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A COMPREHENSIVE ANALYSIS OF URBAN RIVER POLLUTION – THE CASE OF THE HENNOPS RIVER IN GAUTENG PROVINCE, SOUTH AFRICA



UNIVERSITY OF THE
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JOHANNESBURG

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

Johannesburg, 2023

DECLARATION

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

TLetseka

(Signature of candidate)

31st day of October 2023 at University of the Witwatersrand

ABSTRACT

The water quality of rivers is declining at an alarming rate due to pollution from anthropogenic activities associated with urbanization. To ensure ecological restoration and management of rivers, engaging in pollutant source apportionment, evaluation, and monitoring of water quality is of great significance. The study delivers a comprehensive assessment of the state of pollution in the Hennops river catchment facing pollution threats from rapid urbanization. The water quality assessment of the Hennops river was performed through chemical, microbiological, microplastics analysis and ecotoxicological approaches, spanning from upstream region in Tembisa to the downstream Hartbeespoort Dam.

Standard methods were employed to assess physiochemical properties of the river's water. Electrical conductivity and pH fell within the accepted criteria based on the standard water quality guidelines. However dissolved oxygen (DO) levels were below acceptable limits, ranging from 1.53 mg L^{-1} to 6.47 mg L^{-1}. This signifies a substantial demand for oxygen in the river, likely due to the discharge of sewage from leaking pipes and wastewater treatment plants. This sewage introduces a high volume of organic matter, leading to an increased oxygen demand in the water. Microbiological pollution indicators were employed to assess the microbial water quality of the river. The study's findings revealed elevated bacterial counts, with *Escherichia Coli (E. coli)* reaching up to 2 250 cfu mL^{-1} upstream and decreasing to 30 cfu mL^{-1} downstream. These high counts suggest faecal contamination in the river water. Similar trends were observed with total coliform counts, high coliform counts 170 000 cfu mL^{-1} in the upstream which remained detectable even downstream and beyond the Hartbeespoort Dam, despite the dilution effects within the dam. The dam was identified as the primary repository for pollution originating upstream.

Grab sampling followed by solid phase extraction (SPE) and the passive sampling using a Polar Organic Integrative Sampler (POCIS), were employed as sample preparation methods for preconcentration of methocarbamol, etilefrine, nevirapine, carbamazepine and venlafaxine from river water with subsequent analysis on Liquid Chromatography coupled to quadrupole time of flight mass spectrometry. Both methods yielded good figures of merit with limits of quantification in the range of 0.57 to 2.12 ng mL^{-1} for POCIS and 0.19 to 1.82 ng mL^{-1} for SPE. The compounds were detected in the water but at low levels (μgL^{-1}), with detected concentrations of carbamazepine in the range 0.62 ng mL^{-1} – 0.32 ng mL^{-1}, methocarbamol detected in the range 0.11 ng mL^{-1} - 0.14 ng mL^{-1} and venlafaxine 0.50 ng mL^{-1} – 0.44 ng mL^{-1} using POCIS. The detected concentrations using SPE were in the range 0.13 ng mL^{-1} – 0.19 ng mL^{-1} for carbamazepine, while nevirapine and venlafaxine were detected although below limit of quantification. This underscores the advantage of using passive samplers, which enable the detection of fluctuating contaminant concentrations over time, in contrast to the one-time measurements obtained through grab sampling. In the case of microplastics in the

water and sediment samples, five polymer types were identified: polyethylene (PE), polypropylene (PP), high density polyethylene, (HDPE), polyester and polystyrene. The predominant polymer type in surface water was PE (48.6 %), and that in sediment was PP (52.7 %). PE and PP were the most abundant polymer types in both phases, and as these also the leading polymers in plastics production. 80% of the identified microplastics were found to be fibre with most dominant sizes of 1-2 mm for sediments and 0.5-1 mm in water samples.

The conducted tests deemed the river water not suitable for irrigation, drinking or recreational purposes and not capable to support aquatic life.

Keywords: River pollution, water quality assessment, Passive sampling, microplastics

DEDICATION

The thesis is given to the memories and influence to my family; my wife for your everlasting support, patience, and giving me purpose in life, to my father who taught me to be strong and never keep my eyes of the target at all times. It is also dedicated mother's hard work to ensure that I had the best education and upbringing.

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
DSDME	Directly Suspended Droplet Microextraction
EPA	Environmental Protection Agency
EU	European Union
GC-MS	Gas Chromatography Mass Spectrometry
HDPE	High Density Polyethylene
HF-LPME	Hollow Fibre Liquid Phase Microextraction
HLB	Hydrophilic-Lipophilic Balance
LDPE	Low Density Polyethylene
MPs	Microplastics
PAHs	Poly Aromatic Hydrocarbons
PE	Polyethylene
PES	Polyethylene Sulfone
PET	Polyethylene Terephthalate
POCIS	Polar Organic Chemical Integrative Sampler
POPs	Persistent Organic Pollutants
PP	Polypropylene
PTFE	Polytetrafluoroethylene
SBSE	Stir Bar Sorptive Extraction
SDME	Single Drop Micro-Extraction
SEM	Scanning Electron Microscope
SPE	Solid Phase Extraction
SPMD	Semi Permeable Membrane Device
SPME	Solid Phase Microextraction
TAC	Total Aerobic Count
TDS	Total Dissolved Solids
TWA	Time-Weighted Average
UHPLC	Ultra - High Performance Liquid Chromatography
WWTP	Wastewater Treatment Plants
WWTW	Wastewater Treatment Works

CHAPTER 1: INTRODUCTION

A negative projection is predicted for Africa's future water resources and river systems. At the same time, South Africa's rivers also suffer the same fate with additional factors putting further pressure on them. As a country which is rapidly developing, South Africa's rivers encounter several issues linked to development and human activity. The first, and most severe of these problems is the ongoing pollution of rivers across the country. To name but a few of these pollution issues, South Africa's rivers are victims of sewage spills, plastic pollutants, and heavy metal contaminants (Atangana and Oberholster, 2021) (Lebepe et al., 2020; P. J. Oberholster et al., 2008a; Okonkwo and Mothiba, 2005; Sibanda et al., 2015a; Weideman et al., 2020a) This goes above and beyond the impact acid mine drainage and eutrophication have had on water resources across the country as a whole (McCarthy Terence S., 2011; Naicker et al., 2003; Van Ginkel, 2011). Therefore, South Africa's rivers and water resources face the impending and constant threat of climate change, but also face challenges related to an alarming amount of pollution being discharged into them.

The water quality of rivers is depleting at an alarming rate mainly due to pollution from anthropogenic activities, which hinders the supply of clean adequate water to support life (Khatri and Tyagi, 2015a). Rivers that flow around most urban areas are receiving most pollution due to activities associated with urbanization (P. J. Oberholster et al., 2008a). Rapid urbanization has led to the emergence of informal settlements in most countries like South Africa and essential service provision such as proper sanitation and waste management in such communities remains a challenge for government authorities (Schutte and Focke, 2007). Agricultural activities, effluent released from wastewater treatment plants (WWTP) and industries have also been linked to contribution to the discharge of toxic organic compounds into water, among those being emerging contaminants and microplastics (Dalu et al., 2021). Continuous discharge of pollutants into the environment may lead to their accumulation which may endanger the lives of human, plants and animals exposed to those water bodies (Yu et al., 2020; Kay et al., 2018).

Emerging contaminants are defined as a broad range of chemicals are not regularly monitored in the environment but have the likelihood to find way into the environment through point and non-point sources and cause known or suspected adverse human health and/or ecological effects (Stuart et al., 2012). The discharge of these chemicals in the environment may have probably occurred for over an extended period of time but have not been recognised until recent developments in the detection methods of the pollutants in the environment were employed (Abtahi et al., 2019). Other factors that lead to the these compounds being termed as "emerging contaminants" may be recent synthesis of the new substances or changes in use and disposal of existing chemicals which then creates new

sources of contaminants (Mills, 2015). Emerging contaminants in water systems can be broadly classified as, pharmaceuticals, pesticides, industrial compounds and personal care products among others (Montes-Grajales et al., 2017).

1.1 PROBLEM STATEMENT

The Hennops river valley catchment is located between Johannesburg and Pretoria. Its main source originates in Kempton Park in the form of Kaalspruit and joins the Crocodile River then flows into the Hartebeespoort Dam. Within the Hennops River basin, urban settlements, agricultural land as well as industries can be found along the Hennops River and its tributaries. More recently, the state of the Hennops River has become alarming, being reported as heavy polluted and in need of intervention (Tampio et al., 2016). As suggested in some of these media sources and confirmed by research, the Hennops River, like other rivers across the country are affected by the human activity surrounding it. While the river has been reported as polluted, it must be noted that those living round the Hennops River are also adversely affected. To complicate matters further, communities, industries as well as other facilities and land users around the river have been attributed to its pollution (P.J. Oberholster et al., 2008). Therefore, there is an urgent need to engage with key stakeholders and communities around the polluted Hennops River and its basin.

The Hennops river is experiencing a decline in water quality as a result of pollution from upstream activities (Nawn, 2004). This is evident by the death of animal life such as fish, insects and plant life along the river. The riverbed is also coated with black sludge along with vast amounts of solid waste, mostly being plastics. The upstream land use activities around the river catchment include urban development activities such as industries, formal and informal settlements, wastewater treatment works and agriculture. Tembisa and Ivory Park are areas characterized with high population density, with housing consisting of squatter shacks that have very poor sanitation that is not properly maintained which result in periodic leakages that are discharged into the Kaalspruit river that forms Hennops. Other households have improper sanitation in place and have connected toilets directly into the river (Mashazi et al., 2019). This results in faecal matter being directly discharged into the river.

Provision of sanitation is a challenge and such communities resort to other means of sanitation that include open defecation, the use of buckets, plastic bags and improper waste disposal through informal dumpsites which exacerbates the deteriorating water quality status of the Hennops River (Muanda et al., 2020). This introduces pathogenic bacteria and the ingestion of such polluted river water would result in infectious diseases such as cholera, severe diarrhoea and dysentery (Bain et al., 2014). According to a report by the World Health Organization in 2019, it was noted that approximately 485,000 deaths from diarrhea occur worldwide each year due to the consumption of water contaminated with harmful pathogens (WHO, 2019). Illegal littering and dumping introduces

plastic pollution and this has been observed in Kaalspruit catchment that drains Tembisa, which has led to the river being polluted with plastics (Bodenstein et al., 2006).

Industrial activities in Clayville include pharmaceuticals manufacturing, food processing factories, and beverage production around the river basin. This types of industries generate large quantities of waste water that is highly contaminated with both organic and inorganic pollutants (Iloms et al., 2020). The waste discharge from this industrial site is sent for treatment in Olifantsfontein WWTW which discharges treated effluent into the Hennops river ("JR Hoffmann," 2003). Most conventional wastewater treatment plants (WWTP) have been observed to be inefficient in removing of toxic pollutants and during effluent discharges these contaminants are also released into rivers (Szymonik et al., 2017). The Olifantsfontein WWTP discharges approximately 38 to 60 megalitres per day of effluent into the Hennops River. This discharge significantly contributes to the overall flow volume of the river. Consequently, there is a possibility that this discharge could introduce pollutants into the river. (Oberholster et al., 2008). The Sunderland Ridge (WWTP) is located downstream of the river and has also been found to release large quantities of untreated waste into Hennops river (Rimayi et al., 2019).

The Hennops River has undergone a decline in its visual attractiveness and has transformed into a liability instead of the environmental asset with fundamental values to those who are surrounded by it. It has become unfit for both living organisms, agricultural and recreational use due to its poor ecological condition (Nawn, 2004) and measures have to be taken for its rehabilitation and placement of pollution preventative measure. Reported water monitoring approaches conducted around the Hennops River catchment has been primarily focused on determining the physical, chemical or biological properties of water in isolation from one another. This attempt is inadequate to convey the pollution status of an ecosystem. However, a combination of the above properties with ecotoxicological data is argued to be an effective tool in pollution assessment as it reflects the effects of pollutants on aquatic biota (Serpa et al., 2014); (Gheorghe et al., 2016). There is a deficiency of data indicating a comprehensive assessment of the pollution state in the Hennops river by physiochemical, biological and ecotoxicological approaches.

1.2 RESEARCH AIM AND OBJECTIVES

The study was part of an umbrella project entitled – A combination of Chemical Analysis and stakeholders' participation in addressing the Hennops river pollution in Gauteng Province, South Africa. The scope of the study covered mainly the chemical analysis aspects.

The aim of the research project is to assess the water quality state of the Hennops River catchment through the following approaches (1) physiochemical analysis, (2) microbiological analysis, and (3) chemical analysis.

The specific objectives of the project are:

i. To use indicators of microbiological pollution; total aerobic count, total coliforms and *Escherichia coli* to determine the microbial quality of the Hennops river.
ii. To calibrate the Polar Organic Integrative Sampler (POCIS) within controlled laboratory settings through a static depletion approach to determine sampling rates for target organic compounds.
iii. Field deployment of POCIS in the Hennops river for extraction of organic pollutants and performance comparison alongside spot sampling with Solid Phase extraction (SPE).
iv. To study the abundance, physical and chemical properties of microplastics in Hennops river water samples and sediments.

1.3 KEY RESEARCH QUESTIONS

i. What is the water state of pollution in the Hennops river basin?
ii. Which organic pollutants are present in the river and their probable environmental impacts?
iii. What is the extent of microplastic pollution in the Hennops river?

CHAPTER 2: LITERATURE REVIEW

Rivers play an essential role as a mediator between human activities and the natural environment by providing essential functions that include water and food supply, irrigation, and transportation. To ensure ecological restoration and management of rivers, engaging in pollutant source appointment, evaluation, and monitoring of water quality is of great significance (Chen et al., 2018). Water quality is a parameter that is for evaluating the suitability of water for its intended purpose (Khatri and Tyagi, 2015b). This is measured by assessing the chemical, physical and biological attributes of water against established standards, however, water quality monitoring is constrained by the limited test facility and capability in most African countries (Salako et al., 2020)

South Africa remains no exception to the daunting phenomenon of river pollution. A study conducted on the Jukskei revealed that Total Coliform and *Escherichia coli* detected in the river exceeded specifications for recreational, and irrigation activities (Hoorzook et al., 2021). The presence of polyaromatic hydrocarbons in the Jukskei river have also been reported (Sibiya et al., 2013a). The Diep River in the Western Cape has also been found to be under pollution stress from the mushrooming informal settlements in Dunoon that emanate from poor sanitation and improper waste disposal into the river. This has resulted in the levels of *Escherichia coli*, dissolved oxygen (DO), electrical conductivity, salinity, turbidity, chemical oxygen demand (COD), and ammonia surpassing the suggested thresholds (Gqomfa et al., 2022).

The Hennops river has recently caught the attention of researchers and environmental activists as it is undergoing pollution threats due to anthropogenic activities from different land users around it. Such activities include raw sewage pollution and improper disposal of solid waste through illegal dumping and littering that has also led to the introduction of plastic waste, unused household chemicals and pharmaceuticals into the river. Plastic and microplastic pollution has been found to disturb the feeding, fertility, hatching and functioning of fish and zooplankton in water (Jemec et al., 2016). This is evident by the death of biota such as fish, insects and plant life along the river and this may be posed by emerging contaminants.

2.1 Sources of pollutants in urban river systems

Sources of river pollutants are grouped into point sources and non-point sources (Zhou et al., 2016). Point Sources of pollution are described as a discharge directly into a receiving environment from discrete, identifiable locations and can be measured for example industrial and municipal effluents. Non-point sources are those that enter the environment through diffuse, difficult to identify, and quantified sources of pollution as they do not have a single point of entry into the waterbody (Petersen

et al., 2017). The major sources and pathways of organic pollutants in river systems are outlined in Figure 2.1.

2.1.1 Wastewater treatment plants

When Pharmaceuticals are taken into the body of the patient or target consumer, some are converted to metabolites of the drug while others remain as unchanged parent compounds which are released from the body as combined faeces and urine which end up in municipal or hospital sewage that is sent off for treatment in Wastewater Treatment Plants (WWTPs) (Ncube et al., 2018). The process flow is outlined in Figure 2.1.

The other source of pharmaceuticals in the sewage is the disposal of unconsumed drugs which are disposed by individuals by flushing them down the sink or toilet (Tong et al., 2011). Conventional WWTPs are not specifically designed for removal of pharmaceutical compounds, they purify household wastewater mainly by action of bacteria on organic matter, then flocculation to get rid of any suspended solids and phosphates, but pharmaceuticals end up in treated waste as they are not removed (Szymonik et al., 2017). Treated waste is in two forms – sewage sludge and aqueous effluent, the aqueous effluent is discharged into receiving water bodies such as rivers and this is how WWTP serve as point sources of river pollution (Talvitie et al., 2015).

Wastewater treatment in South Africa faces several challenges which may include inadequate or aging infrastructure in many municipalities leads to issues such as leaking pipes, sewer blockages, and inefficient treatment facilities. This can result in untreated or partially treated wastewater being discharged into rivers and other water bodies. Many municipalities struggle with limited budgets for maintaining and upgrading wastewater treatment infrastructure. As a result, some treatment plants may not operate optimally, leading to increased pollution and health risks. As such, the Department of Water and Sanitation in South Africa developed the Green Drop Report as an initiative that aims to assess the performance of wastewater treatment plants across the country.

Wastewater treatment plants play a crucial role in safeguarding public health and protecting the environment by treating and purifying wastewater before it is discharged into natural water bodies. The Green Drop Report assesses various parameters related to the operation and maintenance of these plants, including compliance with regulatory standards, the efficiency of treatment processes, and the overall management of the facilities (Verster and Bouwman, 2020. By conducting regular assessments and publishing the Green Drop Report, the government aims to encourage transparency, accountability, and continuous improvement in the management of wastewater treatment plants (Semalti et al., 2021). The report also serves as a valuable tool for stakeholders,

including policymakers, water authorities, and the public, to monitor the progress and effectiveness of wastewater management initiatives in South Africa.

Two sewage treatment facilities, namely the Olifantsfontein WWTP and the Sunderland Ridge WWTP, are positioned within the Hennops river basin. The Olifantsfontein treatment plant is situated along the Kaalspruit River, which flows into the Olifantspruit river, subsequently merging with the Hennops river. According to the 2022 Green Drop National report, the Olifantsfontein WWTP attained a commendable Green Drop score of 81%, indicating its capacity to minimize the discharge of pollutants into the surrounding waters (Green Drop Report, 2022). Conversely, the Sunderland Ridge WWTP, located along the Rietspruit river, which converges with the Hennops River downstream (as depicted in figure 3.1), registered a Green Drop Score of 66% in the same report, depicting a decline from the 80% recorded in the 2013 report (Green Drop Report, 2022). This decrease in the performance of the Sunderland Ridge WWTP poses a potential hazard, potentially leading to the introduction of contaminants into the receiving waters.

Most WWTPs designed to remove large debris items with a mesh screen of 6 mm or larger in the primary treatment (Dalu et al., 2021). Given the physical properties of some MPs, large quantities of microplastic particles escape the WWTPs and are discharged in great amounts into receiving water bodies such as rivers daily (Mason et al., 2016). A research conducted by (Talvitie et al., 2015) at Viikinmäki WWTP in Helsinki revealed that the number of microfibers in wastewater influent reduced from 180 fibres per litre to between 13.8 and 14.2 fibres per litre after wastewater treatment processed. Although the concentrations of these microplastics are low, the large volumes of water released into the environment from WWTPs per day makes it a concern (Xu et al., 2019). This makes WWTPs one of the major contributors of microplastics in rivers as they receive waste from industries using microplastics and municipal waste-water (Zbyszewski et al., 2014). Microplastics are used as ingredients in personal care products, during bathing and washing with these products containing microplastics are washed down the drain and join municipals sewage which is directed WWTPs (Kurniawan et al., 2021).

2.1.2 Industrial wastewater

The plastic manufacturing and packaging industries serve as direct contributors of MPs into the environment occurring from spills that occur during manufacturing and transport of these products (Verster and Bouwman, 2020). (Karlsson et al., 2018) investigated the release of MPs plastic pellets in Sweden during production and transport and their findings were that between 3 to 36 million pellets are released annually into the surrounding environments. Effluents from textile industries contain dyes which pollute receiving waters and result in death of aquatic organisms (Semalti et al., 2021). Other

industries that have been found to release organic pollutants are paint industries which release polymers and plasticizers into water (Lorton, 1988).

2.1.3 Leakages

Unmanaged sewage mains have become a source of contamination to both ground and surface water through leakages from old, damaged pipes and structural faults. this results in sewage exfiltrating into both ground and river water (Held et al., 2007). Leakage of sewage lines from domestic use may lead to pathogen contamination into water bodies , this may lead to the introduction of nitrates and other nutrients into water that lead to eutrophication (McGrane, 2016). Industrial leakage however poses a greater threat as industrial effluents contain a high content of toxic pollutants (Iloms et al., 2020).

2.1.4 Informal settlements

Service delivery to informal settlements is a challenge because such areas are occupied illegally and are unplanned, this makes it difficult for relevant authorities to offer services to them. Such areas are characterized by lack of proper infrastructure, overcrowding, poor sanitation and poor waste management. Informal settlements are frequently formed in the vicinity of rivers and streams where they can access water and they end up polluting nearby water bodies (Abbott, 2002). In Gauteng the, Jukskei river is one of the highly contaminated rivers from the township Alexander it flows through (Sibali et al., 2013).

There is often no supply of water to informal settlements and the settler's wash their clothes in rivers that are in close proximity to them which introduces detergents and microplastics in river water (Connell et al., 2010). Waste removal is an issue with urban settlers, and the responsibility lies in the hands of individuals for waste disposal. and waste that is not formally collected is disposed in communal dumps and this can be carried into nearby rivers by wind and water run-off into water bodies where these plastics may be fragment to form MPs (Verster and Bouwman, 2020).

2.1.5 Waste Disposal

Potential pathways of microplastics into river systems is due littering, storm overflows, outflows from waste water treatment plants (Barboza et al., 2018). Phthalates are not chemically bound to resins or products when used and can easily be easily released into the environment through industrial effluent discharges and leachate from waste dumps (Abtahi et al., 2019). Unused Pharmaceuticals are disposed in landfills which in leach out then contaminate ground water and surface water and the pathway is indicated in Figure 2.1 (Tong et al., 2011). Following heavy rainfalls, microplastics can be flushed into rivers from poorly managed landfills or illegal waste disposal sites (Ziajahromi et al., 2016).

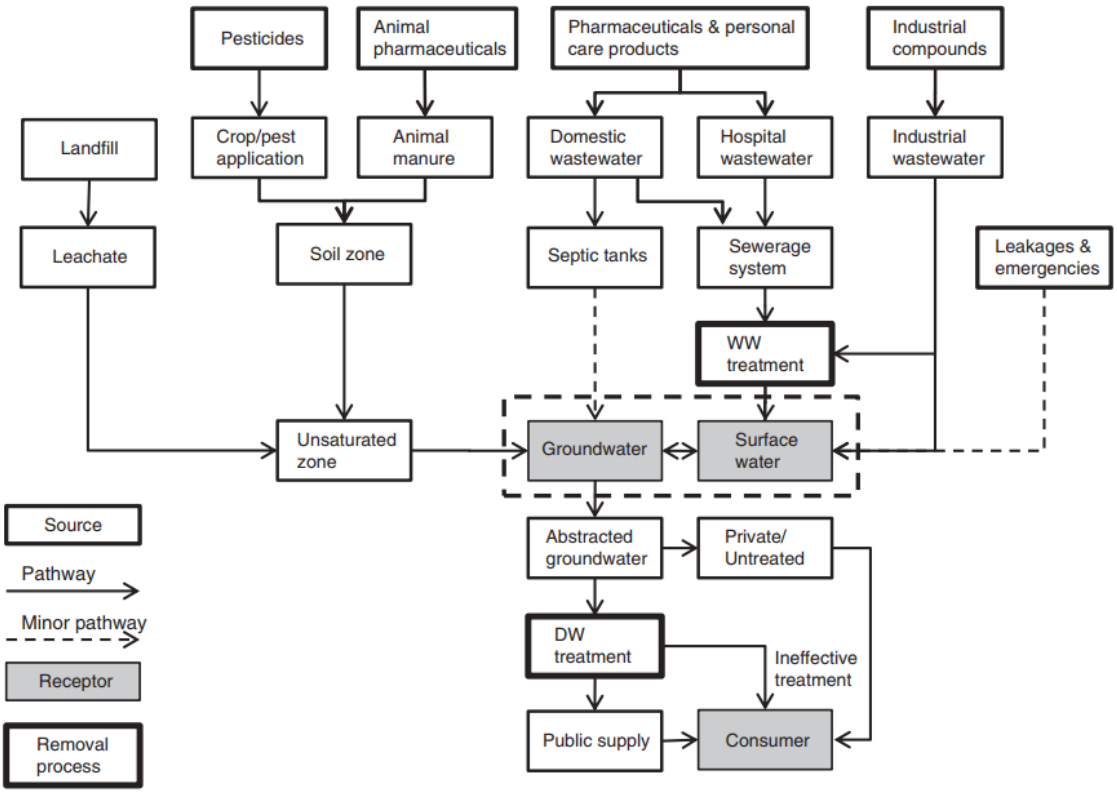


Figure 2.1: Sources and pathways of pollutants in the environment (Stuart et al., 2012)

2.2 Pollutants in urban river systems

2.2.1 Microbiological Contaminants

2.2.1.1 Heterotrophic Bacteria

Heterotrophic bacteria are a diverse group of bacteria that consume organic carbon for growth (McCormick et al., 2016). These entities hold significant roles within diverse ecosystems, encompassing water bodies, soil environments, and even the human body. Additionally, they contribute to nutrient cycling by facilitating the decomposition of organic matter. Through the breakdown of complex molecules, they transform them into simpler forms that can be readily absorbed or consumed by other organisms, thereby supporting the flow of nutrients within ecosystems. High levels of heterotrophic bacteria in surface water can indicate potential contamination from sewage or faecal matter pollution. Heterotrophic bacteria can be used as a measure of pollution in water as their presence can be used associated with organic pollution or nutrient enrichment. Therefore, heterotrophic bacteria can be used as bioindicators in ecological and water quality assessments. The detection of pathogenic heterotrophic bacteria indicates the declining water quality status in freshwater and riverine ecosystems (Primpke et al., 2020). The

Heterotrophic Plate Count (HPC) is a technique employed to quantify all heterotrophic microorganisms capable of growth on a non-selective solid medium, following specific conditions. Evaluating these bacteria is a crucial approach for assessing water quality, as alterations in the microbial community within water bodies can impact their visual attractiveness by influencing characteristics such as taste, odor, and color (Chatanga et al., 2019a). Allowable HPC levels in potable water should be less than 500 CFU/mL and any levels exceeding this permissible limit could indicate a declining water quality. The reliability of this method in determining opportunistic pathogens in aquatic environments remains questionable because non-pathogenic bacteria also grow on the non-selective media. Therefore, this method fails to discriminate between both the pathogenic and non-pathogenic bacteria, but however provides a general sense of the water quality status in aquatic ecosystems (Bartram et al., 2004). As a result, the HPC method is often complemented by other biological and chemical assessment tools to provide a more robust and comprehensive water quality assessment and monitoring approach that presents a true reflection of the water quality status (Mariani et al., 2022).

2.2.1.2 Total coliforms

Total coliforms in surface water constitute a group of bacteria serving as indicators of general microbial contamination and possible faecal pollution within aquatic environments. Despite the fact that not all coliform bacteria are harmful, high total coliform counts in water increases the risk of waterborne diseases. Total coliforms hold significant importance, particularly in safeguarding the safety of drinking water supplies, especially when surface water serves as the primary source of drinking water. The detection of total coliforms signifies potential contamination and underscores the necessity for implementing suitable treatment procedures to ensure that the water adheres to the prescribed drinking water quality standards (Ackers et al., 1998). Faecal coliform bacteria are a sub-group of total coliforms that comprise of enteric pathogens from faeces of human beings and other warm-blooded animals and are also often used as indicators for faecal contamination. For instance, detection of *E. coli* as a faecal coliform bacterium in aquatic systems indicates faecal contamination and possible pathogenic impact within these ecosystems (Ackers et al., 1998; Serpa et al., 2014).

2.2.1.3 Escherichia coli (E. coli)

Escherichia coli is a type of bacterium present in the intestines of humans and other warm-blooded animals. Not all *E. coli* strains are harmful however certain types such as the *E. coli* 0157:H7 can cause fatal illness which is associated with gastrointestinal illness. The primary source of *E. coli* in water bodies is through faecal matter contamination from humans and animals (Dayanti et al., 2018; Gemmell and Schmidt, 2012; Ziajahromi et al., 2016). Leaking sewage pipes and overflows are significant contributors to *E. coli* contamination in surface water. Aging or damaged infrastructure,

including pipes, manholes, or sewer lines can develop leaks. If the leakages occur near water bodies or underground water sources, *E. coli* from sewage can infiltrate the surrounding soil and find its way into surface water through groundwater discharge or direct leakage (Hoorzook et al., 2021).

Untreated or improper treated wastewater discharge have the potential to contain *E. coli* and other pathogens (P. J. Oberholster et al., 2008b). If the wastewater treatment plant does not effectively remove or inactivate these microorganisms, their discharge into receiving water bodies can cause contamination. *E. coli* stands as a more dependable marker for identifying fecal contamination in water, and its significance in causing various illnesses has been well-documented on a global scale. In the case of drinking water, *E. coli* determination is a measure of the efficiency in the disinfection process as there should be no detectable traces of *E. coli* as per SANS 241 (2015) guideline outlined in table 2.1.

The advanced development of uncultured microbiological analysis techniques such as high through-put next generation sequencing has paved way to an in-depth microbial analysis that allows researchers and environmentalists to identify both culturable and non-culturable pathogens in aquatic ecosystems (Rojas et al., 2022). Although this technique has proven to be a more efficient and reliable monitoring tool in the water impact assessment, it is very expensive and therefore difficult to incorporate it in existing water quality monitoring systems, especially in developing countries.

Table 2.1. Specifications for the Standards of Drinking Water Quality SANS-241-1:2015

Water Quality Parameter / Contaminant	**Specifications**
pH at 25 °C	5 – 9.7
Total dissolved solids (mg/L)	1000
Heterotrophic plate count / 1ml	≤ 1000
Total coliforms / 100 ml	Not detected
Escherichia Coli / 100 ml	Not detected

2.2.2 Organic water Pollutants

Most research conducted in water bodies was mainly focused on the termed “priority pollutants” which encompasses persistent organic pollutants, volatile organic compounds, and heavy metals (Lee and Hardy, 1998). As environmental interests and research progressed, so too did technologies result in development of more advanced techniques and the discovery of a new group of emerging contaminants. Emerging contaminants have attracted research attention as these compounds are introduced into the environment mostly by anthropogenic sources (Fischer et al., 2012a). The possibility of presence of emerging contaminants in the environment calls for modern technologies to

identify and quantify the amounts of these pollutants in the environmental bodies to raise awareness and develop legislative measures for their control (Bottoni et al., 2010). There is a myriad of highly toxic emerging pollutants, with partially known chemical properties, that have infiltrated urban surface water bodies mainly due to the rapid industrialization and population growth.

The accelerated economic activities in urban areas such as agricultural activities, manufacturing industries and mining sectors have resulted in elevated levels of emerging pollutants in urban freshwater systems. The rapid population growth in the urban regions has also been accompanied with blooming public health establishments and pharmaceutical companies aimed at maintaining proper health welfare (Sibanda et al., 2015a). As a result, there are large volumes of water contaminated with pharmaceuticals from both the public health facilities and the pharmaceutical companies that continuously pollute the urban surface water bodies (Madikizela et al., 2017). The recalcitrant nature of these pharmaceuticals has resulted in their persistence in urban river water, and this has become a serious health and ecological threat globally. Scientists have since been compelled to identify and determine the prevalence of these pharmaceuticals in urban riverine ecosystems not only for water quality assessment, but also to develop informed interventions aimed at ameliorating this eminent threat (Agunbiade and Moodley, 2014).

2.2.2.1 Pharmaceuticals

Pharmaceuticals are chemical compounds designed to treat or prevent diseases in humans and animals. Pharmaceuticals have found their way into water bodies and the type of pharmaceutical detected relies on economic, social, cultural, and agricultural factors (Zhang et al., 2008). Since these compounds are intended to treat specific diseases and conditions, their unintended consumption by non-target organisms poses crucial threats to the lives of the organisms (Azuma et al., 2016). Most developed Countries such as Australia and some European countries have developed and put in place legislative measures to prevent possible risks associated with pharmaceuticals in aquatic systems (Ngqwala and Muchesa, 2020). This remains a concern in African countries as no legislative measures have been implemented which has resulted in inadequate environmental monitoring of the compounds (Fischer et al., 2012b).

Despite the wide usage of pharmaceuticals, very few studies have been conducted from a South African perspective to investigate their behaviour in aquatic ecosystems. The following types have been detected in aquatic ecosystems in South Africa, antibiotics, antiretrovirals, and non-steroidal anti-inflammatory drugs (Farounbi and Ngqwala, 2020; Madikizela et al., 2017). Antibiotics are a class of chemical compounds that are administered to provide defence against pathogenic bacteria and fungi. They are used to treat infections in humans and animals such as gonorrhoea, cholera, and tuberculosis. Based on their application, these compounds are one of the most highly consumed

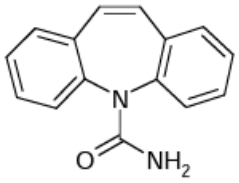
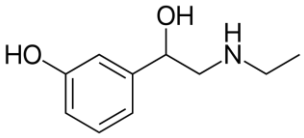
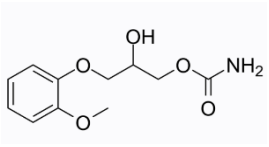
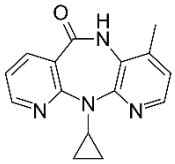
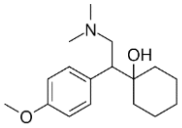
pharmaceuticals globally (Gomes et al., 2020). The physicochemical and biological properties of antibiotics allows them to be persistent in environmental bodies and bio-accumulative environmental contaminants as their rate of degradation cannot offset their accumulation (Matongo et al., 2015).

The threat posed by release of antibiotics into water bodies is the rapid increase of antimicrobial resistance genes and resistance bacteria which limits the effect against bacterial pathogens (Ncibi and Sillanpää, 2015). A surveillance report by (Morrison and Zembower, 2020), demonstrated the occurrence and spread of both antimicrobial resistance genes and antimicrobial resistance bacteria (Morrison and Zembower, 2020). They demonstrated that worldwide 3.6 % of new Tuberculosis cases and 20.2 % of formerly treated cases have multidrug-resistant TB (MDR-TB) which is the most resistant variant and requires extended treatment duration (up to 20 months) with severe side effects.

The project particularly focused on the following pharmaceutical compounds Nevirapine, Methocarbamol, Carbamazepine, Etilefrine, and Venlafaxine which are summarized in table 2.2. South Africa is one of the countries with high infections of the Human immunodeficiency virus (HIV) in the world and has a significant proportion of the population under Antiretroviral (ARV) drug treatment (Swanepoel et al., 2015). Nevirapine is an antiretroviral medication used to treat and prevent HIV infection and was selected in this study to represent the ARV drugs. ARV drugs are used for the treatment of infection by retroviruses, primarily HIV by suppressing the HIV viral load, fighting infections, and enhancing the quality of life for those living with the virus (Wee et al., 2020). Since a large amount of these drugs are being prescribed to HIV patients, they have been detected in aquatic ecosystems. Studies have found the presence of ARV drugs in surface water in South Africa (Wood et al., 2015). As an antiretroviral drug, nevirapine is intended to combat HIV, but its presence in the environment could potentially promote the development of drug-resistant HIV strains if the virus is exposed to suboptimal concentrations of the drug in water bodies (Wood et al., 2015). ARV drugs are found to have endocrine-disrupting properties and their occurrence in surface water systems poses health concerns that call for their monitoring (Zaid and Greenman, 2019).

Venlafaxine is an antidepressant medication that belongs to the class of drugs known as serotonin-noreoineohrine inhibitors (SNIRIs). Venlafaxine can enter water bodies through various pathways, including discharge from wastewater treatment plants, improper disposal of unused medications, or excretion by individuals taking the drug. Once in water, venlafaxine can be persistent and may not easily break down. As an active pharmaceutical ingredient, it can have adverse effects on aquatic organisms and ecosystems. Studies have shown that venlafaxine exposure can disrupt the behavior, reproduction, and development of certain aquatic species.

Table 2.2. Summary of properties of pharmaceuticals under investigation.

Compound	Therapeutic Class	Chemical Structure	Log K_{ow}	pKa	Molecular Weight
Carbamazepine	Anticonvulsant		2.45	15.96	236.27
Etilefrine	Cardiac Stimulant		0.1	9.1	181.23
Methocarbamol	Muscle relaxant		0.6	13.6	241.24
Nevirapine	Antiretroviral		2.5	10.37	266.3
Venlafaxine	Antidepressant		2.9	14.42	277.4

2.2.3 Modern extraction techniques for risk assessment of pollutants in water bodies

Due to the ecological repercussions linked to organic pollutants, the application of scientific methodologies is imperative for their analysis. Within environmental assessment, Sample Preparation plays a pivotal role by enabling the cleansing of samples and elimination of potential disruptions. This step also allows for the concentration of analytes to detectable thresholds and the transformation of analytes into a format compatible with the analytical instrument (Chen et al., 2014; Olisah et al., 2020).

Two main sample preparation techniques have been traditionally used for extraction of organic pollutants based on the physical state of materials used: Liquid based techniques and solid based techniques (Sibali et al., 2013). Liquid-liquid extraction is a liquid-based extraction technique that was traditionally used for the extraction if organic water pollutants. The underlying principle of this technique involves isolating one or more constituents from a liquid mixture through the utilization of a blend of non-polar solvents that are compatible with water. These solvents create a partitioning effect,

causing the target analytes to distribute or partition into the solvent phase. However, this method was associated with several limitations. It involved the use of substantial quantities of expensive and environmentally harmful extraction solvents, which led to pollution concerns. Furthermore, the process was time-consuming, labor-intensive, and lacked automation (Prosen, 2014).

2.2.3.1 Solid phase extraction

In an effort to address the issues associated with liquid-liquid extraction, solid phase extraction (SPE) gained widespread acceptance as a sample preparation method. This technique mitigated the concerns by employing smaller quantities of harmful organic solvents. (Pichon, 2000). The aqueous sample is passed through the stationary phase and the analytes of interest are retained, then an organic solvent is applied for elution of analytes off the column which is then collected and sent for analysis (Kataoka, 2017).

For the analysis of emerging contaminants like pharmaceuticals in water samples using solid phase extraction (SPE), the recommended sorbent is the hydrophilic-lipophilic-balanced (HLB) sorbent(García-Córcoles et al., 2019). This sorbent gained popularity due to its benefits such as high reproducibility, low cost, and rapidness. Madikizela and colleagues applied solid phase extraction to investigate the levels of ketoprofen in river water in Kwazulu-natal, South Africa (Madikizela et al., 2014). Maldaner and co-authors reported the use of solid-phase extraction and analysis by LCMS for selected pharmaceuticals and pesticides (Maldaner and Jardim, 2012).

SPE has been a promising technique but it also had its shortcomings which were associated with classical sorbents used which did not offer selectivity, the other issue was associated with the clogging of the sorbent bed by particles of sample suspended matter and sample carry over (Rawa-Adkonis et al., 2006).

2.2.3.2 Solid phase microextraction (SPME)

Arthur and Pawliszyn (1990) reported SPME as a solvent-free solid phase miniaturization technique for the extraction of volatile chlorinated compounds in water (Arthur and Pawliszyn, 1990). This technique combines sampling, extraction, and preconcentration as one step. SPME has been applied liquid, gaseous and solid samples for the extraction of analytes (Pawliszyn, 2012). The SPME device consists of the syringe assembly and the polymer fibre as its main components.

This is a non-exhaustive method that relies on creation of equilibrium between the target compound and the polymer coated fibre. SPME comprises of 2 steps: the partitioning of analytes between the sample matrix and the desorption of concentrated analytes into the GC injection port and the use of desorption chambers for LC techniques (Kudlejova et al., 2012). This technique is simple and fast

however extraction efficiency depends parameters such as fibre coating material, extraction time, temperature and sample volume among others need to be optimized (Majedi and Lee, 2017).

SPME is performed in either static mode and dynamic modes. Static modes are performed in stirred samples, and this include fibre SPME and stir bar Sorptive extraction (SBSE) rotating disk Sorptive extraction (RDSE) and dispersive SPME.

2.2.3.3 Liquid phase microextraction techniques

To overcome drawbacks associated with this technique, solvent based miniaturization techniques were developed. Three main classes of liquid-based miniaturization techniques were developed: drop based techniques, membrane supported, and dispersed solvent assisted techniques (Sarafraz-Yazdi and Amiri, 2010).

2.2.3.3.1 *Single Drop Micro-extraction (SDME)*

This technique utilizes a single drop that is suspended on a tip of a syringe then suspended in an aqueous sample or exposed to a headspace for extraction of target analytes at a pre-determined time (Liu and Dasgupta, 1995). When extraction is completed, the analyte rich micro-drop is retracted into the micro-syringe then transferred into the chromatographic technique for analysis. This method provided several benefits such as use of very low amounts of solvent (1-5 uL). (George, 2016) applied a modified SDME technique and analysis by GC-MS for the extraction of stilbene hormones in water. Despite the benefits of this method, associated drawbacks such as loss of the drop on the micro-syringe during stirring of the aqueous sample solution, formation of an emulsion and dissolution of the liquid droplet when dealing with dirty samples and volatility of the solvent led to limited use of the method (Sarafraz-Yazdi and Amiri, 2010).

2.2.3.3.2 *Directly suspended droplet microextraction (DSDME)*

The method involves introduction of a small volume of water immiscible solvent in the vortex of a stirred aqueous sample (Wang et al., 2014). The vortex creates a rotating droplet at or close to the centre of rotation which results in mass transfer of analytes. When extraction is completed, the droplet is withdrawn from the aqueous sample solution then sent to a chromatographic instrument for analysis (Yangcheng et al., 2006). The method offers a wide variety of benefits such as, low cost, rapid equilibration time, increased stirring speeds to enhance the mass transfer process and use of increased solvent volume which can make it applicable for HPLC analysis (Kamal Rajabi and Nikserasht, 2018). The application of DSDME for the extraction of fungicides – azoxystrobin, dithiofencarb and pyrimethanil from environmental water samples (Wang et al., 2014).

Associated drawbacks with this extraction method are difficulty of collection of the microdroplet as part of the aqueous solution may be transferred into the micro-syringe (Kamal Rajabi and Nikserasht, 2018).

2.2.3.3.3 *Hollow-fibre liquid phase microextraction (HF-LPME)*

This method makes use of a disposable low-cost polypropylene hollow fibre membranes to support the extraction solvent (Wang et al., 2016). This method was developed to overcome the inadequacy in SDME such as the instability of the solvent and limited use of solvent volume (Pedersen-Bjergaard S, 1997). The organic solvent is immobilized in the pores of the hollow fibre which offers support and during stirring and loss from vibrations.

A short strip of hollow fibre (3 – 10 cm) is dipped in an organic solvent to immobilize the solvents in its pores, the lumen of the hollow-fibre is then filled with an extraction solvent by use of a syringe then introduced in an aqueous sample for analysis (Wang et al., 2016). The analytes of interest are transferred from the donor phase (aqueous phase) to the organic layer in the walls of the hollow fibre then into the acceptor phase in the lumen of the hollow fibre. The solvent in the acceptor phase is then retracted into the micro-syringe then sent for analysis.

The solvent choice used in as the acceptor phase and impregnated in the pores of the hollow fibre result in two modes of HF-LPME. In Two-phase HF-LPME, the acceptor solvent in the lumen is the same as that impregnated in the pores of the hollow fibre (de la Guardia and Armenta, 2011). Three phase HLF-LPME, acceptor phase is selected as an alkaline or an acidic aqueous solution. Associated issues with this method include loss of volatile and less non-polar solvents when extraction times are prolonged. HF-LPME was applied for the determination of five PAHs in Jukskei river South Africa and the concentrations of the compounds were found to be in the range 11 ng/L – 64 ng/L (Sibiya et al., 2013b).

2.2.3.3.4 *Dispersive Liquid-Liquid extraction*

Rezaee and coworkers (2006) designed a technique that made use of cloudy state formed when a mixture few microliter volumes of extraction solvent along with a disperser solvent is introduced into an aqueous sample solution (Rezaee et al., 2006). The dispersed solvent in the aqueous phase creates a large surface area of interaction between both phases leading to a rapid, quasi-instantaneous mass transfer process (A. Lambropoulou, 2010). High density chlorinated organic solvents such as nitrobenzene, chloroform serve as extraction solvents and lower density solvents are used as disperser solvents such as acetonitrile and methanol (Letseka and George, 2016).

The technique involves addition of the predetermined solvent mixture to create the cloudy solution followed by centrifuging of the mixture to result in phase separation of the aqueous and the organic layer, the organic layer rich in analytes is carefully drawn and analysed using GC and LC techniques (Sarafraz-Yazdi and Amiri, 2010).The major drawbacks associated with this technique are that the preconcentration and analysis step are performed separately making it difficult to integrate online and suffers highly of matrix interferences (Prosen, 2014).

2.2.4 Passive sampler approach

The commonly used approach for sampling organic water pollutants is active sampling (bottle or grab sampling). In this approach, the samples are collected from selected sampling points to be processed and analysed (Madrid and Zayas, 2007). However, this approach merely captures the pollutant levels at the time of sampling and is insufficient for identifying intermittent contaminant variations. For a more comprehensive representation of water quality, it is advisable to monitor the concentrations of these organic pollutants over an extended period. Given the typically low concentrations of organic compounds in water, typically in the range of L^{-1} to µg L^{-1} range, this requires the large water samples to be collected or the use of automatic samplers which are expensive and require security measures to be put in place (Vrana et al., 2005a). To overcome the limitations of this technique, several alternative methods and emerging approaches can be employed. These include repeated spot sampling, automated sequential sampling, continuous online monitoring systems, biomonitoring, and the use of passive samplers (Salim and Górecki, 2019).

Passive samplers present a promising solution as environmental monitoring devices for organic pollutants due to their cost-effectiveness, non-mechanical nature, and ease of deployment across various field locations and timeframes. They enable the acquisition of time-weighted average (TWA) concentrations of compounds within the sampled medium or the equilibrium concentrations of compounds on the sampling device itself (Madrid and Zayas, 2007). Passive sampling operates by allowing analytes to migrate freely from the sampled medium to the receiving phase (Górecki and Namienik, 2002). The transfer of analytes between these phases is typically guided by Fick's second Law of diffusion, which states that "the flux moves from regions of high concentration to regions of low concentration, with a magnitude proportional to the concentration gradient." The quantity of the substance, denoted as M, that undergoes diffusion over a duration of time t (in seconds), assuming a linear concentration profile and 100% collection efficiency, can be characterized by the following equation (Górecki and Namienik, 2002):

$$M = U\ x\ t = \frac{DA}{L}\ C_0 t \qquad (2.1)$$

In this context, U represents the rate of diffusive transport (mol/s), D stands for the molecular diffusion coefficient of the substance being analyzed (cm^2/s), A corresponds to the cross-sectional area of the path through which diffusion occurs (cm^2), L denotes the overall length of the diffusion path, expressed in (cm).

The term $\frac{DA}{L}$ is often considered to be the Sampling rate R_S.

Analytes are retained in receiving phase and which can be in different forms such as a chemical reagent or a porous adsorbent.

The interaction speed between a passive sampler and the water phase can be elucidated using a single compartment model based on first-order kinetics:

$$C_s(t) = C_W \frac{k_1}{k_2}(1 - e^{-k_2 t}) \qquad (2.2)$$

In this scenario, $\mathrm{C_s(t)}$ represents the analyte concentration within the sampler at time, (t), while $\mathrm{C_W}$ signifies the analyte concentration in the surrounding aqueous medium, $\mathrm{k_1}$ and $\mathrm{k_2}$ are constants.

While deploying samplers in the field, two primary accumulation patterns are evident in the functionality – the kinetic/linear regime and the equilibrium regime. This distinction also forms the foundation for their categorization,(Salim and Górecki, 2019). In kinetic regime, migration of the analytes occurs endlessly from the sampled phase to the receiving phase until the samplers are withdrawn. The sampler's retained analyte quantity is presumed to correlate with the product of the sampling duration and the analyte concentration. During the initial phase of sampler exposure, the desorption rate of analytes from the receiving phase to water is minimal, allowing equation (2.2) to be simplified to:

$$C_s(t) = C_W k_1 t \qquad (2.3)$$

Which can in turn be arranged into a direct correlation.

$$M_s(t) = C_W R_S t \qquad (2.4)$$

In this context, $M_s(t)$ represents the analyte mass at time (*t*) and R_S stands for the sampling rate.

In the equilibrium regime, the analyte uptake proceeds until the sampler gets to equilibrium with the surrounding concentration. In this case, the exposure duration is sufficiently extended to establish thermodynamic equilibrium between the receiving phase and the water phase(Lee and Hardy, 1998). Equation (2.1) in this case is reduced to:

$$C_s(t) = C_W \frac{k_1}{k_2} = C_W K \quad (2.5)$$

Whereby K represents the partition coefficient between the phases, enabling the estimation of the dissolved analyte concentration.

2.2.4.1 Types of passive sampling devices

Passive samplers are categorized according to their sampling regime, the matrices they are utilized to analyse, and their specific designs (Salim and Górecki, 2019). Across time, passive sampling tools have been created to target particular pollutants and cater to various environmental matrices such as soil, water, and air (Marć et al., 2017).. A compilation of frequently used devices for assessing organic water pollutants will be discussed in the next sections.

2.2.4.1.1 Semi Permeable Membrane Device (SPMD)

Hughkings and team (1990) first reported the design and study of SPMD as a novel technique for monitoring of lipophilic pollutants. The design of SPMD illustrated in Figure 2.2 consists of thick walled (50 – 100 µm) flat polyethylene membrane tube with substantial thickness (ranging from 50 to 100 µm). This tube contains a neutral, high molecular weight lipid, such as triolein. A typical SPMD has dimensions of 2.5 cm in width and 91.4 cm in length, enclosing 1 mL of lipid. The polyethylene membranes used in this design possess cavities or temporary openings in the 5 to 10 Å range, which exclusively permit the diffusion of low-molecular-weight dissolved organic compounds into the enclosed lipid. SPMD proves to be particularly effective for extracting hydrophobic non-polar substances, including compounds like polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and pesticides. It's best suited for pollutants with partition coefficients (log KOW) that fall within the range of 3 to 6 (de la Guardia and Armenta, 2011).

The SPMD sampler can be deployed at extended periods of time to integrate long time data, (Pogorzelec and Piekarska, 2018) successfully applied the SPMD in monitoring of PAHs at different stages of a WWTP at monthly intervals over a year. The major drawback with this method is that following extraction of compounds into the triolein, an extra step is engaged to re-extract the compounds from triolein into organic solvents which increases analysis time (Huckins et al., 1999) and use of large volumes of organic solvents (Nyoni et al., 2010). Application of SPME was demonstrated by (Gilli et al., 2005) for assessing toxicity of polyaromatic hydrocarbons in drinking water.

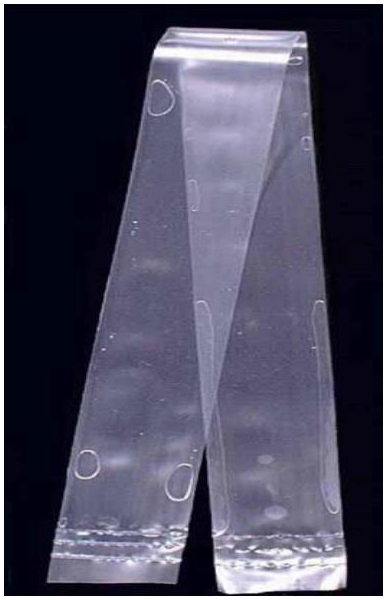


Figure 2.2. Design of SPMD device (Hughkings et al (1990))

2.2.4.1.2 Chemcatcher®

The Chemcatcher® was reported by (Kingston et al., 2000) as a new passive sampling apparatus for TWA concentration determination of organic pollutants in water. The configuration of Chemcatcher® comprises of three components, the polytetrafluoroethylene (PTFE) or polycarbonate support (PC) support device, a diffusion-limiting membrane layer and a receiving phase - solid phase extraction disk. The membrane is mainly used to protect the extraction disk from bio-fouling. Pollutant accumulation into the receiving phase occurs through diffusion during the kinetic or equilibrium phase (Vermeirssen et al., 2009).

Various designs of combinations of diffusion-limiting membrane and receiving phase are present for application of both organic and inorganic pollutants. For low polar to non-polar organic compounds, the styrene divinylbenzene-reverse phase sulphonate (SDB-RPS) disk, styrene divinylbenzene exchange (SDB-XC), C_{18} disks are commonly used receiving phases as they have a high affinity and capacity for the compounds. The receiving phases can be coupled with the cellulose acetate (CA), low-density polyethylene, polysulfone and polethersulfone as diffusion-limiting membranes (Vrana et al., 2005a).

A novel disk configuration, termed the hydrophilic-lipophilic (HLB-L) disk has gained attention as a sorbent for Chemcatcher®. It comprises of a blend of two monomers: hydrophilic N-vinylpyrrolidone and lipophilic divinyl benzene. This composition grants the disk a notable capacity for effectively retaining polar analytes (Castle et al., 2018). One of the notable strengths of the Chemcatcher® lies

in its design, where the receiving phase is firmly affixed to an unreactive polymeric disk matrix. This configuration ensures the prevention of leakage during field deployment and safeguards against material loss during subsequent processing steps (Grodtke et al., 2021a). The incorporation of the HLB disk as the receiving phase in the Chemcatcher® system brings forth a dual advantage. It facilitates the efficient sampling of a broad spectrum of polar organic pollutants, while also capitalizing on the handling benefits inherent to the Chemcatcher® methodology (Petrie et al., 2016).

This type of sampling devices have been found to be ideal for sampling pharmaceuticals, steroids, pesticides, alkylphenols and polybrominated flame retardants. The Chemcatcher® was applied to screen for emerging pollutants in South African rivers (Rimayi et al., 2019). The research revealed the presence of pharmaceuticals, personal care products, and pesticides in the water samples. (Grodtke et al., 2021b) deployed Chemcatcher® in river water and detected triazine herbicides within the concentration range 0.3 – 30.6 ng/L.

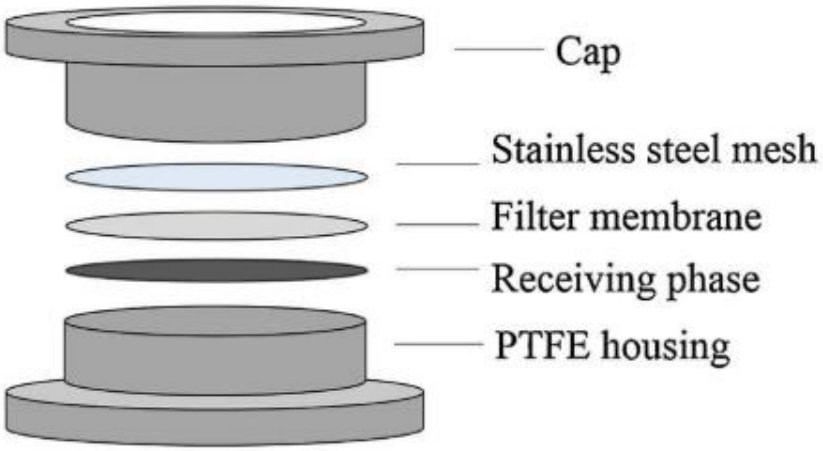


Figure 2.3: Configuration of the Chemcatcher® passive sampler (Gong et al., 2018)

2.2.4.1.3 Diffusive gradients in thin film sampler (DGT)

The use of DGT sampler was first reported for determination of zinc in sea water (Zhang and Davison, 1994). The design of the sampler is a PTFE top and base as outer components enclosing three layers, the filter membrane consisting of resin-impregnated gel layer, diffusive gel and filter membrane stacked on the base as shown in Figure 2.4. The analytes of interest pass through the membrane filter and diffusive gel and concentrate within binding gel (Gong et al., 2018). The inclusion of the diffusive gel layer to regulate analyte transfer makes this sampler unique from other passive samplers (Guibal et al., 2017).

The sampler was widely reported for detection of metals and inorganic compounds in aquatic environments. Chen and colleagues reported the initial instance of passive samplers that incorporates a diffusive hydrogel to sample organic compounds in water (Chen et al., 2012). Guibal and team also

reported the optimization and application of DGT samplers for the analysis of anionic pesticides in aquatic bodies (Guibal et al., 2017).

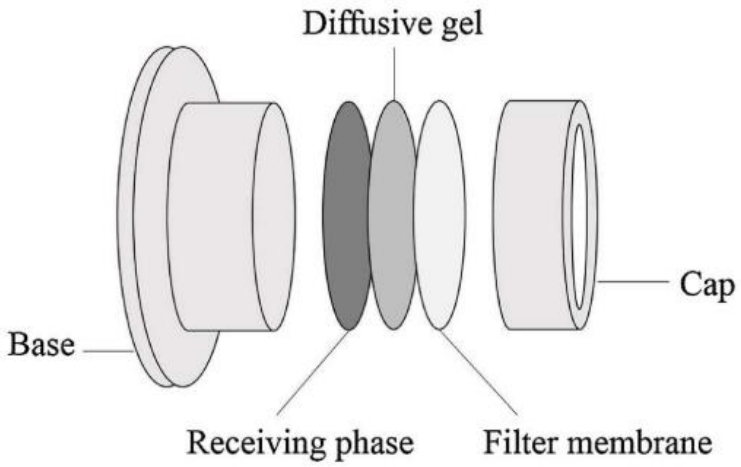


Figure 2.4. Configuration of the DGT sampler (Gong et al., 2018)

2.2.4.1.4 Membrane Enclosed Sorptive Coating (MESCO)

These devices consist of a membrane enclosing polydimethylsiloxane (PDMS) coatings or coarse silicone material that are surrounded in a fluid as sampling phases for target analytes (Paschke et al., 2007). The MESCO sampler has two versions as shown in Figure 2.5, MESCO I that has a stirrer as the receiving phase and the MESCO II that uses a silicone tube as the receiving phase (Paschke et al., 2007). The target organic compounds from the aqueous phase pass through the membrane and are adsorbed onto the PDMS layer on the stir-bar or the silicone phase. The coated stir-bar can be analysed by thermal desorption or solvent back extraction (van Pinxteren et al., 2010).

The benefits of using this sampler include simplicity, loss-free separation of the collector phase and analysis with further processing steps through thermal desorption or solvent microextraction (Paschke et al., 2007). MESCO samples have been found to be ideal for the analysis of pesticides in the environment (Marć et al., 2017). The major drawback about using PDMS-coated stir bar is that the membrane is susceptible to microbial degradation and has relatively poor thermal and chemical stability which results in sampler damage when deployed in environmental eater bodies (Paschke et al., 2007).

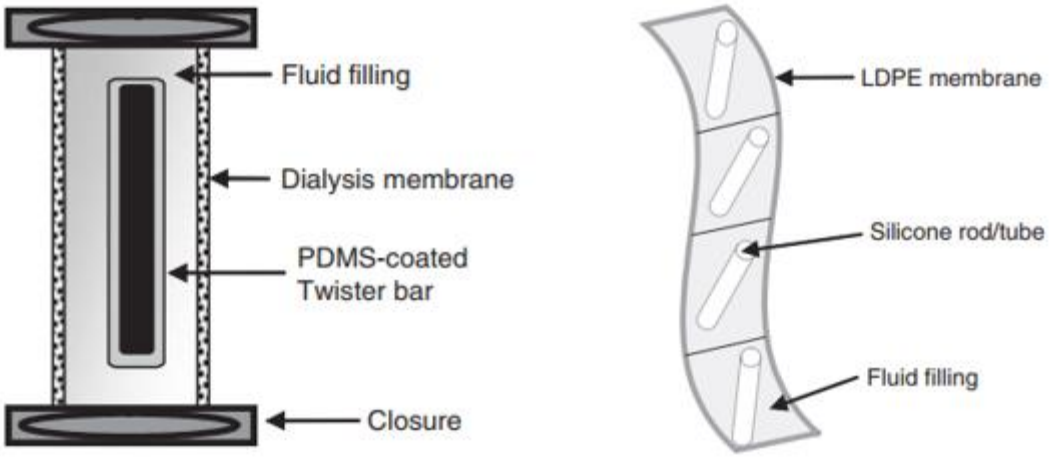


Figure 2.5: Schematic of MESCO sampler designs (MESCO I left and MESCO II right) (Paschke et al., 2007)

2.2.4.1.5 Polar Organic Chemical Integrative Sampler (POCIS)

The structure of the POCIS sampler as shown in figure 2.6 to consist of 3 parts: 100 mg Sorbent, polyethersulfone membranes (pore size 100 nm and thickness 130 nm) and the two stainless steel rings or other rigid inert material to the membrane and sorbent together (Alvarez et al., 2004). The polyethersulfone membranes serve as semipermeable barrier between the aqueous environment and the receiving phase, the membrane prevents the accumulation of solid particles, colloids but allowing the compounds of interest to pass through.

POCIS has 2 commercially available designs: pesticide-POCIS that uses a combination of 3 solid sorbents which has been employed to sample pesticides, hormones. Pharmaceutical-POCIS makes use of one sorbent, the hydrophilic-lipophilic balanced copolymer [poly(divinylbenzene)-co-N-vinylpyrrolidone] for the sampling of pharmaceutical compounds (Gong et al., 2018). The use of commercially available sorbents has allowed for the isolation of compounds with log K_{OW} < 5, which is within range of the target compounds in the study as they have log K_{OW} values in the range 0.1 – 2.9 outlined in table 2.2. POCIS sampler was deployed for the determination of pharmaceuticals and personal care products in waste water (Amdany et al., 2014). For the current study, the POCIS will be employed to analyse pharmaceuticals in Hennops river water.

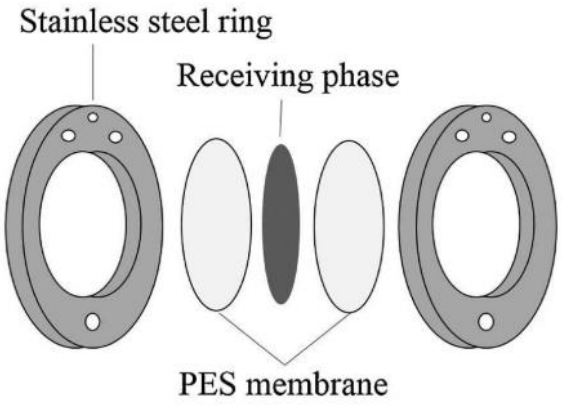


Figure 2.6. Configuration of the POCIS sampler (Gong et al., 2018)

2.2.4.2 Optimisation of passive samplers

The process of calibrating passive sampling devices is crucial for understanding their performance, establishing sampling rates specific to both the chemical and the sampler, and calculating sampler-water partition coefficients to ascertain Time-Weighted Average (TWA) water concentrations of pollutant (Garnier et al., 2020). The rate of chemical uptake relies on analytes physio-chemical properties, sampler design Chemical and environmental variables (Huckins et al., 1999).

Calibration of passive samplers is done by exposing samplers to pre-determined analyte concentrations for stated time frames in designed environmental conditions (Alvarez et al., 2004). Laboratory-based calibrations are performed under set variables such as temperature, flow rate, pH and salinity and the absence of bio-fouling (Aguilar-Martínez et al., 2008). However, when passive samplers are deployed in actual environmental settings, the conditions are often not identical to the laboratory conditions, leading to uptake rates that may deviate from ideal values. To account for these non-ideal uptake rates between water and the receiving phases, Performance Reference Compounds (PRCs) are employed. PRCs help correct for the differences in uptake rates caused by varying environmental conditions (Charriau et al., 2016).

2.2.4.2.1 Static depletion

This is a design in which the passive samplers are exposed to a pre-spiked solution at the beginning of the experiment (Thomatou et al., 2011). Using the first order kinetics (equation 2.1), it was noted that with time that there was a decrease in analyte concentration and the sampling rate R_S could be estimated. Static depletion experiments have been employed in the calibration of SPMDs (Booij et al., 2007). This design is simple, low cost and easy to incorporate environmental variables (Wang et al., 2020).

2.2.4.2.2 Static renewal

In this setup, the spiked solution is regularly removed or renewed at set intervals (Thomatou et al., 2011). This configuration is usually considered in cases where static or continuous flow configurations are not ideal and this instance is likely to arise when the sampler results in excessive depletion of the aqueous phase (Chen et al., 2012). As an attempt to minimize the depletion of analyte concentration, the intervals for renewing the exposure solution should be frequent and the concentration of the solution should be determined at each initial and final renewal stage. When there is less change in analyte concentration during renewals, this allows for a direct modelling of the sampling rate by equation (6) below (Thomatou et al., 2011).

$$C_s(t) = \frac{M_s R_S t}{M_s} \qquad (2.6)$$

2.2.4.2.3 Continuous Flow design

This design is mainly aimed to stop the excessive consumption of the aqueous solution by offering a consistent inflow of freshly spiked solution into samplers are under constant hydrodynamic conditions. During calibration, it's crucial to ensure minimal sorption of dissolved organic or inorganic substances, as this prevents potential overestimation of analytes in the aqueous solution. The samplers are constantly retrieved to determine the analyte sampling rate. As the number of samplers are reduced, the flow rate may be lowered if the hydrodynamic condition is held. When it's feasible to maintain constant aqueous concentrations, the R_S and $C_s(t)$ may be obtained by curve fitting of equation (2.6). If it is not possible to maintain aqueous concentrations, a second order polynomial function outlined in equation (2.7) could be assigned to account for concentration change in the sampler.

$$\frac{C_s(t)}{K_{mw}} = \left(C_0 - \frac{C_1}{k_e} + \frac{2C_2}{k_e^2}\right)[1 - exp(-k_e t)] + \left(C_1 - \frac{2C_2}{k_e}\right)t + C_2 t^2 \quad (2.7)$$

Where C_0, C_1 and C_2 are polynomial function constants.

2.2.4.3 Applications of passive samplers

2.2.4.3.1 Chemical Monitoring

Enhancing water quality involves overseeing the existence of pollutants within aquatic settings to confirm adherence to limits established by regulatory bodies (Miège et al., 2015). Passive samplers serve as effective tools for the integrative collection of contaminants. They can be deployed for

extended durations, ranging from weeks to months, within water bodies. These samplers offer a cost-efficient means of monitoring that supplies the necessary information for evaluating water contamination. Tapie and co-authors employed POCIS for monitoring the release of pharmaceuticals, pesticides, phenols and hormones in Baïse River (Garonne basin, southwest of France) (Tapie et al., 2011).

2.2.4.3.2 Quantification of Concentrations in water

Passive samplers offer the capability to assess Time-Weighted Average (TWA) concentrations of pollutants within the aqueous phase (Vrana et al., 2007). Contaminant concentrations in the environment are not constant and can fluctuate depending on their sources over time and this can be achieved effectively with the application of passive as opposed grab sampling. In the study conducted by (Ahrens et al., 2018), three different passive samplers were employed to determine pesticide concentrations that were in the National pesticide monitoring programme of Sweden's two catchment areas that were characterized by agricultural activities.

2.2.4.3.3 Estimate of organism Exposure

Passive samplers can determine the truly dissolved contaminant fraction of contaminants. The dissolved fraction of contaminants is the most bioavailable (Morrone et al., 2021). Monitoring the concentrations of contaminants in aquatic environments provides direct evidence for bioavailability and the contaminant's potential for ecological and human health risks (Joyce et al., 2016). Biomimetic sampling using passive samplers can mimic the partitioning of pollutants between the aqueous phase and the organism (Vrana et al., 2005a).

2.2.4.4 Relationship between sampling rate Rs and Log Kow

Researchers have attempted to establish a connection between their measured laboratory retention factors (Rs) and the logarithm of the octanol-water partition coefficient (log Kow) of molecules. This endeavour was driven by the goal of predicting Rs values and circumventing the need for individualized laboratory calibrations for each studied molecule (Morin et al., 2012). A linear correlation between Rs and log Kow within the range of 0.07 to 4.80 for organic compounds (anticonvulsants, antidepressants, an antihistaminic, and a stimulant). Similarly, a linear relationship among 14 fundamental compounds (encompassing antibiotics, antidepressants, and beta-blockers) with log Kow values ranging from 0 to 4 was recognized (Li et al., 2010).

Nevertheless, it was pointed out that beta-blockers and four acidic compounds (anti-inflammatories), Rs adhered to a Gaussian model in relation to log Kow (ranging from 0 to 4.5) (MacLeod et al., 2007).

Notably, the highest Rs values occurred around log Kow values of 3 or 4 (with an impressive r^2 value of 0.99 for beta-blockers) (MacLeod et al., 2007).

2.2.4.5 Comparison of sampling Rates

The literature provides specific POCIS sampling rates which are elaborated upon in Table 2.3. Furthermore, laboratory Rs were categorized based on the 'standard conditions,' which are commonly utilized in existing literature. This pertains to the established configuration of POCIS with a surface area of 45.8 cm^2 and 200 mg of the absorbing material. The calibration was carried out in freshwater, encompassing temperatures that spanned from 15 °C to 25 °C, under uniformly maintained stirring conditions. Various factors including the calibration technique, deployment duration, and agitation speed were employed, allowing for a more refined classification that aids in identifying the sources of Rs variability. In general, Rs values tend to be below 1 L d^{-1} as the sampling rates for POCIS spanned from 0.0118 1 L d^{-1} for nadolol to 0.894 L d^{-1} for venlafaxine (Bayen et al., 2014). When considering individual compounds, laboratory Rs may differ substantially across studies due to variations in conditions. For instance, for carbamazepine, Rs span from 0.1199 1 L d^{-1} under field-calibrated conditions to 0.235 1 L d^{-1} using a semi-static approach in laboratory conditions. Disparities in Rs between different substances may arise due to elements including POCIS type and size, agitation, temperature, and physicochemical properties (such as pH, and conductivity), as well as biofouling (Miège et al., 2015). Furthermore, aspects like the calibration system itself, experimental duration, concentrations of the substances under investigation, and calculation methods are all believed to influence Rs values (Morin et al., 2012).

Table 2.3: Implementation of POCIS for surface water pharmaceutical monitoring

Pharmaceutical compound	**Log Kow**	**Region or country**	**Sampling rates (L d^{-1})**	**Calibration method**	**Deployment time (days)**	**Agitation speed/flow rate**	**Reference**
Carbamazepine	2.45	Canada	0.235	Static depletion	8	60 rpm	(Li et al., 2010)
		France	0.14	Continuous flow	30	1 ml $hour^{-1}$	(Belles et al., 2014)
		Singapore	0.60	Static depletion	7	3 – 5 cm sec^{-1}	(Bayen et al., 2014)
		Czech Republic	0.11	In situ	28	N/A	(Vrana et al., 2021)
Venlafaxine	2.9	Singapore	0.894	Static depletion	7	3 – 5 cm sec^{-}	(Bayen et al., 2014)
		Canada	0.104	Static depletion	8	60 rpm	(Li et al., 2010)
		Czech Republic	0.23	In situ	28	N/A	(Vrana et al., 2021)
Atenolol	0.16	France	0.025	Continuous flow		N/A	(Morin et al., 2012)
		Singapore	0.051	Static	7	3 – 5 cm sec^{-}	(Bayen et al., 2014)
		Canada	0.073	Static		60 rpm	(Li et al., 2010)

Pharmaceutical compound	**Log Kow**	**Region or country**	**Sampling rates (L d^{-1})**	**Calibration method**	**Deployment time (days)**	**Agitation speed/flow rate**	**Reference**
Nadolol	0.85	France	0.114	Continuous flow		N/A	(Morin et al., 2012)
		Canada	0.0118	Static depletion	8	60 rpm	(Li et al., 2010)
Acetaminophen	0.46	Canada	0.139	Static depletion	8	60 rpm	(Li et al., 2010)
		Singapore	0.048	Static depletion	7	3 – 5 cm sec^{-1}	(Bayen et al., 2014)
Naproxen	0.65	Canada	0.135	Semi-static	25	N/A	(MacLeod et al., 2007)
		Italy	0.031	Semi-static	3	1300 rpm	(Magi et al., 2018)
		France	0.118	Semi-static	14		(Morin et al., 2012)

2.2.5 Analytical methods for identification and quantification of organic pollutants in water

Environmental matrices are intricate and encompass an array of organic compounds present in trace levels and over the years, more emerging contaminants have been identified in the environment with the advancement of analytical methods (Madikizela et al., 2020). Chromatographic techniques such as liquid chromatography (LC) and gas chromatography (GC) coupled to mass spectrometry (MS) are the most employed methods for the determination of emerging contaminants in environmental matrices as they offer sensitivity and selectivity for the detection of these compounds at µg/L to ng/L levels (Clarke, 2017).

Mass spectrometry is the most powerful analytical technique that is used for the identification of unknown organic compounds, the determination of structures of complex molecules, and the quantitation of low concentrations of known compounds (Clarke, 2017). Mass Spectrometers function by changing the analyte molecules into an ionizable form and then analyse the formed ions and any fragmented ions through their mass-to-charge ratio (*m/z*) and modern configurations are based on the speed of analytes, time and rate of reaction (Pitt, 2009). The choice of ionization technique has to be taken into consideration and this mostly relies on the physio-chemical properties of the compounds of interest (Alves et al., 2013).

Electron spray impact (ESI) is a technique commonly used in commercial LC-MS systems in which a stream of liquid containing the sample is passed through a narrow capillary set at a specific voltage. This process causes nebulization at the capillary's tip, resulting in the formation of a fine spray of charged drops. (Banerjee and Mazumdar, 2012). The droplets are swiftly dessolvated by heating to a set temperature leaving the residual charge to the analytes (Banerjee and Mazumdar, 2012). Subsequently, the ionized analytes are directed to the mass analyzer through a sequence of minor apertures and focusing voltages. This technique applies to polar and non-polar analytes such as pharmaceuticals, perfluorinated compounds (Fischer et al., 2012b). This is referred to as a “soft” ionization source as low energy is transported to the analyte which results in low fragmentation (Banerjee and Mazumdar, 2012). ESI is susceptive to matric effects which affects the analysis, it is essential for elimination or compensation of matrix effects when developing methods for this source (Pitt, 2009).

Atmospheric pressure chemical ionization (APCI) is also an LC-MS ionization technique similar to ESI, however, APCI uses a corona pin adjacent to the capillary to ionize gas and solvent molecules through are a charge transfer reaction (Banerjee and Mazumdar, 2012). This technique is highly applicable to thermally stable molecules that cannot be ionized well with ESI and less polar and non-polar analytes such as hormones and polybrominated diphenyl ethers. The limitations with APCI are that it results in

singly charged ions which limits the effective mass range and is undesirable for compounds that can be thermally broken down or displaced (Nguyen, 2018).

Atmospheric pressure photo-ionization (APPI), the solvent is initially vapourised by a nebulizing gas such as nitrogen under atmospheric pressure to form an aerosol (Banerjee and Mazumdar, 2012). This is subjected to a UV light source that emits photons possessing enough energy to ionize the specific target compounds. Dopants such as (toluene and acetone) are introduced into the sample before MS detection to improve the ionization efficiency. The APPI source can ionize compounds that both ESI and APCI cannot ionize and non-polar compounds such as polycyclic compounds, steroids, and some mycotoxins (Primpke et al., 2020).

Electron ionization (EI) is an ionization process used in most GC-MS systems, the ionization takes place in the gas phase through interactions of molecules with accelerated electrons from a resistively heated filament (Desfontaine et al., 2017). Energy is transferred to the organic compounds to form a positively charged molecule and additional energy results in the fragmentation of molecular ions to form fragment ions that are exclusive to the compound under those specified conditions (Pitt, 2009). This technique applies to volatile samples that are thermally stable with low molecular weight. The major drawback of this technique is that extensive fragmentation occurs resulting in little or no molecular ions (Banerjee and Mazumdar, 2012).

Chemical Ionization (CI) is widely employed in GC-MS systems, this technique uses interactions between a reagent gas (such as methane or isobutane) and sample molecules in the gaseous phase (Smith, 2013). The reagent gas undergoes electron ionization and releases molecular ions which react with the sample molecules leading to its ionization through adduct formation. This is a relatively softer gas-phase ionization technique compared to electron ionization (Clarke, 2017).

The selectivity of a mass spectrometer is manifested through its mass resolution, indicating its capacity to differentiate between ions with minute variance in their m/z (Sandau et al., 2003). Mass spectrometers are versatile and can be a simple quadrupole that makes use of an electron multiplier up to a high-resolution mass spectrometer with the capability to collect and distinguish compounds over a large *m/z* range (Fischer et al., 2012b). An MS instrument that achieves a resolution exceeding 10,000 is categorized as a high-resolution mass spectrometer (HRMS)and it is favoured as they offer increased mass accuracy and can discriminate between compounds with similar mass (Clarke, 2017).

The quadruple mass analyser has four metal rods positioned between the ion source and the detector. The triple quadruple mass spectrometer in which a collision cell is placed between first and second quadrupole mass analysers, which provides specificity of the analysis in a single mass analysis (Clarke, 2017). Because of their low cost, this are the most common mass analysers. The single

quadrupole is the most simple, cheap and robust and requires relatively low maintenance. The major limitation with this type of mass analyser is that is the limitation to unit mass resolution and has lower mass accuracy which results in various chemical formulas and false identification (Taylor et al., 2020).

Time of flight (TOF) operates by pushing ions through a high voltage. The ion's velocity and the time required to travel through the flight tube to get to the detector rely on their *m/z* values. TOF mass analysers are considered as high-resolution mass spectrometer (HRMS) and are also characterised by high scan rates and dynamic range (Oetjen et al., 2017).

Hybrid Mass Analysers use a combination of two or more different mass analysers in design and are therefore termed as “hybrid designs” (Clarke, 2017). When the last quadrupole in a triple quadrupole Mass spectrometer is replaced by a Time of flight, the result is a hybrid quadrupole time-of-flight (QTOF) mass spectrometer. In this design, the first quadrupole performs precursor ion selection then the Time of Flight mass analyser completes the product ion analysis (García-Córcoles et al., 2019).

2.2.5.1 High Pressure Liquid Chromatography – Mass Spectrometry (HPLC-MS)

LC-MS techniques have allowed determination of high polar to non-polar contaminants without the need for derivatization through the use of varying ionization sources (Pérez-Fernández et al., 2017). LC-MS methods offer the capability to monitor a wide array of emerging contaminants. In the context of water bodies, liquid LC-MS techniques have been employed to establish the concentrations of pharmaceuticals and pesticides. For compounds existing in low concentrations, multiple reaction monitoring (MRM) has been employed to validate and quantify their presence in water (Maldaner and Jardim, 2012). Ultraperformance liquid chromatography (UHPLC) coupled to a quadrupole time of flight mass spectrometer (Q-TOF) for target screening of emerging contaminants in water. This approach enabled them to efficiently identify 101 suspect compounds and confirm 40 target compounds. LC-HRMS is considered to be the best method for target and non-target screening of emerging pollutants in water bodies for its ability to assign molecular formulae for unknown compounds and added confidence for positive identification in quantitative work (Bataineh et al., 2021).

2.2.6 Gas Chromatography-Mass Spectrometry

Gas chromatography (GC) is used for the reparation of volatile, semi volatile and thermally stable organic compounds in samples (Manirakiza et al., 2002). While it can also accommodate non-volatile compounds, an additional derivatization step becomes necessary. However, this supplementary step can lead to time-consuming processes, introduce undesired side reactions, and complicate the overall

identification process (Morales et al., 2012). Different groups of emerging contaminants have been successfully analysed using GC-MS approaches.

GC-MS methods have allowed for analysis of pesticides in water samples, Gakuba and colleagues (2019) used GC-MS for the detection of organochlorine pesticides in river water. They applied single ion monitoring (SIM) mode monitoring 3 target ions for each compound for the identification of pesticides (Gakuba et al., 2019). The presence of extensive libraries in GC allows for confirmation of unknown compounds in samples (Bataineh et al., 2021).

2.2.7 Microplastics

Plastics continue to offer a wide range of advantages in our everyday lives; however, it is essential to acknowledge the significant environmental issues connected to them. The growing demand for plastics has led to an increase in their production, and inadequate management of plastic waste has resulted in a rise in the improper disposal of plastics that ultimately end up in water bodies. Plastics can be classified into three categories: macroplastics, microplastics, and nanoplastics (Kurniawan et al., 2021). This categorization allows for the assessment of their possible sources, the way they behave in the environment, their effects, and the identification of measures to mitigate their environmental impact. Microplastics (MP) refer to plastic particles between 0.05 - 5 mm in diameter (Xu et al., 2021). Microplastics can be considered as the tiniest form of plastic debris, posing a grave threat to aquatic organisms. Due to their size, they have the potential to be ingested by various species inhabiting diverse habitats, which can have fatal consequences.

In recent years, there has been a notable surge in global research focused on microplastics, particularly in most developed countries (Saad et al., 2022). However, it is evident that there is a significant scarcity of research papers addressing the presence of microplastics in Africa (Naidoo et al., 2015). Microplastic research conducted in South Africa is dominantly focused on the marine environment with few studies focused on the freshwater environment (Dahms et al., 2020).

The sources and pathways of microplastics in river water and sediments are diverse and can vary depending on the location of the water body. Improper waste management and disposal has led to plastics finding their way into the aquatic environment where they accumulate (Dalu et al., 2021). Microplastics are one of the major water pollutants and in water bodies including oceans, rivers, lakes however studies on microplastic abundances in rivers is limited relatively to marine environment (Xu et al., 2021). The threat of plastic pollution in water bodies arises from the composition and characteristics of plastics such as toxic chemical additives have a possibility to leach out when plastics are disposed (Chen et al., 2017). Common additives to microplastics are phthalates, bisphenol A and

polybrominated diphenyl ethers (PDBE), these compounds have drastic effects to living organisms and they are commonly classified as endocrine disruptors (Windsor et al., 2019).

The pathway of microplastics into aquatic biota is through ingestion direct or indirect ingestion. Direct ingestion of microplastics by aquatic organisms occurs where microplastics are identified as food by aquatic organisms. This in turn causes mechanical disturbances in the bodies of the organism such as clogged intestines or penetrating into the intestine wall (Yu et al., 2020). MPs polymer cannot also be acted upon by the organism's enzymes, this leads to blockage of the digestive system or sending of false signals to the brain that the stomach is full. This leads to nutritional associated problems such as weight loss, reproductive disruption, growth reduction and energy resource depletion (Eltemsah and Bøhn, 2019).

Microplastics have been found to have the potential to interact with other water pollutants and gradually gather pollutants on their surfaces and serve as a concentrated source of pollutant which may pose fatal effects to the consumer (Eltemsah and Bøhn, 2019). A study was conducted by (Rios et al., 2007) to determine whether MPs have the potential to trap persistent Organic Pollutants, analytical results revealed that PCBs ranged from 27 to 980 ng/g; DDTs from 22 to 7100 ng/g and PAHs from 39 to 1200 ng/g, and aliphatic hydrocarbons from 1.1 to 8600 µg/g. MPs are difficult to detect in the environment have a potential to accumulate in the their bodies aquatic organisms and pose both acute and chronic toxicity (Kurniawan et al., 2021).

Primary plastics are used as raw material in the plastic industry, synthetic textiles, personal care products, and electronic equipment and are released in water bodies in their micro or nano size (Kurniawan et al., 2021). Secondary microplastics are formed through the fragmentation of larger plastics into smaller fragments by photolysis, thermo-oxidation, and thermo-degradation (McCormick et al., 2016). Microplastics can also be described and characterized based on their polymer composition and the commonly identified are polyethylene (PE), polypropylene (PP), polyethylene terephthalene, Polyamide and Polyurethane (Duis and Coors, 2016a). MPs have been found in the environment with varying morphologies and shapes such pellets, sheet, film, fragment, fibre, and foam (Duis and Coors, 2016a).

2.2.7.1 Microplastics sampling and sample preparation methods in river water and sediments

Although numerous research studies have been carried out on microplastics, there is still a lack of standardized methodologies. The process of analysing microplastics typically consists of four main stages: collecting samples, preparing samples, extracting and isolating, and finally identifying and quantifying the samples.

2.2.7.1.1 Sample Collection

The selection of microplastics sampling techniques relies on the specific objectives of the study, aiming to gather a comprehensive range of samples to obtain precise and thorough insights into the distribution and abundance of microplastics. The chosen methods for microplastics sampling should strike a balance between simplicity, cost-effectiveness, precision, and accuracy, all while minimizing the potential for contamination. During river sampling, various approaches can be employed to collect samples from different locations, including the water surface, water column, bottom sediment, or riverbank sediment. These sampling techniques allow researchers to gather microplastics from different areas within the river ecosystem.

Two primary methods are commonly employed: Bulk sampling/grab sampling and volume reduced sampling. During bulk sampling a complete specimen is extracted from the environment and this approach is commonly applied to sediments and water (Tanaka and Takada, 2016). This is a simple approach that makes use of buckets, pumps, and glass bottles for collection of water with further sample processing at the laboratory. To collect sediment bulk samples from the riverbed, researchers can use simple tools like spoons, spatulas, or soil augers. However, for a more representative sample of the riverbed, Ekman bottom grab samplers have been employed. These specialized samplers ensure a more comprehensive and accurate collection of sediment samples from the riverbed.

Volume-reduced sampling is a commonly used method for collecting microplastics in various aquatic environments. It involves the use of fine mesh nets or sieves to capture microplastic particles suspended in the water. Net sampling can be conducted at different depths within the water column, ranging from surface sampling to vertical trawling. During net sampling, a net with a specific mesh size, usually between 100 to 500 µm, is towed through the water to collect the microplastic particles from a boat or stationery from the riverbank or bridge (Saad et al., 2022). The net is carefully handled to prevent contamination from external sources. Once the sampling is complete, the collected material is carefully transferred to containers for further analysis.

Net sampling allows for the collection of microplastics of various sizes, including both larger plastic fragments and smaller microplastic particles (Vasilopoulou et al., 2021). This method provides valuable information about the abundance, distribution, and types of microplastics present in the water body being sampled. It is particularly useful for studying the surface waters of rivers, lakes, and oceans. Different configurations of devices have been documented in various studies for microplastics sampling. Some commonly mentioned ones include: Neuston nets are designed are used to collect microplastics present in water. These nets typically have a wide mouth opening and fine mesh to capture floating microplastics. Plankton nets have widely been adopted for sampling

microplastics in the water column, targeting both surface and subsurface layers. These nets are similar to those used for plankton sampling and are equipped with fine mesh of particle size 0.3 mm to capture microplastic particles. (Naidoo et al., 2015) sampled microplastics along the river estuaries in feeding into the Indian ocean by zoo plankton net with a mesh size of 0.3 mm as outlined in table 2.3.

Manta trawls also known as high-volume surface trawls, are larger net systems with a distinct "manta ray" shape. They are towed at the water surface and have a large mouth opening, allowing for the collection of a high volume of water. Manta trawls are effective in capturing larger microplastic debris and are commonly used in studies focusing on microplastics as well. Studies that reported the use of manta trawls employed nets with 0.333 mm or 0.350 mm mesh size as suggested by the National Oceanic and Atmospheric Administration to preconcentrate topwater samples as microplastics can be collected in this range (Aragaw, 2021). (Naidoo et al., 2015) reported the use of manta trawls for the water sampling along the Kwazulu-Natal coastline. Weideman reported that the limitation associated with the use of manta trawls is the ineffectiveness to sample during dry seasons where water levels are low.

These different devices and configurations provide researchers with flexibility in sampling microplastics in various aquatic environments, enabling them to collect samples from specific depths, locations, or size ranges. The selection of the appropriate device depends on the objectives of the study and the specific sampling requirements. During net sampling, water flow rates are recorded using mechanical or electronic flow meters. These instruments help measure the velocity of water passing through the sampling area. This information is crucial for calculating the sample volume, which is obtained by multiplying the water flow rate by the net opening area and the duration of the sampling.

To achieve more precise velocity measurements throughout the water column, some studies have employed Acoustic Doppler Current Profilers (ADCPs). ADCPs utilize sound waves to measure water velocity at multiple depths, providing a more accurate representation of the flow profile. By incorporating ADCP data, the accuracy of velocity measurements can be improved and, subsequently, the estimation of sample volumes. The sample volume is a critical parameter for determining microplastic concentrations and loads in the river. By accurately measuring the water flow rates and multiplying them by the net opening area and sampling duration, a reliable estimate of the volume of water from which microplastics were collected can be obtained. This allows for more accurate assessments of microplastic pollution in rivers.

To eliminate systematic errors during sampling and sample preparation, certain practices such as: wearing cotton lab coats to reduce the risk of introducing synthetic fibres or microplastics from

clothing into the samples should be adhered to. It is also important to refrain from the use of plastic materials during sampling and transportation to prevent contamination from additional microplastics, this can be achieved through the use of alternative materials such as glass or metal. Timely cleaning of sampling containers and tools after use helps prevent cross-contamination between samples and ensures the accuracy of subsequent analyses. By implementing these measures, researchers can minimize the introduction of external microplastics and maintain the integrity of the sampling process.

2.2.7.1.2 Sample preparation

Following sample collection, it is essential to separate the microplastic particles from the surrounding water or sediment matrix, which is done through a process known as sample preparation. Sample preparation is a vital and indispensable stage in analysing microplastics, involving pretreatment steps to extract and concentrate the microplastic particles from the collected samples from the sampling techniques. Samples may undergo filtration or sieving processes as the initial step to eliminate any remaining water, solid residues, organic structures, such as leaves, as well as larger plastic pieces.

The pretreatment steps for the samples were divided into two main stages: organic matter digestion and density separation. The first step, organic matter digestion, involves breaking down the organic components present in the samples. This process typically uses chemical agents to digest the organic matter, such as leaves or biological material, that might interfere with the analysis of microplastics. By digesting the organic matter, it becomes easier to separate and concentrate the microplastic particles. The commonly used digestion reagent is 30 % hydrogen peroxide with the addition of ferrous sulfate ($FeSO_4$) as a catalyst. In addition to the previously mentioned digestion agents, other oxidizing agents such as potassium hydroxide (KaOH), sodium hydroxide (NaOH), and nitric acid (HNO_3) have been utilized for digestion purposes. The choice of oxidizing agent depends on factors such as the nature of the sample, the type of organic matter present, and the specific requirements of the analysis being conducted. Each oxidizing agent may have different strengths and properties, and the appropriate one is selected based on the specific needs of the digestion process.

To further enhance the digestion process, the solution can be stirred while applying heat. Stirring the solution helps in promoting the mixing and contact between the digestion agents and the organic matter present in the samples. The application of heat increases the reaction rate and accelerates the breakdown of organic materials, facilitating their digestion. This combination of stirring and heat helps to improve the effectiveness and efficiency of the organic matter digestion step in preparing the samples for subsequent analysis.

The second step, density separation, is employed to isolate microplastics from the remaining sample matrix, which often includes inorganic materials and sediment. Density separation utilizes the principle that different substances have varying densities. By creating a density gradient or using a specific density medium, the microplastic particles can be separated and recovered from the sample matrix, which predominantly consists of inorganic material. The commonly used flotation agents for the isolation of microplastics is NaCl (sodium chloride) with a density of 1.2 g/ml due to its low-cost availability and has a relatively high density which allows for the separation of microplastics from the surrounding matrix (Aragaw, 2021). Saturated NaCl solutions were reported by (Weideman et al., 2020a) for density separation for microplastics in water and sediments. Microplastics polymer types such as polyvinyl chloride and polyoxymethylene that have higher density cannot be easily separated by saturated NaCl solution and require and require alternative flotation agents such as NaI (sodium iodide), and $ZnCl_2$ (zinc chloride). (Saad et al., 2022) prepared a NaI solution of density 1.8 g cm^{-3} for the density separation of microplastics in sediments collected from the Vaal River.

The choice of flotation agent depends on factors such as the desired density gradient, the size and type of microplastics being targeted, and the specific analytical requirements of the study. An alternative method to chemical flotation agents for the separation of microplastics is oil extraction. This method is considered safe, low cost, and environmentally friendly. In oil extraction, a suitable oil is used to selectively attract and capture the microplastic particles. The oil should have a higher affinity for microplastics than the surrounding sample matrix. By mixing the sample with the oil, the microplastics adhere to the oil phase while other materials remain in the aqueous phase.

After mixing, centrifugation or filtration can be used to separate the oil phase containing the captured microplastics from the remaining sample matrix. The separated oil phase can then undergo further processing to extract and concentrate the microplastics for analysis. Oil extraction offers several advantages as an alternative method to chemical flotation agents. It eliminates the need for potentially hazardous chemicals, making it safer for both operators and the environment. It is also a cost-effective option as oils can be readily available and affordable. Additionally, oil extraction has shown good efficiency in capturing microplastics of various sizes and types.

However, it is important to consider the potential limitations and challenges associated with oil extraction, such as the choice of the appropriate oil, optimization of extraction parameters, and potential interference from co-extracted organic matter. It is crucial to conduct proper validation and optimization studies when implementing this method for microplastic analysis.

Density separation is accomplished by observing the formation of two distinct layers, and the solution can be decanted with care to separate them. After decanting, the separated layers can be further processed by filtration. The choice of filter is crucial in retaining the microplastic particles while

minimizing any interference with subsequent identification methods. When selecting a filter, it is important to consider its pore size and material composition. The filter should have a pore size small enough to retain the microplastic particles, preventing their loss during filtration. At the same time, the filter material should not introduce any additional contaminants or interfere with the subsequent identification methods.

Commonly used filters for microplastic analysis include those made of materials such as cellulose nitrate, glass fibre filters, mixed cellulose ester, or polycarbonate membranes. These filters typically have precise pore sizes and are designed to effectively capture microplastic particles while allowing the passage of other unwanted materials. Preparing blank samples is an important step when filtering microplastic particles to account for any potential contamination introduced during the filtration process. Blank samples are essentially control samples that do not contain the target microplastics but undergo the same filtration procedure as the actual samples.

2.2.7.1.3 Identification, quantification and characterization techniques of microplastics

The next step following the extraction and isolation of microplastics is the identification and characterization of microplastics. These are essential to develop understanding for the sources, distribution and potential environmental impacts of microplastics, which further aids in the extent of microplastics pollution and designing effective mitigation strategies. It is beneficial to employ multiple techniques and approaches to ensure comprehensive and accurate identification and characterization of microplastics. Visual identification is the preliminary step in the identification and characterization of microplastics in which particles are visually identified with an unaided eye or under a microscope. Key features of isolated particles such as size, shape, colour and transparency are studied.

The criteria proposed by Noren (2007) aims to avoid misidentification of particles during the process of microplastic analysis. These criteria are as follows: 1) No visible organic structures on particles/fibers: This criterion suggests that microplastic particles should not have visible organic structures attached to them. Organic structures, such as biological matter or natural fibers, may interfere with the identification and characterization of microplastics. 2) Equally thick fibers with 3-dimensional bending: This criterion emphasizes that microplastic fibers should have a consistent thickness throughout their length and exhibit 3-dimensional bending. This characteristic helps distinguish microplastic fibers from natural fibers, which often have variations in thickness and show different bending patterns. 3) Clear and homogeneously coloring of particles: Microplastic particles should have a clear and uniform coloration. Any variation or mottling in colour may suggest the presence of additives, fillers, or impurities, which can indicate non-microplastic materials.

These criteria serve as guidelines to ensure accurate identification of microplastics and reduce the chances of misidentifying other particles as microplastics. However, it is important to note that relying solely on visual criteria may not be sufficient for comprehensive microplastic analysis. Additional analytical techniques, such as pyrolysis gas chromatography with tandem mass spectrometry, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FT-IR) and Raman spectroscopy have been employed to confirm the presence of microplastics and accurately characterize them.

The commonly reported approaches to microplastic identification are FT-IR and Raman spectroscopy which involves the excitation of particles that allows for structure specific vibrations to be detected. The formed vibrational bands are characteristic fingerprint regions that allow for specific characterization between plastic and non-plastic materials. The formed spectra can also be used for polymer identification by comparing with a known reference spectre.

2.2.7.1.1 The occurrence and abundance of microplastics in rivers South Africa

Rivers, whether they are small or large, are natural watercourses that meander through channels on the earth's surface. Eventually, they merge into other bodies of water like oceans, lakes, seas, and even sometimes into groundwater reservoirs. In comparison to other aquatic systems, rivers play a crucial role in transporting various forms of waste, notably plastic waste. This makes them significant contributors of microplastics (MPs) into marine environments (Aragaw, 2021). The quantification and characterization of MPs have been investigated in diverse rivers situated across various geographical regions in South Africa as summarized in Table 2.4. Several rivers, including Jukskei River, Orange-Vaal River, Vaal River, and Plackenburg, were the studied. In the case of the Jukskei River, research has focused on evaluating MP pollution levels in water and sediment samples collected from urban streams. The recorded values varied across different sampling sites, with mean MP concentrations reaching 705 particles m-3 in water and 166.8 particles kg-1 in sediment samples (Dahms et al., 2020).

An investigation of the Orange-Vaal River revealed that the average MP abundance was 1.7 ± 5.1 particles L-1, with over 99% of these particles being fibers. This was consistent across various sampling sites and indicated a high level of pollution within the river system (Weideman et al., 2020b). The Plackenburg River in the Western Cape, South Africa was studied with MP abundance recorded as 5.13 ± 6.62 particles per L-1 during the wet spring season and 1.52 ± 2.54 particles per L-1 (Apetogbor et al., 2023). Overall, findings from the examined river water samples across different geographical areas consistently underscored the substantial presence of MP particles, signifying widespread pollution.

Table 2.4: Studies on microplastics sampling, processing, identification and quantification techniques in aquatic ecosystems.

Water body name and location	**Fresh water system**	**Sample collection**	**Purification**	**Identification**	**Abundance of particles**	**Reference**
Mdloti, uMgeni, Durban harbour, ilLovu Estuaries, South Africa	Surface water	Conical zooplankton net – (300 µm mesh size, 30 cm mouth diameter,	10 % KOH	Microscopic identification	159 ± 271.2 particles per 500 ml 41.7 ± 23.0 particles per 500 ml 47.6 ± 22.8 particles per 500 ml	(Naidoo et al., 2015)
Braamfontein Spruit, South Africa	Surface water	25 L container	10 % KOH	Visual and microscopic identification	705 particles m^{-3}	(Dahms et al., 2020)
Orange-Waal River	Water	Neuston nets	NaCl (density 1.2 g cm^{-3})	Microscope	0.04 ± 0.16 particles m^{-2}	(Weideman et al., 2020b)
Bizerte Lagoon, Tunisia	Sediment	Stainless steel spatula used to remove 250 cm x 250 cm quadrants	NaCl Solution (density 1.13 g cm^{-3})	Visual and microscopic identification	2340 ± 227.15 particles kg^{-1}	(Toumi et al., 2019)
Kwazulu-Natal Coastline	Sediment	Stainless steel manta trawl – 333 µm nylon mesh	Physical separation using forceps	Dissecting microscope	4.01 ± 3.28 particles m^{-2}	(Naidoo and Glassom., 2019)

Water body name and location	**Fresh water system**	**Sample collection**	**Purification**	**Identification**	**Abundance of particles**	**Reference**
Plakenburg river, South	Surface water	10 L bucket	NaCl solution	Visual observation and FTIR	5.13 ± 6.62	(Apetogbor et al., 2023)
Vaal River, South Africa	sediment	500 ml Van Veen grab	NaI	Stereomicroscope, Raman spectroscopy and Scanning electron microscope	463 particles kg^{-1}	(Saad et al., 2022)

2.3 CHALLENGES WITH URBAN POLLUTION

2.3.1 Economic challenges

The improper design and operation of wastewater treatment plants in most developing countries still remains an issue to date as conventional methods are still being used for treatment of incoming waste. Most conventional wastewater treatment plants have been found to lack the capacity to remove all the contaminants in water, especially the class of emerging contaminants. It is therefore important for implementation of modern technologies in WWTP, and the decision about their improvement is highly influenced by direct capital and operation costs (Awad et al., 2019).

The Arad urban wastewater treatment plant in Romania was found to discharge effluent into a highly sensitive stretch of the Mures River which was a nutrient sensitive area designated for special protection of birds. Upgrades on seven treatment plants, rehabilitation of reservoirs and sewage network cost EUR 18 million, this resulted in significant reduction of organic and nutrient pollution load entering the river (EEA, 2021).

2.3.2 Health Concerns

The introduction of raw and/or untreated faecal waste into surface waters which are sometimes sources of drinking water. This introduces pathogens into water and resulted in water-borne diseases such as gastroenteritis, cholera, typhoid fever, and dysentery, these diseases are the root cause for high child mortality rates (Jabeen et al., 2015). Water contamination also results in fish contamination, fish are primary food sources to human, and the consumption of contaminated fish has been reported to result in food ailments depending on (Deb, 2018).

Irrigation by contaminated water may introduce foodborne pathogens to fruits and vegetables and this is the case in most developing countries that suffer water scarcity and resort to untreated or insufficiently treated wastewater for irrigation (Steele and Odumeru, 2004). An investigation conducted by (Ackers et al., 1998) when residents in Montana were laboratory-confirmed with *Escherichia coli* 0157:h7 infections revealed that patients had consumed lettuce from farmyards using contaminated water for irrigation. (Sahota, 2018) emphasizes that the consumption of ready to eat crops and vegetables that are irrigated with sewage contaminated water pose consumers to faecal coliforms, *Escherichia coli* and diarrheagenic *Escherichia coli*. The Hennops river is contaminated with both raw and treated sewage (P. J. Oberholster et al., 2008a), use of this water for irrigation is a health concern.

2.3.3 Degradation of aquatic biodiversity

Water pollution is one of the main causes of loss of biodiversity in river water (Betts et al., 2020). The introduction of emerging contaminants and microplastics may lead to acute exposure of contaminants to the aquatic species leading to their death and some contaminants have been linked to the alteration in the reproduction capability of fish, this hinders the rate at which fish multiply therefore leading to their extinction (Sibanda et al., 2015b). Sewage contamination in rivers causes eutrophication due to algal blooms depletes oxygen in water thus killing aquatic organisms such as fish and amphibians (Subramaniam et al., 2018).

CHAPTER 3: RESEARCH METHODOLOGY

STUDY AREA

Sampling points from identified major potential point and non-point sources of pollution into the Hennops River catchment were investigated as shown in Figure 3.1. The upstream area is classified as the areas around the origin of the Hennops river and some of the tributaries that flow into the river. Sampling site S1 (25° 59' 13.94" S, 28° 13' 59.18" E) is located along Olifantspruit stream which runs through the Thembisa, where there is with build-up of municipal waste which is not efficiently removed and sewage leakages that are rarely attended. The sampling site S2 (25° 57' 35.93" S, 28° 13' 23.49" E) is a stream that runs opposite Clayville Industrial area which has pharmaceuticals manufacturing industries, food processing factories and beverage production companies that joins into the Hennops river. The effluent from the Olifantsfontein wastewater treatment plant discharges as a point source of pollution into the Hennops river, the Irene farm sampling site S3 (25° 54' 56.19" S, 28° 13' 58.96" E) is located below the discharge point along the river as it continues to flow further north.

Sampling site S4 (25° 52' 49.89" S, 28° 14' 42.91" E) was located along the Sesmylpruit which finds its source as the Ritevlei Dam which joins the Hennops river. The river continues to flow through urban land use sites such as the Centurion Golf Estate, between Centurion Cricket stadium and a recreational water park, sampling point S5 (25° 49' 49.43" S, 28° 08' 41" E) is located near the Royal elephant hotel. As the Hennops river flows downstream, it is joined by the Rietspruit which the Sunderland Ridge wastewater treatment discharges effluent into. Below the confluence of both rivers in Erasmia, there are commercial crop production activities taking place along the riverbed which make use of the Hennops river for irrigation and this location is marked the Vegetable Garden sampling point S6 (25° 49'10.09" S, 28° 04' 12.43" E). Sampling point S7 (25° 49' 46.39" S,28° 08'9.91" E) was located below along the Crocodile River to determine the pollution effect of the Hennops and Crocodile Rivers entering into the Hartbeespoort dam. Sampling point S8 is western channel of the dam mainly from agricultural area upstream and S9 is after the Hartbeespoort Dam wall near Mount Amanzi resort.

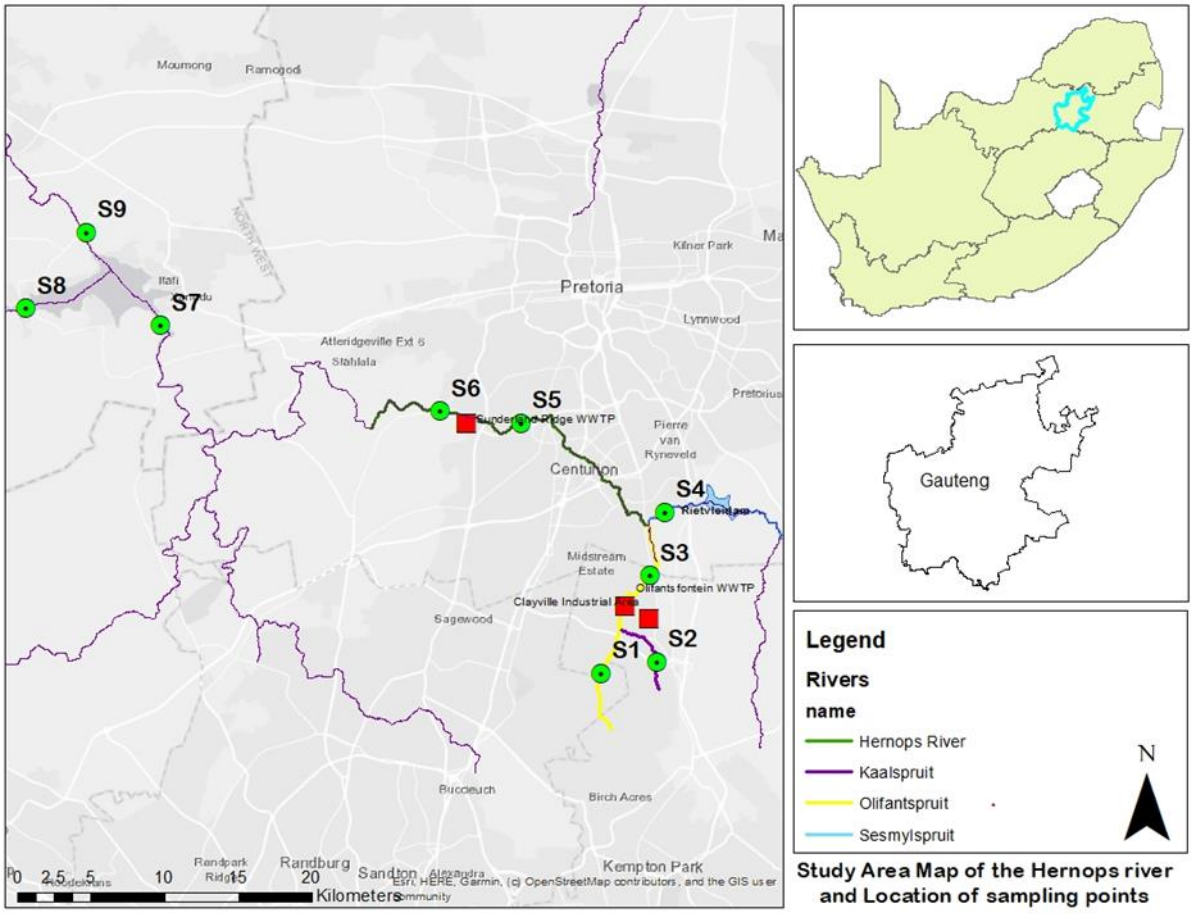


Figure 3.1. Area Map showing the study area sampling sites along the Hennnops river catchment in Gauteng, South Africa.

3.1 Chemicals and reagents

Analytical grade nitric acid 65% (w/w), UHPLC Grade Acetonitrile (≥ 99.9 %), methanol (≥ 99.9 %) and Formic acid (≥ 99.9 %) were purchased from Merck (Johannesburg, South Africa). Analytical grade venlafaxine hydrochloride (≥98%), methocarbamol, etilefrine hydrochloride, nevirapine, and carbamazepine, p-vinylbenzoic acid (97%) were purchased as single components in powder form from Sigma- Aldrich, Johannesburg

3.2 Instrumentation and apparatus

Ultrapure water was produced inhouse using the Millipore DirectQ 3 UV water purification system from Millipore (Massachsetts, USA). Three component Polyetetrafluoroethylene (PTFE) POCIS bodies were manufactured by University of The Witwatersrand School of Physics (Johannesburg, South Africa). The passive sampler device design consisted of PTFE base Figure 3.2a and cap that screwed together to seal the membrane Figure 3.2c and the disk based on a design reported by (Vrana et al.,

2005b). The base had a diameter of 49 mm and screw cap was open in both ends with the front window having a diameter of 55 mm while the back internal diameter was 40 mm and tread depth of 5mm.

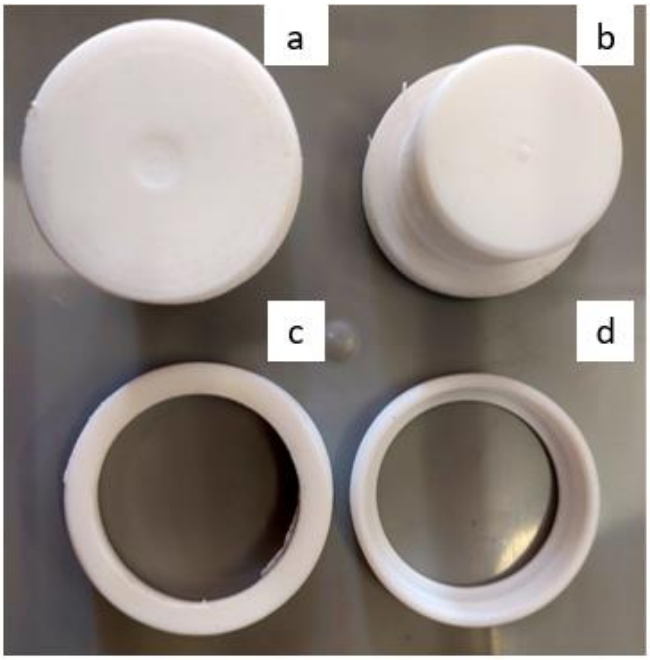


Figure 3.2. Components of the POCIS body (a and b represent front and back of the base, c and d represent front and back of the screw cap). (Photograph: T. Letseka)

Affinisep Hydrophilic-lipophilic balance (HLB) receiving phase disks (47 mm) were purchased from Anatech (Johannesburg, South Africa) and 0.22 µm PES membranes (47 mm) were purchased from Merck (Johannesburg, South Africa). A Solid Phase Extraction (SPE) setup consisted of Supel-Select Hydrophilic-lipophilic balance (HLB) sorbent tubes (500 mg) purchased from Sigma Aldrich (Johannesburg, South Africa) mounted on a vacuum pump procured from Pall Corporation (Fribourg, Switzerland) and manifold set-up purchased from Phenomenex (California, USA).

A Dionex Ultimate 3000 UHPLC Thermo Fisher Scientific (Bremen, Germany) instrument coupled to a Bruker Maxis Impact II ESI-Q-TOF high resolution tandem mass spectrometer equipped with an electrospray ionisation source (Bruker Daltonics, Bremen, Germany) was used for analysis of organic pollutants from both grab sampling and passive samplers. A 20 µL injection volume was used for samples and standards with separation performed on a Luna Omega C18 column (50 mm x 4.6 mm x 3 µm) from Phenomenex (Torrance, CA, USA). The mobile phases reservoirs were filled as follows; Solvent (A) 0.1 % formic acid in water, and solvent (B) 0.1 % formic acid in acetonitrile. The gradient method was set at initially 5 % B for a 1 minute then ramped to 55 % B for 6 minutes and maintained constant for 2 minutes. The amount of solvent B was further increased to 95 % at 10 minutes then held constant for 2 minutes thereafter allowed to equilibrate to initial conditions giving a total run-time of 14 minutes. Instrument operating parameters are shown in Table A1.

3.3 Sample Collection

3.3.1 Physiochemical, Ecotoxicology and Microbiological sampling

Grab samples for physicochemical, ecotoxicological and microbiological analysis were collected in sterilized 1 L glass bottles. Powder free nitrile gloves were used to handle samples and containers to prevent contamination. Sediment samples were collected using a sediment auger and stored in plastic containers. The samples were brought to the lab in a cooler box then stored at 6 °C.

3.3.2 Organic water pollutants sampling

PTFE Components of the POCIS body were thoroughly rinsed with deionized water and the HLB disks was conditioned by soaking the discs in 50 mL methanol for 30 minutes followed by 100 mL ultrapure water, the PES membranes were treated in a similar approach. The POCIS components were connected by placing the HLB disk on the body followed by carefully placing the PES membrane on top of the HLB disk to make sure than there were no air bubbles between the two surfaces. The assembled samplers were submerged in ultrapure water prior use to prevent drying out of the HLB disk before deployment and during transport. Samplers were deployed at specific points based on accessibility and safety for deployment over a period of 10 days as shown in Figure 3.3. The POCIS body assemblies were collected and enclosed with the PTFE transport lid, leaving some water at the top of the device. A field blank sampler was left in distilled water and handled in a similar manner as for the field-exposed devices. Where passive samplers were deployed, grab samples were collected in 1 L polyethylene terephthalate (PET) bottles for solid phase extraction.



Figure 3.3. Field deployment of POCIS passive samplers (left), POCIS placed inside a cage (right) (Photograph: T. Letseka)

3.3.3 Microplastics sampling

River water was sampled using a 50-micron mesh plankton net (50 cm mouth diameter) that was deployed in the river for 3 hours as shown in figure 3.4. The net was secured by a string on a stable platform such as bridge or tree and suspended to a depth of 50 cm into the river and the water column was sampled horizontally. A flow meter attached to the net to determine the volume of water sampled. The contents of the net were washed from the outside of the net with river water into glass sample jars and capped with aluminium foil lined lids. Jars were rinsed 3 times prior sampling with distilled water in the lab then capped and rinsed with river water at the time of sampling. Sediment samples were collected along the riverbank using a sediment auger from different points into 500 mL glass jars that were cleaned in a similar manner as of water samples. The collected samples were transported to the lab for further preparation and analysis.



Figure 3.4. Microplastics Sampling procedure (Photograph: T. Letseka)

3.4 Sample Analysis

3.4.1 Physiochemical water quality

The conductivity, pH, dissolved oxygen (DO) and total dissolved solids of the samples were analysed and recorded onsite using a Hanna Multiparameter meter (Bucharest, Romania) that was calibrated in accord with the manufacturers' instructions.

3.4.2 Microbiological Analysis

The microbiological analysis was performed at ARC-Irene analytical services. The method followed was adapted from the work done by Gemmell and Schmidt (Gemmell and Schmidt, 2012) and Chatanga and colleagues (Chatanga et al., 2019b). In this regard, 10 mL of the collected river water sample was pipetted with a sterile pipette tip into a sterile conical flask and diluted to a total volume of 100 mL by quarter-strength Ringer's solution followed by 10 times dilution using the same solution (Gemmell and Schmidt, 2012). Thereafter, aerobic plate counts of the sample were conducted according to the South African National Standards (SANS, 2007) procedure 4833 employing the plate count agar as specified in the procedure (SANS, 2007). These were done in triplicate. For calculation of total and faecal coliforms, the most probable number (MPN) method was used.

Firstly, 10, 1 and 0.1 ml of water samples were inoculated into a series of tubes containing 9 ml of lactose broth. Thereafter, a loop full of the suspension from positive gassing lactose broth tubes was transferred into tubes containing 9 ml of 2% brilliant green lactose bile broth and incubated at 35 °C (Chatanga et al., 2019b). Lastly, confirmation of the presence of total coliforms in the water sample was done through observation of gas production in the brilliant green lactose bile broth tubes within 48 h of the analysis (Chatanga et al., 2019b). The calculations of the most probable number were done only for the lactose broth tubes which confirmed the presence of the coliforms. The *E. coli* was enumerated by inoculating gas-positive Lauryl Sulphate Tryptose broth (LST) tubes into E. coli broth 4-methylumbelliferyl-β-D-glucuronide substrate that is hydrolysed by β-D-glucuronidase enzymes for positive *E. coli* detection. Positive *E. coli* test and growth was confirmed by the increase in fluorescence and the production of gas.

3.4.3 Organic water pollutants analysis

3.4.3.1 Solid phase extraction

A Solid Phase Extraction (SPE) setup shown in Figure 3.5 was assembled for the extraction of pharmaceuticals from grab water samples. An optimized SPE method reported by Madikizela and co-

workers was employed, the HLB sorbent was pre-conditioned with 5 mL of methanol followed by 5 mL ultrapure water. Measured 500 mL volume of water samples were loaded on the SPE cartridge and allowed to flow though at a steady flow (1 – 2 drops/sec) by use of the adjustment knob on the vacuum manifold. The SPE cartridge was then washed with 5 mL of methanol:water (10:90 %v/v) then allowed to dry for 1 minute under vacuum. The analytes were eluted with 5 mL of 2 % formic acid in water:methanol (20:80 %v/v) mixture which collected and evaporated to near dryness under a stream of nitrogen then reconstituted with 2 mL of water:methanol (90:10 %v/v). The extract was filtered 0.22 µm PTFE syringe filters then transferred into 2 mL autosampler vials for LC-qTOF-MS analysis.

Recovery experiments were conducted by spiking river water samples to a concentration of 0.05 µg ml^{-1} as a matrix matched approach. The spiked samples were allowed to go through the SPE procedure as the unadulterated samples.

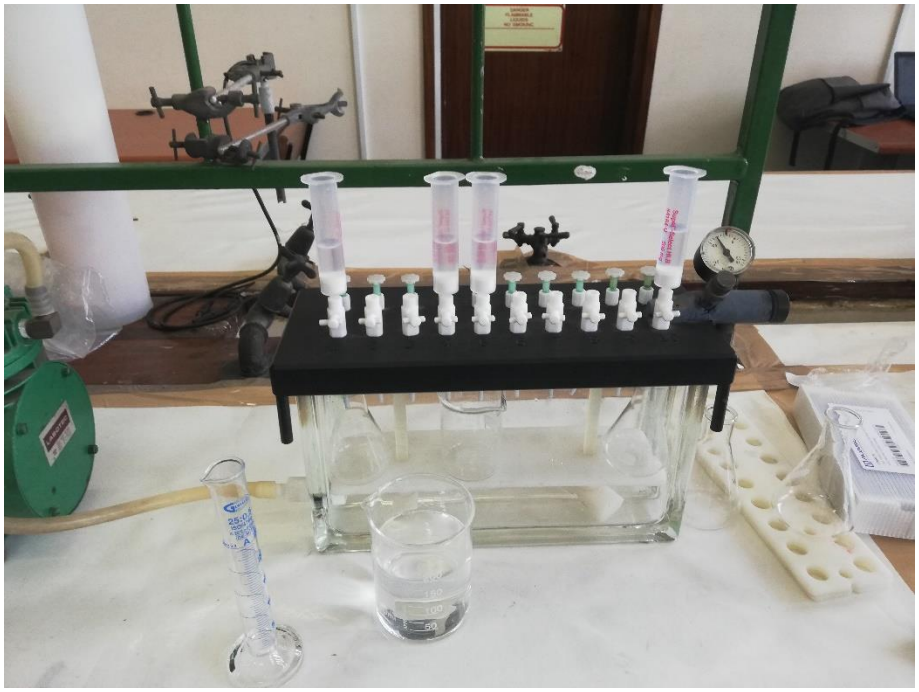


Figure 3.5. Solid Phase extraction setup (Photograph: T. Letseka)

3.4.3.2 Passive sampling by Polar Organic Chemical Integrative Sampler (POCIS)

Laboratory-based calibration

Laboratory scale calibration of the passive samplers was over a period of 6 days in order to understand the accumulation of the analytes on the sampler during the time of deployment. After which sampling rates (Rs) for each targeted compound could be estimated. The estimation of sampling rates was done using Equation 3.1.

$$R_S = \frac{M_S}{C_w\, t} \qquad (3.1)$$

where R_s is the sampling rate (L d^{-1}), M_s is the mass of analytes accumulated in the receiving phase (ng), C_w is the mean water concentration (ng L^{-1}), t is the deployment time (days).

Components (PTFE) of the POCIS body were cleaned by soaking overnight in a detergent solution then thoroughly rinsed with deionized water. Conditioning of the HLB disk was performed in order to activate and open pores of the sorbent and to ensure optimum intake of analytes. The HLB disks were conditioned by soaking in 50 mL methanol for 30 minutes followed by 100 mL ultrapure water, the PES membranes were treated in a similar approach. The devices were assembled by placing the HLB disk on the body followed by carefully placing the PES membrane on top of the HLB disk ensuring that no air bubbles were trapped between the two surfaces. POCIS calibration was performed in a 5 L-capacity beaker containing deionized water which was spiked to a final concentration of 5 ng mL^{-1} of each analyte.

The solution was allowed to equilibrate for 30 minutes at a stirring rate of 3000 rpm using a magnetic stirrer to mimic environmental river flow. Four samplers were loaded into the tank with each sampler removed at regular intervals (1 day, 2 days, 4 days and 6 days), after retrieval of each sampler a POCIS body (without membrane and HLB disk) was placed at the initial position of the sampler to maintain flow kinetics of the system. The target compounds were extracted from the dried disks using 40 mL methanol on a glass extraction funnel manifold assembly under gravity. The analyte rich methanol was collected into clean 50 mL centrifuge tubes then the solvent was evaporated to near dryness under a stream of nitrogen then reconstituted with 2 mL of water: methanol (90:10 % v/v).

Field deployment of POCIS

Samplers were prepared for field deployment in a similar manner as for lab calibration and on the day of deployment the retaining ring was then screwed over the two components to secure them in place. The assembled samplers were submerged in ultrapure water prior use to prevent drying out of the HLB disk before deployment and during transport. Samplers were deployed at specific points based on accessibility and safety for deployment over a period of 10 days. Upon retrieval, the POCIS body assemblies were resealed with the PTFE transport lid to prevent the HLB disk from drying out during transportation. A blank sampler was exposed to deionized water and treated in the same manner as that of field-exposed devices.

Upon retrieval of samplers from the field in the laboratory, the POCIS were carefully disassembled then PES membrane was discarded while the HLB disk was allowed to dry on a solvent-rinsed aluminium foil for 24 hours at room temperature. The target compounds were extracted from the dried disks using 40 mL methanol on a funnel manifold assembly under vacuum. The analyte rich methanol was collected into clean 50 mL centrifuge tubes then the solvent was evaporated to near dryness under a stream of nitrogen then reconstituted with 2 mL of water:methanol (90:10 %). The sample

solutions were transferred into 2 mL autosampler vials for LC-qTOFMS analysis. The calculation of the time-weighted average (TWA) concentration of the analytes throughout the deployment period was achieved using Equation 3.2.

$$C_w = \frac{C_s M_s}{R_s\, t} \quad (3.2)$$

C_w represents the average concentration of the target compounds (TWA) in the water during the time of deployment in (ng L^{-1}), C_s stands for the quantity of analytes gathered on the adsorbent material (ng g^{-1}), M_s is the adsorbent mass (g), R_s is the sampling rate (L d^{-1}) and t represents the overall deployment time (days).

3.4.4 Microplastics Extraction

3.4.4.1 Extraction of Microplastics from water samples

Water samples were digested with 30 ml of 0.07M $FeSO_4 \cdot 7H_2O$ and 30 ml of 30% (v/v) H_2O_2 were added stirred at 10 revolutions per minute for 24 hours at room temperature covered with aluminium foil in a closed laminar flow cabinet. To the mixture, 45 ml of saturated NaCl (1.2 g cm^{-3}) was initially dispensed then 45 ml of NaI (1.8 g cm^{-3}) was added. The mixture was let to stand for 24 hours for density separation and filtered through a 47 mm glass microfiber filter then let to dry at room temperature for 24 hours in covered glass petri dishes.

3.4.4.2 Extraction of microplastics from Sediments

Sediments were dried in the oven at 40 °C for 7 days and the dry mass was obtained. To the dried samples, 10% (w/v) KaOH solution was added at a ratio of 1:3 (w/v). The mixture was placed in a laminar flow fume hood, covered with aluminium foil then placed on a hot plate set to 40 °C and stirred at 10 revolutions per minute for 24 hours. Sodium iodide solution (1.8 g cm^{-3}) was mixed with the digestate at a ratio of 1:3 (v/v) and stirred for 5 minutes. The mixture was let to stand in a laminar flow fume hood for 24 hours for density separation to obtain a two separate layers. The solution was filtered through a 47 mm glass microfiber filter then let to air dry for 24 hours in glass petri dishes.

3.4.4.3 Morphological Characterization

The “break test” was employed to differentiate between plastic and non-plastic particles. In this test, a sharp stainless-steel pin was used investigate individual particles. Plastic particles are flexible and do not break upon prodding but rather bounce. Non-plastic particles that shattered when prodded were disqualified. Visual identification was performed under a Nikon stereomicroscope (Nikon MET SMZ745T) with up to 50× magnification, equipped with an imaging source camera (TIS) USB 3.0. The

length (size) of individual plastic particles was analysed by NIS Elements-D imaging software Ver 5.30 (Nikon).

A TESCAN Vega scanning electron microscope (SEM) was employed to produce high-resolution images of morphological surface structures of different microplastic shapes. The specimens were affixed to carbon tape and subsequently coated with a layer of gold. Scanning electron microscopy (SEM) images were assessed with a TESCAN Vega TC device, employing an acceleration voltage of 2 kV. The analysis was conducted using the VEGA 3 TESCAN software.

3.4.4.4 Polymer Composition

Polymer composition of suspect particles was analysed by a Horiba LabRam HR Raman spectrometer (Tulln, Austria) equipped with an Olympus BX41 microscope. LabSpec (version 5) software was used for data acquisition and analysis.

3.4.4.5 Quality control measures

Sampling and sample preparation was performed using glass and metal equipment and containers. Cotton lab coats and gloves were worn during sampling and sample processing with all experiments conducted under a closed laminar-flow hood. The extracted microplastics were stored in cleaned covered glass petriI-dishes. All containers were covered with aluminium foil to prevent any airborne contamination. During the microscopic analyses, glass petri dishes were used and rinsed several times before any solution was added for counting. The petri dish was kept closed as much as possible and was only opened to remove microplastic particles or to test them. Cross contamination was controlled by the use of blank control experiments during sampling and sample preparation. No microplastics were detected in the blank samples indicating low potential of contamination from sampling apparatus and the laboratory environment. Any material that resembled the lab coat, clothing or the containers used, was not included in the results section to provide a conservative estimation on the number of microplastics found.

3.5 Data validity and reliability

Sampling of water and sediments was conducted consistently by skilled and competent personnel to ensure consistency. Positive controls were used to ensure the reliability of the results for all methods. Where possible, replicate analysis was performed to ensure accuracy. Water quality parameters were measured using calibrated equipment.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Physio-chemical and microbiological water quality

The results show the status of the Hennops river at the start of the Project before intervention measures that were part of a separate project that is beyond the scope of this study were implemented that was conducted from (August 2021 – November 2021) and the status of the pollution at the end of the project that was conducted from (July 2022 – November 2022).

Table 4.1 indicates the initial physiochemical water parameters, the lowest pH value recorded was 6.9 recorded at S2 and the highest value was 7.4 that was recorded at S7. The final status results in Table 4.2 had the lowest pH value of 7.87 recorded at S3 and the highest value of 8.18 at S8. The trend in pH observed during the initial and finals status showed an increase in pH from upstream to downstream, and the pH of the water was more basic in the final sampling. The pH of the Hennops river water was found to be within tolerable limits as per SANS-241 specifications.

Electrical Conductivity values for both the initial and final status assessment were within tolerable limits. Conductivity values for the initial status assessment ranged from 242 $\mu S.cm^{-1}$ recorded at S2 to 785 $\mu S.cm^{-1}$ recorded at S1. For the final assessment conductivity values ranged from 163.7 $\mu S.cm^{-1}$recorded at S7 to 376.0 $\mu S.cm^{-1}$ recorded at S9. The conductivity values were lower in the final assessment. Total Dissolved Solids (TDS) values for the initial assessment ranged from 115 mg/L recorded at S2 to 399 $\mu S.cm^{-1}$ recorded at S1. For the final assessment conductivity values ranged from 91.5 mg/L recorded at S7 to 164.6 mg/L recorded at S9. The conductivity values have dropped during the final assessment in comparison to the initial assessment.

Dissolved Oxygen (DO) levels were found to be below the ideal quality range for fresh water of 9.1 mg L^{-1}. S3 revealed the lowest DO value of 1.53 mg/L and S7 had the highest DO value of 6.47 mg/L which is tolerable. The DO content is drastically low in the upstream areas as they are point sources of sewage discharge from leaking pipes and wastewater treatment plants which introduces a high content of organic matter leading to a high oxygen demand in the water. DO measurements were only conducted in the initial water quality status assessment.

The data in table 4.1 and 4.2 further shows indicates that river water is under microbiological contamination at all sampling points and is unfit for drinking, irrigation, and recreational use as the specifications by DWAF are exceeded tremendously. There is generally high microbiological contamination in the upstream area that arises from the raw sewage spew into the river, site S3 which is located below the Olifantsfontein wastewater treatment plant which discharges effluent into the river

has the highest Total Aerobic Count (TAC) 20 000 000 CFU mL^{-1} and S2 had the highest Coliform Count of 900 000 CFU mL^{-1}.

Microbiological indicators however show a worsening status of the Hennops river as the total aerobic counts are very high in the range 1 500 000 - 400 000 000 CFU mL^{-1} which are higher than those observed in the initial sampling that reached a maximum of 20 000 000 CFU mL^{-1} in the upstream area. Total coliform count was in the range 78 333 CFU – 180 00 CFU mL^{-1} while the *E. coli* count were in the range 225 - 14 500 CFU mL^{-1}. The microbiological analysis data shows that the Hennops water quality is still in a poor state that most likely arises from uncontrolled sewage leakages in the upstream areas and faecal contamination. Results also suggest that the Hartbeespoort is currently acting as a major sink for upstream pollution as the amount *E. Coli* detected after the dam was low but still present indicating that some pollution still escapes the dam. This is supported by repeated covering of the dam with water hyacinth because of continued pollution from upstream. Interesting, the western channel of the dam has high conductivity and also after the dam wall.

Table 4.1 Initial Water quality parameters measured at each site of the Hennops river.

Sampling Site	**pH**	**TDS (mg L^{-1})**	**Conductivity (µs cm^{-1})**	**DO (mg L^{-1})**	**TAC (CFU mL^{-1})**	**Coliform Count (CFU mL^{-1})**	***E. Coli* Count (CFU mL^{-1})**	***E. Coli* Detection**
S1	7.06 ± 0.01	399 ± 13	785 ± 60	1.82	2 800 000	670 000	8 600	Positive
S2	6.90 ± 0.03	115 ± 70	242 ± 12	2.87	3 900 000	900 000	20 000	Positive
S3	6.92 ± 0.01	364 ± 15	743 ± 40	1.53	20 000 000	853 333	20 000	Positive
S4	7.12 ± 0.02	379 ± 10	758 ± 16	4.66	3 300 000	82 333	1 200	Positive
S5	7.13 ± 0.01	343 ± 13	690 ± 50	5.04	1 500 000	78 333	225	Positive
S6	7.15 ± 0.02	379 ± 22	758 ± 90	4.37	1 956 667	24 500	225	Positive
S7	7.40 ± 0.03	376 ± 11	768 ± 40	6.47	310 000	8 500	20	Positive

Table 4.2. Water quality parameters measured at each site of the Hennops river after clean-up.

Sampling Site	**pH**	**TDS (mg L^{-1})**	**Conductivity (µs cm^{-1})**	**TAC (CFU mL^{-1})**	**Coliform Count (CFU mL^{-1})**	***E. Coli* Count (CFU mL^{-1})**	***E. Coli* Detection**
S3	7.87	153.4	306.6	320 000 000	170 000	2 250	Positive
S5	8.00	95.4	190.9	400 000 000	180 000	1 150	Positive
S7	8.12	91.5	183.7	3 150 000	195 000	1 450	Positive
S8	8.18	187.8	275.8	1 700 000	495	30	Positive
S9	8.16	184.6	376.0	90 000	2 650	4	Positive

4.2 Organic water pollutants analysis

4.2.1 Method Performance and Validation

Method validation for SPE was performed on river water samples spiked at 5 ng mL^{-1} and POCIS method validation was performed on deionized water spiked with 5 ng mL^{-1} of the target analytes. The method performance and validation parameters of each technique are summarized in Table 4.5. The correlation coefficient (r^2) was in the range of 0.989 to 0.999 and the linearity was determined in the range 1 – 100 ng mL^{-1} for methocarbamol, carbamazepine, nevirapine, venlafaxine and 5 - 100 ng mL^{-1} for Etilefrine. The POCIS and SPE methods yielded recoveries ranging from 76 to 92% and 73 to 89% for all target compounds, respectively. Despite the recoveries of SPE being lower than POCIS, the standard deviation of the SPE results was lower than that of POCIS indicating that the method offers better reproducibility. The sensitivity of the methods was evaluated through the method detection limits (MDL) and the method quantification limits (MQL). The MDL and MQL for POCIS were in the range of 0.16 to 1.14 ng mL^{-1} and 0.57 to 2.12 ng mL^{-1}, respectively, whilst they were in the range of 0.1 to 1.64 ng mL^{-1} and 0.19 to 1.82 ng mL^{-1}, respectively, for SPE.

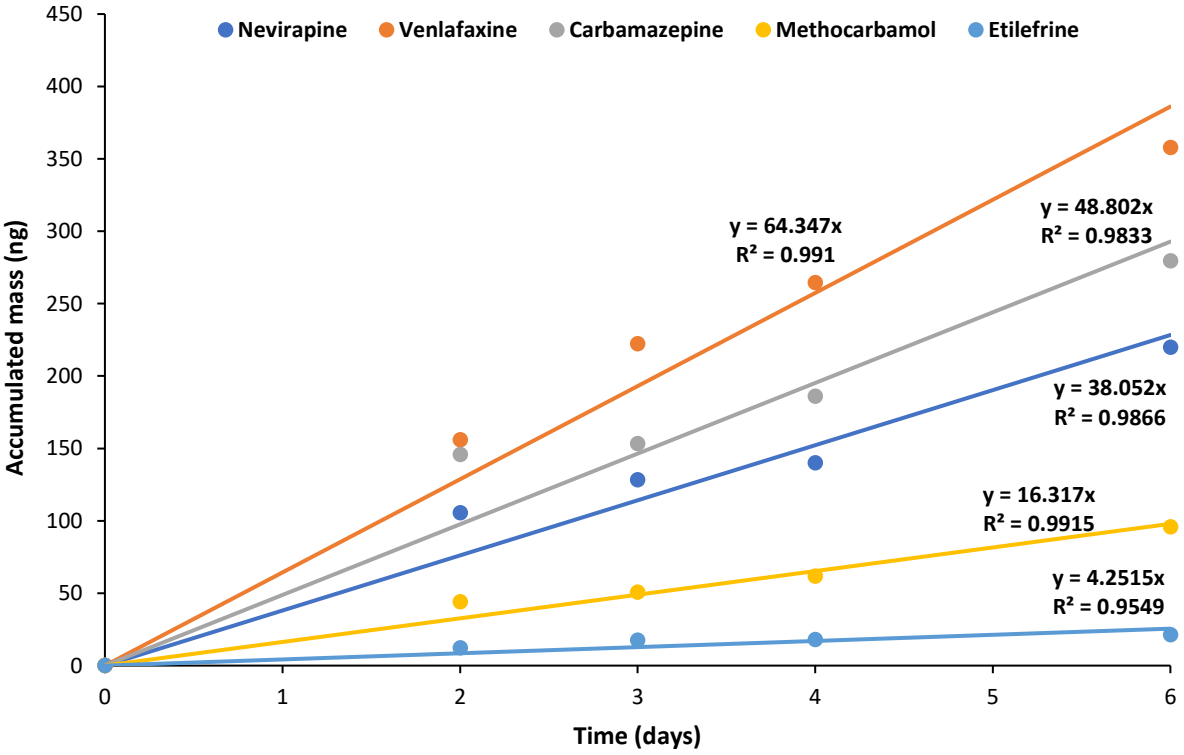


Figure 4.1. Uptake curves showing relationship between accumulated mass of Nevirapine, venlafaxine, Carbamazepine, methocarbamol and Etilefrine over 6 days.

Figure 4.1 depicts that the uptake curves for all targeted compounds assumed linear uptake models in the range in which the calibration was conducted. The data obtained from the uptake curves was used to calculate the sampling rates (Rs) reported in Table 4.4. The experimental sampling rate obtained from this work for carbamazepine was 0.067 L d^{-1} which was found to be lower than the literature values that were in the range 0.11 – 0.235 L d^{-1} (Bayen et al., 2014; Belles et al., 2014; Li et al., 2010; Vrana et al., 2021) , whilst venlafaxine had an experimental sampling rate of 0.87 L d^{-1} that was comparable to 0.894 L d^{-1} reported by Bayen et al., (2014) who employed a static depletion approach for the calibration of samplers The sampling rate obtained by Vrana et al., (2021) was lower than the obtained value in this study of 0.870 L d^{-1} and this could be attributed to the fact that field calibration was employed in their study which is prone to matrix effects such biofouling that limit the diffusion of analytes into the adsorbent leading to lower sampling rates (MacLeod et al., 2007).

There were no literature sampling rates for nevirapine, methocarbamol and etilefrine using POCIS passive sampler. As such, comparison was done with literature values obtained with compounds with similar or closer Log Kow values as a linear relationship has been established between log Kow and sampling rates (Morin et al., 2012). Etilefrine has a log Kow value of 0.1 and was compared to atenolol with log Kow value of 0.16, the sampling rate determined in the study was found to be 0.041 L d^{-1} which was closer to 0.051 L d^{-1} reported by (Bayen et al., 2014). Methocarbamol has a log Kow value of 0.6 and was compared to acetaminophen with log Kow value of 0.46, the sampling rate determined in the study was found to be 0.260 L d^{-1} which was higher than the reported values 0.0118 L d^{-1} and 0.114 L d^{-1} (Li et al., 2010; Morin et al., 2012). Nevirapine has a log Kow value of 2.5, it was compared to naproxen with log Kow value of 3.18, the sampling rate determined in the study was found to be 0.022 L d^{-1} which was closer to the reported value 0.031 L d^{-1} reported by Magi et al., (2018) but five times lower than that reported by Morin et al., (2012).

Table 4.3. Estimated sampling rates (Rs) obtained from literature and the current study for the target pharmaceuticals.

Pharmaceutical compound	**Log Kow**	**Sampling rates (L d^{-1})**	**Calibration method**	**Reference**
Carbamazepine	2.45	0.235	Static depletion	(Li et al., 2010)
		0.14	Continuous flow	(Belles et al., 2014)
		0.60	Static depletion	(Bayen et al., 2014)
		0.11	In situ	(Vrana et al., 2021)
		0.067	**Static depletion**	**This study**
Venlafaxine	2.9	0.894	Static depletion	(Bayen et al., 2014)

Pharmaceutical compound	Log Kow	Sampling rates (L d^{-1})	Calibration method	Reference
		0.104	Static depletion	(Li et al., 2010)
		0.23	In situ	(Vrana et al., 2021)
		0.870	**Static depletion**	**This study**
Atenolol	0.16	0.025	Continuous flow	(Morin et al., 2012)
		0.051	Static	(Bayen et al., 2014)
		0.073	Static	(Li et al., 2010)
Etilefrine	0.1	**0.041**	**Static depletion**	**This study**
Nadolol	0.85	0.114	Continuous flow	(Morin et al., 2012)
		0.0118	Static depletion	(Li et al., 2010)
Methocarbamol	0.6	**0.260**	**Static depletion**	**This study**
Acetaminophen	0.46	0.139	Static depletion	(Li et al., 2010)
		0.048	Static depletion	(Bayen et al., 2014)
Naproxen	3.18	0.135	Semi-static	(MacLeod et al., 2007)
		0.031	Semi-static	(Magi et al., 2018)
		0.118	Semi-static	(Morin et al., 2012)
Nevirapine	2.45	**0.022**	**Static-depletion**	**This study**

4.2.2 Application of SPE and POCIS in monitoring of target pharmaceuticals in river water

Table 4.5 shows SPE and POCIS sampling data for monitoring target pharmaceutical compounds along the Hennops River Catchment. The is no data obtained for S1 – S4 through passive sampling as these sites were not suitable for deployment of passive samplers based on safety and security. The general trend observed from the data obtained from both methods is that the data obtained through SPE is slightly lower than the data obtained from POCIS. This can be due to several factors. 1) The passive samplers are able to offer average concentrations of the compounds during the entire time of deployment which means that it is able to cater for any sporadic events of contamination e.g. release of effluent, burst effluent pipes etc. On the other hand, SPE is only able to offer contaminant concentrations at the time of sampling which means that if the sampling is done before any

contamination event, then the results are bound to be lower than those of passive sampling. 2) The other cause could be attributed to the filtering of samples prior to solid phase extraction as some of the compounds could be attached to dissolved solids in the sample.

There were no detected traces of etilefrine in all sites through both POCIS and the SPE. This implies that if there was any etilefrine present in the Hennops River at the time of sampling, it may have been present at very low concentrations i.e. below detection limits. Carbamazepine was detected in all sites through both approaches, whilst methocarbamol and venlafaxine were detected in all sites except for S1 and S2. Nevirapine was detected only in three sampling sites, i.e. S3, S8 and S9 whilst it was detected in the sites through passive sampling. This could be attributed to the fact that passive sampling devices only measure the bioavailable fraction of concentration whereas SPE measures the total concentration in the sample. The general trend in concentration of compounds is observed to be low in the upstream areas from data collected through grab sampling. Although there are identifiable point and non-point pollution sources in the upstream areas, the high flow rate of the river rapidly flushes pollutants downstream where there is more river meandering that reduces flow rate and allows for settling of pollutants. This gives enough contact time between samplers and water. The concentration of pollutants is generally low in the upstream from S1 where it is below quantification limits then increases as flow rate decreases as observable in S7.

The presence of these pharmaceutical compounds may be a result of sewage contamination in the river from leaking sewage pipes and wastewater treatment plants. Sampling point S5 that was introduced to determine the pollutants from the Rietvlei Dam coming into the Hennops River had the compounds carbamazepine, nevirapine and venlafaxine detected at concentrations 0,04 ng mL^{-1}, 0,10 ng mL^{-1} and 0,04 ng mL^{-1}, respectively, through SPE.

Table 4.4: Analytical Method performance of SPE and POCIS

Compound	Calibration range (ng mL^{-1})	Correlation Coefficient (r^2)	SPE Limits (ng mL^{-1})			POCIS Limits (ng mL^{-1})		
			MDL	MQL	Recovery %	MDL	MQL	Recovery %
Methocarbamol	1 – 100	0.999	0.16	0.37	73 ± 1	0.08	0.10	92 ± 6
Carbamazepine	1 - 100	0.999	0.19	0.41	84 ± 3	0.17	0.28	87 ± 4
Etilefrine	5 – 100	0.999	1.14	2.12	68 ± 4	1.64	1.82	76 ± 8
Nevirapine	1 – 100	0.994	0.74	0.97	89 ± 3	0.12	0.47	86 ± 5
Venlafaxine	1 - 100	0.989	0.27	0.91	75 ± 2	0.19	0.11	85 ± 8

Table 4.5: Average concentrations of pharmaceuticals at different sampling points along the Hennops river

Sampling Site	Carbamazepine (ng mL^{-1})		Methocarbamol (ng mL^{-1})		Nevirapine (ng mL^{-1})		Venlafaxine (ng mL^{-1})	
	POCIS	SPE	POCIS	SPE	POCIS	SPE	POCIS	SPE
S1	-	< LOQ (0.06)	-	ND	-	ND	-	ND
S2	-	< LOQ (0.02)	-	ND	-	ND	-	ND
S3	-	< LOQ (0.17)	-	0.18 ± .0.02	-	< LOQ (0.01)	-	0.16 ± 0.07
S4	0.60 ± 0.12	< LOQ (0.04)	< LOQ (0.05)	0.10 ± 0.07	N.D	N.D	0.50 ± 0.02	< LOQ (0.04)

Sampling Site	Carbamazepine (ng mL^{-1})		Methocarbamol (ng mL^{-1})		Nevirapine (ng mL^{-1})		Venlafaxine (ng mL^{-1})	
S5	0.51 ± 0.13	0.19 ± 0.04	0.11 ± 0.09	0.10 ± 0.09	N.D	N.D	1.19 ± 0.83	< LOQ (0.08)
S6	0.40 ± 0.06	0.12 ± 0.07	0.18 ± 0.10	0.10 ± 0.03	N.D	0.10 ± 0.03	0.20 ± 0.04	< LOQ (0.03)
S7	0.32 ± 0.04	0.13 ± 0.03	0.14 ± 0.03	0.14 ± 0.09	N.D	< LOQ (0.01)	0.44 ± 0.07	< LOQ (0.07)

(–) No data collected

N.D denotes non-detected in the sample (below LOD)

4.3 Microplastics analysis

4.3.1 Abundance and distribution of Microplastics in surface water and sediments

The was no data obtained for S7 – S9 due to limited accessibility for sampling at those points. A total of 352 and 409 particles were detected in water and sediment samples collected at all sampled points as shown in figure 4.2 and figure 4.3. their abundances ranged from 2 - 11 particles/m^3 and 5 – 30 particles/g in sediments and water. The most recorded number of microplastic particles was in the upstream area from S1 to S2 where there is extreme occurrence of solid waste dumping and sewage leakages. The establishment of litter traps in water has also allowed for accumulation of plastics which forms microplastics by physical or chemical degradation. Sampling point S6 also showed a spike in the number of particles which may be contributed by the Sunderland ridge wastewater treatment plant. Tables 4.6 and 4.7 show examples of microplastics collected at different sampling points in water and sediment samples.

Table 4.6. Types of Microplastics found along the Hennops river water.

Sampling Point	Microscope results	Physical Characteristics
S1		Shape: Fragment Colour: Black Size: 3210 µm
S2		Shape: Fragment Colour: Red Size: 1815 µm

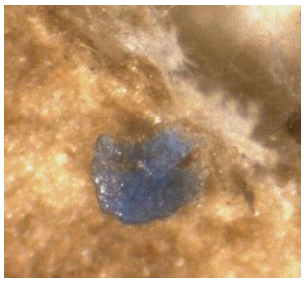
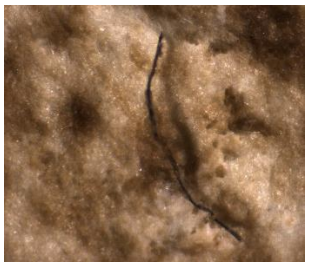
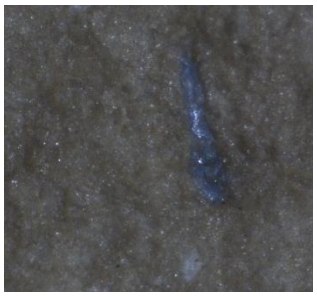
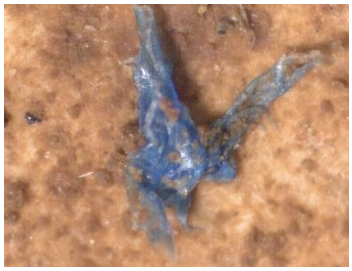
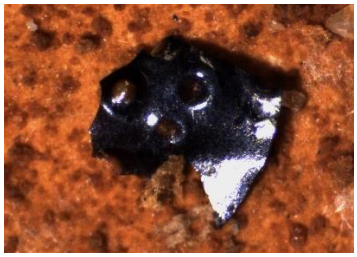
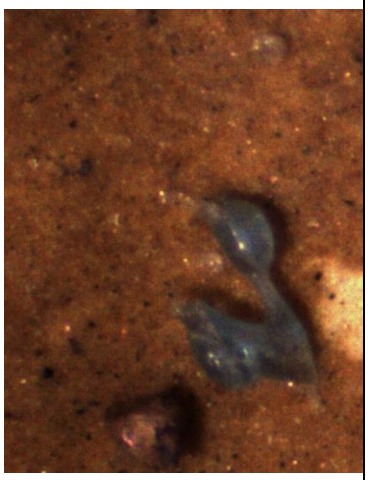
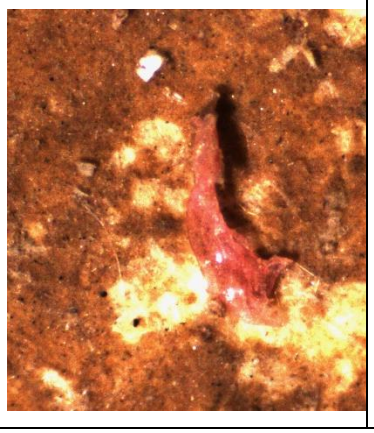
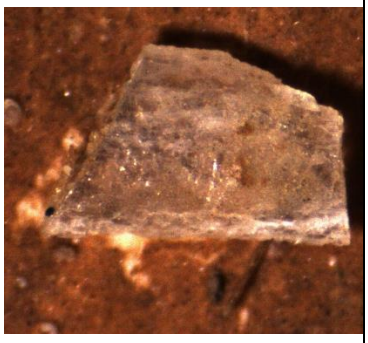
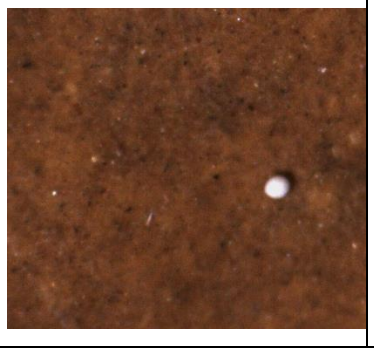
Sampling Point	Microscope results	Physical Characteristics
S3		Shape: fibre Colour: Black Size: 1850 µm
S4		Shape: Fragment Colour: Blue Size: 870 µm
S5		Shape: Fibre Colour: Black Size: 1916 µm
S6		Shape: Fragment Colour: Blue Size: 540 µm

Table 4.7. Types of Microplastics found along the Hennops sediments

Sampling Point	Microscope results	Physical Characteristics
S1		Shape: Film Colour: Blue Size: 4028µm
S2		Shape: Fragment Colour: Black Size: 1827 µm
S3		Shape: Fragment Colour: Blue Size: 1658 µm
S4		Shape: Fragment Colour: Red Size: 3210 µm

Sampling Point	Microscope results	Physical Characteristics
S5		Shape: Fragment Colour: Transparent Size: 2510 µm
S6		Shape: Pellet Colour: White Size: 720 µm

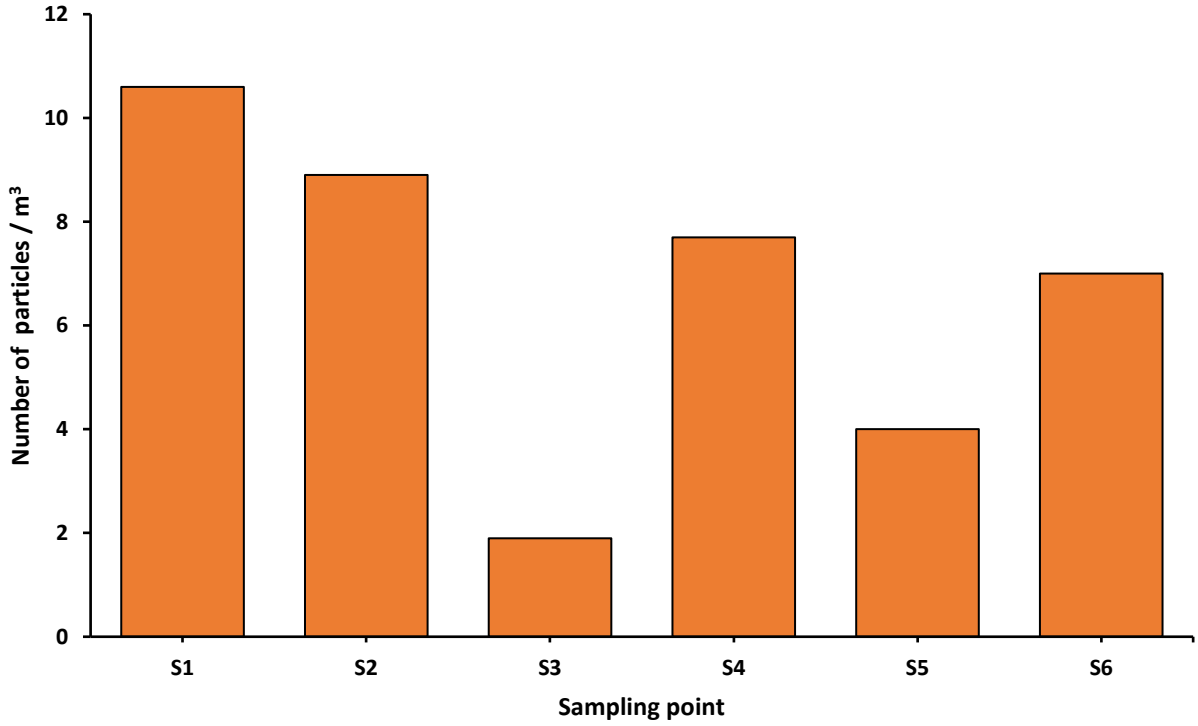


Figure 4.2. Number of microplastics in Water samples collected at different sampling points.

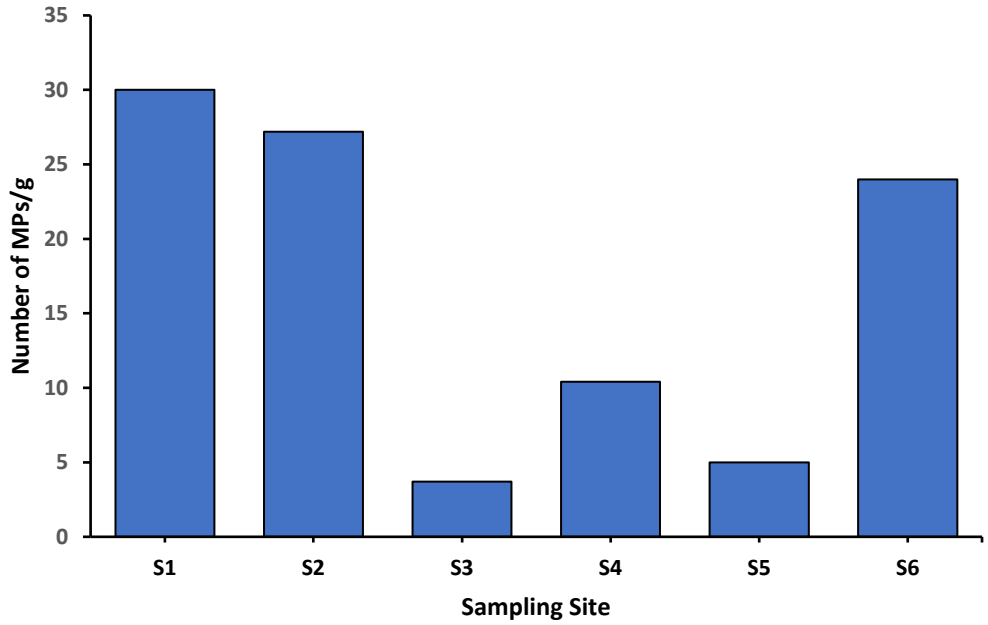


Figure 4.3. Number of microplastics in Sediment samples collected at different sampling points.

4.3.2 Shapes of Microplastics

Identified particles were classified based to their shapes: fragments, pellets, fibers, films, and foams their proportions are shown in figure 4.4. The predominantly detected shape of microplastics in water and sediment samples were fibres accounting for 80 and 79 % respectively. The was a seldom occurrence of fragments (7 and 10 %) and films (3 and 8 %) with pellets being the least detected (4 and 6 %) in water and sediments respectively. Microplastic fibres in the environment are introduced by textile industries and domestic sewage, of which textiles and domestic emissions should be the main source of MPs in surface water (Almroth et al., 2018). Film and fragment particles may be formed from the physical and thermal degradation of larger plastics that are improperly disposed on the riverbanks.

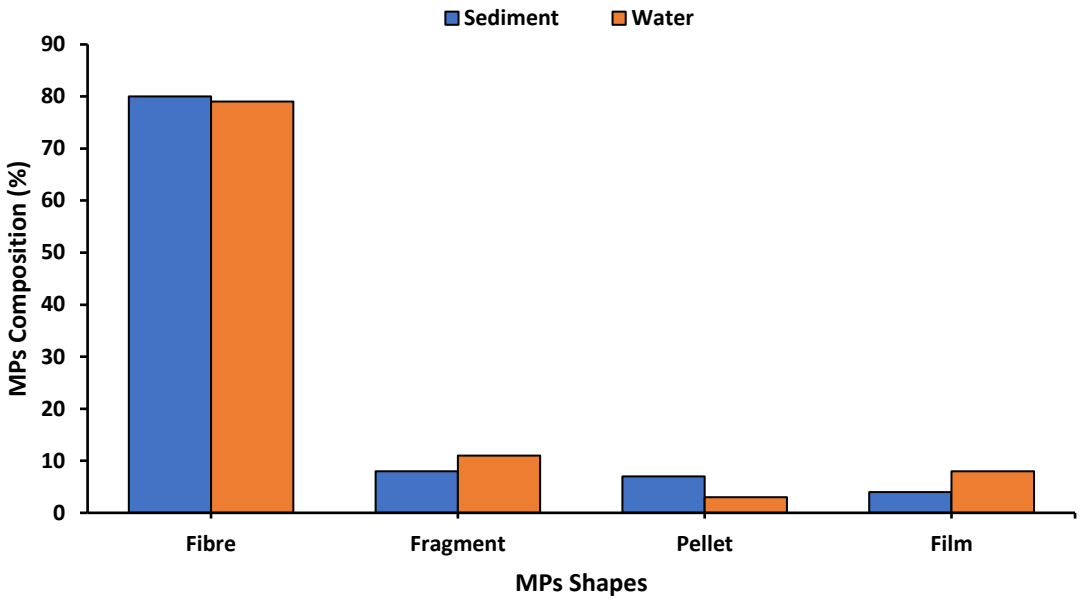


Figure 4.4. shapes of microplastics in water and sediment samples.

4.3.3 Size of Microplastics

Microplastics were also categorized into six different groupings based on their sizes: less than 0.5 mm, 0.5 –1 mm, 1 – 2 mm, 2 – 3 mm, 3 – 4 mm, and 4–5 mm as shown in figure 4.5. Microplastic particles abundance in water samples based on the size distribution was in the order: 0.5 –1 mm > less than 0.5 mm > 1 – 2 mm > 2 – 3 mm > 3 – 4 mm > 4–5 mm and the abundance order of microplastic particles in sediments was 1 – 2 mm > 0.5 –1 mm > less than 0.5 mm > 2 – 3 mm > 3 – 4 mm > 4–5 mm.

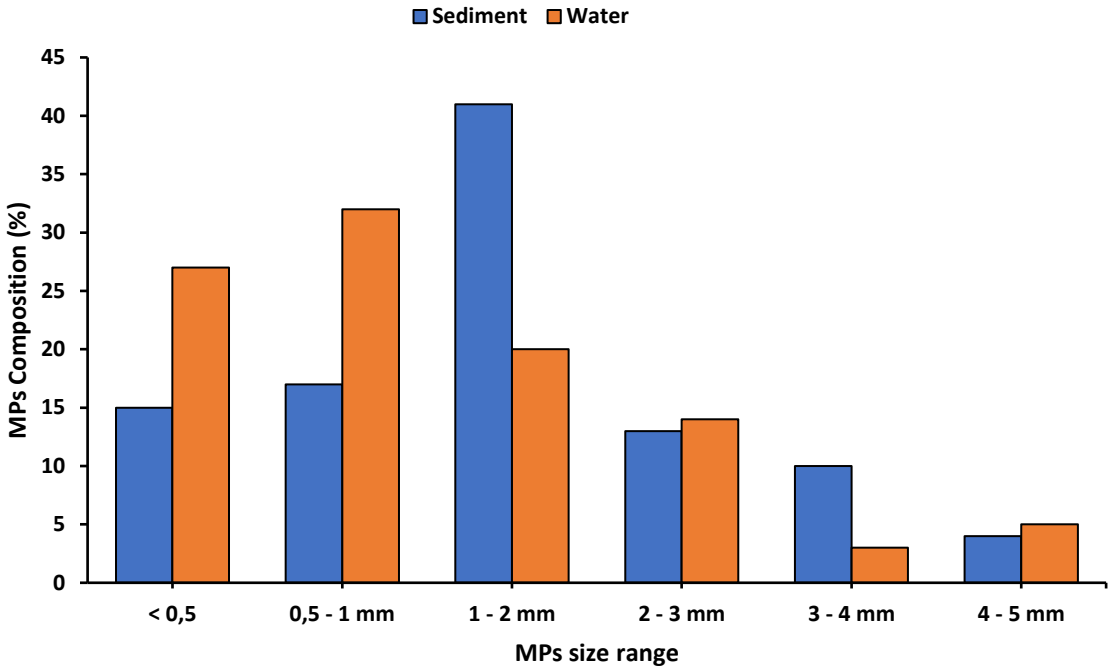


Figure 4.5. Size distribution of microplastics in water and sediment samples.

4.3.4 Colour of Microplastics

MPs were categorized into color groupings: blue, black, red, green, white, and transparent, as those were the dominating colors; other colors (brown, purple and pink) that were present in low amounts were assigned as “others” and are represented in figure 4.6. Blue coloured microplastics particles took dominance in water 40 % and 35 % in the sediments. Black coloured microplastic particles were also frequently present accounting for 25 % and 30 % in water and sediment samples respectively. MPs in colours white, red, green and transparent were seen less frequently, which made up almost 35 % of MPs in water and sediments.

Research has shown that the surface of dyed plastic particles contains toxic trace metals, persistent organic pollutants and pathogens (Duis and Coors, 2016b), and that have a potential to be released in water. Furthermore, aquatic organisms have been found to be more likely to ingest bright coloured plastic particles that had similar colour characteristics to their natural foods (Hidalgo-Ruz et al., 2012).

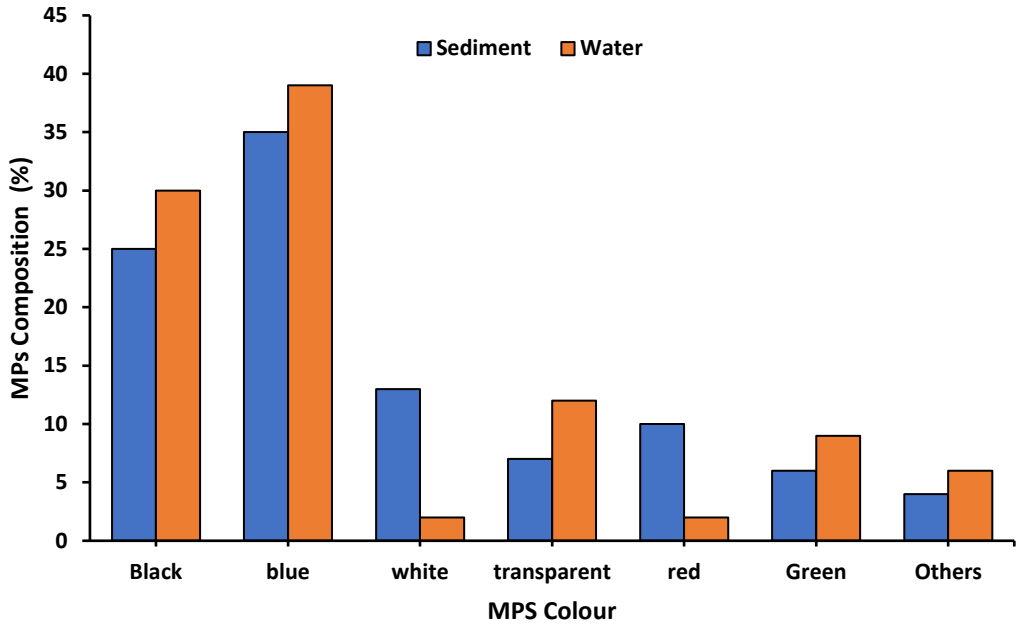


Figure 4.6. Colour of microplastics in water and sediment samples.

4.3.5 Surface morphology

The surface morphology of suspect particles of various shapes was explored trough SEM analysis as shown in (figure 4.7 – figure 4.9). Figure 4.7 shows fractures on a filament particle, that may have been formed by corrosion cracking. As the river flows plastic particles can be dragged on the riverbed which scratches them resulting in formation of grooves in figure 4.8 and a fragment in figure 4.9.

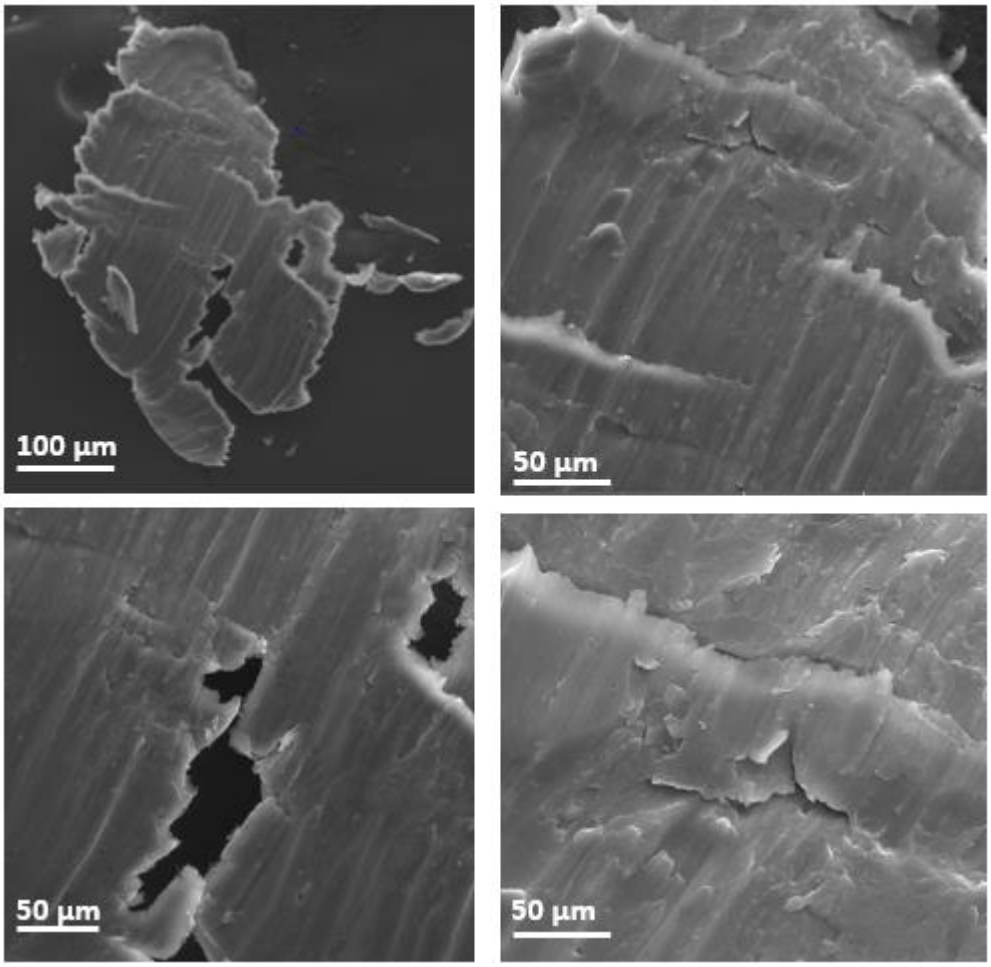


Figure 4.7. SEM images of a microplastic film

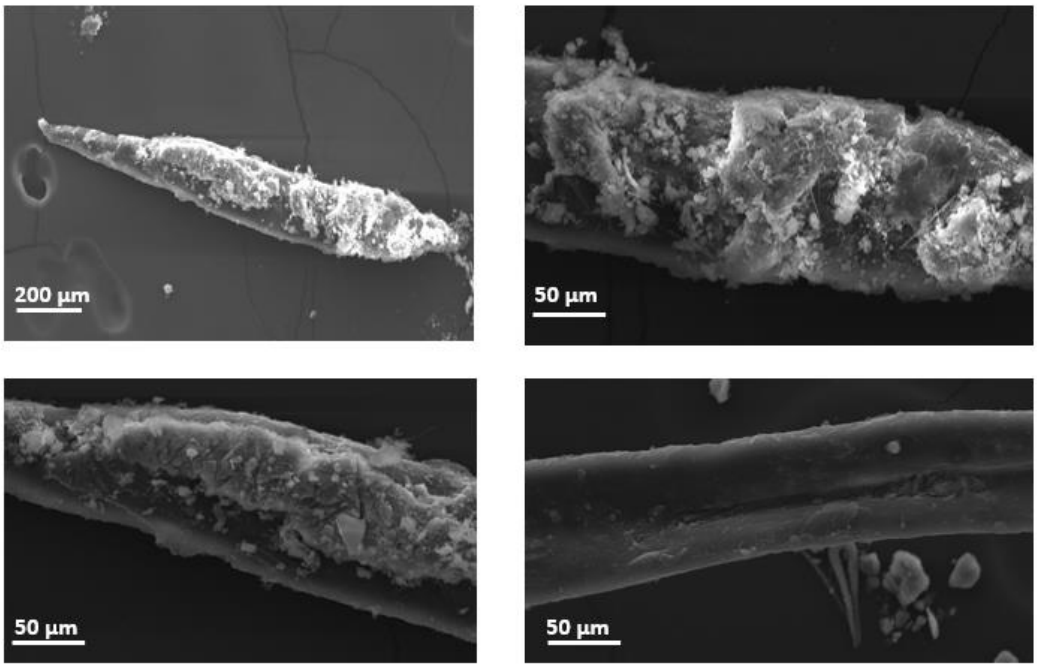


Figure 4.8. SEM images of a microplastic fibre

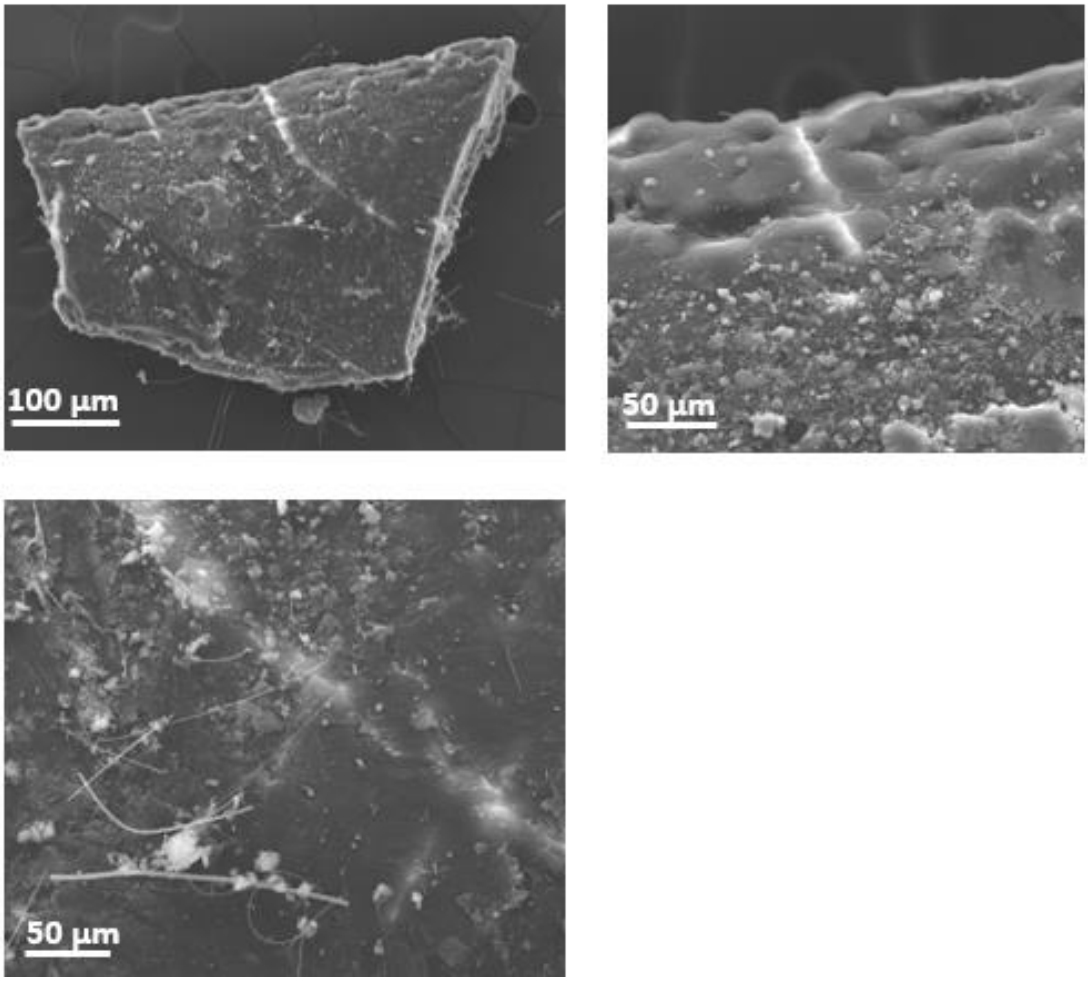


Figure 4.9. SEM images of a microplastic fragment

4.3.6 Chemical composition of Microplastics

Selected microplastic particles were analysed by Raman Spectroscopy and Figures 4.10 – 4.12 shows Raman spectra of common polymers analysed in microplastics, the sample spectra are plotted in blue while the corresponding reference spectra are shown in orange. Five polymer types were identified polyethylene (PE), polypropylene (PP), high density polyethylene, (HDPE), polyester and polystyrene. The major polymer type water samples was polyethylene that accounted for (48.6 %), and that in sediment was polypropylene (52.7 %).

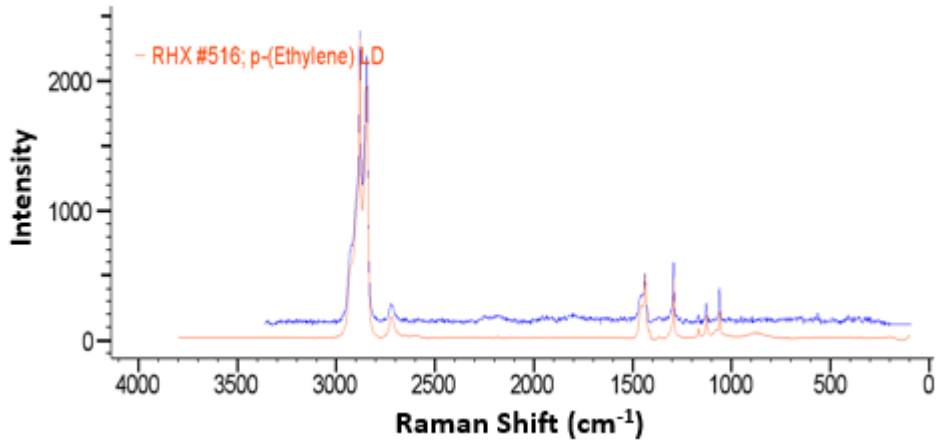


Figure 4.10. Raman spectra of low-density polyethylene (LDPE)

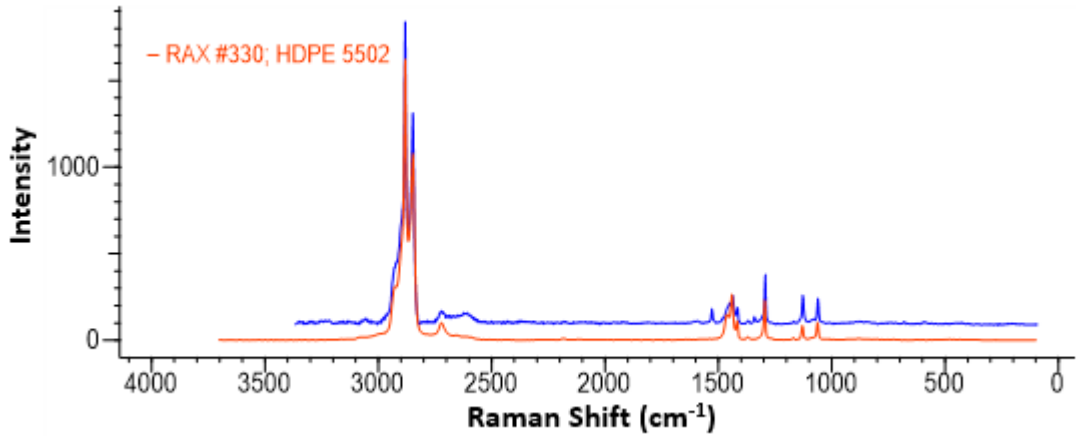


Figure 4.11. Raman spectra of High-density polyethylene (HDPE)

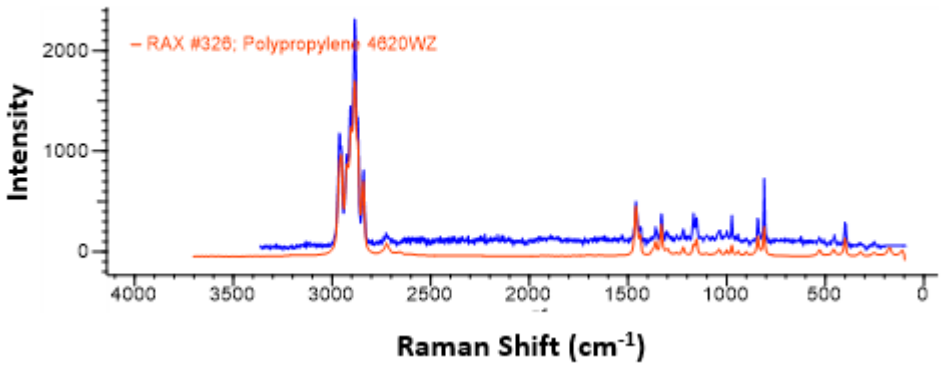


Figure 4.12. Raman spectra of Polypropylene

4.3.7 Microplastics discussions

The research has confirmed the existence of microplastics in the Hennops River, a water body surrounded by residential areas and urban zones within a significant South African city. These microplastics have been identified in the water and sediment of the river. However, similar to other microplastics research, this study can only provide cautious estimates due to the diverse methodologies used to determine microplastics in water and sediments. Throughout the study, the primary form of microplastics discovered consisted of coloured fibres. These fibres, which are considered secondary microplastics, mostly originate from synthetic textile materials like polyester, nylon, and acrylic. They also result from the degradation of larger plastic particles, as mentioned by (Almroth et al., 2018). A study conducted by (Napper and Thompson, 2016) revealed that a single clothing item being washed could release a substantial number of microplastic fibres into wastewater, potentially accounting for the high presence of fibres in the samples.

The Hennops River faces significant contamination from sewage in its upstream where it is still in the form of the Kaalspruit from surrounding neighbourhoods of Ivory Park and Thembisa. Domestic wastewater introduces microplastics from laundry activities, with small fibres shedding from synthetic fabrics and subsequently entering the municipal sewage system. Moreover, improper solid waste disposal exacerbates the issue. Clothing items, mats, and rugs are discarded along the riverbanks and deteriorate over time due to natural weathering processes, including exposure to sunlight, wind, and water, as described by (Zbyszewski et al., 2014). The SEM images in figure 4.7 indicate that microplastic fragmentation through mechanical weathering is one of the possible pathways of microplastic formation.

Microplastic particles found upstream at sampling site S1 along the Olifantspruit stream exhibited the highest quantities in both sediment and water, with a total of 11 particles per cubic meter and 30 particles per gram collected, respectively. These elevated figures were a consequence of contamination from solid waste and sewage in the vicinity. A similar situation occurred at sampling point S2, situated along the Kaalspruit, which traverses through informal settlements in Ivory Park characterized by inadequate sanitation. Likewise, at sampling site S4 along the Sesmylspruit originating from the Rietvlei dam—a tributary of the Hennops river—there was a notable uptick in the number of gathered particles within both sediment and water

samples. This behavior of tributaries contributing to the dispersion of microplastics into larger rivers is corroborated by a study conducted by Nel et al., (2018) on the Bloukrans River.

A reduction in the count of microplastics was observed in water samples and sediments at sampling point S5, which could be attributed to decreased river flow and the broadening of the river channel, allowing particles to scatter and settle. In contrast, sampling site S6 revealed a significant surge in microplastics within both water and sediment samples. This location lies downstream of the confluence with the Rietspruit, where the Sunderland Ridge wastewater treatment plant has been identified as a source of untreated sewage discharge into the river. Consequently, this discharge could potentially introduce microplastics into the river ecosystem as well. A total of 352 and 409 particles were detected in water and sediment samples in the Hennops river. A river that flows through urban suburbs just as the Hennops, the Braamfonteinspruit recorded higher microplastic oncentrations in water 705 particles m^{-3} in water and but lower microplastics concertation in sediment samples (Dahms et al., 2020).

Microplastics that were recovered from water and sediment samples fibrous, in small sizes (0.5 –1 mm) and coloured (dyed). These characteristic nature of microplastics are a concern as aquatic organisms are attracted and consume small, fibrous microplastics as they have a resemblance to their prey. Smaller sized microplastics have also been found to end up in aquatic organisms' muscles and liver and their fibrous nature allows them to be embedded in the tissue and digestive systems of organism.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

A comprehensive assessment of the Hennops River through use of physiochemical, chemical, microplastics analysis and ecotoxicological approaches was employed. Sampling was performed on river water and sediment samples from the upstream in Tembisa to downstream Hartbeespoort Dam. Physiochemical parameters: pH and conductivity were within the DWAF and WHO recommended limits. However, the dissolved oxygen (DO) levels in the upstream were found to be too low and are likely to cause detrimental effects on aquatic biota which justifies the absence of aquatic organisms.

Microbiological analysis revealed that all water samples were found to contain high levels of *E. coli* which decreased as one moves downstream to the Hartbeespoort Dam. The dam was found to be the major sink for pollution that occurs upstream.

Various amounts of pharmaceuticals were detected in the waters but at low levels (µg L^{-1}) and showed little increase in concentration downstream. This could suggest varied inputs along the river from various sources. In this study, both passive sampling and solid phase extraction techniques were used to extract the pharmaceuticals from water at various points along the river followed by UHPLC-MS analysis. For microplastics in the water and sediment samples, five polymer types were identified: polyethylene (PE), polypropylene (PP), high density polyethylene, (HDPE), polyester and polystyrene. The predominant polymer type in surface water was PE (48.6 %), and that in sediment was PP (52.7 %). PE and PP were the most abundant polymer types in both phases, and these also are the leading polymers in plastics production. 80% of the identified microplastics were found to be fibrous with most dominant sizes of 1-2 mm for sediments and 0.5-1 mm in water samples. Blue was the most dominant colour of the microplastics in both sediments and water samples. Results obtained at the start of the project and that at the end showed no decrease in various forms of pollution in the river, be it microplastics, *E.coli* or pharmaceuticals.

Although there is no single method for integrating the complex state of pollution in the Hennops river that arises from variable sources, an integrated scheme combining physiochemical, chemical microbiological analysis with an ecotoxicological approach can be used to assess the water quality of urban river systems. The overall results indicate that sustainable water resource management measures must be put in place to address the pollution in the river to prevent further degradation of the natural resource. There should be continuous monitoring of the pollution state in the Hennops river, and this should be linked to any Hennops river management and upstream activities aimed at reducing pollution in the river.

5.2 RECOMMENDATIONS

The Hennops river continues to be in a vulnerable state as a result of the ongoing pollution and is incapable of sustaining the lives of aquatic biota, and the consequences downstream in Hartbeespoort Dam are increasing becoming dare. Current efforts are being done to take care of plastic pollution in the river is through the use of litter traps and the water hyacinth in the dam is through army of insects. But that is dealing with symptoms and thus is a misdirected effort. Most of the focus should be upstream to stop the pollution from the source. If the situation is

left unchecked, fish in the dam will start dying especially in dry months when there is little dilution of fresh water from rains. Thus, a study needs to be engaged to determine how dissolved oxygen levels fluctuates in the downs stream parts of the river close to the dam and inside the dam itself. Other physical chemical parameters that could impact oxygen availability should be determined as oxygen availability in the dam is now a ticking time bomb because of continued input of raw sewage from upstream.

APPENDIX

Table A1: UPLC – qTOF MS Conditions

Parameter	Details	
LC flow gradient	**Retention /min**	**% Composition B**
	0	5.0
	1.0	5.0
	6.0	55.0
	8.0	55.0
	10.0	95.0
	12.0	95.0
	13.0	5.0
	14.0	5.0
Flow rate	0.3 $ml.min^{-1}$	
Column temperature	25 °C	
Capillary Voltage	2500 V	
End plate offset	500 V	
Nebulizer pressure (N_2)	2 Bar	
Drying gas (N_2)	8 $L.min^{-1}$	
Drying Temperature	300 °C	
Mass range	30 – 1000 Da	

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