenburg River. The physicochemical characterization of the river water was done onsite. The ecological and human health risks of microplastics in the Plankenburg River were conducted in the laboratory. Water samples (10 L) were collected in triplicates and filtered through a 250 µm mesh onsite using a metal bucket. Extraction of MPs from water in the laboratory was by density separation. From the chosen locations, sediment samples were also obtained, oven-dried, and tested for microplastics in the laboratory. Sampling was carried out over four seasons - spring, summer, autumn and winter. Microplastics were classified by visual observation using stereomicroscope and Fourier Transform Infrared Spectroscopy (FTIR-ATR). Test organisms were exposed to the environmental samples, Milli-Q water and polyethylene microspheres in the laboratory and endpoints were measured. The three test organisms used were *Daphnia magna*, *Raphidocelis subcapitata* and *Tetrahymena thermophila*. Primary microplastics, polyethylene microspheres (40-48 µm) were used in the experiment. The genotoxicity of surface water samples was carried out with a mutagenicity test over the abovementioned four seasons. *S.typhimurium* strain TA98 (frameshift mutagen indicator) with metabolic activation (S9 induction by β-naphthoflavone/phenobarbital) mutagenicity assay was used for the investigation. The seasonal distribution of MPs in the surface water samples varied across all sites. However, spring samples had the highest MPs occurrence (5.13 ± 6.62 MPs/L) and the least in autumn (1.52 ± 2.54 MPs/L). MPs in sediment samples were observed in abundance in spring at 1587.50 ± 599.32 MPs/kg. Fibres were the most dominant type of microplastic particles (shape), with a size range of 500–1000 µm at different sites in water and sediment. The infrared spectroscopic analysis confirmed the dominant polymer type to be polyethylene. The selected physicochemical parameters, temperature, pH, dissolved oxygen, electrical conductivity, total dissolved solids, redox potential, and chemical oxygen demand were within the Department of Water Affairs & Forestry (DWAF) or World Health Organization (WHO) guidelines for water quality. However, BOD was not within the regulatory threshold in three seasons (summer, autumn, and winter). No significant correlations were reported between microplastics

distribution and pH, total dissolved oxygen, electrical conductivity, redox potential, temperature, biochemical oxygen demand and chemical oxygen demand. However, there was a strong negative correlation between MPs distributions and dissolved oxygen. The battery of bioassay tests showed a variation in the level of toxicity of river samples over the four seasons. The most sensitive organism for the bioassay experiments using river water samples without virgin PE-MP was *T. thermophila*. The highest toxicity was recorded in summer and autumn, with high acute hazard (class IV) at PR4 and PR2. The simulated climate change experiments showed that an increase of 0.5°C exhibited a similar pattern for all three bioassays. Mutagenicity was observed for the Plankenburg River water samples tested. This is both a human and ecological health concern for human exposure and the ecosystem structure and function.

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DEDICATION

To Jireh, my parents, Mr Ayawovi Apetogbor, Mme Tchotchovi Apetogbor, to my late aunt Seraphine Aku Apetogbor, the entire Apetogbor family and my lovely fiancé Mbha Bayari Charlie Jessica.

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GLOSSARY

Acronym	Definition
BOD	Biochemical oxygen demand
COD	Chemical Oxygen Demand
CPUT	Cape Peninsula University of Technology
DO	Dissolved oxygen
DWAF	Department of Water Affairs & Forestry
EC	Electrical conductivity
ECx	Effect concentration
EDC	Endocrine-disrupting chemicals
FTIR-ATR	Fourier transform infrared
GST	Glutathione S-transferases
HOCs	Hydrophobic organic chemicals
LCx	Effective lethal concentration
LOEC	Lowest observed effect concentration
MPs	Microplastics
NOEC	No observed effect concentration
OD	Optical density
ORP	Oxidation-reduction potential
PE	Polyethylene
PE	Percentage effects
PET	Polyethylene terephthalate
PP	Polypropylene
PR	Plankenburg River

PS	Polystyrene
PVC	Polyvinylchloride
SD	standard deviation
SI	International Standard
TDS	Total dissolved solids
T°C	Temperature
WHO	World Health Organization
WWTP	Wastewater treatment plants

CHAPTER 1:INTRODUCTION

1.1 Background

Anthropogenic activities have been identified as the cause of water quality issues globally. Water pollution is one of the most complex problems of the 21st century due to the various toxic chemical compounds and solid waste released into water bodies. Plastics have numerous benefits within society and registered a production increase from 0.5 million tonnes in 1950 to over 360 million tonnes in 2018 (Plastics Europe, 2019). However, the accumulation of plastic debris in the terrestrial environment is considered one of the contributors to water pollution. The presence of plastics in the environment is currently a priority research area in environmental sciences globally.

Plastic pollution has been reported to affect about 660 marine species. Adverse effects of plastic wastes in aquatic ecosystems include damage to energy intake, hormone secretion, growth rate, and reproductive capacity (Su et al., 2018; Qu et al., 2018). Large plastics waste items undergo fragmentation under physical action, ultraviolet radiation, oxidative properties of the atmosphere and hydrolytic properties of water bodies (Andrady, 2011; Webb et al., 2012; Auta et al., 2017; Xu et al., 2021) and are progressively broken down into smaller particles known as microplastics. Engineered microplastics may also be produced for use in household cleaning and personal care products, among others.

1.2 Research problem

Microplastics pollution has recently become a threat to the aquatic environment due to its wide distribution in freshwater ecosystems. Microplastics can be a source of toxic chemicals or a sink for persistent organic pollutants or metals. Their presence and accumulation have been detected at different trophic levels of the biological chain and raised concerns about adverse effects at molecular, individual, population and community levels. A few studies on microplastic pollution in marine systems have been reported in South Africa. However, there is a dearth of information about microplastic contamination in freshwater systems in the country.

The Plankenburg River flows through different land use practices in Stellenbosch. It receives agricultural pollutant inputs from its source through formal and informal residential neighbourhoods to industrial activities. Although the river is not used for potable activities, it is important for the ecological health of the system as well as possible urban agriculture. Previous studies on the river revealed contamination by organics. This current study provides additional information on the health status of the river. This study, therefore, aims at understanding the MP burden of the Plankenburg River, as well as the ecological and human health risk assessment of MPs in the river.

The Plankenburg River flows through Stellenbosch and Kayamandi township in Western Cape, South Africa. The river receives greywater, polluted stormwater and effluent overflow from Kayamandi and Enkanini Informal Settlement (Infrastructure news, 2020). The largest of these communities and one of the most important sources of the grossly high level of coliform bacterial pollution of the Plankenburg River comes from the township of Kayamandi and Enkanini Informal Settlements. With the slow flow rate of the water during the year, visible solid waste pollution such as plastic bags or bottles accumulate in the riverbed (Barnes J M, 2003). According to reports, the Plankenburg River is severely contaminated by urban overflow from Enkanini, Kayamandi, and other industrial regions. Because there is no formal sewage infrastructure in place, sewage mixes with rainfall and surface runoff flow into the Plankenburg River (Infrastructure News, 2020).

At the point of confluence with the Eerste River, the water is diverted for agricultural use, mainly for the irrigation of crops (Barnes, 2003). Hence, it is essential to study the MPs' contamination and assess their associated risks to the ecological health of the Plankenburg River system. In this work, we will investigate MPs' pollution in the water and sediment samples of the Plankenburg River. The research focus will be on MPs' abundance, distribution patterns, and characteristics, as well as the physicochemical parameters of the river water. The ecological risks of MPs will be evaluated by conducting eco-toxicological tests using bioassays and probabilistic risk assessment models.

1.3 Research questions

1. To what extent is the Plankenburg River contaminated with microplastics?

2. To what extent is the relationship between the physicochemical characteristics of the Plankenburg River water samples and the occurrence of microplastics in the river?
3. What are the potential ecological and human health risks of microplastics in the Plankenburg River system?

1.4 Aims and objectives

The aim of this study is to investigate and evaluate the occurrence of microplastics particles with a view to determining the potential ecological and human health risk in Plankenburg River.

Specific objectives will be to:

1. Evaluate the physicochemical characteristics of the Plankenburg River water samples;
2. Assess the possible occurrence of microplastics in the Plankenburg River;
3. Investigate the spatial and temporal variations of microplastics distribution in the Plankenburg River and
4. Assess possible ecological and human health risks of microplastics in the Plankenburg River.

1.5 Significance of study

This study provides valuable information on the possible occurrence of microplastics in the Plankenburg River. It also provides data on the effects of microplastics on model freshwater organisms and human health. The result of this study provides baseline data for subsequent monitoring of microplastics in freshwater systems for informed decision-making processes.

1.6 Delineation

This study was carried out on the Plankenburg River, Stellenbosch, Western Cape. Due to logistical problems, the mesh size for on-site filtration was limited to 250 µm. *D. magna*, *R. subcapitata*) and *T. thermophila* are the three test models that were used for ecotoxicological assessment. *S.typhimurium* mutagenicity assay was the only health assessment test conducted.

CHAPTER 2:LITERATURE REVIEW

2.1 Classification of microplastics in the aquatic environment

Microplastics are defined as polymers with different densities and are classified into two different types, which include large microplastics ranging from 1 to 5 mm (Figure 2.1.a) and small microplastics from 20 µm to 1 mm (MSFD, 2013; Wagner et al., 2014; Erni-Cassola et al., 2017) in the environment. They occur as macroplastics, mesoplastics, microplastics and nano-plastics in the aquatic environment based on particle size, but there is currently no International Standard (SI) size definition of microplastics (Pagter et al., 2018).

Microplastics can further be categorised into primary and secondary microplastics (Ma et al., 2016; Jingyi Li et al., 2018; Issac & Kandasubramanian, 2021). Primary microplastics are intentionally manufactured in small sizes with small spherical pellets, which are mainly used in textiles, medicines and other personal care products such as facial and body scrubs (Cole et al., 2011; Browne et al., 2013), and pre-production pellets (nurdles) (Storck, F. R. & Kools, 2015; Pereao et al., 2020).

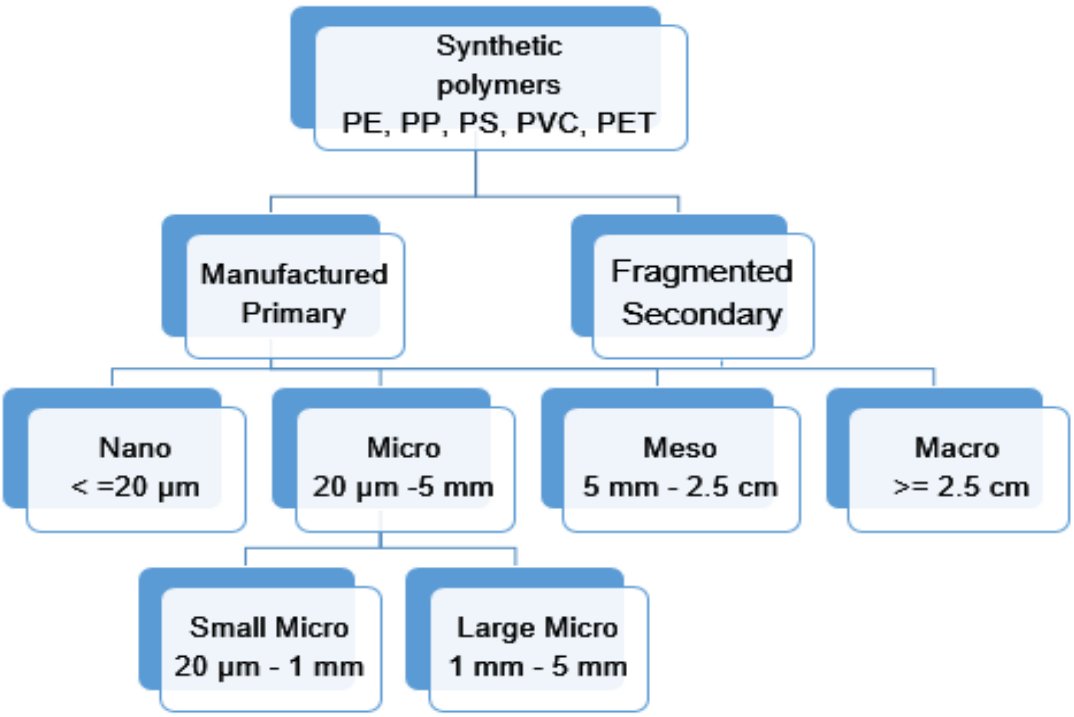

Figure 2.1.1:Size classification of microplastics in environmental samples

Secondary microplastics, such as fibres, fragments, and flakes, are derived from large plastic debris fragmentation due to photo-degradation, physical, chemical and biological interactions (Thompson et al., 2009; Galgani et al., 2013), and from other mechanisms, such as ultraviolet (UV) light and hydrolysis (Guo et al., 2020). Other sources of secondary MPs may be mismanaged plastic litter, industrial resin pellets, washing of synthetic textiles (Browne et al., 2011; Eerkes-Medrano et al., 2015; Boucher & Friot, 2017), tyre debris, and road marking paints (Boucher & Friot, 2017). Eriksen et al. (2013) reported that the majority of microplastics in the freshwater environment are secondary MP, and this number would increase along with an increase in the input of large plastic debris from different origins due to the continuous transformation of secondary microplastics (Cole et al., 2011).

Microplastics are defined by their size, origin, shape, polymer composition, and colour (Wagner et al., 2014; Jiang et al., 2018) and are usually reported as fragments (rounded, angular), pellets (cylinders, disks, spherules), filaments (fibres), and granules (MSFD, 2013) (Figure 2.1.22). The most identified microplastics in the environment are polypropylene (PP), polyethylene (PE, high and low density), Polyvinylchloride (PVC), polyethylene terephthalate (PET) and polystyrene (PS) (Sorolla-Rosario et al., 2022) as well as polyamide fibres (nylon) (Wagner et al., 2014; Fan et al., 2021).

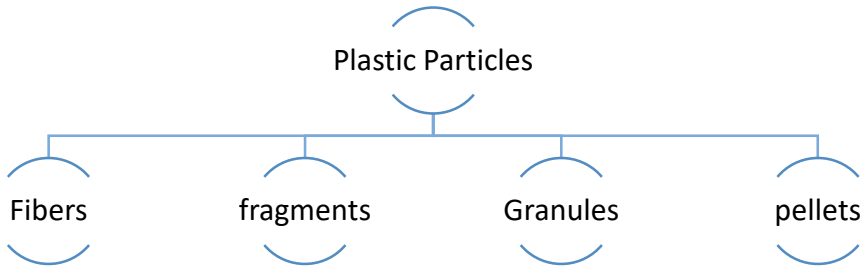

Figure 2.1.2: Morphological classification of microplastics found in environmental samples

2.2 Sources and occurrence of microplastics in the aquatic environment

Plastics increasingly constitute the majority of marine waste and represent a serious threat to aquatic ecosystems (Green, 2016). According to Hu *et al.* (2019), microplastics are ubiquitous

in the environment, frequently utilized, reasonably affordable, and durable but persistent in the environment (Lin *et al.*, 2020). They get into rivers through the wind, storm sewers and wastewater treatment plants. Rivers are sinks for microplastics since most wastewater treatment plants do not effectively remove microplastics from effluents. Plastic waste ubiquity in marine and the freshwater environment has detrimental impacts on the ecological systems, biodiversity and human health.

Several studies on microplastics have been conducted in the marine environment but are still limited to the freshwater system in Africa and, specifically, South Africa (Nel et al., 2018; Migwi et al., 2020; Weideman et al., 2020a). Most plastics are manufactured, used and deposited in the terrestrial environment, with rivers as a primary pathway (source) of microplastics to the ocean (Horton, 2017). Mani et al. (2015) identified the closeness of urbanisation to rivers as sources of microplastics from activities (effluent discharge, road runoff, littering and atmospheric deposition) to aquatic ecosystems. The most significant source of freshwater pollution is wastewater effluent from sewage treatment plants and runoff from road surfaces caused by the breakdown of road markings and tyre debris (Eriksen et al., 2013; Horton, 2017).

Wastewater treatment plants (WWTPs) are an important point source of freshwater environmental secondary microplastics due to domestic waste (in the form of microbeads from personal care products and microfibers from the laundry), industrial input and stormwater (Leslie et al., 2017; Mintenig et al., 2017). WWTPs are reasonably effective at removing between 96 – 99.9% of microplastics (Ziajahromi, Neale, et al., 2017; Talvitie et al., 2017) from the wastewater stream and tertiary treatment can also remove some fine particles of a size larger than 10 µm (Wardrop et al., 2016). Ineffectively removed microplastic particles will still be discharged into the freshwater ecosystem. Microplastic particles removed from wastewater are retained in the sludge, which more plastics than the effluent (Magnusson & Norén, 2014; Mintenig et al., 2017; Rasmussen et al., 2021).

WWTP sludge is considered a source of microplastic pollution when used as agricultural land fertilizer or deposited on landfills via surface runoff or drainage. Microplastics may be transported to water bodies (rivers, lakes and ultimately river basins) and into the marine system (Wagner et al., 2014; Leslie et al., 2017). Microplastics also enter rivers through storm drainage systems during storm events and periods of high rainfall, allowing runoff from roads and urban areas to enter directly into the river.

During heavy rainfall, microplastics enter rivers through combined sewage overflows designed to discharge untreated sewage overflow directly into the river Litter represent another source of microplastics to the rivers, either directly into the water or washed in from the bank or surrounding land (Horton, 2017). The study of Weideman et al. (2020b) on litter loads in urban stormwater run-off from an urbanised city (Cape Town) in South Africa showed a vast variation in the number of plastics loads in stormwater.

Microplastic burden from different sources varies and may account for their presence in the environment. For instance, the industrial area contributes about 78%, commercial 49% and residential 40% which established industrial areas as a source of plastics pollution. The fragmentation of these plastics into secondary microplastics represents a large amount of microplastics transported to and in freshwater systems. The microplastic pollution burden in aquatic ecosystems continues to grow with the increasing production and consumption of plastic materials.

The presence of microplastic is worrying due to the potential threats to the suitability of water for human use (Bouwman et al., 2018). Several studies have been conducted on MP pollution in marine and freshwater ecosystems to understand the occurrence of microplastics in water bodies.

Scientists have become interested in marine environmental microplastics (Maryani & Wibowo, 2020), which prompted the demand for knowledge on the impact of microplastics as marine pollutants (Lusher, 2015). The presence of microplastics was reported globally in all marine systems, oceans, from the Arctic to the Antarctic, water columns and seabed sediments, and at sea surfaces (Claessens et al., 2013; Lusher, 2015; Abayomi et al., 2017; Isobe et al., 2017; C. Zhang et al., 2017). Sharma & Chatterjee, (2017) reported the accumulation and persistence of microplastics in the Mediterranean Sea in the range of 1.000 and 3.000 tonnes. According to Law et al. (2010), wind current and geostrophic circulation were responsible for the distribution and accumulation of MPs. Turbulence and oceanographic effects (Ballent et al., 2012; Turra et al., 2014) accounted for the plastic pollution load of marine ecosystems.

Microplastics are potentially bioavailable to various organisms like zooplankton, lobsters, worms, fishes, birds and mammals; the ingestion of MPSs by organisms is a concern due to their potential for increased bioaccumulation with decreasing size (Wright et al., 2013a; Sharma & Chatterjee, 2017). Freshwater is a primary human need for survival; it was identified

by Lebreton et al. (2017) and Schmidt et al. (2017) as one of the pathways to marine microplastic pollution. Horton, (2019) reported microplastics in different freshwater ecosystems, rivers and lakes. The freshwater hydrologic system (flow rate, depth and topography) contributes to the accumulation of microplastics in freshwater ecosystems (Tibbetts et al., 2018), which leads to more questions about microplastics source apportionment and occurrence of ecological adverse effects in the freshwater systems.

The variation in occurrence and abundance of microplastics in freshwater systems is dependent on sampling points, anthropogenic activities, sampling approaches, the water flow rate (Eerkes-Medrano et al., 2015; Jia Li et al., 2018), water surface area, depth, wind, currents and density of particles (Eriksen et al., 2013; Fischer et al., 2016). Weideman et al. (2020a) investigation reported variable values for microfiber presence in wet and dry seasons, suggesting that seasons might play an influential role in microplastic abundance and occurrence through water flow in the river system. Microplastic particles were found to be 65% higher in the populated urban section of the River Tame compared to populated rural sites of the River Tame (Tibbetts et al., 2018). However, Weideman et al. (2020a) reported the abundance of microplastics in the upper, middle and lower reaches of the Orange-Vaal River in South Africa. Microplastics have been identified in surface water samples from the Yongjiang River (China) with an average concentration of 2345 ± 1858 particles per cubic metre (n/m^3). The midstream of the Yongjiang river had an average concentration of 3675 ± 2361 n/m^3 microplastics particles while upstream and downstream had 1300 ± 477 n/m^3 and 1617 ± 560 n/m^3, respectively (Zhang et al., 2019).

Studies of freshwater microplastics have increased rapidly, indicating their importance to the water sector. Reported microplastic values in freshwater ecosystems are equivalent to the observed amounts in the marine environment (Peng et al., 2017; Ma, Wang, et al., 2019) , with a highly heterogeneous distribution in different areas (Wagner & Lambert, 2018; Jingyi Li et al., 2018) as shown in Table 2.2.1.

Table 2.2.1: Examples of microplastics distributions in the freshwater systems

Location	Sampling Point/Region	MP Occurrence Average/range	References
Yongjiang River, South China	Midstream	3675 ± 2361 n/m^3	(Zhang et al., 2020)
	Upstream	1300 ± 477 n/m^3	

	Downstream	1617 ± 560 n/m^3	
Mega-cities, Shanghai, China	Park, Caohejing river	1535 ± 771 kg^{-1}/ dw	(Peng et al., 2018)
	Residential, Beishagang river	1600 ± 191 kg^{-1}/ dw	
	Rural, Jiangjiagang river	1120 ± 56 kg^{-1}/ dw	
	Park, Yujiabang river	410 ± 127 kg^{-1}/ dw	
River Tame, Birmingham, UK	urban section, River Tame	350 particles/ kg^{-1}	(Tibbetts et al., 2018)
	rural section, River Tame	20 particles/ kg^{-1}	
Naivasha Lake, Kenya	Malewa River mouth	0.633± 0.067 particles/m^2	(Migwi et al., 2020)
	Hippo Point	≅ 0.17±0.2 particles/m^2	
River Kelvin sediment, UK	SE1 (December 17, 2015)	220±448 items /kg dw	(Blair et al., 2019)
	SE2 (February 15, 2016)	161±432 items /kg dw	
Poyang Lake sediment, China	Upstream reaches of Raohe	3153 items/kg dw	(Liu et al., 2019)
	Najishan National Nature Reserve	11 items/kg dw	
Rhine-Main, Germany	R3 (Mainz-Kastel)	30106 particles/m^2	(Klein et al., 2015)
	R6 (Walluf)	1784 particles/m^2	
Kallavesi Lake, Finland	Site 7 (city harbour)	0.66 MPs/m^3	(Uurasjärvi et al., 2020)
	Site 2 (highway bridge)	0.037 MPs/m^3	

Great Lakes, US	Detroit River plume	1,910,562 particles km^{-2}	(Cable et al., 2017)
	Lake Huron	126,933 particles km^{-2}	
Subalpine Lakes, Italy	Lake Iseo	40000 particles/km^2	(Sighicelli et al., 2018)
	Lake Maggiore	39000 particles/km^2	
	Garda Lake	25000 particles/km^2	
Orange- Vaal Rivers, SA	Upper Orange (Wet/ Dry)	0.6 ± 0.4 N. L^{-1}/ 1.0±1.2 N. L^{-1}	(Weideman, et al., 2020)
	Lower Orange (Wet/ Dry)	17.1±17.4 N. L^{-1}/ 1.3±1.3N. L^{-1}	
	Upper Vaal (Wet/ Dry)	0.4 ± 0.3 N. L^{-1}/ 2.3±3.2 N. L^{-1}	
	Lower Vaal (Wet/ Dry)	0.7 ± 0.7 N. L^{-1}/ 0.8±0.8 N. L^{-1}	

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

2.3 Effects of plastic pollution in aquatic systems

Plastic products are widespread in the environmental matrices with wide application in industrial production systems, commercial entities, and domestic products. Lozoyaa et al. (2015) reported that plastics are widely used in many products. The prevalence of microplastics

and their detrimental effects on the natural system emphasize the need for further knowledge on risk assessment. However, there is very little and limited study on the ecotoxicology of microplastics in freshwater and their exposure to the environment (Zhang et al., 2020). Plastic pollution in an aquatic ecosystem may exert either physical stress or biochemical processes disruption through leachate of chemicals additives of the breakdown of the plastic polymer

Adverse effects of plastic wastes in aquatic ecosystems include damage to energy intake, hormone secretion, growth rate, and reproductive capacity (Su et al., 2018; Qu et al., 2018). They have been identified as Endocrine-disrupting chemicals (EDCs) and may also cause choking, internal or external wounds, ulceration, blocked digestive tracts, false sense of satiation, debilitation and death (Eerkes-Medrano et al., 2015). Negative consequences of plastics include adverse effects on aquatic organisms, bioaccumulation with resulting bio-magnification in the food web, and loss of aesthetic value of water bodies, among others. Land-based plastic wastes were also reported to contribute to the plastic pollution burden of aquatic systems (Lozoyaa et al., 2015).

Several studies in the marine environment have reported ingestion of microplastics in the range of 250-1000 µm by the Wild Gudgeon fish (Sanchez et al., 2014) and by fish larvae (Steer et al., 2017). The ingestion of microplastics by marine organisms may cause physical and chemical damage to organisms (Zhao et al., 2017); it may affect growth, mortality rate, metabolism, reproduction and health (Von Moos et al., 2012; Lusher et al., 2013; Xu et al., 2017). Bioaccumulation of microplastics in the food chain could further affect human health as the highest trophic level consumer (Barboza et al., 2018; Wang et al., 2018).

It was widely reported that microplastics are ingested by some aquatic species (Horton, 2017; Anbumani & Kakkar, 2018), such as pelagic fish (Rummel et al., 2016), shrimp, mussels, polychaete larvae and ciliates (Setälä et al., 2016), and invertebrates, as well as, filter feeders, lugworms and detritivores (Besseling et al., 2013). Ingested microplastics may translocate to tissue and liver, causing adverse effects such as inflammation and lipid accumulation (Lu et al., 2016), reduced growth (Au et al., 2015), immobilisation (Rehse et al., 2016), and mortality (Jemec et al., 2016). Scherer and co-workers reported that 39 freshwater species (4 species of fish and 35 species of invertebrate) ingested microplastics in their study (Figure 2.3.1) (Scherer et al., 2018). Other studies made similar observations for *Tubificid worms, Gammarus pulex* and *Hyalella Azteca* (Hurley et al., 2017; Redondo-Hasselerharm et al., 2018; Weber et al., 2018).

According to Farrell & Nelson, (2013); Setälä et al. (2014); and Remy et al., (2015), microplastics can be ingested and transferred to all food chains, where they can bioaccumulate, affect the gastrointestinal tract (Gall & Thompson, 2015), or transport toxic substances and toxic metals (Andrady, 2011). They can also act as a vector of microbial and pathogen transport to the environment (Zettler et al., 2013). Organisms that have ingested microplastics may experience some physical harm, blockage of the gut, internal or external abrasion or inflammation or suffocation caused by blockage of gills (Von Moos et al., 2012; Wright et al., 2013a).

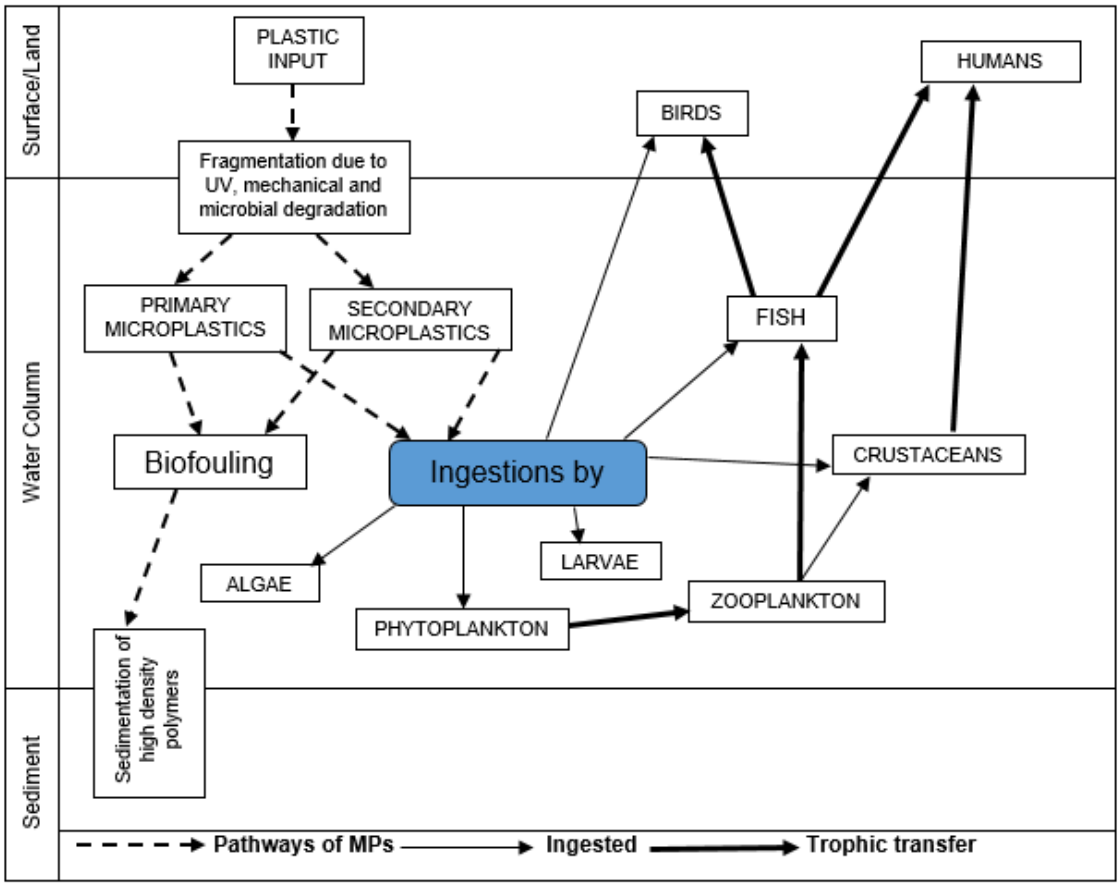

Figure 2.3.1: Microplastics ingestion by freshwater organisms

Most studies on the effects of microplastics on aquatic ecosystems are on marine organisms, but there is a dearth of information on the microplastic's fate in freshwater ecosystems (Elizalde-Velázquez et al., 2020). *D. magna* has been observed to ingest microplastics (Besseling et al., 2014; Rehse et al., 2016), at a high rate under laboratory conditions (Rosenkranz et al., 2009). According to Horton, (2017), consumption can take place by direct ingestion from water or prey that ingested microplastics and accumulated along food chains (Cole et al., 2013; Farrell & Nelson, 2013; Setälä et al., 2014).

The ingestion and transfer of microplastics may harm ecosystems and human health (Wright et al., 2013a) because microplastics may leach and transfer toxic additives (Phthalates, nonylphenol, polybrominated diphenyl ethers (PBDE)), and adsorbed persistent organic pollutants (POPs) from the environment to the biota (Anbumani & Kakkar, 2018; Prinz, 2020). Microplastic ingestion may also release pollutants initially sorbed on the surface of microplastics with potential availability to human beings through bioaccumulation and bio-magnification, often exacerbated at low pH and high-temperature values (Jingyi Li et al., 2018).

Another study showed that the desorption rate of sorbed contaminants in organisms was accelerated when compared to the marine system (Bakir et al., 2014). Until recently, studies were mostly focused on freshwater organisms' ecological toxicity of microplastics exposure with little information on toxicological effects (Wagner et al., 2014; Eerkes-Medrano et al., 2015; Ma, Wei Wang, et al., 2019). The toxicity impacts of microplastics on freshwater organisms are manifested by morphological damage, clogging of the intestinal tract, and reduction of nutrients. These impact the growth and development of freshwater biota, feeding rate, fecundity capacity and gene expression (Jingyi Li et al., 2018).

Ingested microplastics may harm freshwater organisms similarly to marine organisms (Wright et al., 2013a; Scherer et al., 2018) with comparable food acquisition strategies (Eerkes-Medrano et al., 2015; Ziajahromi, Kumar, et al., 2017). Lithner et al. (2011) with comparable food acquisition strategies.

Studies on freshwater species exposed to microplastics showed some adverse effects. For example, Au et al. (2015) investigated the exposure of the freshwater amphipod *Hyalella Azteca* to polypropylene fibre and polyethylene particles. Reported chronic exposure of *Hyalella azteca* to microplastic (10 µm polyethylene particles) included damaged digestive function leading to a decrease in growth and reproduction. Zebrafish (*Danio rerio*) and nematode (*Caenorhabditis elegans*) exposure to microplastic particles led to an accumulation in the gills, liver, and gut (Lu et al., 2016), intestinal damage (Ma et al., 2019) and the inflammation of the liver (Lu et al., 2016). Quinn et al. (2017), found that microplastics (polyethylene flakes) can accumulate in the gut of freshwater *Cnidarian Hydra* attenuate and reduce food intake. Similarly, after 48 h of exposure of daphnids to microfibers from textile weathering and washing, 300 and 1400 µm synthetic fibres were ingested and found in the gut of daphnids.

Mortality was observed only in daphnids that did not feed on algae before exposure, while no lethality was found in daphnids fed with algae (Anbumani & Kakkar, 2018). The mortality recorded following exposure to daphnids could be due to a clogging effect in the intestinal part instead of the release of chemicals from the fibres. Although the water body was more polluted with secondary microplastics, primary microplastics were used in the laboratory for exposure studies (Phuong et al., 2016; Connors et al., 2017; Potthoff et al., 2017). Ogonowski et al. (2016) compared the primary and secondary microplastics' chronic exposure to *D. magna* based on their feeding and reproductive performance, and they noted that there was a significant reproduction impact on *D. magna* only when exposed to secondary microplastics.

Lei et al. (2018) work on the water column and sediment-dwelling worm's exposure to microplastics showed significant inhibition of the survival, growth and reproduction rate and an increase in Glutathione S-transferases (GST) enzyme levels. The primary effects in the sediment-dwelling worm were caused by a microplastic particle that induced oxidative stress. Microplastics can release addictive toxic substances into the aquatic environment with potential antagonistic or synergistic effects with other chemical pollutants present that can lead to toxicological effects on the ecosystem (Fonte et al., 2016; Ma et al., 2016).

However, Ma et al. (2019) noted that there is a dearth of toxicity research on freshwater organisms relative to marine species. The ingestion of the combination of microplastics (polyethylene) pellets and organic pollutants in water by Japanese medaka (*Oryzias latipes*) induced hepatotoxicity (liver cell damage) (Oliveira et al., 2013; Rochman et al., 2013a) and genetic damage to (Rochman et al., 2014). Furthermore, the exposure of *Clarias gariepinus* to a combination of microplastics and phenanthrene was associated with inhibition of protein synthesis (Karami et al., 2016).

Hydrophobic organic chemicals (HOCs) in combination with microplastics have been indicated to have a toxicity effect on aquatic ecosystems due to greater bioavailability to organisms (Rochman et al., 2013b; Avio et al., 2015; Chen et al., 2018). HOCs are removed from the water by their strong binding to microplastics, but they do not enter living things when consumed. Furthermore, clean microplastic ingestion can absorb HOCs from the body tissues of a previously contaminated organism (Koelmans et al., 2013). It is also important for further study to investigate the type of microplastic polymers that can absorb HOCs efficiently from the body tissues. The exposure conditions laboratory studies on microplastic pollution are often

unrealistic relative to actual exposure conditions in the environment. Hence, more studies need to be done better understand the effects of exposure of freshwater organisms to microplastics.

Biological concerns over microplastics may be exacerbated by microorganisms that form biofilms (Ma et al., 2019); biofilms may quickly adhere to the surface of microplastics (Ivleva et al., 2017), thereby changing the partitioning and availability in the environment. Microplastics may be deposited underwater to form composites with sediment due to the change of physical properties, increased density and the reduction of hydrophobicity caused by the biofilms (Zettler et al., 2013; Ivleva et al., 2017). Ma et al. (2019) reported the presence of several types of microbes on microplastics, such as harmful dinoflagellates *Ostreopsis sp.*, *Coolia sp.*, *Alexandrium sp*. These pathogens carried by biofilms may pollute freshwater ecosystems (McCormick et al., 2016) via geographical transfer. The entry of microplastics into freshwater systems has been shown to pose ecological health risks (Wright et al., 2013b; Besseling et al., 2014). Hence, biofilm communities' geographical transfer of microorganisms attached to microplastics and their interaction with persistent organic pollutants are still not understood.

CHAPTER 3: MATERIALS AND METHODS

3.1Study area

The Plankenburg River is approximately 10 km long and provides ecosystem services for residential, agricultural, and industrial sectors (clothing factory, cheese factory, wineries, and dairy factories) and agricultural activities (irrigation of crops) of the area. The river runs through Stellenbosch (Western Cape Province), popular for its wine estates and the fourth largest province in South Africa with a geographical location lying approximately on latitude and longitude coordinates of 33° 56′ 12″ S, 18° 51′ 41″E (Udebuani et al., 2021). It runs adjacent to the Kayamandi Informal Settlement, which comprises a population of over 24.645 inhabitants, and the township lacks proper sanitation (Alegbeleye et al., 2016). Stormwater and sewage pipes from the settlement drain into the river, this reduces the water volume and quality rendering it unsafe for domestic, agricultural, and recreational purposes. The sampling points were selected based on possible routes of microplastics entry, including human and industrial activities (Figure 3.1.1). Table 3.1.1 shows the four sampling sites GIS Coordinates:

Table 0.1: GIS Coordinates of the Plankenburg River sampling sites

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

Figure 0.1: Sampling sites on the Plankenburg River

3.2Water and sediment sampling

Onsite water sampling

Water samples were collected in glass jars with a 2.5 L storage capacity, pre-cleaned by washing with non-ionic detergents, washed with tap water, treated with hydrochloric acid (1:1), and then treated with Milli-Q water. The bottles were cleaned three times with sample water before sampling. Sampling was carried out by submerging each sample bottle beneath the water's surface and directing the mouth of the bottle opposite to the flow. The samples were transferred to the Environmental Toxicology laboratory at the Cape Peninsula University of Technology in coolers with ice and stored in the dark in the refrigerator (+4 °C) before chemical analyses and performance of bioassays.

Onsite and laboratory water quality parameters measurement

Temperature (T°C), pH, total dissolved solids (TDS), redox potentials (ORP), dissolved oxygen (DO), and electrical conductivity (EC) were measured in situ at each sampling location using multi-parameter equipment (SensoDirect 150, Lovibond® Water testing and Tintometer® group, Germany). All in-situ measurements were repeated in the laboratory the day after sampling. Other physicochemical parameters like chemical oxygen demand (COD) and

Biochemical oxygen demand (BOD) were measured in the laboratory within 24 h using the Photometer-System MD 100 (Lovibond® Water testing and Tintometer® group, Germany) and BOD-System BD 600 (Lovibond® Water testing and Tintometer® group, Germany) respectively.

3.2.3 Water sampling for microplastics analyses

Sampling was undertaken over four seasons (summer, spring, autumn, and winter). Three replicates of 10 L water samples were filtered through a 250 μm stainless steel sieves onsite in each of the selected sampling locations. The metal bucket was immersed below the surface water opposite the water flow direction. The residual particles on the sieve were transferred and stored in small glass jars and taken to the laboratory for further analysis. At each sampling site, the metal bucket and stainless sieve were cleaned carefully with Milli-Q water to reduce cross-contamination and rinsed thoroughly with water from the site prior to sampling. A 12 L sample of surface water was collected at each sampling site, transported to the laboratory for further analysis, and filtered through a 20 μm stainless steel sieve. The 12 L brought into the laboratory was filtered in three replicates of 4 L per site.

3.24 Sediment sampling for microplastics analyses

A 100 g sediment sample was collected using metal scoops to approximately 5 cm depth at each sampling site to achieve three replicates, 2 m apart, which were randomly collected and pooled as one sample per site. Sediment samples were individually stored in sealed plastic bags, placed in an ice chest, and transported to the Cape Peninsula University of Technology Microplastics Laboratory. Samples were oven-dried at 50^{o}C for 24 h and covered with aluminium foil to avoid contamination.

3.3 Microplastics extraction by density separation

3.3.1 Microplastics extraction from water samples

The collected samples were processed to isolate the microplastics based on the adapted method from GESAMP, (2019). MPs were extracted from the 12 L offsite sample and taken to the laboratory by filtration through a vacuum pump system with 20 μm mesh. Alkali digestion was used to degrade organic matter before the extraction process (Maes et al., 2017). Residues of samples processed onsite were stored in glass jars and labelled appropriately. The residues were

transferred into a 500 ml glass beaker with 10% Potassium hydroxide (KOH) and covered with aluminium foil for 24 h in the oven at a temperature of 50°C. Hypersaline solutions (NaCl 360 $g \cdot \ell^{-1}$) were added to the digested sample, stirred vigorously for 2 min, allowed to settle for 15 min and then filtered through a vacuum pump system with 20 µm mesh. For each sample, this extraction procedure was carried out three times. The filters were then placed on fresh Petri dishes for additional examination. The density fractionation approach, as described by Di & Wang, (2018) with a few minor modifications, was used to recover microplastics from sediment samples.

3.1.1 Microplastics extraction from sediment samples

Sediment samples were dried in the oven at 50 °C for 48–72 h to constant weight, then 20 g of dry sediment from each replicate was transferred into a 500 ml glass beaker with 10% KOH and placed in the oven at 50°C for 24 h. Saturated NaCl solution (360 $g \cdot \ell^{-1}$) was added to the digested sample, which was then stirred with a clean stainless spoon for 2 min and allowed to settle for 30 min. Dried sediment in the beaker was always covered with aluminium foil to avoid air-bone microplastic contamination. The supernatant of the solution containing floating particles were vacuum filtered through a 20 µm nylon mesh pre-washed with Milli-Q water, which was repeated three times for each sample. For additional investigation, the mesh was place in sterile Petri dishes.

3.2 Microplastics identification and quantification

The dried meshes in the pre-cleaned petri dishes were examined for microplastics based on physical appearance under a stereomicroscope (BS-3060CT, Bestscope, China). Particles were categorised based on their morphological characteristics (size, shape, and colour) as previously described by Hidalgo-Ruz et al. (2012) and Masura et al. (2015). Microplastics were classified and measured using a microscope by their sizes into six categories: <63 µm, 63 - 500 µm, 500 - 1000 µm, 1000 – 2000 µm, 2000 – 5000 µm and >5000 µm. Particles were classified based on their shapes (fibre, fragment, Sphere and film) according to Baldwin et al. (2016) and Zhang et al. (2017). All suspected microplastic particles were photographed using a BestScope BHC3E-1080P HDMI Digital Camera (China) connected to the microscope. Microplastics abundance and mass were recorded in terms of MPs/L and MPs/Kg dry sediment. A minimum of 10% of randomly selected microplastic particles larger than 500 µm were analysed using Fourier Transform Infrared (FTIR-ATR). A spectroscopy Perkin Elmer Two FTIR-ATR

Spectrometer system was used to analyse microplastic particles. With a resolution of 4 cm^{-1} and a data interval of 1 cm^{-1}, spectra in the wavenumber range of 5000 to 450 cm^{-1} were captured. Before each sample analysis, background scans were performed, and the ATR crystal was cleaned with 70% propanol before usage. Using a pair of fine tweezers and a force of at least 80 N, particles (Filaments and fragments) >500 µm in size were squeezed against the diamond head. Polymer identification was done by comparing spectral scans with the ST Japan Library and a Perkin spectral library provided by the supplier (Perkin Elmer).

3.3 Exposure studies using environmental water samples and microplastics microspheres under climate-changing conditions

3.3.1 Microplastics particles stock preparation for bioassay experiments

Studies have shown that MPs particles are commonly ingested by *D. magna,* as they represent a similar size range of their food (Rehse et al., 2016; Rist et al., 2017; Jaikumar et al., 2019). Polyethylene microspheres of size range 40-48 µm with a density of 0.94 g/mL purchased from Sigma-Aldrich were used for microplastics exposure studies. A stock suspension of 1000 mg/L of microplastics was prepared with distilled water. The stock solution was agitated in a shaker (NUVE BM 30, Turkey) for 2 h at 150 rpm and kept in storage for a week at room temperature. The stock solution was diluted with distilled water to the final concentrations of 400, 200, 100 and 20 mg/L.

3.3.2 *Raphidocelis subcapitata* 72 h growth inhibition test

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

inhibition of *R. subcapitata* after exposure to water samples and microplastics contaminated water. Data analysis was performed using ToxRat® Professional software to determine toxicity endpoints.

3.3.3 *Daphnia magna* 48 h acute immobility test

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

3.3.4 *Tetrahymena thermophila* 24 h chronic toxicity test

A short-term assessment of chronic toxicity was conducted using *T. thermophile* –a freshwater ciliate protozoa obtained as Protoxkit FTM (Microbiotest Inc., Belgium). The Protoxkit assay is a 24 h multi-generation growth test that covers 5–6 generations. The experiment depends on the conversion of substrate into ciliate biomass. The proliferating cell cultures cleared the substrate suspension while the growth inhibited culture remained turbid. The optical density measurement of the turbidity using a spectrophotometer (Jenway 6300) at a wavelength of 440 nm provided information on the degree of inhibition. Sample dilution series were prepared according to standard procedures. There were six treatments, control and each treatment had two replicates. Holding trays of experimental cells were incubated in darkness at 30°C for 24 h. Experiments with temperature increases of 0.5ºC,1ºC and 1.5ºC were also conducted to assess climate change variabilities. Optical densities were measured at the beginning and at the end of the experiment.

3.3.5 Toxicity evaluation of Plankenburg River water samples and microplastics standards suspension

Toxicity and lethal concentration/effect concentration (LC/EC) values are inversely related, and the percentage effects (PE) are used to describe concentration-based toxicity measures. According to Kaza et al. (2007) and , the data for toxicity of non-diluted river water samples have been expressed as PE of mortality or inhibition of growth and reproduction, depending on the effect criterion of the respective test procedure scoring system. Acute hazard is used to express acute hazard of concentration-based toxicities. According to Persoone et al. (2003), the Acute hazard classification system includes no acute hazard (class I) when PE ≤20%; class II when slight acute hazard 20% ≤ PE < 50%; class III when acute hazard 50% ≤ PE < 100%; class IV when high acute hazard in at least one test PE = 100% and class V PE = 100% very high acute hazard in all tests. Class weight scores were evaluated by the allocation of a test score for the effective results of each test of the battery according to equations 1 and 2 (Kaza et al., 2007; Szklarek et al., 2021).

a) Calculation of weight scores

Score 0 = No significant toxic effect, PE ≤ 20%
Score 1 = Significant toxic effect, 20% ≤ PE < 50%
Score 2 = Toxic effect, 50% ≤ PE < 100%
Score 3 = 100% = PE

b) Calculation of the class weight score

Class weight score = $\sum all\ test\ scores/n$ ……...equation 1

n = is the number of tests performed

c) Calculation of the class weight score as a percentage

% Class weight score = Class score/maximum class weight score x 100……...equation 2.

3.4 Human health risk assessment

3.4.1 Ames fluctuation test

Ames test was performed based on Ubomba-Jaswa et al. (2010) method using the bacteria MutaChromoPlateTM (EBPI Inc., Mississauga, Ontario, Canada). The tester *S. typhimurium*

strain TA98 +S9 and strain –S9 mix were conducted. Lyophilised bacteria were transferred into the nutrient broth and grown overnight for 16 to 18h. The liquid reaction medium consisted of Davis-Mingioli salts, D-glucose, D-biotin, L-histidine and bromocresol purple, sterile distilled water and *S. typhimurium* TA 98. The S9 mix consisted of MgCl2 + KCL solution, Glucose-6-phosphate, NADP, Phosphate buffer, sterile water and S9 fraction (hydrate with 2.1ml of sterile H2O). River water samples were added to the reaction medium and to the S9 mix (strain TA98 +S9). Strain TA98 –S9, were prepared with the river samples and the reaction medium. A 96-well microplate with 200 µl in each well was then filled with the suspension for each test. In order to prevent evaporation, plates were incubated at 37°C for 6 days. All yellow, partially yellow or turbid wells were considered positive, and all purple wells were recorded as negative. All yellow, partially yellow or turbid wells were considered positive, and all purple wells were recorded as negative. For each experiment, a blank, positive control and background (negative control) were run. The blank was used to ensure the sterility of the experiment; all wells in the blank were expected to be purple. The standard mutagen sodium azide (0.5µg/100µl) was used for the positive control, and all wells were expected to be yellow. The number of spontaneous reversions that would take place in the bacterial population was estimated using DMSO (dimethyl sulfoxide) as a negative control.

3.5 Quality assurance and quality control

Preventive measures were undertaken to minimise potential contamination of microplastic particles during sampling and laboratory processing using Wang et al. (2017). Microplastics equipment was rinsed thoroughly with distilled water before use. Plastic items used were minimized in the laboratory, but when used, it was rinsed with Milli-Q ultra-pure water. Nitrile gloves were always worn during the whole process, and solutions were covered with aluminium foil for the duration of the process. Saturated NaCl solution and KOH were filtered through a 10 µm size mesh under vacuum. Airborne contamination control was conducted for the duration laboratory sessions by putting petri dishes containing a wet mesh on workbenches. If microplastics were detected on the control mesh, the amount detected was subtracted from the values obtained. The Wind direction was considered when sampling to avoid contamination. A total of nineteen fibres were recorded from the negative controls for the whole study and the data was adjusted accordingly. Extraction efficiencies were done by filtering known quantities of fibres to ensure the reliability of the data obtained in this study.

The experimental process showed an efficiency rate of 95% fibres. When the daily coefficient growth rates in the control cultures for the test (days 0-1, 1, 2, and 2-3) did not exceed 35%, the test results for *R. subcapitata* were considered valid. Results from *D. magna* were acceptable when the observed mortality rate was under 10% for the wells. Test results for *T. thermophile* were considered valid when the optical density (OD) of the T0 controls decreased by at least 60% after 24 hours of incubation and when the T24 value was 40% or less of the OD at T0. The Ames test was validated if: the blank wells are purple, the background is ≥ 0 and ≤ 30 revertant wells and the positive control is ≥ 50 revertant wells per 96-well section on day 3. If on or all the three of these criteria are not met, the entire test is invalid.

3.6 Data analyses

The microplastics abundance, in mean and standard deviation (SD) from water and sediment, were expressed as MPs/L and MPs/kg (dw), respectively. Based on the characteristics of the distributed data, non-parametric analyses were conducted on the data using the Kruskal–Wallis test for analysis between microplastics abundances in surface water and sediment. The significant differences between the groups, were determined by the Mann–Whitney U test with a <0.05 significance level. Post hoc analyses for significant differences between sites were conducted using pairwise comparisons of the Kruskal–Wallis analysis. The physicochemical parameters' data were analysed for means, and SD and correlated to MPs data parameters using the IBM Statistic Package for Social Science (SPSS) 28. The statistical design for ecotoxicology and genotoxicity experiments were based on hypothesis testing (NOEC) and regression (ECx). Results were analysed statistically using ToxRat Professional 3.2 Software.

CHAPTER 4:RESULTS AND DISCUSSION

4.1 INTRODUCTION

The microplastics burden of the river was assessed spatially and temporally. A total of 1756 microplastics were recorded from water and sediment samples collected from the Plankenburg River. Two different environmental matrices (water and sediment) were collected and analysed. Six sampling events were conducted rather than eight due to the COVID-19 pandemic restrictions nationally and at Cape Peninsula University of Technology (CPUT). Two sampling events were carried out for spring and autumn but only one for each summer and winter seasons. The results of exposure studies using MP standards and the Plankenburg River water samples are also presented in this chapter.

4.2 Physicochemical properties of the Plankenburg River water samples

The physicochemical parameters of the Plankenburg River observed results are presented n Table 4.2.1 to provide an overview of surface water quality. The values were compared to the South African national standard and water quality for inland waters by the Department of Water and Forestry, (DWAF, 1996) and World Health Organization (WHO, 2006) guidelines for water quality.

Table 4.2.1: Physicochemical parameters values (mean ±SD) of the Plankenburg River water samples over four seasons

Parameter	Season	PR1	PR2	PR3	PR4	Mean	DWAF	WHO
T°C	Spring	19.75±1.63	19.25±2.05	18.85±0.78	19.50±2.26	19.34±1.39		
	Summer	21.40*	20.70*	19.40*	22.10*	20.90±1.15		
							≤25	N/A
	Autumn	16.75 ± 2.47	16.65±2.62	16.25±2.76	16.20±2.97	16.46±2.07		
	Winter	14.20*	13.90*	14.30*	13.90*	14.08±0.21		
pH	Spring	7.89±0.81	8.45±1.37	8.75±1.17	7.53±0.18	8.16±0.91	6.50 – 9.00	6.50– 8.50

	Summer	8.12*	8.47*	8.23*	9.90*	8.68±0.83		
	Autumn	9.70 ±0.11	8.44±0.88	9.82±0.04	9.37±0.76	9.33±0.73		
	Winter	8.82*	8.49*	8.58*	9.39*	8.82±0.40		
DO (mg/L)	Spring	3.25±0.78	4.40±1.13	4.75±0.49	4.20±1.41	4.15±0.97		
	Summer	3.80*	4.80*	17.70*	7.70*	8.50±6.35		
							6 - 9	4 -10
	Autumn	6.40±4.10	5.90±3.68	10.35±4.03	5.90±1.70	7.14±3.32		
	Winter	3.80*	4.80*	7*	5.30*	5.23±1.34		
EC (µS/cm)	Spring	817±67.88	857±100.41	418±22.63	678±38.18	692.50±190.08		
	Summer	840*	885*	147*	101*	493.25±427.18		
							0-1500	N/A
	Autumn	753.50±75.66	779.50±65.76	300.50±187.38	478.50±147.79	578±234.10		
	Winter	743*	757*	388*	589*	619.25±171.93		
TDS (mg/L)	Spring	551±43.84	565±70.71	287.5±13.44	444±28.28	461.88±123.35		
	Summer	569*	606*	95*	69.80*	334.95±292.19		
							0 - 450	1000
	Autumn	513±39.60	516.50±43.13	201±124.45	314.50±94.05	386.25±157.10		
	Winter	493*	504*	258*	396*	412.75±114.01		
ORP (mV)	Spring	-500.50±696.5	-34 ± 31.11	51±31.11	42.5±17.68	-110.25±359.02		
	Summer	73*	92*	115*	91*	92.75±17.21		
							N/A	≤700
	Autumn	-110.50±0.71	-25.50±53.03	44.50±21.92	48±9.90	-10.87±72.44		
	Winter	83*	84*	106*	92*	91.25±10.63		

COD (mg/L)	Spring	62.45±45.04	47.30±24.04	28.80±1.13	30.30±7.07	42.21±24.41		
	Summer	48.5*	49.33*	20*	24.5*	35.58±15.51		
							≤20	≤100
	Autumn	56±12.97	46.75±6.72	19.22±4.79	20.42±14.02	35.60±18.94		
	Winter	42*	39.30*	24.30*	24*	32.40±9.59		
BOD (mg/L)	Spring	13.87±13.05	5.62±2.10	2.72±2.43	2.45±1.07	6.16±7.10		
	Summer	27.15*	125*	12.10*	5.36*	42.40±55.81		
							10	N/A
	Autumn	30.39±21.87	22.71±13.36	16.10±9.50	50.93±66.16	30.03±30.46		
	Winter	90.92*	7.97*	48.1*	28.13*	43.78±35.44		

*Samples were collected once. PR1: Kayamadi Informal Settlement/Commercial activities, PR2: Industrial and recreational park, PR3: Krom River, PR4: Plankenburg River mixed with Krom River.

Temperature is used to assess the water quality of the freshwater system as it can affect the rate of metabolic activities in organisms and oxygen solubility. (Chatanga et al., 2019). Table 4.2.1 shows temperature levels at each sampling point obtained over the four seasons ranged from 13.90 to 22.10°C. Winter had a lower seasonal mean temperature of 14.08°C, while summer had a higher one of 20.90°C. The ambient temperature on the sample day may have contributed to the temperature level measured at site PR4 (22.10°C), the mixed point of the Plankenburg and Krom Rivers. However, the seasonal water temperature of the Plankenburg River was below DWAF (≤25°C) and WHO (≤ 37°C) water quality limit for the freshwater system. The overall temperature obtained over the seasonal water sampling of Plankenburg River is similar to the one reported by Nephale, (2021) in the monitoring pollution status of urban rivers in Limpopo, South Africa. Chigor et al. (2013) seasonal temperature result in the Buffalo River in the Eastern Cape Province was higher in winter (range, 22.6– 25.8°C) and lower in summer (range, 13.1– 17.7 °C), and these results are similar to the one obtained in this study. Britz et al. (2013) results ranging from 9.9 – 21.8°C were comparable to those from this study.

pH as a water quality parameter influences chemical and biological processes in the aquatic system (Raghav et al., 2022). The mean pH value was higher in summer at PR4 (9.90) and lower in spring at PR4 (7.53), the mixed point of the Plankenburg and Krom Rivers (Table 4.2.1). High pH in the summer at PR4 may have been caused by water evaporation,

which results in the loss of half-bound CO_2 and mono-carbonate precipitation, or by phytoplankton taking in more dissolved inorganic carbon through photosynthetic processes (Olaniran et al., 2014). Nevertheless, seasonal mean pH was the highest in autumn with 9.33 and lowest in spring with 8.16 (Table 4.2.1). Mean pH in autumn compared to the other three seasons is above DWAF and WHO threshold for aquatic systems and classified as alkaline river water. The mean pH value of the Plankenburg River was within the DWAF (6.50 - 9.00) and WHO (6.50 - 8.50) water quality limits for spring at all four sites. Mean pH in autumn compared to the other three seasons was above DWAF and WHO threshold for aquatic systems and classified as alkaline river water. The pH results obtained by Britz et al., (2013) on the Plankenburg River (5.78 – 7.24) are lower than the results of this study. Similar results to this study were obtained by Ohoro et al. (2021) in surface water and sediment of Buffalo and Sundays River estuaries, South Africa.

Dissolved oxygen (DO) is essential in the oxidation and reduction of organic and inorganic matter. The natural elimination of the pollutant load in the aquatic system is facilitated significantly by DO (Karolina et al., 2022). Anthropogenic activities such as domestic and industrial waste disposal and agricultural waste discharge into the river might cause a decrease in dissolved oxygen concentration. According to Edokpayi et al. (2017), DO concentrations below 5 mg/l affect aquatic organisms. The presence of plastic particles and solid waste in the freshwater environment may affect the amount of oxygen present and the river organisms. DO recorded range from 3.25 to 17.70 mg/L with a higher value at PR3 (Krom River) and a lower at PR1 (Kayamandi Informal Settlement/Commercial activities). Seasonal mean DO levels were reported high in summer at 8.50 mg/L and low in spring at 4.15 mg/L (Table 4.2.1). The seasonal results of DO were within the targeted limit of WHO but did not meet DWAF's targeted limit. The low level of DO reported in spring at PR1 might be due to the occurrence of plastics and solid waste from Kayamadi Informal Settlement, and the high level of DO at PR3 in summer can be due to low turbidity, which indicates fewer microorganisms in Krom River (Table 4.2.1). The seasonal variations reported for DO in this study are similar to the summer result obtained by Ohoro et al. (2021), in surface water and sediment of Buffalo and Sundays River estuaries, South Africa. Also, Karolina et al. (2022) results on Water Quality Analysis at Komering River Kayuagung City Ogan Komering Ilir Regency were below the results of this present study.

The electrical conductivity (EC) is a valuable indicator of salinity with total salt content (Pereao et al., 2021). High EC indicates runoff of wastewater and anthropogenic activities impact the

water bodies. These activities include domestic waste, industrial sewage and agricultural waste discharged into the river (Korkanç et al., 2017). The mean EC value was higher at PR2 (885 µS/cm) and lower at PR4 (101 µS/cm), both in the summer (Table 4.2.1). The seasonal mean value of EC was recorded higher in spring at 692.50 µS/cm and lower in summer at 493.25 µS/cm (Table 4.2.1). The recorded value at PR2 in the Industrial and recreational park can be due to the higher amount of dissolved inorganic substances in ionized form. According to Agoro et al. (2018), EC is mainly attributed to pollution and dissolved ions from the decomposed plant matter. The low EC value recorded at PR4, the mixed point of the Plankenburg and Krom Rivers, might be due to the use of salts, organic and inorganic matter by phytoplankton and other aquatic organisms. The obtained EC values were within the permissible DWAF standard limit for the freshwater system. The overall results (10 – 890 µS/cm) of Britz et al. (2013), the Plankenburg River were similar to this study. The results of EC all over the four seasons were comparable to Edokpayi et al. (2015) study on the Mvudi River, South Africa.

The amount of total dissolved solids (TDS) is a measure of inorganic salts and dissolved organic matter in water and is directly related to EC. Total dissolved solids high levels could come from intrusion, mining, irrigation water, oil field refinery, and domestic wastewater (Sharma et al., 2017; Awe et al., 2020). High TDS levels impact the odour and colour of water and the growth rate of aquatic organisms (Sharma et al., 2017). As recorded in Table 4.2.1, during the sampling period, the mean variation of TDS through the different sampling sites ranged from 69.80 mg/L to 606 mg/L in the summer. Seasonally, TDS was higher in spring at 461.88 mg/L and lower in summer at 334.95 mg/L (Table 4.2.1). Values recorded at PR1 (Kayamadi Informal Settlement/Commercial activities) and PR2 (Industrial and recreational park) over the four seasons did not fall into the DWAF water quality threshold, while all recorded values were in the general limit of WHO (1000 mg/L). The highest values obtained at PR2 might be due to the dissolution of sediment and small rocks in the river and wastewater from the industrial area. These results are higher than the one reported by Nephale, (2021) during the study of urban rivers in Limpopo, South Africa. Also, Awe et al. (2020) results in water samples of the Diep River, South Africa, were higher than in this study.

Redox potential or oxidation-reduction potential (ORP) is a measure of the ability of a river to break down contaminants and dead plants and animals. Redox potential is measured in addition to dissolved oxygen because it can provide additional information on the water quality and degree of pollution. Its high levels in water are related to a high amount of oxygen in the water.

The mean ORP value over the four sampling sites varied between - 500.50 mV and 115 mV (Table 4.2.1). The ORP value was recorded as high in summer at PR3 (Krom River) and low in spring at PR1 (Kayamadi Informal Settlement/Commercial activities). Seasonal analysis of the values recorded during the four seasons revealed that the average ORP value was higher in summer. The low values observed across the four seasons at different sampling sites were due to a lower presence of oxygen in the river system. The highest value at PR3 around the Krom discharge point into Plankenburg River might be due to the high level of oxygen in the river water. The ORP values of the Plankenburg River were within the permissible limit of WHO ≤700 mV. The ORP values in this study are below the obtained results on the Fenghe River Basin, China: assessment and source analysis by Luo et al. (2021).

Chemical oxygen demand (COD) is the oxygen necessary for the chemical oxidation of organic materials with the aid of a potent chemical oxidant. Chemical oxygen demand can increase due to bacterial cell death in the aquatic system (Anhwange et al., 2012). As recorded in Table 4.2.1, the COD value over the sampling sites varies from 19.22 mg/L to 62.45 mg/L. Seasonally, the COD level was higher in spring at 42.21 mg/L and lower in winter at 32.40 mg/L. The measured COD values were in the target water quality range of DWAF (≤20 mg/L) only at PR3 in summer and autumn over the four seasons, while all recorded values were in the general limit of WHO (≤100 mg/L). The elevated levels of mean COD at PR1 (56 mg/L and 62.45 mg/L) around Kayamandi Informal Settlement/Commercial activities in spring and autumn, respectively, can increase the quantity of biologically active substances such as bacteria in the Plankenburg River probably due to organic pollutants that are available for oxidation in this part of the river that is polluted by human activities (the inflow of domestic, livestock and industrial waste). The lower level of COD at PR3 in autumn at 19.22 mg/L (Table 4.2.1) can be a sign of good water quality, while a high level may cause harm to aquatic life. This study's results are lower compared to Raghav et al. (2022) study on the water quality index of polluted water of River Yamuna. The results of this study are comparable to Britz et al. (2013), who reported COD values between 0 – 421 mg/L.

Biochemical oxygen demand (BOD) indicates the extent of organic pollution in the river that adversely affects water quality (Chatanga et al., 2019; Raghav et al., 2022). The mean BOD levels ranged between 2.45 and 125 mg/L (Table 4.2.1). The increase in BOD level over the sampling points may be due to the increased amount of organic waste discharged from industrial waste and the informal settlement (Raghav et al., 2022). A high BOD level indicates that bacteria are consuming the oxygen in the water, which harms aquatic life in the river. The

BOD levels over the sampling points fall within the DWAF threshold in spring at PR2, PR3 and PR4 (5.62, 2.72 and 2.45 mg/L, respectively), and in summer and winter at PR4 (5.36 mg/L) and PR2 (7.97 mg/L), respectively (Table 4.2.1). The highest level of BOD at PR2 near the Industrial and recreational park might be due to the higher pollution burden from the informal settlement population that uses the recreational park. The results obtained from this study are lower than Anhwange et al. (2012) report for a seasonal variation study on the Benue River, Makurdi Metropolis. Raghav et al. (2022) result recorded in summer for the water quality index of polluted water of River Yamuna is consistent with the observations in this study.

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

The distribution of microplastics particles in the river provides an understanding and information on MP occurrence in the freshwater ecosystem. Results of microplastic distribution over four seasons in the Plankenburg River are presented in Table 4.3.1. The spatial and temporal distributions of MPs using different mesh sizes showed a different recovery overview.

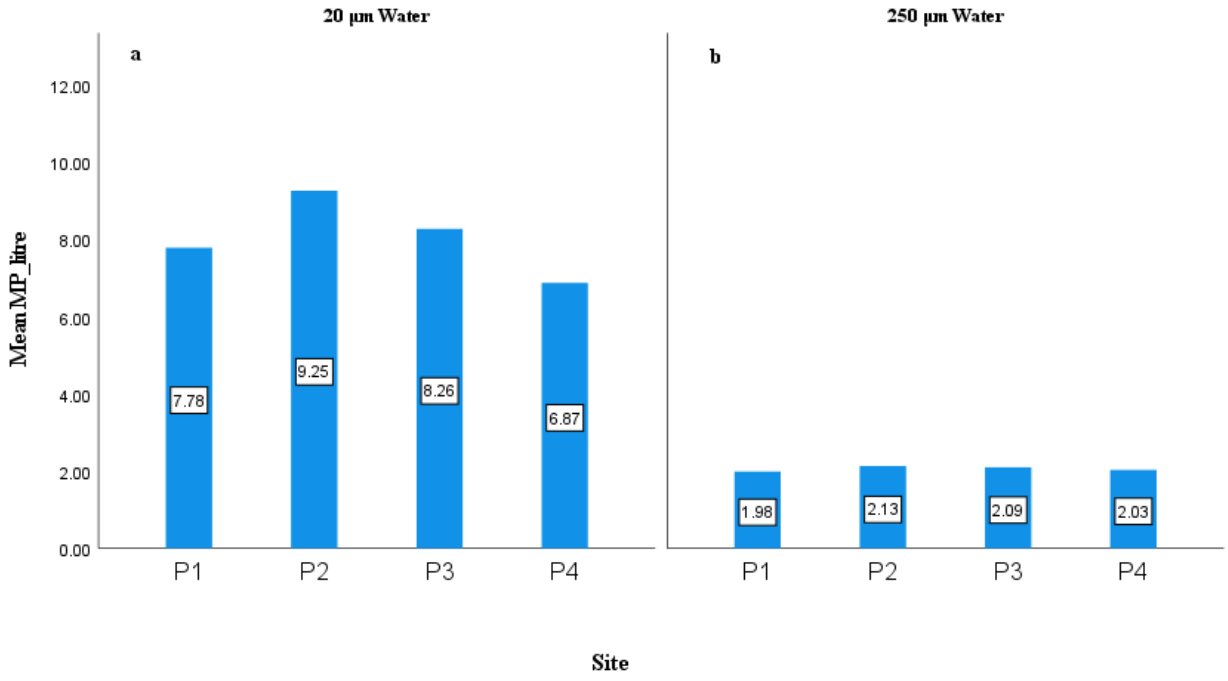

Figure 4.3.1:Abundance and distribution of microplastic particles in the Plankenburg River water sample using 20µm (a) and 250µm (b) mesh sizes

The MPs from 20 µm and 250 µm were extracted and results presented (Figure 4.3.1). The recovery efficiencies for two different mesh sizes during filtration were compared, and the results revealed that the 20 µm mesh had a better extraction efficiency than the 250 µm. MPs abundances were calculated for each site and presented as the number of MPs/L (N = 72). For microplastics processed under 20 µm, there were no significant differences in microplastic occurrences between sampling points analysed (Kruskal-Wallis; $p > .05$). The highest mean occurrence of microplastics over the four sampling sites was recorded at PR2 (9.25 MPs/L), and the lowest across the four sampling sites was recorded at PR4 (6.87 MPs/L) (Figure 4.3.1a).

Table 4.3.1: Abundance and distribution of microplastics particles in the Plankenburg River water sample (mean ± SD)

Site	Season			
	Spring	Summer	Autumn	Winter
PR1	0.47 ± 0.70	0.83±0.96	0.58±0.90	0.77±1.23
PR2	0.78 ± 1.27	0.31±0.47	0.54±0.82	0.59±0.93
PR3	0.48 ± 0.74	0.74±1.13	0.57±0.85	0.49±0.76
PR4	0.59 ± 0.90	0.49±0.80	0.63±1.03	0.69±1.21
Seasonal mean	0.58 ± 0.92	0.57±0.85	0.58±0.89	0.63±1.01

PR1: Kayamadi Informal Settlement/Commercial activities, PR2: Industrial and recreational park, PR3: Krom River, PR4: Plankenburg River mixed with Krom River.

There was no significant difference in the microplastic burden of samples processed through the 250 µm mesh across sampling points (Kruskal-Wallis; $p > 0.05$). The highest mean occurrence of microplastics was in summer and winter at PR1 (0.83 ± 0.96 and 0.77 ± 1.23 MPs/L, respectively), in spring (PR2: 0.78 ± 1.27 MPs/L) and autumn (PR4: 0.63 ± 1.03 MPs/L), and the lowest across the four seasons were recorded in summer at PR2 (0.31 ± 0.47 MPs/L) (Table 4.3.1). Seasonally, MPs were in higher in winter at 0.63 ± 1.01 MPs/L and lower in summer at 0.57 ± 0.85 MPs/L (Table 4.3.1). The mean abundance in the 20 µm and 250 µm water samples were respectively 7.98 ± 4.61 MPs/L and 1.72 ± 0.67 MPs/L. The results of the occurrence of MP in this study are more than the observed abundance of 0.23 ± 0.27 items·L^{-1} obtained by Weideman et al. (2019) in the Orange–Vaal River system in South Africa. This study's findings are comparable to those reported by Alam et al. (2019), who found 5.85 ± 3.28 particles/litre of microplastics.

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

The results of microplastic distribution in sediment samples over four seasons in the Plankenburg River are presented in Table 4.4.1. A total of 72 sediment samples were collected for this study. Three replicates were collected per site over the four seasons in six sampling events.

Table 4.4.1: Abundance and distribution of microplastics particles in the Plankenburg River sediment sample (mean ± SD)

Site	Season			
	Spring	Summer	Autumn	Winter
PR1	2133.33 ± 898.15	1183.33 ± 301.39	1066.67 ± 415.53	1666.67 ± 189.30
PR2	1358.33 ± 320.03	616.67 ± 160.73	1250 ± 370.14	1333.33 ± 275.38
PR3	1500 ± 251	783.33 ± 57.74	691.67 ± 159.43	983.33 ± 160.73
PR4	1358.33 ± 432.92	950 ± 86.60	866.67 ± 338.62	1686.33 ± 860.72
Seasonal mean	1587.50 ± 599.32	883.33 ± 266.57	968.75 ± 379.02	1416.67 ± 499.24

PR1: Kayamadi Informal Settlement/Commercial activities, PR2: Industrial and recreational park, PR3: Krom River, PR4: Plankenburg River mixed with Krom River.

From the results presented in Table 4.4.1, MPs ranged from 616.67 ± 160.73 to 2133.33 ± 898.15MPs/kg in the sediment samples. The distribution suggests that in winter, there were more deposits of microplastics. The abundance of microplastics was calculated for each site and presented in microplastics per kilogram (N = 18). Results are reported as means (±SD) and significances set at $p < 0.05$. There were no significant differences in microplastic abundances across all sites for the six sampling periods (Kruskal-Wallis; $p > 0.05$). The highest mean values of MPs were recorded in spring, summer, and autumn at PR1 (2133.33 ± 898.15, 1183.33 ± 301.39 and 1066.67 ± 415.53 MPs/kg) and in winter at PR4 (1686.33 ± 860.72 MPs/kg). The lowest across the four seasons were recorded in Summer (PR2; 616.67 ± 160.73 MPs/kg) (Table 4.4.1).

The seasonal distribution of microplastics in sediment from the Plankenburg River is summarized in Table 4.4.1. The spring samples had the highest MP abundance (1587.50 ± 599.32 MPs/kg). The mean abundance in the sediment samples of 1235.42 ± 552.23 MPs/kg of dry sediment is higher than the MPs recorded by Maheswaran et al. (2022), who recorded

an abundance of 699 ± 66.00 items/kg from the West Dongting Lake. The results from this study result is lower than the one recorded by Xia et al. (2021), where the recorded abundances of MP were 33200 items/kg of dry sediment. An assessment of the Bloukrans River, South Africa, showed microplastic contamination ranging from 6.3 ± 4.3 and 160.1 ± 139.5 kg^{-1} particles, respectively, in the summer and winter seasons (Nel et al., 2018b), lower than the values recorded in the present study in summer and winter with a similar abundance trend. However, the lower burden of MPs in summer and autumn can be attributed to the low level of water in the river. The high concentrations of microplastics found at the PR1 sampling site may be suggested to runoff from informal settlements. The research done by Huang et al. (2021) also shows how human activity affects river pollution.

4.5 Characterization of microplastics in the Plankenburg River

4.5.1 Microplastics shape

The shapes of MPs filtered from water and sediment are shown in Figure 4.5.1. MPs shapes were categorized into five: foam, fragment, Sphere, film, and fibre (Figure 4.5.2). At all four sampling sites, fibres (PR4: 98.40%) and films (PR2: 2.71%) were recorded as the dominant forms of plastic particles in the Plankenburg River system. Because of the wide application of plastic fibres, they occupy a large proportion of many studies, especially in freshwater environments (Yin et al., 2022). As very close to human activities, the Plankenburg River received a large amount of fibre. According to Migwi et al. (2020), the high informal settlement population increases pressure on the waste disposal systems, posing a threat to the river's health. Thus, the dominance of fibres and films in the Plankenburg River could be an indication of household and industrial effluent discharge into the river breakdown of household fibres.

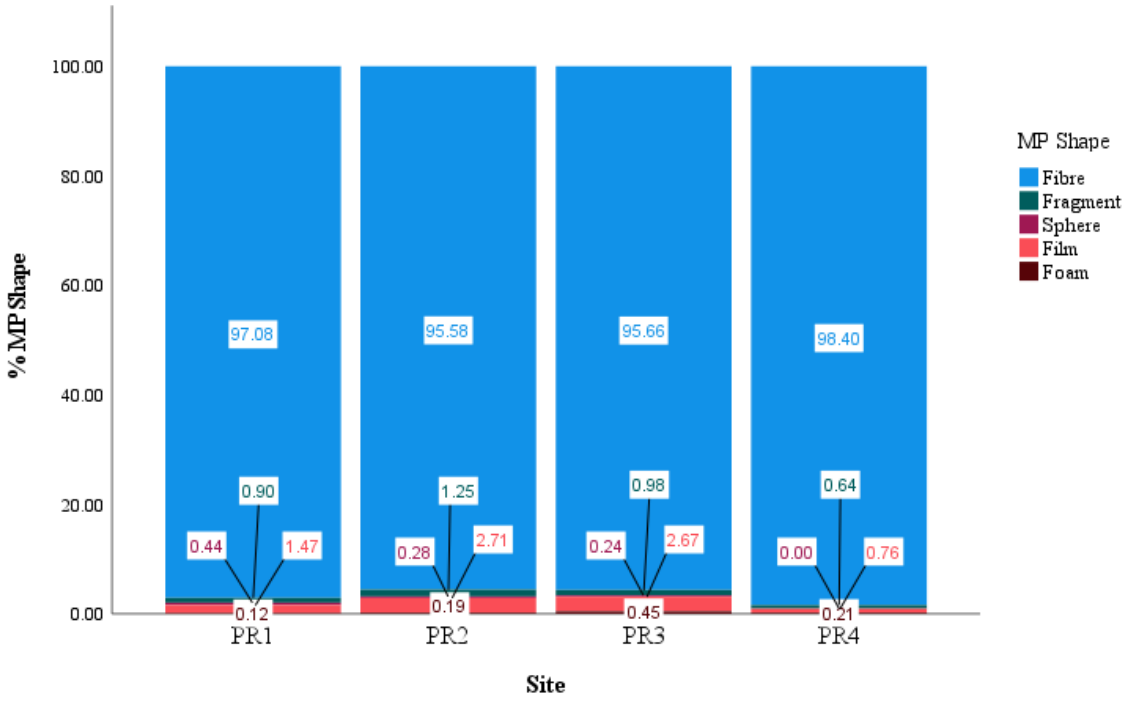

Figure 4.5.1: The Plankenburg River microplastics particles distribution by shape

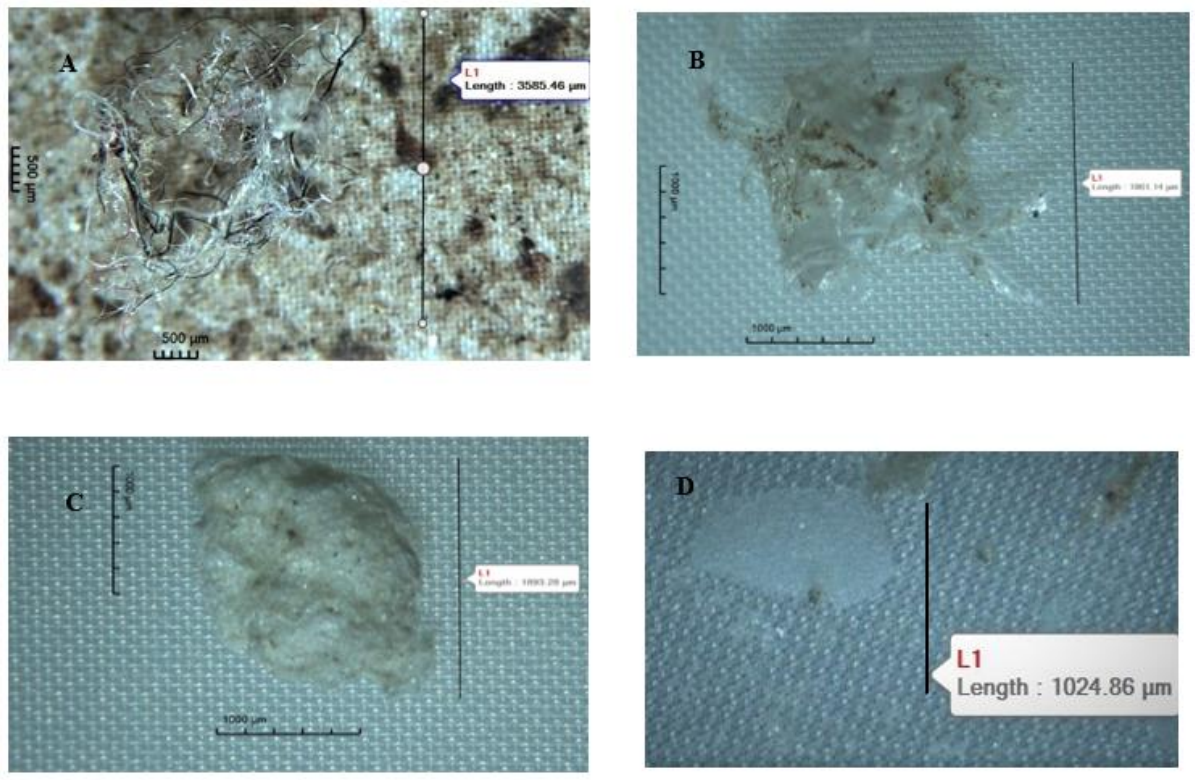

Figure 4.5.2: Microplastic particle types found in the Plankenburg River (A) fibre, (B) Film, (C) foam, (D) fragment.

4.5.2 Microplastics colour

White, yellow/brown, black/grey, translucent, blue/green, and red/pink were among the colours of MPs that were found (Figure 4.5.3). The highest percentage of MPs at PR1 (80.62%) and

the lowest percentage at PR2 (75.51%) were transparent. According to the previous research, transparent and blue/green MP was the dominant colour in the study of the freshwater system by Zhang et al., (2020). The colour of MPs is mainly due to the contamination of plastic materials from urban wastewater. Transparent and white MP may be caused by the discolouration of coloured MPs and weathering, while red/pink MP may be formed from coloured manufactured clothing (Maheswaran et al., 2022). Coloured MPs particles could easily be mistaken for food by freshwater biota, colour is a potential health hazard to the freshwater ecosystem, and its blocking effect of light can affect plant photosynthesis and animal activity (Silva et al., 2020). According to He et al., (2020), some colourless or transparent MPs particles could potentially be overlooked during extraction or identification processes.

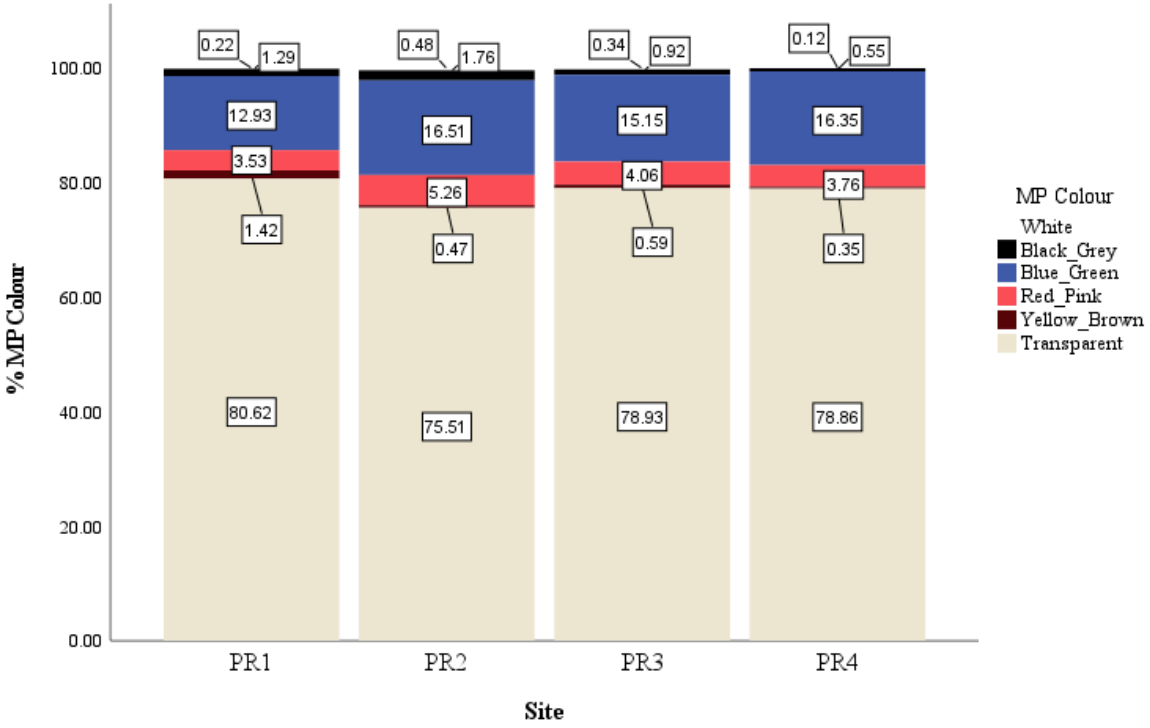

Figure 4.5.3: Colour distribution of microplastics particles in the Plankenburg River

4.5.3 Microplastics size

The identified MPs were classified into six categories according to their sizes: <63 µm, 63 - 500 µm, 500 - 1000 µm, 1000 – 2000 µm, 2000 – 5000 µm and >5000 µm. Figure 4.5.4 presented a high proportion of MP particles size between 500 – 1000 µm at the different sites with 61.87% at PR4, similar to Yin et al., (2022) in Xiangjiang river. In the current study, MPs <63 µm particle size was substantially smaller than that reported by Maheswaran et al., (2022).

Macro-plastic can break down into multiple small-size plastics that are considered to pose the most serious potential threats to both aquatic organisms and ecosystems. MPs with relatively lower density and smaller sizes have increased potential for transportation by wind and water. Consequently, MPs hazard impacts, compared to larger plastic debris, MP have more serious toxic effects and a higher probability of being mistakenly ingested by organisms (He et al., 2020; Yin et al., 2022).

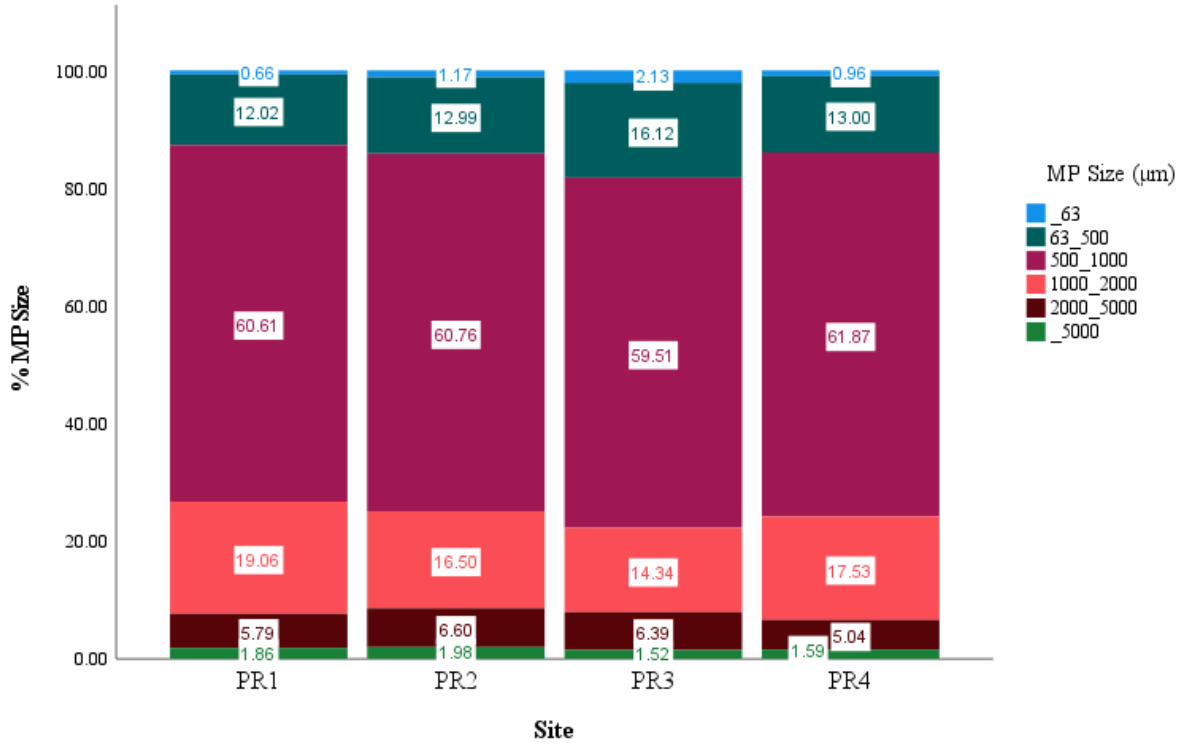

Figure 4.5.4: The Plankenburg River microplastics occurrence by size

4.6 Chemical analyses of microplastics in the Plankenburg river

4.6.1 Polymer distribution of microplastics in the Plankenburg River

FTIR-ATR was used to conduct chemical analyses of plastic particles to identify the various polymer types detected in the freshwater system. The primary polymers found in the Plankenburg River were PE (57.86%), Cotton (29.84%), PP (6.68%%), and PET (5.62%) (Figure 4.6.1).

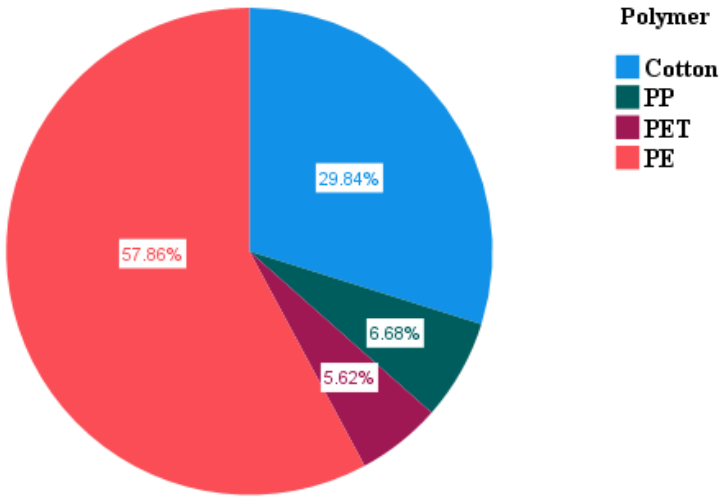

Figure 4.6.1: Microplastics types identified in Plankenburg River

Based on the proportion of the density of each type of MP, different percentages were recorded in water and sediment, and PE is the most predominant type of MPs in water and sediment, followed by cotton (Figure 4.6.2). According to Xia et al., (2021), PE and PP are widely used due to their lightweight and stable characteristics as packaging bags in food, fishing and agricultural sectors. However, PE has been reported in the previous research literature as one of the most common polymer types with wide distribution in freshwater depending on its quantity (He et al., 2020; Kukkola et al., 2021). Due to landfill operations by the people who live along the river, the Plankenburg River contains cotton, which is used in the production of fabrics, whereas PET is the most recycled thermoplastics and is mainly used for rigid and flexible packaging. In addition, the small amounts of PET may be more significant than large PE based on their differences in toxicity in aquatic systems.

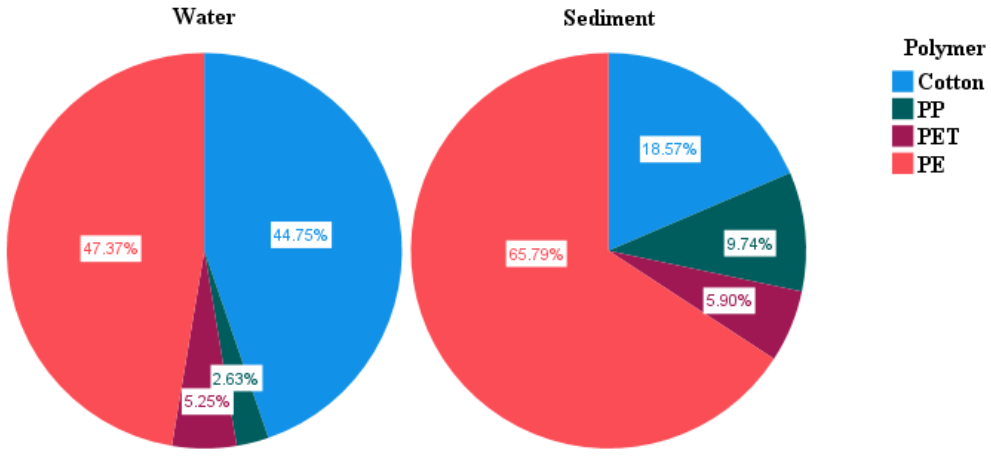

Figure 4.6.2: Polymer type distribution in water and sediment of the Plankenburg River

Fibres were the most common shape of MP particles investigated in this study using FTIR-ATR. Fibres were categorised as Cotton (44,35%); PE (37,37%), PET (8,35%) and PP (9,93%) particles, whilst films were identified in PE particles only (Figure 4.6.3a). Fibre polymer identification all over the four sites indicated that Cotton was the predominant polymer from site PR1 to PR 3 and PE at site PR4 (Figure 4.6.3b), and this can be due to the various endeavours and activities within the vicinity of the river and the proximity to the informal settlement.

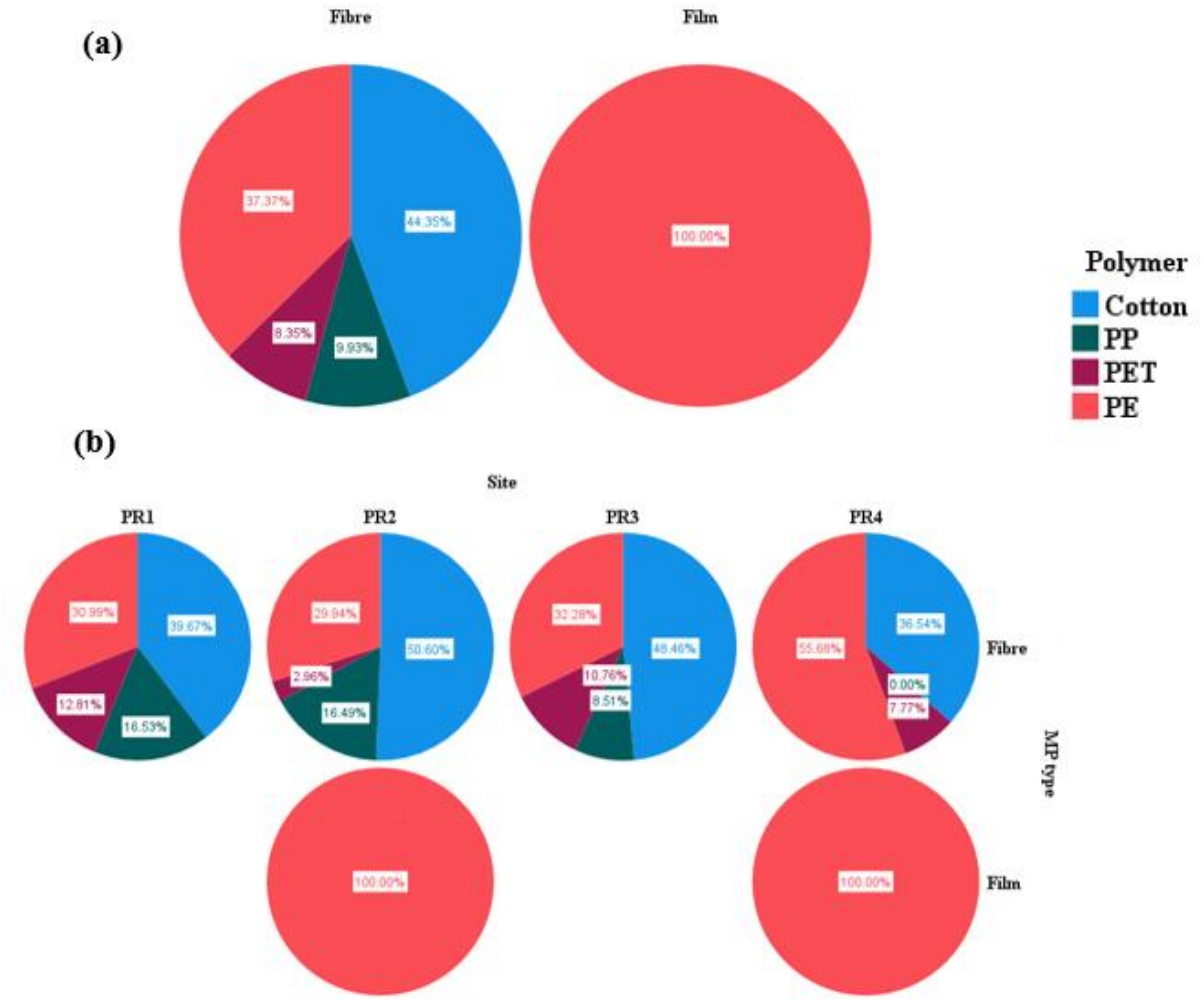

Figure 4.6.3: Polymer types in water and sediment (a), and types per sampling site (b)

Among the films analysed, PE was the only polymer identified at PR2 and PR4 (water and sediment). Based on the results obtained, MP observed in Plankenburg River might be due to the microplastics released from the daily life of the informal settlement, surface runoff, and discharge from the industrial and agricultural wastewater.

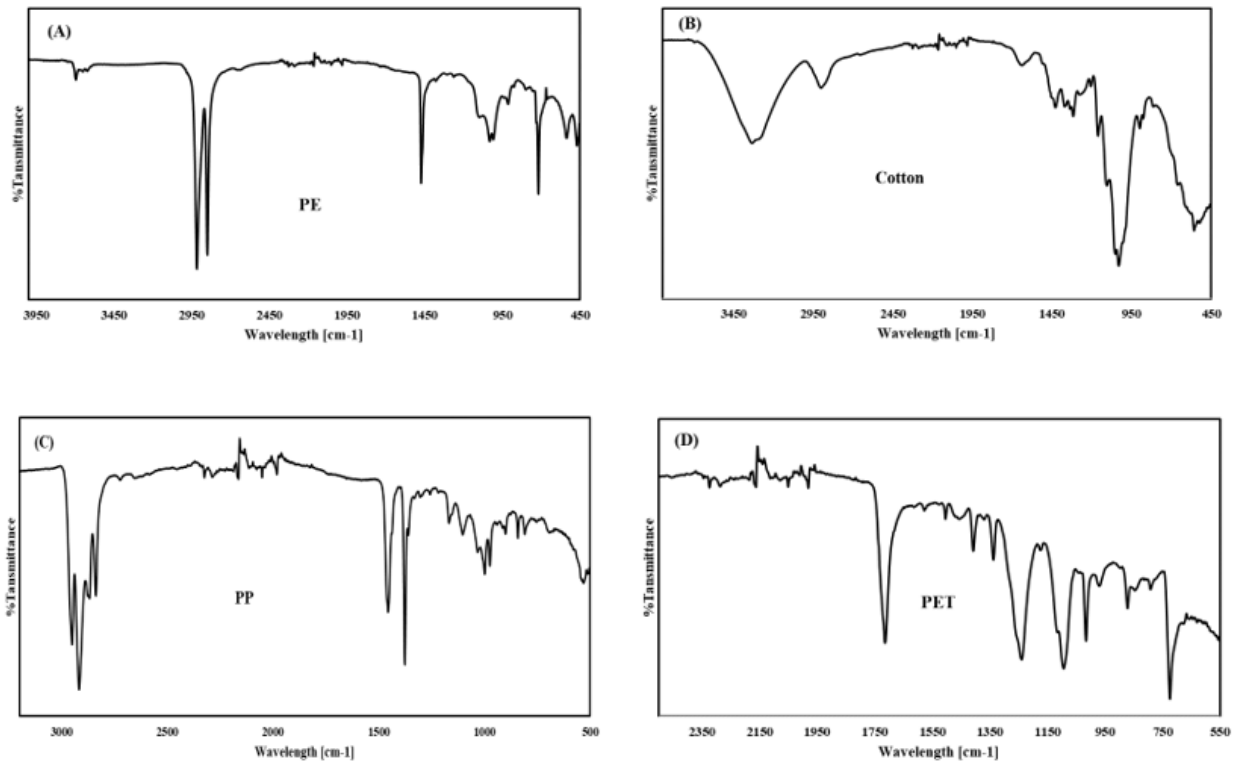

Figure 4.6.4: FTIR spectra of selected MP particles from the Plankenburg River

FTIR microplastics spectra found from Plankenburg River were compared with reference IR spectrums and were mainly composed of cotton, PE, PP and PET (Figure 4.6.4). PE characteristic spectra for the four sites display peaks around 717, 1472, 2916 and 2849 cm^{-1}. Cotton produces peaks around wave number regions 556, 1030, 2900 and 3327 cm^{-1}. PP spectra from the study river show peaks around 1376,1456 and 2917 cm^{-1}, and PET display peaks around 725, 1016,1238 and 1715 cm^{-1}. These results are consistent with those described by other authors (Rodrigues et al., 2018; Maheswaran et al., 2022).

4.7 Significance of microplastics occurrence and water quality parameters

The relationship between the physicochemical parameter and the amounts of microplastics reported in the Plankenburg River has been investigated. A Spearman's correlation analysis (*r*) was conducted to assess possible associations between the physicochemical properties of water, microplastics occurrence and possible ecological effects of microplastics in the waterbody (Table 4.7.1).

Table 4.7.1: Spearman's correlation matrix for water physicochemical parameters and microplastics occurrence in water and sediment

	pH	DO	EC	T °C	TDS	ORP	COD	BOD	250 µm	20µm	Sediment
pH	--										
DO	.630**	--									
EC	-.484*	-.666**	--								
T °C	-.465*	-.430*	.218	--							
TDS	-.477*	-.655**	.998**	.212	--						
ORP	.018	.303	-.433*	-.091	-.441*	--					
COD	-.346	-.475*	.832**	.231	.841**	-.514*	--				
BOD	.403	.316	.130	-.431*	.152	.190	.210	--			
250 µm	-.030	-.126	.061	.203	.032	.037	.015	-.199	--		
20 µm	-.348	-.258	.135	.245	.119	.068	.032	-.479*	.030	--	
Sediment	-.425*	-.594**	.380	.042	.370	-.235	.368	-.259	.223	.463*	--

*. Correlation is significant at the 0.05 level: **. Correlation is significant at the 0.01 level.

The results showed that the parameters have an inconsistent correlation relationship, and there were no significant correlations between microplastic distributions and pH, total dissolved oxygen, electrical conductivity, redox potential, temperature, biochemical oxygen demand and chemical oxygen demand (Table 4.7.1). Nevertheless, there was a strong negative correlation between microplastic distributions and dissolved oxygen. The significant positive correlation between DO and pH (r=0.630; p<0.01) and between 20 µm and sediment (r =0.463; p <0.01) demonstrated that sediment might be observe as a sink for microplastics. There is a strong inverse correlation between DO and each of TDS (r=−0.655; p<0.01), sediment (r=−0.594; p<0.01) and EC (r=−0.666; p<0.01). Significant (p<0.05) inverse relationships existed between pH and sediment (r=−0.425). In a study by Tien et al. (2020) on the relationship between microplastic and aquatic factors, the results showed a positive correlation between chemical oxygen demand and microplastic occurrence. However, Migwi et al. (2020) studies revealed that pH, dissolved oxygen, electrical conductivity, total dissolved oxygen and temperature parameters did not affect microplastic distribution and occurrence in freshwater systems.

4.8 Bioassay tests of the Plankenburg River water samples

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

4.8.1 *Raphidocelis subcapitata* 72 h growth inhibition test

The mean growth inhibition for *R.subcapitata* exposed to different sampling sites of Plankenburg River samples represented in Figure 4.8.1 ranged from -1.40% to 22.50 %. In spring, no significant difference in mean growth inhibition values was observed between sampling points PR1, PR2 and PR4, except PR3, where a significant decrease in microalgae growth (PE < 0%) was observed. The maximum PE was obtained at PR2 (12.50%), which means that in spring, the river samples had no significant toxic effect on *R. subcapitata* and can be scored 0. In summer, the mean growth inhibition value observed at PR1 (0.80%) was the lowest compared to PR2, PR3 and PR4, with the highest PE obtained at PR4 (12.80%), meaning that in summer, the river samples had no significant toxic effect on *R. subcapitata* and can be score 0 (Figure 4.8.1). In autumn, a significant difference in mean growth inhibition was observed between PR1, PR2 and PR3, PR4. A significant increase in microalgae growth was observed at PR1 with 22.50%, meaning that in autumn, the river sample at PR1 had a significant toxic effect (score 1), except the other three sites, which had no significant toxic effect (score 0). In winter, the highest mean growth inhibition was obtained at PR2 (12.50%), and a significant decrease in microalgae growth (PE < 0%) was observed at PR3 (- 1.40%). The river water samples in winter at the four sites had no significant toxic effect (score 0).

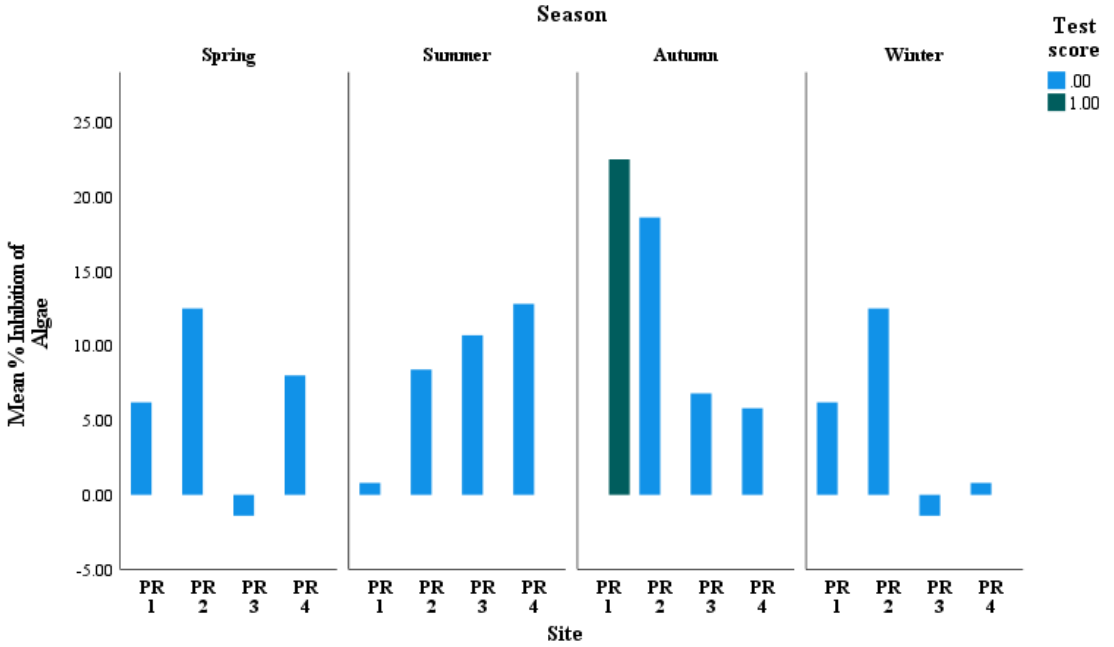

Figure 4.8.1: Mean toxic response (growth inhibition) of *R. subcapitata* for Plankenburg River sites. PE values < 0% indicate growth stimulation.

In general, the river samples demonstrated no significant toxic effect for *R. subcapitata* (Figure 4.8.1), where the mean inhibition was below 20%, except PR1 in autumn, with a mean inhibition of 22%. The results of *R. subcapitata* exposure to the Plankenburg River might be due to the limited amount of nutrients over the four seasons at the different sites. However, the significant toxic effect observed in autumn at PR1 might be due to a consequence of water pollution led by an increase of complex compounds, that provided nutrients for algal proliferation. The algal bloom might also be due to the informal settlement in the vicinity of the sampling site. The obtained results confirmed the value of *R. subcapitata* as a sensitive bio-indicator in ecotoxicological assessment of aquatic systems. It should be expected that complex compounds enriched in river waters would stimulate *R. subcapitata* growth. The results are similar to Serpa et al. (2014), where *R. subcapitata* exposed to the Cértima river did not cause any algal growth inhibition.

The exposure studies of the spring samples with virgin polyethylene microspheres (PE-MP) revealed that there was no significant difference in the mean growth inhibition of *R. subcapitata* at different sampling sites (Figure 4.8.2a). The percentage growth inhibition for PR2, PR3, PR4 and PR1 were 29.20%, 28.50%, 32.20% and 39.10%, respectively. The percentage effect at PR1 was the highest, and so, it had a significant toxic effect (score 1). This observation is consistent with Li et al. (2020) who reported that exposure to microplastics resulted in the growth inhibition of microalgae.

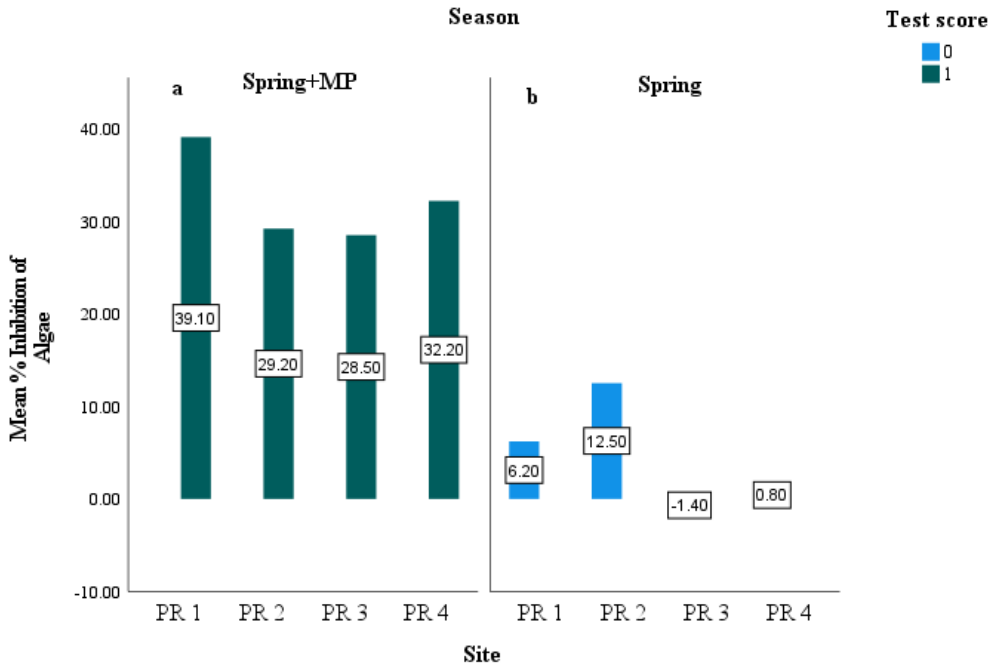

Figure 4.8.2: Mean toxic response of algae for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b).

The results of the *R. subcapitata* exposed to PE-MP in Figure 4.8.2a revealed a significant difference in growth inhibition and scoring compared to the results obtained in spring for same samples without MPs (Figure 4.8.2b). The virgin PE-MP had a negative effect on the microalgae observed as proliferation. This implies the that the mixture had euthrophic potential in the freshwater ecosystems. According to Davarpanah & Guilhermino. (2015), the results of this test might be due to the size of the plastic particles tested with the concentrations tested PE-MP. The results of this study are similar to Casado et al. (2013) results obtained on the eco-toxicological assessment of silica and polystyrene nanoparticles assessed by a multi-trophic test battery.

4.8.2 *Daphnia magna* 48 h acute immobility tests

Daphnia magna acute toxicity test for all samples taken from Plankenburg River is presented in Figure 4.8.3. In spring, the mean mortality rates of the exposed *D. magna* showed very low toxicity only at PR4 with 5% neonates mortality, while no neonates mortality occurred at sites PR1 to PR3. According to the later findings, the river samples in the spring exhibited no discernible harmful effects on *D.magna* and can therefore be scored 0. The river samples exposed to *D. magna* in summer and winter presented a 5% mortality rate at PR2 and PR4, respectively and a 10% mortality rate at PR1 in both seasons (Figure 4.8.3). The PE mortality

in both seasons was scored 0 as they presented no significant toxic effect on *D. magna* after 48 h of exposure.

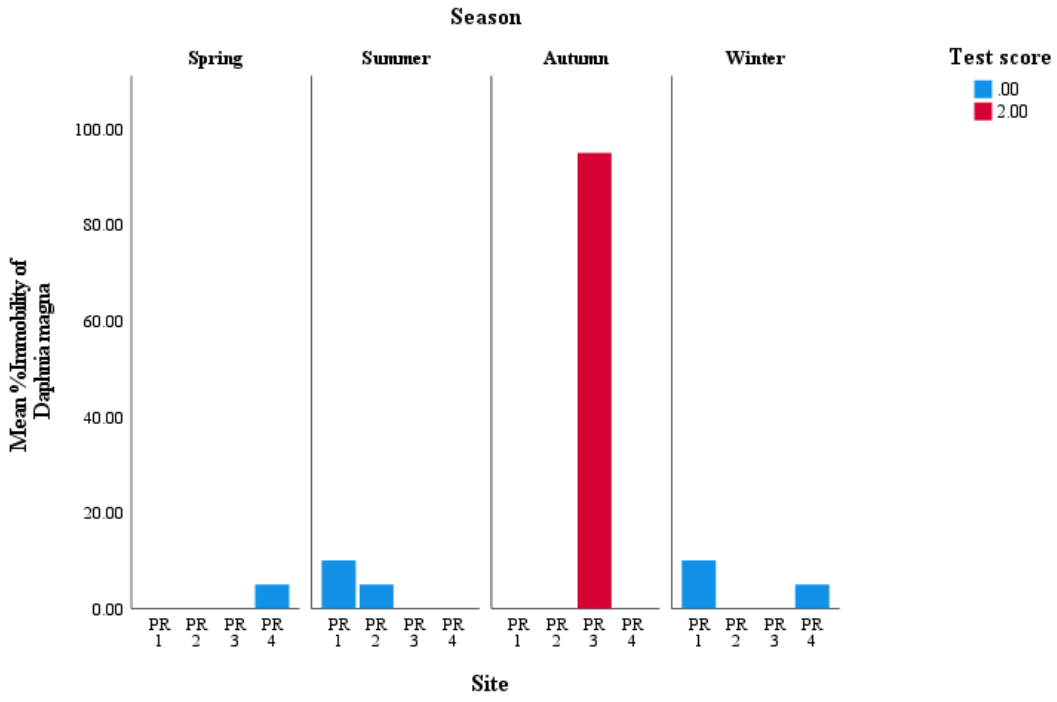

Figure 4.8.3: Mean toxic response (mortality) of *D. magna* for Plankenburg River sites

A toxic effect (score 2) to *D. magna* was observed in autumn with a 95% mortality rate at PR3 after 48 h of exposure, while no neonates mortality was obtained at the other three sites (Figure 4.8.3) and river water impacted the mortality rate at site PR3 in autumn. The presence of high nutrient availability in the river can favour daphnids mobility which means that the opposite could account for this mortality rate observed at PR3 in autumn. The results obtained are lower compared to the observation by Szklarek et al. (2021) where mortality rate in samples from a river in Poland was assessed over the four seasons.

The toxicity test of *D. magna* exposed to the samples collected in spring with PE-MP is presented in Figure 4.8.4a. The mean mortality rates of the exposed *D. magna* showed very high toxicity only at PR2 with 20% neonates mortality, while 5% neonates mortality occurred at sites PR3 and PR4. The latter results mean that the river samples with PE-MP had no significant toxic effect (score 0) on *D. magna*, which is similar to the test score obtained without PE-MP in Figure 4.8.4b. The result of this study might be related to the size of the particles used (40-48 mm), and according to Castro et al. (2020), this size class is larger than the size class of PE-MP that had effects on daphnids immobility.

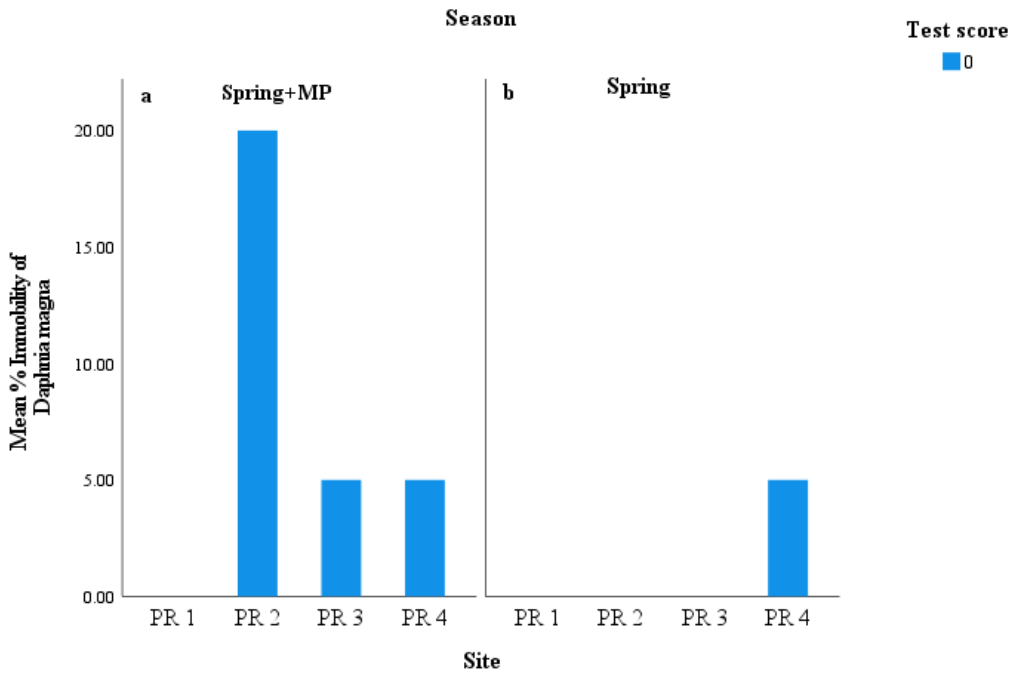

Figure 4.8.4: Mean toxic response of *D. magna* for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b)

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

4.8.3 *Tetrahymena thermophila* 24 h chronic growth inhibition test

The mean toxicity of Plankenburg River samples exposed to the ciliate *T. thermophila* presented in Figure 4.8.5 shows a significant mean percentage growth inhibition over the four seasons. *Tetrahymena thermophila* bioassay showed a high sensitivity response to the river samples with PE < 0% in autumn and winter at PR1 and PR4, respectively. In spring, no significant difference in mean growth inhibition value was observed between sampling points PR1, PR2 and PR3, except PR4, where a small exposure response to *T.thermophila* was observed at 10.98% (Figure 4.8.5). The river sample at PR4 (spring) had no significant toxic effect (score 0) on *T. thermophila*, while a toxic effect (score 2) was recorded from PR1 to PR3 in the same season with a maximum PE value of 73.49% at PR3. In summer, there was a significant difference in mean growth inhibition value between sampling sites, with a high PE value at PR4 (100%) and a low value at PR1 (28.35%). The river samples had a significant

toxic effect (score 1) from PR1 to PR2, a toxic effect (score 2) at PR3 and a PE= 100% (score 3) at PR4.

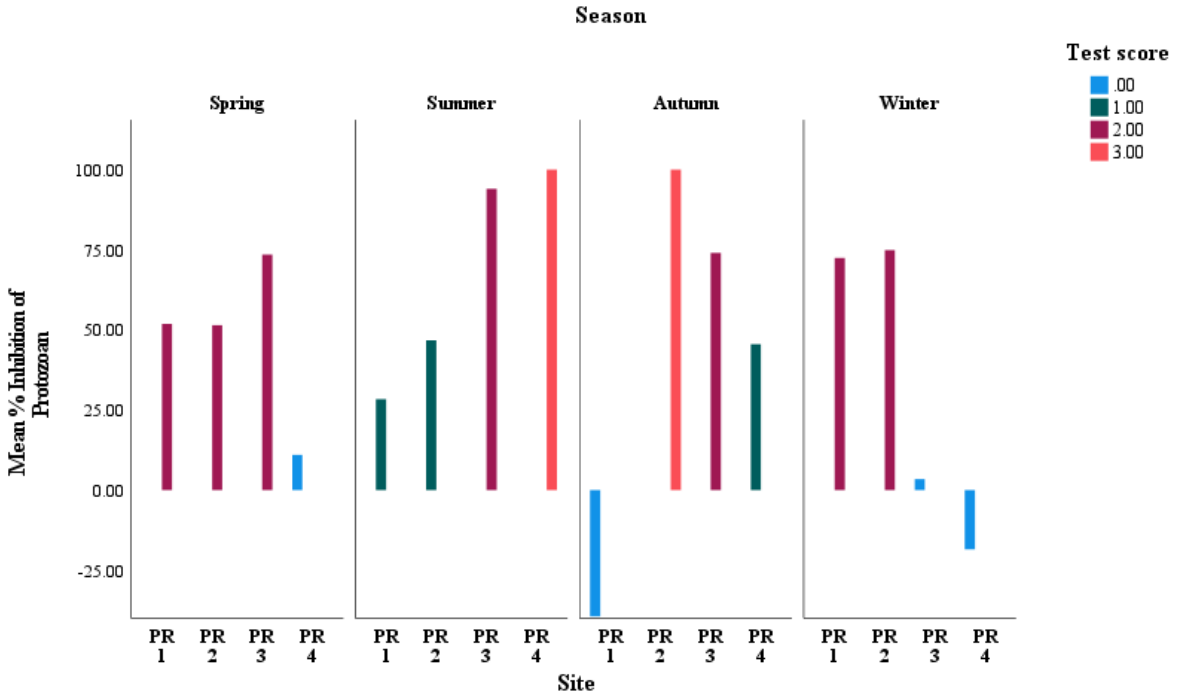

Figure 4.8.5: Mean toxic response (growth inhibition) of *T. thermophila* for Plankenburg River sampling sites

In autumn, there was a significant difference in mean growth inhibition value between sampling sites, with a high PE value at PR2 (100%) and a low value at PR1 (- 39.44%). *T.thermophila* exposure to the river samples presented different responses at each of the four sampling sites, and the highest score (score 3) was obtained at PR2 and the lowest one at PR1 (score 0) (Figure 4.8.5). In winter, no significant difference in mean growth inhibition value was observed between PR1 and PR2 and presented a toxic effect (score 2) on *T.thermophila* exposure to the river samples at these sites. Very low growth inhibition was observed at PR3 and PR4 with PE < 0%. The river samples at PR3 and PR4 had no significant toxic effect (score 0) on *T.thermophila*. The high effect of *T.thermophila* exposure to the river samples in autumn and winter might be due to the suspended solids or microbial growth in the river water during the two seasons. According to Pereao et al. (2021) the presence of suspended solids or microbial growth in water can affect cell division and cause a slower growth of *T.thermophila*. Based on the results obtained, the presence or absence of ciliates can be correlated to a specific environmental condition and can be used as a biological indicator of pollution in river water. Thus *T.thermophila* could be an excellent tool to assess toxicity and pollution in aquatic systems. The results obtained in this study are high compared to Szklarek et al. (2021), where *T.thermophila* was exposed to river samples in Poland.

The spring mean toxicity of Plankenburg River with PE-MP samples exposed to the ciliate *T.thermophila* presented in Figure 4.8.6a shows a significant mean percentage growth variation over the four sites. A significant difference in mean growth inhibition value were observed at PR3 (60%), whereas the remaining three sites showed a significant decrease in microalgae growth with PE < 0% (Figure 4.8.6a). The river sample at PR1, PR2, PR4 had no significant toxic effect (score 0) on *T.thermophila*, while a toxic effect (score 2) was recorded at PR3.

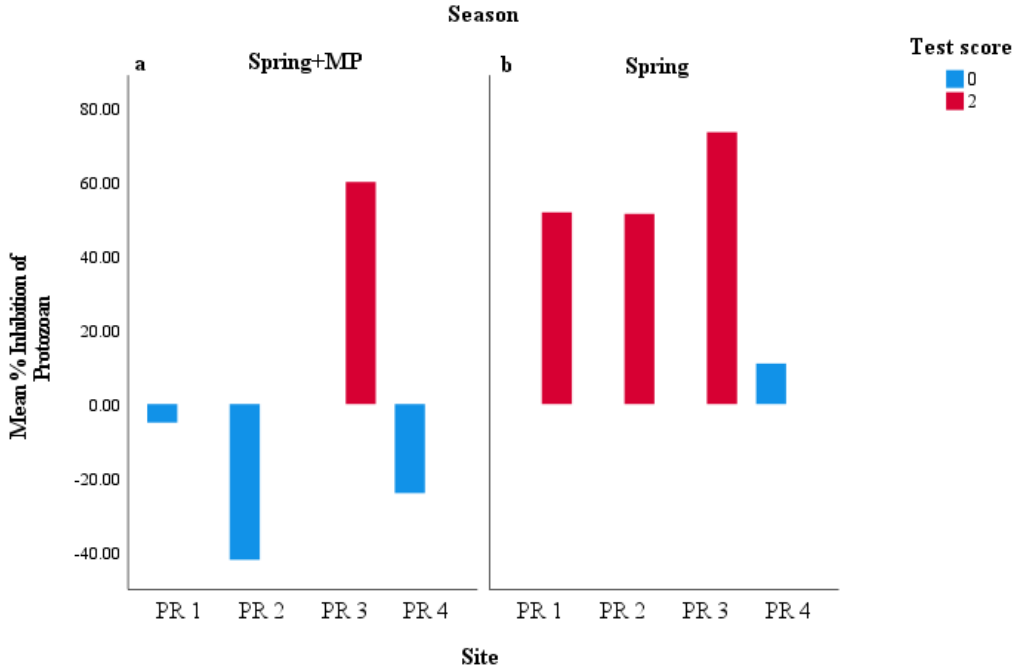

Figure 4.8.6: Mean toxic response (growth inhibition) of *T. thermophila* for Plankenburg River sample in Spring with virgin PE-MP (a) and without virgin PE-MP (b).

Results from samples with virgin PE-MP show a significant change in *T.thermophila* growth inhibition after 24 h of exposure compared to samples without PE-MP (Figure 4.8.6b). In this study, the presence of PE-MP impacted the toxicity effect of *T.thermophila*. The result of this study is similar to Wu et al. (2021) study on the sensitivity of *T.thermophila* when exposed to microplastics.

According to Casado et al.(2013), eco-toxicity studies on the sensitivity of trophic level of samples with microplastics when compared to algae and *D. magna*, algae is expected to be the most sensitive trophic level. The results presented in this study have *R.subcapitata* > *Daphnia magna* > *T.thermophila* as sensitive trophic level, which is consistent with Casado et al.(2013) conclusion.

4.8.4 Acute Hazard Classification of Plankenburg River water samples

The proposed toxicity classification based on a battery of microbiotests presented in Figure 4.8.7 was applied by Mankiewicz-Boczek et al.(2008) and Szklarek et al. (2021), on river samples. Biological analyses were based on three bioassays' sensitivity of the surface-water samples from Plankenburg River over the study period. The river samples that were taken in the spring at the sampling sites PR1, PR2, and PR3 are classified as Class III (Acute hazard) since one of the bioassays examined (*T.thermophila*) yielded a 50% PE 100% result, but no other tests revealed toxic effects. The 20% threshold was not exceeded in any of the three tests, hence the sample for site PR4 falls under the category of no acute hazard (Class I).

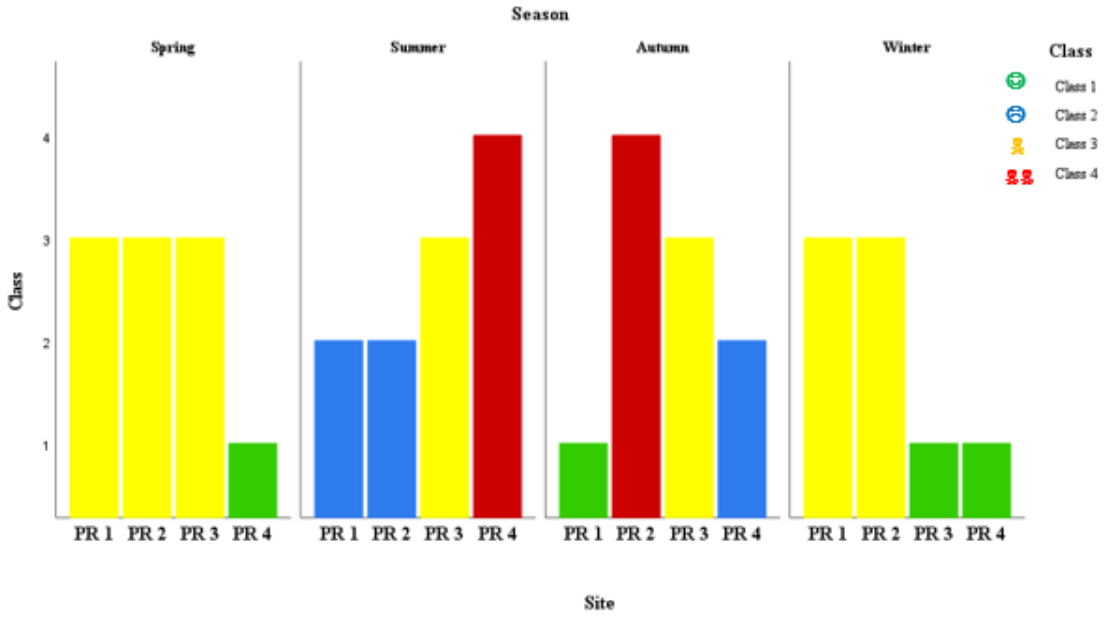

Figure 4.8.7: Seasonal toxicity classification for Plankenburg River

The percentage of the class weight score in spring presented in Table 4.8.1 is low at sites PR1, PR2 PR3 (33.5%) and very low at site PR4 (0%), and it is concluded that Plankenburg River samples contain virtually lower to no toxic chemicals, as against the physicochemical analyses parameters which indicated no pollution.

Table 4.8.1: Plankenburg River class weight score and percentage

Season	Site	class weight score	class weight score as a percentage
Spring	PR1	0.67	33.5
	PR2	0.67	33.5
	PR3	0.67	33.5
	PR4	0	0

	PR1	0.33	33
Summer	PR2	0.33	33
	PR3	0.67	33.5
	PR4	1	33.3
	PR1	0.33	33
Autumn	PR2	1	25
	PR3	1.33	66.5
	PR4	0.33	33
Winter	PR1	0.67	33.5
	PR2	0.67	33.5
	PR3	0	0
	PR4	0	0

The summer samples shown in Figure 4.8.7 are classified as Class II (Slight Acute Hazard) at sites PR1 and PR2 because only one test (*T.thermophila*) exceeded the 20% threshold while no other tests revealed harmful effects. Site PR3 samples are classified as Class III (Acute hazard) since only one of the bioassays conducted (*T. thermophila*) exceeded the 50% threshold for toxic effects. The samples from Site PR4 fall under Class IV (High Acute Hazard) since only one of the evaluated bioassays (*T. thermophila*) yielded PE 100%, while no other tests revealed toxic effects. The percentage of the class weight score in summer presented in Table 4.8.1 is between 33% and 33.5%, and it is concluded that Plankenburg River samples contain virtually lower toxic chemicals. The samples shown in Figure 4.8.7 are from the fall and are considered to pose no acute hazards (Class I) at site PR1 because none of the three tests surpassed the 20% threshold. Site PR2 samples fall under Class IV (High Acute Hazard) since only one of the assessed bioassays (*T. thermophila*) produced PE 100%, while no other tests revealed toxic effects. Site PR3 samples fall into the category of acute hazard (Class III) since two of the examined bioassays (*T. thermophila* and *D. magna*) exceeded the 50% threshold but no other test demonstrated a toxic effect. The samples collected at site PR4 fall under Class II (Slight Acute Hazard) since only one test (*T. thermophila*) exceeded the 20% threshold while no other tests revealed harmful effects. The percentage of the class weight score in autumn presented in Table 4.8.1 is 25% (PR2), 33% (PR1, PR4) and 66.5% (PR3), and it is concluded that Plankenburg River at PR1, PR2, and PR4 contains virtually lower toxic chemicals, and at PR3 the river samples can be considered seriously hazardous and acutely toxic to aquatic flora and

fauna. The winter samples shown in Figure 4.8.7 are classified as an acute hazard (Class III) at sites PR1 and PR2 because one of the evaluated bioassays produced a result of 50% PE 100%. (*T. thermophila*). A Low (33.5%) percentage of the class weight score is shown in Table 4.8.1, indicating a less toxic chemical. The Plankenburg River at the sampling sites PR3 and PR4 are classified as no acute hazard (Class I) because the 20% threshold was not exceeded in all three tests. The very low percentage class weight score at sites PR3 and PR4 in Table 4.8.1 can be an indication of no toxic chemicals, as against the physicochemical analysis parameters, which indicated no pollution.

An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix A-1.

Figure 4.8.8a presented the toxicity classification of Plankenburg River samples collected in spring with virgin PE-MP. Two types of classes were observed after the exposure of the samples with the three test models bioassays used in this study. Sites PR1, PR2, and PR4 are classified as a slight acute hazard (Class II), as only one test (*R. subcapitata*) are above the 20% threshold for toxic effects. Whereas at site PR3, one of the examined bioassays (*T. thermophila*) exceeded the 50% threshold, and the sample is in the no acute hazard category (Class I). The results of the toxicity classification of Plankenburg River in spring with virgin PE-MP (Figure 4.8.8a) is completely different from the result obtained in

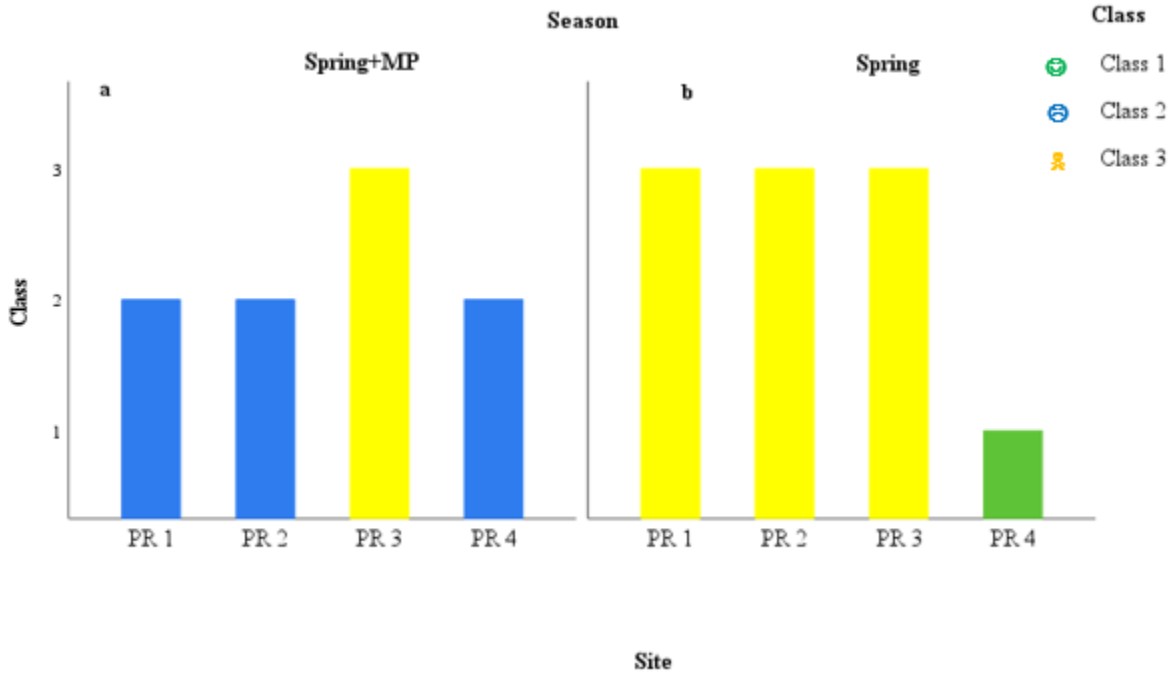

Figure 4.8.8: Toxicity classification for Plankenburg River with virgin PE-MP (a) and without virgin PE-MP (b).

Figure 4.8.8b without the virgin PE-MP. A decrease in Classes was observed at PR1 and PR2, with an increase at PR4 and constancy at PR3. The presence of virgin PE-MP impacts the toxicity classification of the freshwater system. Therefore, it will be important to investigate other parameters or chemicals that may influence the impact of virgin PE-MP on freshwater organisms.

Table 4.8.2:Plankenburg River with virgin PE-MP class weight score and percentage

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

The percentage of the class weight score of the sample in spring with virgin PE-MP presented in Table 4.8.2 is low at sites PR1, PR2 and PR4 (33.3%) and relatively high at site PR3 with 50%. It is concluded that Plankenburg River samples in spring with virgin PE-MP contain toxic chemicals at PR3 and lower toxic chemicals at the other three sites. The samples at PR3 can be considered hazardous and acutely toxic to aquatic flora and fauna.

An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix A-2.

4.9 Microplastics and temperature variability exposure studies

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

4.9.1 *Raphidocelis subcapitata* growth inhibition study

The toxicity of the analysed Milli-Q water with virgin PE-MP at three different temperatures (0.5°C, 1°C and 1.5°C) was determined. The mean growth inhibition of *R. subcapitata* exposed to different temperatures shown in Figure 4.9.1 ranged from 30.10% to 33.20%. No significant difference in mean growth inhibition values was observed between the three temperatures. It can be observed from this study that the increase in temperature might affect aquatic organisms, and an adaptation at high temperature (1.5°C) of *R. subcapitata* can be possible. The results of

this study suggest that increased temperature negatively affected aquatic organisms and but *R. subcapitata* has adaptation potentials.

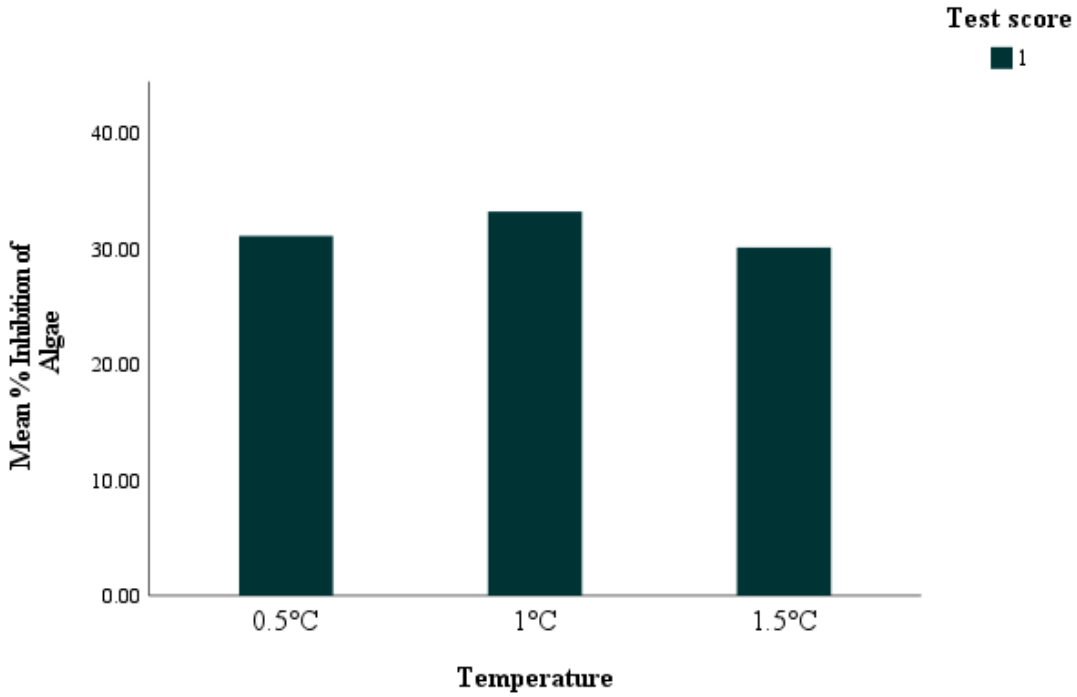

Figure 4.9.1: Mean toxic response of algae for Milli-Q water with virgin PE-MP

4.9.2 *Daphnia magna* immobility study

The immobility test for *D. magna* exposed to Milli-Q water with virgin PE-MP at increases of 0.5°C, 1°C and 1.5°C from optimal ambient temperatures are presented in Figure 4.9.2. The mean mortality rates of the exposed *D. magna* showed high toxicity at the 0.5°C temperature increase with 35% neonates mortality, while 20% and 25% neonates mortality occurred at 1°C and 1.5°C, respectively. The Milli-Q water with virgin PE-MP was rated 1 with a significant toxic effect on *D. magna*. The result of this study revealed the negative effect of temperature increase on *D. magna*. The implication of this is that temperature increases of 0.5^{o}C in combination with PE-MP could be significantly dangerous to the aquatic fauna.

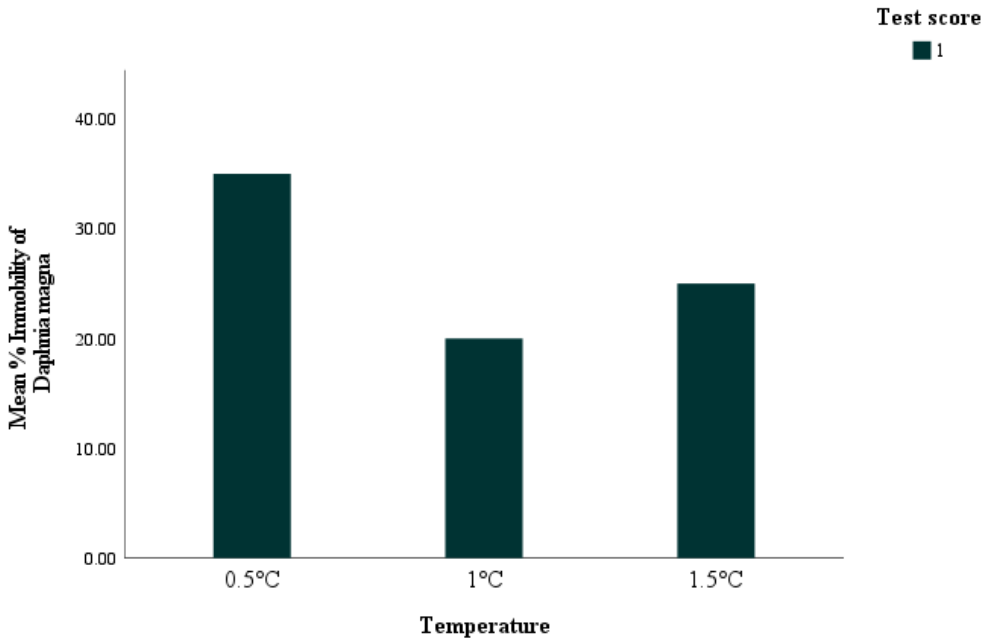

Figure 4.9.2: Mean toxic response of *D. magna* for Milli-Q water with virgin PE-MP

4.9.3 *Tetrahymena thermophila* growth inhibition study

The mean percentage inhibition of Milli-Q water with PE-MP exposed to ciliate, *T. thermophila* at three different temperatures (0.5°C, 1°C, 1.5°C) shown in Figure 4.9.3. There were significant variations in the percentage of growth inhibition over the different temperatures. Cell proliferation was observed at 1°C and 1.5°C with -673.585 and -10.3 percentage of growth, respectively. The Milli-Q water with PE-MP at 0.5°C and 1°C had PE= 100% (score 3) and 1.5°C had a significant toxic effect (score 1) on *T. thermophila*.

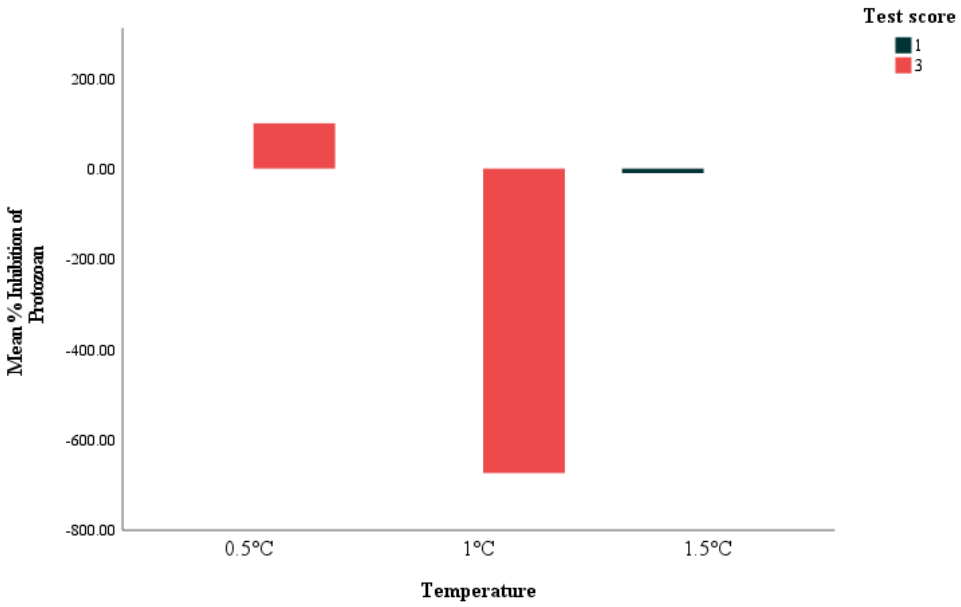

Figure 4.9.3: Mean toxic response of *T. thermophila* for Milli-Q water with virgin PE-MP

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

The proposed toxicity classification using the battery of tests presented in Figure 4.9.4 was applied on Milli-Q water with virgin PE-MP. Two types of classes were observed after the exposure of the samples with the three test models bioassays used in this study. Water samples with temperatures of 1.5°C increase was categorised as Class II (slight acute hazard) because the 20% threshold was exceeded in two tests (*R. subcapitata* and *D. magna*), but no other test showed a toxic effect. At a temperature of 0.5°C and 1°C a PE of 100% was obtained in at least on bioassays tested and classified as high acute risk (class IV).

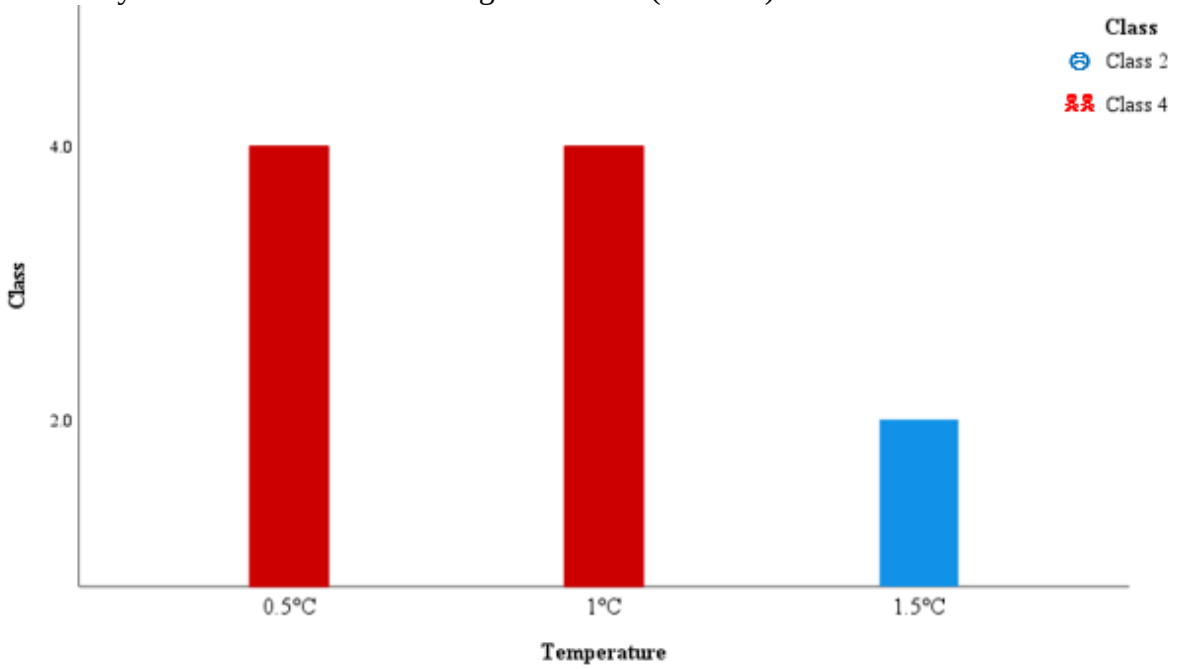

Figure 4.9.4: Toxicity classification for Milli-Q water with virgin PE-MP

The percentage of the class weight score presented in Table 4.9.1 is between 100% and 55.67%, and it is concluded that Milli-Q water with virgin PE-MP can be considered hazardous and acutely toxic to aquatic organisms.

An overview of the PE, the Class weight score and class weight score as a percentage was presented in appendix A-3.

Table 4.9.1: Milli-Q water with virgin PE-MP class weight score and percentage

Temperature	class weight score	class weight score as a percentage
0.5°C	1.67	55.67
1°C	1.67	55.67
1.5°C	1	100

4.10 Mutagenicity of the water samples

Mutagenicity, which alters the DNA structure permanently, has become a growing problem in recent years. According to Liu et al. (2015), an organism's genetic material can modify how it functions in ways that are heritable. The measurement of mutagenic risks has been made possible by the use of short-term bioassays, which can identify a wide range of chemicals that may cause genetic damage. To assess the mutagenicity of the water from the Plankenburg River, the S. typhimurium TA98 strain was employed.

4.10.1 Ames fluctuation test

Maron & Ames, (1983) posited that *Salmonella* assay is a generally accepted biotest to detect mutagenicity of individual compounds and environmental samples. Ames test with TA98 strain was applied for assessing the mutagenicity of the water samples of the Plankenburg River. The Ames test results of the Plankenburg River water samples showing LC10, LC20, and LC50 values are presented. No statistically significant concentration was found ($p(F) > 0.05$); the selected effective concentrations (LCx) of the reversion *S. typhimurium* rate TA 98 (frameshift mutagen indicator) and the obtained LOEC and NOEC at a 95% confidence limit based on Fieller's theorem (Table 4.10.1).

Table 4.10.1: Results of a 48 h of the reverted analysis for Plankenburg River Ames test

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

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

The strain TA98+S9 exposed to the Plankenburg River for 6 days showed mutagenic activity at LOEC (≤ 0.194) in the Ames test results from sites PR2 and PR4, however, LC50 was not detected at both sampling sites. When the strain TA98-S9 was exposed, a sample obtained at PR2 showed a positive mutagenic response (LOEC of 0.571) with an average LC50 value of 0.850, while at site PR4 mutagenic activity was reported with a LOEC of ≤ 0.286. It is suggested that the Plankenburg River water body's potential for environmental mutagenesis is strongly influenced by the mechanism of reading-frame shifting of genetic macromolecules

(Roveri et al., 2021)). This study's findings are comparable to those reported by Roveri et al. (2021), who did find genotoxicity activity in Guarujá surface runoff water.

An overview of the number of revertant cells over a period of 6 days was presented in Tables 4.10.2 and 4.10.3.

Table 4.10.2: Results of Plankenburg River exposure to strain 98+S9 for a period of 6 day

Plate	Concentration	Day 2	Day 3	Day 4	Day 5	Day 6
Blank (sterile water)	-	0	0	0	0	0
Blank PR2	100,00%	73	96	96	96	96
Blank PR4	100,00%	94	96	96	96	96
Background	-	1	2	8	10	12
Positive Control	-	36	96	96	96	96
P2a	100,00%	96	96	96	96	96
P2b	51,61%	96	96	96	96	96
P2c	19,35%	85	96	96	96	96
P4a	100,00%	96	96	96	96	96
P4b	51,61%	96	96	96	96	96
P4c	19,35%	96	96	96	96	96

Table 4.10.3: Results of Plankenburg River exposure to strain 98 - S9 for a period of 6 day

Plate	Concentration	Day 2	Day 3	Day 4	Day 5	Day 6
Blank (sterile water)	-	0	0	0	0	0
Blank PR2	100,00%	2	2	5	9	13
Blank PR4	100,00%	60	94	94	96	96
Background	-	0	1	2	4	4
Positive Control	-	0	51	90	91	95
P2a	100,00%	3	59	69	81	82
P2b	57,14%	2	23	42	72	91
P2c	28,57%	1	3	19	32	55
P4a	100,00%	7	31	36	71	94
P4b	57,14%	7	23	57	61	78
P4c	28,57%	2	10	14	28	33

CHAPTER 5:CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Studies on microplastics pollution and its possible effects on aquatic ecosystems have increased recently, but there are still knowledge gaps on this topic, particularly concerning South African freshwater ecosystems. This research study evaluated MP's distribution, abundance and characteristics in the surface water and sediments of the Plankenburg River in the Western Cape and provided insights into their physicochemical parameters. Pre-treatment methods used to extract the microplastics included density separation, filtration, and alkaline digestion to ensure efficient extraction from water and sediment samples. MPs in the surface water were in abundance in spring at 5.13 ± 6.62 MPs/L, while the occurrence of MPs in sediments was highest in spring at 1587.50 ± 599.32 MPs/kg. Infrared spectroscopy analysis confirmed that the dominant polymer type was polyethylene (57.86%), and fibres (98.40%) were recorded as the dominant forms of microplastic particles, while the size range of 500 to 1000 µm (61.87%) was the dominant MPs at different sites. Spatial and temporal distributions showed differences based on anthropogenic activities, and the COD and BOD levels in the river showed to be above the South African water guideline threshold.

The battery of biotests showed a variation in the level of toxicity of river samples over the four seasons, which confirms the need to set up biotest analyses in addition to the physicochemical assessment of the river. Such a strategy guarantees the establishment of a complete cause-and-effect study and identifies practical remedies to improve the quality of water resources. The eco-toxicity assessment of the river freshwater presented some adverse impacts on the battery of biotests that were not obtained with physicochemical parameters. Plankenburg River study without virgin PE-MP showed high sensitivity on *T. thermophila* compared to the other bioassays tested. The highest toxicity level was recorded in Summer and Autumn, with high acute hazard (class IV) at PR4 and PR2, respectively. The exposure of bioassays to the Plankenburg River with virgin PE-MP presented a total different sensitivity to biological organisms used in this study. The samples were more sensitive to *R. subcapitata*, with Class II (Slight Acute Hazard) being the lowest and Class III being the greatest (Acute hazard). The presence of virgin PE-MP in the river may have contributed to the degradation of river water quality, which might have a toxicological impact on freshwater organisms.

The experiment on the effects of climate change at three different temperatures with an increase of 0.5°C showed similar sensitivity results obtained with the spring samples and the virgin PE-MP exposed to bioassays. Temperature increases negatively affected test organisms. The genotoxicity results revealed that the Plankenburg River water had mutagenic activity- a concern for human health and the aquatic ecosystem. This study demonstrated that the microplastic pollution occurrence in the investigated freshwater systems was predominantly due to human activities in the vicinity of the sampled sites.

This study provides a baseline data for future monitoring and assessment of water and sediment in the South African freshwater systems.

5.2 Recommendation

This study showed the need for further investigations to the effect of runoff, wind, and season on the occurrence of microplastics in freshwater ecosystems. Toxicological assessment of freshwater systems requires deeper research into microplastics bioassay exposure studies. Genetic toxicology tests, such as the Ames test, can be applied as a routine measure to monitor and/or screen the freshwater ecosystems.

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APPENDICES

Appendix A

Appendix A-1: Seasonal hazard scoring system of Plankenburg River

Season	Site	Microalgae (*Pseudokirchneriella subcapitata*)		Crustaceans* (Daphnia magna)		Protozoa* (Tetrahymena thermophila		Class
		% effect	Test score	% effect	Test score	% effect	Test score	
Spring	PR1	6.2	0	0	0	51.84	2	III
	PR2	12.5	0	0	0	51.44	2	III
	PR3	-1.4	0	0	0	73.49	2	III
	PR4	0.8	0	5	0	10.98	0	I
Summer	PR1	6.2	0	10	0	28.35	1	II
	PR2	8.4	0	5	0	46.71	1	II
	PR3	10.7	0	0	0	94.04	2	III
	PR4	12.8	0	0	0	100	3	IV
Autumn	PR1	22.5	1	0	0	-39.44	0	I
	PR2	18.6	0	0	0	100	3	IV
	PR3	6.8	0	95	2	74	2	III
	PR4	5.8	0	0	0	45.5	1	II
Winter	PR1	6.2	0	10	0	72.43	2	III
	PR2	12.5	0	0	0	74.89	2	III
	PR3	-1.4	0	0	0	3.41	0	I
	PR4	0.8	0	5	0	-18.52	0	I

Appendix A-2: Plankenburg River with virgin PE-MP hazard scoring system

Season	Site	Microalgae (*Pseudokirchneriella subcapitata*)		Crustaceans* (Daphnia magna)		Protozoa* (Tetrahymena thermophila		Class
		% effect	Test score	% effect	Test score	% effect	Test score	
Spring	PR1	39.1	1	0	0	-5	0	II
	PR2	29.2	1	20	0	-42	0	II
	PR3	28.5	1	5	0	60	2	III
	PR4	32.2	1	5	0	-24	0	II

Appendix A-3: Milli-Q water with virgin PE-MP hazard scoring system of Plankenburg River

Temperature	Microalgae (*Pseudokirchneriella subcapitata*)		Crustaceans* (Daphnia magna)		Protozoa* (Tetrahymena thermophila		Class
	% effect	Test score	% effect	Test score	% effect	Test score	
0.5°C	31.1	1	35	1	100	3	IV
1°C	33.2	1	20	1	-673.585	3	IV
1.5°C	30.1	1	25	1	-10.3	1	II

Appendix B

Appendix B-4: Krom River

Appendix B-5: Kayamadi Informal Settlement/Commercial activities

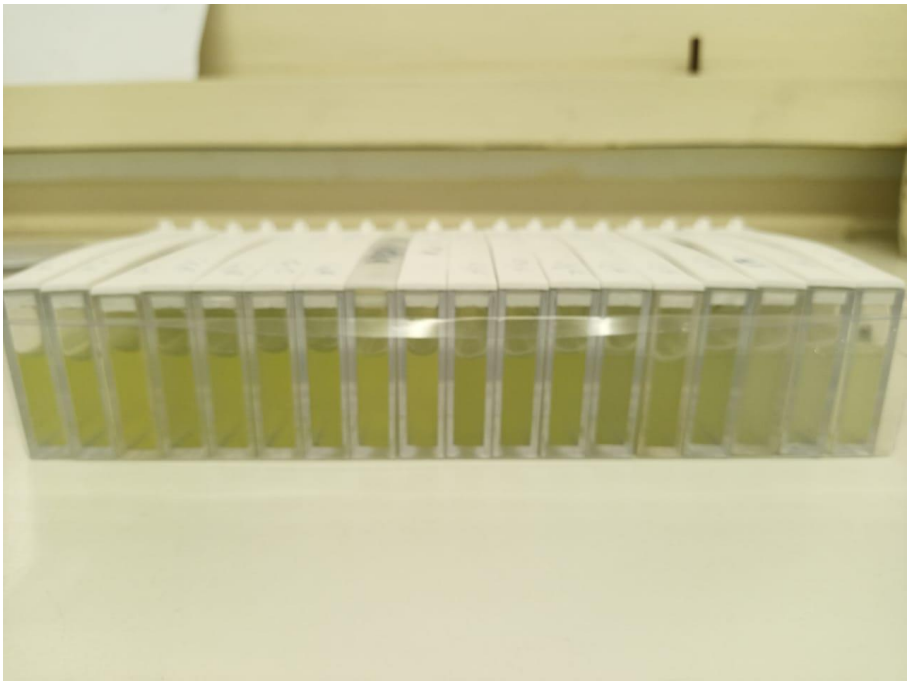
Appendix B-6: Plankenburg River mixed with Krom River

Appendix B-7: Industrial and recreational park

Appendix C

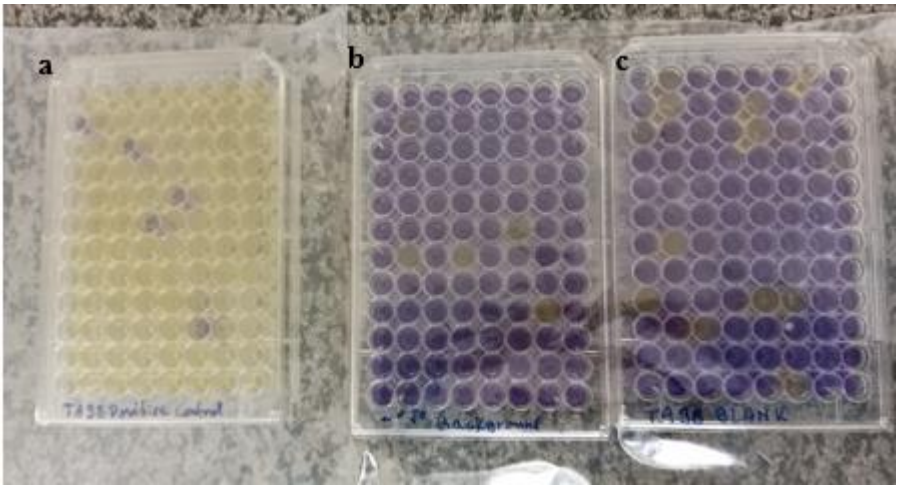
Appendix C-8: Dried sediments mixed with KOH and NaCl

Appendix C-9: FTIR Instrument for polymer analyses



Appendix C-10: River sample exposed to *R. subcapitata* after 72h incubation

Appendix C-11: Sample exposed to *D. magna* after 48h incubation



Appendix C-12: Ames test at day 6 (Positive control (a), Background (b) and blank (c))