# A MECHANISTIC AND TRAIT-BASED APPROACH TO INVESTIGATING MACROINVERTEBRATES DISTRIBUTION AND EXPOSURE TO MICROPLASTICS IN RIVERINE SYSTEMS

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

OF

RHODES UNIVERSITY


BY

ENAHORO KENNEDY OWOWENU

https://orcid.org/0000-0003-3003-030X


FEBRUARY 2024

Supervisors: Prof ON Odume, Dr CF Nnadozie and Dr X Siwe Noundou

**ABSTRACT**

Microplastics in rivers pose an ecological risk. Hydraulic biotopes form distinct flow patches that vary longitudinally along the river, potentially influencing the transport dynamics of microplastics. Macroinvertebrates exhibit adaptations to different hydraulic biotopes through their unique traits. These traits can mediate their exposure to microplastics, thereby imposing selective pressures on these organisms. Different taxa often demonstrate preferences for specific hydraulic biotopes characterized by distinct flow regimes. Understanding the transport dynamics of microplastics mediated by hydraulic biotopes and the potential exposure of macroinvertebrates at the hydraulic biotope scale is important for determining the fate of riverine microplastics and detecting species at risk. Both empirical and theoretical studies have highlighted the interconnectedness of hydrology, geomorphology, and microplastic transport in rivers, yet, there remains a gap in understanding how a hydro-geomorphological approach could enhance the understanding of the microplastic transport process. Little is known about the role of traits in driving macroinvertebrate exposure to microplastics at a scale relevant to ecological dynamics. This study addressed these gaps by applying a hydro-geomorphological approach to investigate the distribution of microplastics at the hydraulic biotope scale and assessed the potential exposure of macroinvertebrates using a trait-based approach. This study also explored the relationship between microplastic abundance and selected water physicochemical properties, as well as the influence of adjacent land use types. By integrating these aspects the research provided a comprehensive understanding of microplastics dynamics in river systems, shedding light on both environmental factors shaping their distribution and the potential impacts on aquatic organisms.

The study was conducted over the wet and dry seasons (October 2021 – July 2022) at 10 sites located in the upper, middle, and lower reaches of the Swartkops and Buffalo River systems in the Eastern Cape Province of South Africa. The hydraulic biotopes (i.e., pools, runs, riffles) were grouped into two conceptualised forms, namely, sink and flush hydraulic zones and were characterized by hydraulic indices such as the Froude number and the Reynolds number. The flush hydraulic zone represents hydraulic biotopes where microplastics can potentially be remobilized quickly into suspension, and the sink represents biotopes where microplastics can potentially accumulate and remobilisation is far slower. Fast-to-moderate flowing hydraulic biotopes were conceptualised as microplastics flush zones while slow-flowing to still biotopes as microplastic sink zones. Samples were collected at different depths in each hydraulic zone to quantify suspended and settled forms of microplastics. Microplastics targeted in this study

ranged in size from 0.063 mm to less than 5 mm. Classification was achieved through microscopic observation, and confirmation via Fourier Transform Infrared Spectroscopy (FTIR-ATR) was conducted for samples ranging from 0.5 mm to less than 5 mm.

At the site level, settled microplastics showed statistically significant spatial and temporal variations between the sites, and between the seasons ($P < 0.05$). The suspended microplastic varied only spatially. Fibres and fragments were the dominant microplastic shape, while polyethylene and polypropylene were the dominant microplastic polymers. Suspended microplastics showed statistically significant variation between urban land cover and other land cover categories (industrial, agricultural, rural, and natural land cover). Microplastics abundance was associated with high levels of turbidity, total suspended solids, total inorganic nitrogen, higher temperatures and increasing electrical conductivity.

At the hydraulic biotope scale, the mean occurrence of suspended microplastics (1.76 ± 1.44 items/L; mean + SD) in the flush hydraulic zone was higher than that in the sink zone (1.54 ± 1.46 items/L), while settled microplastics were more abundant in the sink hydraulic zone (1.82 ± 1.98 items/L) than the flush hydraulic zone (1.32 ± 1.49 items/L). This observation was in line with the prediction in this study. The mean suspended and settled microplastics concentrations were higher during the wet season across the flush and sink hydraulic zones than in the dry season. Global multivariate analysis of variance (MANOVA) and two-way analysis of variance (ANOVA) revealed significant spatial and temporal variations in settled microplastics abundances between the flush and sink hydraulic zones. The results indicated that geomorphologically defined units such as riffles and moderate to fast runs (flush) generally contained lower amounts of settled microplastics compared to pools and backwaters (sink). However, this distinction between the flush and sink microplastic zones was observed only for settled microplastics and not for suspended microplastics. Suspended and settled microplastics showed a statistically significant relationship with the Froude number index. The generalised additive model indicated that settled microplastics abundance distribution decreased significantly with increasing Froude number value in the flush zone. Suspended microplastics decreased at low Froude number values and showed an increasing trend at higher Froude number values of about 0.75. The results indicate the usefulness of the hydraulic biotope scale microplastic monitoring approach in detecting microplastic hotspots and explaining variations in microplastics abundances driven by instream hydraulics.

Four traits and ecological preferences of macroinvertebrates including body size, gill type, feeding habit, and velocity preferences were selected and resolved into 17 trait attributes. The sink hydraulic zones such as pools were indicated to favour exposure to and ingestion of microplastics compared to the flush zones such as riffles and fast runs. Large body size macroinvertebrates were associated with the sink zone. Taxa with a very small body size had a higher likelihood for microplastics ingestion than taxa with other body sizes. Collector-gathering macroinvertebrates taxa that have operculate gills with small body sizes were more prone to exposure to microplastics in hydraulic biotopes with slow to very slow velocities. Fibres were the most abundant plastic ingested by macroinvertebrates preferring the flush zone while fibres and fragments were mostly ingested by those preferring the sink zones. The binomial logistic model revealed a highly significant result for the likelihood of operculate gill shape to clog in the sink hydraulic zone. The result of the binomial logistic regression indicates the usefulness of the trait-based approach for predicting exposure to microplastics.

Overall, the study reveals the influences of hydro-geomorphological features on the transport dynamics of microplastics and the usefulness of the trait-based approach in the ecological study of microplastics in riverine systems.

## TABLE OF CONTENTS

## LIST OF TABLES (SHORT TITLES)

## LIST OF FIGURES (SHORT TITLES)

## LIST OF APPENDICES

## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ANOVA | Analysis of variance |
| APHA | American Public Health Association |
| BOD | Biochemical Oxygen Demand |
| CMA | Catchment Management Agency |
| CPOM | Coarse particulate organic matter |
| DCA | Detrended correspondence analysis |
| DEA | Department of Environmental Affairs |
| DEA | Department of Environmental Affairs |
| DO | Dissolved oxygen |
| DWAF | Department of Water Affairs |
| DWAF | Department of Water Affairs and Forestry |
| DWS | Department of Water and Sanitation |
| FDR | False discovery rate |
| FPOM | Fine particulate organic matter |
| FTIR | Fourier Transform Infrared Spectroscopy |
| HFC | Habitat filtering concept |
| HSD | Honesty significance difference |
| HTC | Habitat template concept |
| MANOVA | Multivariate analysis of variance |
| MC | Management class |
| MD | Mean depth |
| MP | Microplastics |
| MV | Mean velocity |
| NOAA | National Oceanic and Atmospheric Administration |
| NWA | National Water Act |
| NWRS | National Water Resource Strategy |
| PA | Polyamide |
| PC | Polycarbonate |

| | |
|---|---|
| PE | Polyethylene |
| PERMANOVA | Permutational multivariate analysis of variance |
| PET | Polyethylene terephthalate |
| PEVA | Polyethylene vinyl acetate |
| PP | Polypropylene |
| PU | Polyurethane |
| PVC | Polyvinyl chloride |
| RDA | Redundancy analysis |
| RDM | Resource directed control |
| RHMC | River Hierarchical Morphological Classification |
| RHP | River Heath Programme |
| RQOs | Resource quality objectives |
| RSA | Republic of South Africa |
| SASS5 | South African Scoring System version 5 |
| SDC | Source directed control |
| TBA | Trait-based approach |
| TIN | Total inorganic nitrogen |
| TSS | Total suspended solids |
| USEPA | United States Environmental Protection Agency |
| VDR | Velocity depth ratio |
| WRCS | Water Resources Classification System |
| WSA | Water Service Act |
| WWTW | Wastewater treatment works |

## ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my lead supervisor Professor O.N. Odume whose guidance, mentorship, inputs, and encouragements served as a pillar upon which this entire project rested from start to completion.

I sincerely appreciate every member of my supervisory team, Dr C.F. Nnadozie, Dr X.S. Noundou and Dr F.C. Akamagwuna for their guidance, corrections, assistance, suggestions, and encouragement.

To all staff and students at the Institute for Water Research, especially students and staff of the Centre for Environmental Water Quality, for all your support in different capacities, thank you. I appreciate the support of the interns, Siyabonga Mazibuko and Aphiwe Magwala for their support both in the field and in the laboratory. Thanks to the administrative staff led by Ms Juanita McLean for all their wonderful assistance.

Special thanks to the technical staff at the IWR, Khaya Mgaba for her very supportive roles in the field and in the laboratory.

I am sincerely grateful to the African Water Resources Mobility Network (AWaRMN) for providing the platform, opportunity and funds for this entire project and my stay at the Institute for Water Research. Special recognition and thanks to the Intra-African Academic Mobility Scheme of the European Union for providing the AWaRMN network with all required funds.

To my dear friend Clinton Egbe for his firm support and encouragement and for always looking out for me, thank you.

To my siblings and parent, thank you for all your support.

To my lovely wife Dorcas, for all her support during this arduous venture. She has had to wear many hats during this period including wife, mother, therapist, teacher, housemistress, instructor, and motivator. She has been very supportive, understanding and patient. To my lovely children, Nihra and Noohra for always being obedient and understanding.

Finally, to God Almighty for divine providence, be all the glory.

## DEDICATION

This project is dedicated to everyone everywhere around the world who is giving their best in every capacity to make the world a better place for all and sundry.

## CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

### 1.1 Introduction

*The following paper has been published based on this chapter.*

Owowenu, E. K., Nnadozie, C. F., Akamagwuna, F., Siwe, X. N., Uku, J. E. & Odume, O.N. (2023). A critical review of environmental factors influencing the transport dynamics of microplastics in riverine systems: implications for ecological studies. *Aquatic Ecology*, *57*, *557–57*(0123456789). https://doi.org/10.1007/s10452-023-10029-7

The occurrence of microplastics (MP), usually defined as particle sizes less than 5 mm in length (Frias & Nash, 2019), in riverine ecosystems represents an ecological risk of concern. Laboratory and field-based studies have shown effects of microplastics on different riverine organisms, including macroinvertebrates (Welden & Cowie, 2016; Scherer *et al.*, 2017; Akindele *et al.*, 2019), fish (McNeish *et al.*, 2018; Kühn *et al.*, 2020; Sequeira *et al.*, 2020) and birds (Lavers *et al.*, 2019). Commonly reported effects of microplastics include gut filling, abrasion, entanglement, and release of potentially toxic chemicals into the environment (Kwon *et al.*, 2017; Ma *et al.*, 2019; López-Rojo *et al.*, 2020; Issac & Kandasubramanian, 2021). Adverse effects on the reproduction, behaviour, survival, growth, and metabolism of aquatic organisms after microplastic ingestion, have also been documented (Blarer & Burkhardt-Holm, 2016; López-Rojo *et al.*, 2020; Tongo & Erhunmwunse, 2022).

Monitoring and evaluation studies have demonstrated that freshwater systems (e.g., rivers) are essential sinks and regulators of microplastic transport to marine systems (Klein *et al.*, 2015; Mani *et al.*, 2015; Hoellein *et al.*, 2019). Although, lentic and marine habitats have been described as the most probable ultimate sinks of microplastics (Berlino et al., 2021; Y. Tang et al., 2021), rivers act as conduits and microplastics accumulators (Hoellein *et al.*, 2019). Most plastics that leak into the environment never make it to the ocean; only a fraction of plastics that are found in the terrestrial and aquatic compartments of river systems finally reach the marine environment (Newbould *et al.*, 2021; van Emmerik *et al.*, 2022). Accordingly, monitoring and evaluation studies have now started considering the terrestrial environments and freshwater systems. Monitoring studies are particularly on the rise for freshwater systems, especially rivers, as rivers are highly susceptible to inputs from primary sources as a result of closer proximity to point sources of microplastics pollution (McCormick *et al*., 2016).

In river systems, the transport dynamics of these microplastic particles may be regulated by riverine hydro-geomorphology (Kiss *et al*., 2021). However, despite the rise in freshwater microplastic research, the role of hydro-geomorphology has been largely overlooked in studies investigating microplastic contamination in rivers. The microplastics regulatory role of riverine hydro-geomorphology may potentially create a situation of "flush" and "sink" zones for microplastics (i.e., flushes – flow patches with potential for high microplastic remobilization and suspension within the water column; sinks – flow patches with potential for high particle deposition and settling). The patchy distribution of microplastics in rivers would imply that sampling protocols and monitoring studies be designed and calibrated to integrate hydro-geomorphological considerations for an adequate assessment of microplastic risk with more detailed field-based evidence of microplastic concentrations, distribution, and hotspots within river corridors.

According to the river geomorphological hierarchical classification, spatial organisations of morphological and hydraulic units/biotopes form different types of physical habitats or flow patches which change longitudinally along the river as a function of flow, depth, nature of substratum, and channel morphology (Thomson *et al*., 2001; Lehotský, 2004; Pastuchová *et al*., 2008). A hydraulic biotope is a spatially distinct instream flow environment in which species assemblages or communities live (Wadeson & Rowntree, 1998). Riffles, runs, glides, pools, and backwaters are examples of spatial organisations at the morphological and hydraulic biotope scale which are characterised by distinct hydraulic patterns. Under normal conditions, hydrological and hydraulic biotope features such as flow velocity, flow depth, river discharge, and channel morphology interact with microplastics' physical properties (e.g., size, shape, density) to influence the mobilisation, transport and deposition of microplastics within riverine systems (de Carvalho *et al*., 2021; Kumar *et al*., 2021; Yan *et al*., 2021a; van Emmerik *et al*., 2022). For instance, hydraulic biotopes, such as pools characterised by greater depth and subcritical-tranquil flow, have been documented as influencing microplastic settling, resulting in higher concentrations of settled microplastics (Mani *et al*., 2015; Tibbetts *et al*., 2018; Dahms *et al*., 2020a). Conversely, hydraulic biotopes with critical to supercritical turbulent flows such as rapids, riffles and fast-runs have been demonstrated to have higher concentrations of suspended microplastics (Kumar et al., 2021; X. Lu et al., 2023). The hydraulic biotope represents an ecologically relevant spatial scale for classifying and understanding river hydrodynamics and the interaction of aquatic biota with the physical environment (Wadeson & Rowntree, 1998; Thomson *et al.*, 2001) and thus, an adequate understanding of the influences of hydraulic biotopes on microplastic transport dynamics is critical for uncovering hotspots, detecting ecosystems at risk and for predicting the exposure of biota to microplastics.

Through their traits, macroinvertebrates are adapted to different hydraulic biotopes (Schmera *et al.*, 2022), which may impose on them different levels of exposure to microplastics in the sink and flush zones. Studies have shown that aquatic macroinvertebrates are associated with a gradient of flow velocity, depth and substrate elements (Jowett & Richardson, 1990; Jowett *et al.*, 1991; Karaouzas *et al.*, 2019; Wegscheider *et al.*, 2023). The association of macroinvertebrates with different hydraulic biotopes implies that the degree and mode of exposure to microplastics would be dependent upon the hydraulic biotopes for which they show preference. Thus, examining the effect of the underlying relational mechanism between microplastics (MP) and mechanistically linked traits that adapt macroinvertebrates to their preferred hydraulic biotope can provide a means for diagnosing exposure to microplastics.

A trait is any measurable characteristic (morphological, physiological, behavioural or phenological) of an organism measurable at the individual level or other relevant level of organisation (Violle *et al.*, 2007; Dawson *et al.*, 2021). The trait-based approach is theoretically embedded in the habitat template concept (HTC) of Southwood (1977, 1988) and the habitat filtering concept (HFC) of Poff (1997). The HTC and HFC emphasise that species distribution and abundances and local community composition are constrained by the prevailing environmental characteristics so that only species with the right combination of traits are adapted to a particular environment or habitat. In this regard, hydraulic biotope features may act as filters which constrain macroinvertebrates such that only macroinvertebrate species with the right combination of traits are adapted to the various hydraulic biotopes. The differential concentrations of microplastics in different hydraulic biotopes may further constrain macroinvertebrate species such that in hydraulic biotopes with high concentrations of microplastics, species traits sensitive to microplastics pollution will be excluded, while tolerant traits will dominate. The trait-based approach thus enhances causal diagnosis and prediction by providing mechanistic linkages of biotic responses to environmental conditions (Culp *et al.*, 2011; Pilière *et al.*, 2016).

***This study then draws on hydraulic and geomorphological concepts to determine the distribution of microplastics at the hydraulic biotope scale and uses the trait-based approach***

***to determine the potential exposure of macroinvertebrate.*** This is the first time a hydraulic, geomorphological, and trait-based approach is being explicitly applied concurrently to study the distribution of microplastics and macroinvertebrate exposure at an appropriately relevant ecological scale within riverine systems. This study thus makes an original contribution to the field in this regard.

The rest of this chapter one provides a critical literature review of water resources management in South Africa, the National Water Act, and the National Water Resource Strategy. It also presents the notion of rivers as physical habitats and gives a background to the instream behaviour of microplastics by exploring the river geomorphological concept. A detailed review of microplastics transport in rivers is provided, starting with river-related factors, followed by microplastic characteristics, and allochthonous factors. A further review of the influences of trait and hydraulic biotopes on macroinvertebrate distribution and potential exposure to microplastics is also provided. The chapter concludes with the rationale and significance of the study, aim, objectives, guiding research questions and thesis structure.

## 1.2 Water Resources Management in South Africa

The Department of Water and Sanitation (DWS), acting through the Minister, is the custodian of water resource management and policy implementation in South Africa. The DWS and the entire water sector draw their primary mandate from three pieces of legislation, that is, the National Water Act, 1998 (Act No 36 of 1998) as amended, the Water Services Act, 1997 (Act No 108 of 1997), and the Water Research Act, 1971 (Act No 34 of 1971). Sustainability, equity and efficiency are the central values guiding the management of water resources in South Africa (de Wet & Odume, 2019). Striving towards achieving these three central values, within the context of limited water resources exacerbated by a growing demand, has been the main driver of all water resource policies and programmes in South Africa. The National Water Act (NWA) was promulgated to provide the legislative and legal framework that guides the sustainable and equitable management of water and related resources, while the Water Services Act (WSA) provides for the rights of access to basic water supply and sanitation. The Water Research Act establishes the Water Research Commission and the Water Research Fund to promote water-related research and the use of water for agricultural, industrial, and urban purposes. However, the NWA sets out the ground rules, procedures, principles, and overreaching framework for effectively protecting, using, developing, conserving, managing, and controlling water resources in South Africa.

### 1.2.1 The National Water Act (No. 36 of 1998) as amended.

The National Water Act as amended in 1999, emphasises sustainability, efficiency, and equity as the central guiding values that govern the protection, use, development, conservation, management, and control of water resources for the benefit of all people in South Africa (Republic of South Africa [RSA], 1998). It identifies the need to establish suitable institutions to achieve its purpose. To give effect to and realise its objectives, the NWA provides for the development of a National Water Resource Strategy (NWRS), and the requirement of each Catchment Management Agency (CMA) to develop a catchment management strategy for the water resources within its jurisdiction (RSA, 1998). The National Water Resource Strategy is a document that provides the roadmap for the realisation of the intent of the NWA. The NWA also mandates the Minister of the Department of Water and Sanitation (DWS) to review and revise/update the NWRS at intervals of not more than five years (RSA, 1998).

### 1.2.2 The National Water Resource Strategy

The National Water Resource Strategy (NWRS) is a legal instrument and is recognised as the primary mechanism to manage water across all sectors of society towards achieving the National Government's development objectives in South Africa. The first and second editions of the NWRS were published in 2004 and 2013, respectively. The second edition of the NWRS (NWRS 2) builds on the first edition while the third edition of the NWRS (NWRS 3) is currently underway (DWS, 2013, 2022). The NWRS contains strategies, plans, and procedures on how water and related resources are to be protected, used, developed, conserved, managed, and controlled in accordance with the provisions of the NWA. To give effect to the interrelated objectives of sustainability, efficiency, and equity enshrined in the NWA, the NWRS adopted two complementary strategies for managing South Africa water resources. These complementary strategies are the Resource Directed Measures (RDM) and the Source Directed Controls (SDC).

Resource directed measures (RDM) are directed at the protection of water resources by setting goals and objectives for the desired condition of water resources in aquatic ecosystems while ensuring socio-economic growth and development (DWS, 2013). The RDM recognise the increasing stress and anthropogenic impacts (e.g., pollution, population growth, poor agricultural practices) on the health of aquatic ecosystems and provides tools and approaches for their management. The core of the RDM is the determination of a Management Class (MC) which prescribes what the quality and overall health of the water resource should be. The MC is defined in terms of the resource quality (i.e., water quantity and quality, character and condition of the instream and riparian habitats, and the characteristics, condition, and distribution of the aquatic biota) that must be maintained. Management classes are determined using the Water Resource Classification System (WRCS). The RDM is comprised of the following components i) the classification system ii) the classification of significant water resources iii) the Reserve determination and iv) setting of Resource Quality Objectives (RQOs).

The classification system provides a set of nationally consistent rules to guide decision-making about water resources classification. Water resources are classified into three management classes which reflect the desired levels of use and protection: Class I) water resources that are minimally used and the ecological condition minimally altered from its natural condition; Class II) water resources that are moderately used and the ecological condition is moderately altered; and Class III) water resources that are heavily used, resulting in significant deviation from its natural conditions. The third component of the RDM is the determination of the Reserve. The Reserve provides for the quantity, quality, and reliability of a supply of water required to satisfy basic human needs (human need Reserve) and for the functioning of aquatic ecosystems (ecological Reserve). The aim of the Reserve is to secure ecologically sustainable and responsible development and use of the water resources in South Africa (King & Pienaar, 2011). The basic human need Reserve provides for the requisite needs of individuals (e.g., water for drinking, preparing food and for personal hygiene) while the ecological Reserve relates to the quality and quantity of water required to protect and maintain the aquatic ecosystem (King & Pienaar, 2011). The Reserve, therefore, accords rights to both humans and the aquatic ecosystem. The last component of the RDM is the setting of the RQOs. The RQOs provide measurable quantitative and qualitative descriptors of the water resources aligned with MCs. The RQOs relate to all components (i.e., quality, quantity, and biota) of the water resources and are expressed as numeric or descriptive goals for resource quality within which a water resource must be managed. The RQOs establish comprehensive water quality management targets that relate to the relevant water quality.

The second complementary strategy in the NWRS2 is the SDC. Source Directed Controls (SDCs) specify criteria for controlling water resource use activities and their impacts on aquatic ecosystems. The SDCs, in combination with the RDM, define, impose limits, and restrict the use of water resources, with the primary purpose of ensuring the objectives that have been set for the water resource are achieved. The SDC tools include regulatory mechanisms such as water quality standards for wastewater discharges, pollution prevention measures, registrations, licenses, authorisation, and permits. These tools usually reflect best national management practices, site-specific requirements, and source-related requirements. Progressive implementations of self-regulation using both punitive and economic incentives are encouraged by the DWS. Source Directed Controls are the essential links between protecting water sources and regulating their use.

The RDM and SDC are therefore two strategies used in the management of water resources in South Africa. They provide different tools and approaches for monitoring water quality to realise the overreaching objectives of concurrent use and protection of water resources. However, approaches and tools for monitoring, mapping, and benchmarking acceptable limits for emerging pollutants, such as microplastics, are not currently enshrined in the two strategies, the RDM and SDC, mainly because microplastics are an emerging pollutant that has been poorly studied across the African continent relative to other continents of the world, such as Asia, Europe, and North America (Chen *et al*., 2020; Aragaw, 2021). Across Africa, microplastic research has only recently gained attention. Although most of the plastic research in Africa has been conducted predominantly in South Africa and Nigeria (Aragaw, 2021), local data to inform decisions, policies and management strategies are still scanty. An important step to managing microplastic pollution is to understand the instream behaviour within a river system. To do that, in the next section, I draw on the river geomorphological concept to give relevant context to microplastics' instream behaviour.

## 1.3 Rivers as physical habitats

Rivers are complex biophysical and hydrogeochemical systems (Dollar *et al*., 2007). Viewed spatially, rivers can be construed as hierarchical systems with smaller units (e.g., hydraulic biotopes) nested within larger ones (e.g., morphological units) (Frissell *et al*., 1986; Thomson *et al*., 2001; Kondolf *et al*., 2016). Drawing from the river morphology hierarchical classification by Lehotský (2004), rivers are characterised by seven spatial levels (Figure 1.1), that is, catchment-drainage network, zone, segment, channel-floodplain unit, river reach, morphological unit (i.e., geomorphic unit) and morphohydraulic unit (i.e., hydraulic biotope or hydraulic unit). The hydraulic biotope is the lowest spatial level of the hierarchical model. This level of the hierarchy provides the link between catchment geomorphology and lotic ecology. It is the key component to be considered in conservation programmes which strive to maintain the ecological integrity of fluvial environments (Wadeson, 1994, 1996).

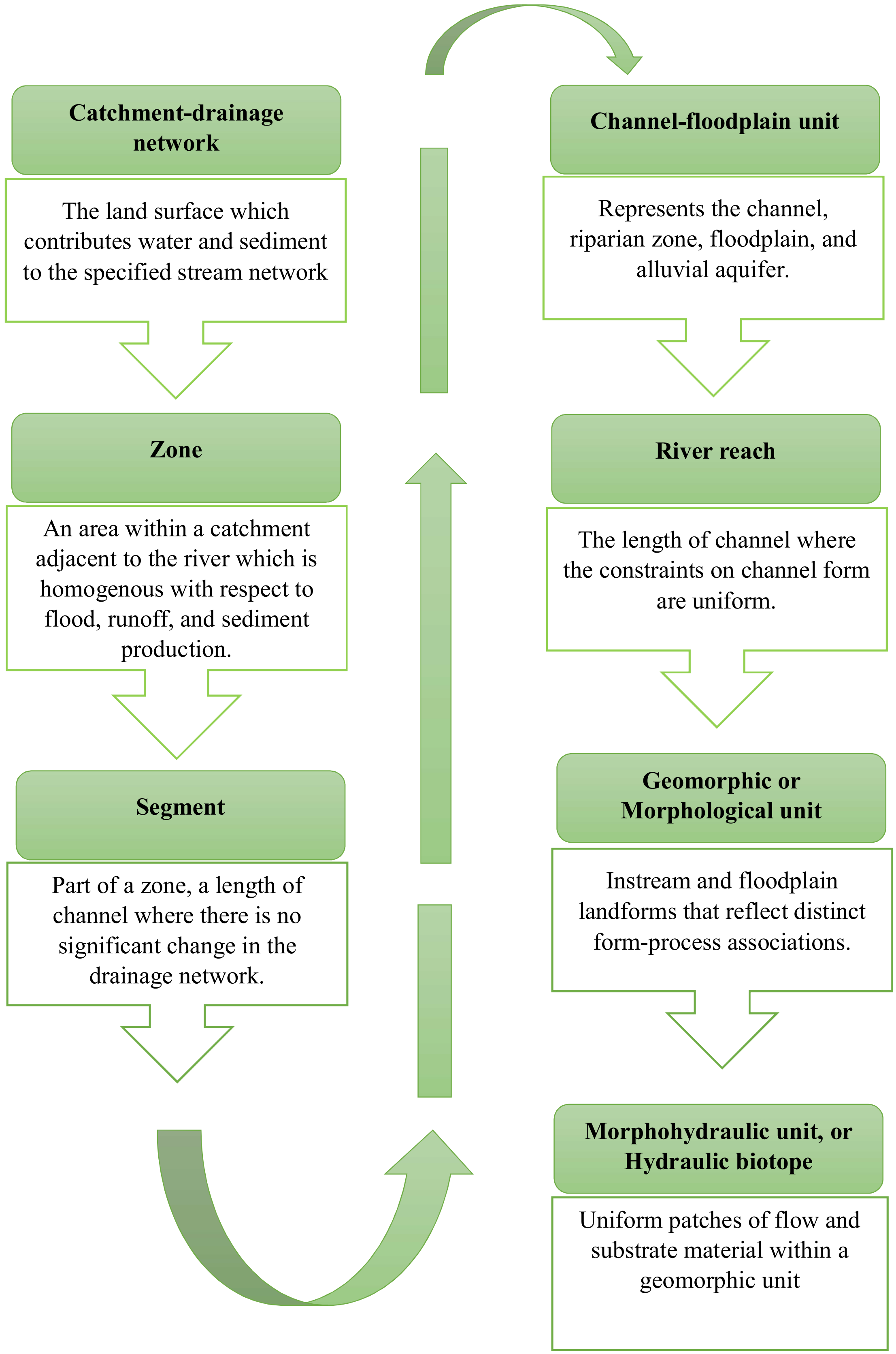


**Figure 1.1.** River morphological hierarchical classification (Lehotský, 2004)

Further, the smaller spatial levels are constrained by the larger levels (Thomson *et al*., 2001). For instance, the morphological units are the basic structures or building blocks comprising the channel morphology and may either be erosional or depositional features, while the hydraulic biotope, usually contained within the morphological unit, provides the essential scale-based link between the physical characteristics of the river and the aquatic biota which respond to changes in substratum and flow conditions (Wadeson, 1994; Thomson *et al*., 2001; Lehotský, 2004; Gurnell *et al*., 2016). In the current study, the term 'hydraulic biotope' was preferred to 'hydraulic unit' or 'morphohydraulic unit' because it brings into sharp focus the interaction between biology and hydraulics. A hydraulic biotope is therefore defined as a spatially distinct instream flow environment (e.g., run, pool, rapid), characterised by specific hydraulic attributes, that provides the abiotic environment in which species assemblages or communities live (Wadeson & Rowntree, 1998). Hydraulic biotopes (e.g., run, pool, rapid) are flow-dependent and are characterised by distinct hydraulic patterns that result from the interaction between flow, substrate, and channel morphology (Wadeson & Rowntree, 1998; Thomson *et al*., 2001), which are three important factors that impact the distribution of MPs in river systems (Kiss *et al*., 2021; Kumar *et al*., 2021). ***It is important therefore, to unravel how the hydraulic biotope impacts the patchy distribution and transport dynamics of MP in rivers.***

### 1.3.1 River hydrodynamics and microplastic behaviour

At the reach-scale, the variety of hydraulic biotopes present in a morphological unit and their distinct hydraulic patterns give a picture of the hydrodynamic conditions of the river environment (Jowett, 1993; Wadeson, 1994, 1996; Rowntree & Wadeson, 1999). The distinct hydraulic patterns in hydraulic biotopes (e.g., laminar, and turbulent flow conditions), which are a function of the interaction between flow, substrate, and channel morphology, together with the physical properties of microplastics, determine the hydrodynamic behaviour of microplastics (i.e., deposition, suspension, aggregation, remobilization) (Kumar *et al*., 2021; Yan *et al*., 2021a). The hydrodynamic behaviour of microplastics eventually determines the fate of microplastics in the river systems (Yan *et al*., 2021a). Therefore, at the reach-scale, the morphological unit and associated hydraulic biotope(s) are critical considerations for understanding the behaviour and eventual fate of microplastics.

Dimensionless indices such as the velocity/depth ratio (*VDR*), Froude number (*Fr*), Reynolds number (*Re*), shear velocity (*U**), and 'roughness' Reynolds number (*Re**) have been used to distinguish and describe the mean motion flow in the water column and near-bed hydraulics in hydraulic biotopes (Jowett, 1993; Rowntree & Wadeson, 1999; Newson & Newson, 2000; Biswas & Chandra Das, 2016). These indices thus have several implications for microplastic behaviour (e.g., deposition, suspension, remobilisation, or horizontal transport, etc.). For instance, in pools with subcritical tranquil flow (i.e., slow, and streaming flow), the movement of suspended microplastics and the remobilisation of settled microplastics is likely to be slow compared to rapids and other biotopes with super-critical turbulent flows (i.e., fast, and turbulent flows) where the rate of remobilisation of settled microplastics and the translocation of suspended microplastics are likely to be higher. It is important to note that hydraulic biotopes in both erosional and depositional morphological units change spatially and temporally as a result of increased flow velocity occasioned by extreme events such as flooding (Wadeson & Rowntree, 1998), thus, the patchy concentrations and behaviour of microplastics within river reaches may be driven more by these biotopes than by input from a potential source.

Understanding the hydrodynamic conditions in morphological units by characterising associated hydraulic biotope(s) is useful for the study of microplastics in riverine systems in several ways. First, at the reach-scale, disparity in the concentrations and density of microplastics that exist within rivers, or different river morphological units can be interpreted based on the hydrodynamic characteristics of the hydraulic biotopes within a particular morphological unit. Second, the hydraulic biotope and type of morphological unit where they occur, whether depositional or erosional, offer an ecologically useful way of comparing sites in terms of their microplastic concentration and transport dynamics. This way, studies can be standardised in terms of collecting technique, sampling sites, and measurement of hydraulic indices for inter-laboratory calibration and study comparison. Third, the hydraulic biotopes present the most straightforward scale within riverine ecosystems where the impact of hydro-morphological variations on the occurrence and transport of MP can easily be understood and related to the biotic community. ***Despite the role of hydraulic biotopes in shaping biological assemblage distribution in streams, and their perceived role in the transport dynamics of microplastics, limited consideration has been given to it in microplastic research. Hence, this study investigates the influences of hydraulic biotopes on the distribution and transport of microplastics in river systems.***

To better understand the way in which hydraulic characteristics impact microplastic transport, the next section is devoted to a review of microplastic transport in riverine systems.

### 1.4 Microplastic transport in rivers

Rivers serve as crucial pathways for transporting microplastics from terrestrial areas to marine ecosystems (Boos *et al*., 2021; Frei *et al*., 2019; He *et al*., 2021). Both theoretical (e.g., modelling) and empirical studies have contributed to our understanding of microplastic transport dynamics. For instance, He *et al*. (2021) employed a three-dimensional hydrodynamic and particle transport model to investigate the dispersal and transport processes of polyethylene (PE), polypropylene (PP), polyamide (PA), and polyethylene terephthalate (PET) microplastics in river sediments. The authors reported that microplastic density played a critical role, with lower density types (e.g., PE, PP) generally showing greater mobility, while higher density counterparts (e.g., PA, PET) tend to remain closer to their entry points into river systems. Studies also reveal a size-dependent relationship in microplastic transport, where smaller particles (e.g., 10 – 149 µm) exhibit higher concentrations during times of elevated suspended particulate matter and discharge rates (Moses *et al*., 2023). Modelling studies is based on the fundamental assumption that the same physical controls on soil erosion and natural sediment transport, for which model calibration and validation are possible, also control microplastic transport and storage (Nizzetto *et al*., 2016).

Further, experimental findings indicate that microplastics  can infiltrate riverbed sediments to depths up to twice the amplitude of bedforms, with maximum infiltration depth decreasing as particle size increases (Boos *et al*., 2021, 2024). During periods of high flow, increased discharge rates can effectively flush microplastics from sediments, highlighting the dynamic nature of microplastic transport influenced by flow velocity (Boos *et al*., 2021, 2024). Nizzetto *et al*. (2016) also noted that microplastics less than 0.2 mm are generally not retained in rivers, regardless of their density. Larger microplastics with densities marginally higher than water can instead be retained in sediments. However, in the Roter Main River sediment, microplastics were detected down to a depth of 0.6 m below the streambed in low abundances (Frei *et al*., 2019). Highest abundances were measured for small size microplastics (20 – 50 µm) in the hyporheic zone of the river. Most authors agree that high water flow can remobilize the pool of microplastics from sediment and that more microplastics can also be transported from source points (Boos *et al*., 2021, 2024; He *et al*., 2021; Nizzetto *et al*., 2016).  These studies highlight the interplay of particle size, density, sediment dynamics, and flow conditions in shaping the transport and distribution of microplastics in river environments. In the current study, microplastics ranging from very small sizes (e.g., 63 µm) in the micron range to larger sizes (e.g., less than 5 mm) were targeted to investigate variations in transport behaviour influenced by riverine hydro-geomorphology.

Three forms of transport patterns can be deduced for microplastics in riverine systems. These are horizontal transport, vertical transport, and lateral transport. Horizontal transport occurs when microplastics follow the natural upstream and downstream river flow, whereas vertical transport is driven primarily by gravity, density, and turbulence, allowing settling or remobilisation of settled microplastics. Lateral transport is driven primarily by allochthonous factors such as wind, runoffs, or discharges from the adjacent landscape and it can be bidirectional.

Several important biophysical and microplastic characteristics play an important role in influencing each of the transport mode. For example, horizontal transport is influenced by hydraulic factors, such as flow velocity, turbulence; climatic factors, such as rainfall; biological factors, such as vegetation patchiness; microplastic characteristics, such as size and density, and river anthropogenic alterations, such as dams (He *et al.*, 2020; Kumar *et al.*, 2021; Yan *et al.*, 2021a). Regarding vertical transport for instance, polymer density and size play an important role. Often polymers denser than water tend to sink to the river bottom and may remain settled (settled microplastics) or be remobilised into suspension (suspended microplastics) by hydraulic factors such as flow velocity. Ballent *et al.* (2012) demonstrated that plastic density, followed by size, markedly influences vertical displacement. Besides particle density and size, particle shape may also play a part in the sinking of microplastics (Kowalski *et al.*, 2016). Further, biofilm colonisation of microplastics has implications for the density of the plastic particle. Owing to biofilms, microplastics usually considered less dense than water may become denser and then sink to the riverbed (Miao *et al.*, 2021) where they may be consumed by benthic macroinvertebrates. The development of biofilms tends to increase the efficiency of forming aggregates of more prominent and denser particles (Miller & Orbock Miller, 2020), thus impacting vertical movement. Although buoyant, turbulent forces also transport microplastics vertically in the water column (Zhang, 2017), vertical movement generally favours denser microplastics in biotopes with sub-critical to critical tranquil flow where both the level of disturbance (i.e., turbulence) and speed of flow is markedly reduced.

Microplastics found in rivers originate from a broad range of terrestrial sources, including land cover and several anthropogenic activities in proximity to freshwater bodies. Terrestrial sources of microplastics can be both point and non-point sources (Kataoka *et al.*, 2019a). In particular, population density and urban ratio have been correlated with microplastic abundance in rivers (Kataoka *et al*., 2019b; Li *et al*., 2021). Microplastics from land-based sources are transported to freshwater environments by several pathways, such as precipitation, runoff (Talbot & Chang, 2021), rainwater in roadside gutters, wind, and storms (Kataoka *et al*., 2019b).

Plastics littered on the open landfill near rivers may be fragmented into microplastics and released into rivers by rainfall and strong wind (Kataoka *et al*., 2019b). Once in the aquatic environment, microplastics are subjected to hydrodynamic processes which may influence their accumulation or deposition (Kumar *et al*., 2021; Yan *et al*., 2021b). However, the volume of precipitation and runoff, the intensity of wind that drives this lateral movement of microplastics, the impact of windbreaks and other terrestrial debris barriers, and how they influence the eventual input into freshwater systems need to be clearly understood. In the next section, key factors that influence microplastic transport in riverine systems are reviewed in detail. First, river-related factors were reviewed, followed by microplastic characteristics, and lastly, allochthonous factors.

### 1.5 River-related factors influencing microplastic transport dynamics

Autochthonous river factors that may impact the transport of microplastics in river systems include channel morphology (e.g., channel width, channel slope, watershed pattern, substrate character including bed roughness, and clast size), hydraulic and hydrological factors (e.g., flow velocity, water depth, discharge, and stream power) (Kumar *et al*., 2021; Newbould *et al*., 2021; Yan *et al*., 2021b). These factors and their potential influence on microplastics transport are reviewed below.

#### 1.5.1 Channel morphology and hydraulic factors

The behaviour of microplastics (e.g., deposition, suspension, remobilisation, aggregation, etc.) is impacted by different hydraulic patterns and channel morphology, especially within the flow-dependent hydraulic biotopes. For instance, Miller and Orbock (2020) stated that microplastic concentrations, transport and storage in river sediments are highly susceptible to changes in flow conditions over a range of temporal scales. Wang *et al.* (2021) observed a positive correlation between river channel width and microplastics larger than 2 mm, and a negative correlation with smaller microplastics. Little is known about the level of resistance posed by different substrate types to flow in relation to microplastics transport. The shear stress that must be surpassed within the terminal sub-layer in different biotopes to remobilise settled microplastics also remains an important area of research for a better understanding of microplastics hydrodynamic behaviour in riverine systems.

Variations in flow velocity and depth are critical factors influencing the mobilization and deposition of microplastics in rivers (Dahms *et al.*, 2020a). For instance, Newbold *et al.* (2005) conducted experiments showing that particulate organic matter of different size classes exhibits varying rates of deposition and resuspension. Model-estimated residence times and migration velocities from these experiments suggest that a significant proportion of particles exported from upstream locations can travel long distances, potentially reaching larger riverine or marine environments before undergoing mineralization. In other studies, Minshall *et al.* (2000) found that the transport distance of fine particulate organic matter was strongly influenced by the cross-sectional area of transient storage zones and water uptake length, while Cushing *et al.* (1993) observed rapid exchange of particles between surficial sediments and the water column, with downstream migration occurring at several kilometers per day during alternating deposition and resuspension events. These findings underscore the longitudinal connectivity of river systems, where organic matter from upstream reaches can be transported over considerable distances for storage or eventual export to estuaries and marine systems. Given that microplastics undergo similar entrainment processes as natural particles, their residence times, deposition patterns, and resuspension rates are likely to mirror those observed for natural particles. The durability and prolonged lifecycles of microplastics will further enhance their ability to persist in aquatic environments, potentially leading to increased accumulation in marine environments over time. Although, Thomas *et al.* (2001) argued that local hydrological and benthic conditions can only establish a minimum rate of particle deposition, this principle likely applies to smaller size classes of microplastics (i.e., less than 50 µm). Larger microplastics may behave differently due to their greater mass and size, as the authors rightly noted that gravitational forces affect deposition differently for particles with diameters of 50 µm and above.

Rowntree *et al.* (1999) suggested that flow velocity and depth are two hydraulic factors that may act independently or in combination through some hydraulic indices which describe either the mean flow (average conditions in the water profile) or near-bed conditions. These hydraulic indices describe both the mean flow motion in the whole water column, and the microflow environment in which many aquatic communities live either on or near the channel bed (Gordon, *et al.*, 1992; Wadeson & Rowntree, 1998; Thomson *et al.*, 2001). The velocity/depth ratio (mean), Reynolds number ($Re$), and the Froude number ($Fr$) describe the mean flow in the water column (Gordon, *et al*., 1992; Jowett, 1993; Thomson *et al*., 2001). In contrast, near-bed hydraulic conditions have been described by hydraulic indices such as the shear velocity ($U^*$) and the 'roughness' Reynolds number ($Re^*$) (Gordon, *et al*., 1992; Wadeson, 1994; Wadeson & Rowntree, 1998). The role of these indices in describing the situation in terms of flow hydraulics is critical to understanding the hydrodynamic behaviour of microplastics in different flow environments. This is because the inherent density of microplastic particles and the hydrodynamic conditions of the river partly govern the transport of microplastic particles (Kumar *et al*., 2021).

The Reynolds number (Re) gives an indication of whether the flow is laminar, transitional or turbulent by representing the ratio of inertial forces to viscous forces (Gordon, *et al*., 1992; Wadeson & Rowntree, 1998). Kumar *et al*. (2021) noted that, depending upon the *Re,* either laminar or turbulent flow can impact the flow dynamics of microplastics concerning the river depth profile; laminar regime at $Re < 1$, turbulent regime at $10^3 < Re < 10^5$, and the transitional regime, in-between (at $1 < Re < 10^3$). At low Re, the water is highly viscous, mixing is impeded, and the transport of materials is slowed (Rowntree & Wadeson, 1999). The implication is that high-density microplastic particles can dominate the river bottom in laminar flow conditions, while low-density microplastic particles are in suspension. The low-density microplastic particles travel for long distances in turbulent flow conditions, while the high-density microplastics can either be in suspension (suspended microplastics) or settle on the river bottom (settled microplastics) after a certain translocation distance (Kumar et al., 2021; X. Lu et al., 2023). However, very little is known about the rising and settling velocities of different microplastic polymers in different flow conditions.

The *Fr* is a dimensionless number derived from the ratio of inertial to gravity forces and is used to differentiate between sub-critical flows (where $Fr < 1$; i.e., slow, or tranquil flow), and super-critical flows ($Fr > 1$; i.e., fast, or rapid flow) (Gordon, *et al*., 1992; Bravard & Petit, 2009). In sub-critical flows, the role played by gravity forces is more pronounced, so that flow has a low velocity relative to depth (Gordon, *et al*., 1992; Rowntree & Wadeson, 1999; Bravard & Petit, 2009). The behaviour and movement of microplastics is impacted depending on the flow type (i.e., either sub-critical or super-critical). For example, sub-critical flow ($Fr < 1$) can potentially increase the sinking and settlement of microplastics. In contrast, super-critical flows, that is, rapid flow and flooding events ($Fr > 1$) can potentially increase the remobilisation, horizontal transport and aggregation of microplastics (Kataoka *et al*., 2019a; Kumar *et al*., 2021; Liro *et al*., 2020).

Bed shear stress is a force per unit area of the bed (in N $m^{-2}$) and increases with flow depth and steepness of the channel (Biswas & Chandra Das, 2016). A critical shear stress is required to set a particle in motion (Gordon, *et al.*, 1992). Shear stress typically increases with discharge, and is unevenly distributed within a channel (Gordon, *et al.*, 1992). The coarse particles lying on riverbeds are set in motion during floods when critical shear stress is passed (Bravard & Petit, 2009a). Thus, laminar and turbulent flow can induce critical shear stress, in which uneven riverine bottom surfaces can play a significant role in the resuspension of microplastics (Kumar *et al.*, 2021).

The 'roughness' Reynolds number (*Re*)* can be developed using shear velocity (*U*)* and the roughness height (k). The roughness of the substrate material also influences flow. The height of the roughness elements relative to the thickness of the laminar sub-layer (i.e., the region close to the bed where flow is entirely laminar) is an essential determinant of flow conditions near the bed (Gordon, *et al.*, 1992; Rowntree & Wadeson, 1999). If roughness height extends above the laminar sub-layer, it affects the surface flow. River roughness depends mainly on the nature of bed material, on vegetation that can clutter the channel, on bedform succession, and the presence of sinuosity (Bravard & Petit, 2009a). River roughness impacts stream power as velocity decreases with increasing bed roughness and decreasing slope (Rowntree & Wadeson, 1999). Sediments of river sections experiencing low stream power are likely hotspots for the deposition of microplastics (Nizzetto *et al.*, 2016). The *Re**, therefore, combines the effects of velocity and substrate type. Thus flow near the riverbed will be disturbed either when the roughness elements increase in height or when the velocity increases, causing the laminar sub-layer to become smaller than the height of the projections (Gordon, *et al.*, 1992). Low *Re** will, therefore, potentially increase the transport of more microplastics than high Re.

In river systems, areas with low velocity and increased depth have been reported to facilitate the vertical transport of microplastics (Tibbetts *et al.*, 2018; Dahms *et al.*, 2020b) with implication for benthic macroinvertebrates that may feed on microplastics settled on the riverbed. Hydraulically smooth areas with shallow depth and increased velocity will experience microplastic flushing compared to areas cluttered with vegetation and where substrates protrude above the terminal sublayer, imposing higher critical shear stress on settled particles. The implication is that areas experiencing microplastics flushing will have more particles in the water column than areas with low VDR. These indices (*Fr, Re*, *Re*, U* VDR*) show good promise in terms of providing critical insights for microplastic transport dynamics. The integration of hydrodynamic conditions indicated by the different hydrodynamic indices in river-related microplastics field studies will enhance the understanding of microplastic distribution patterns and concentration configurations in rivers.

### 1.5.2 Stream power

The concept of stream power was first used in fluvial geomorphology to study sediment transport in river channels. It was later applied for other purposes such as bedload, suspended load, channel instability, bank erosion, thresholds in channel patterns, and floodplain dynamics (Kondolf *et al*., 2016b). Stream power (Ω) is the rate at which a stream performs its geomorphologic work and is a good measure of its capacity to erode and transport materials (Biswas & Chandra Das, 2016). It determines the capacity of a given flow to entrain and transport sediment; thus, stream power increases with increasing gradient and flow discharge (Rowntree & Wadeson, 1999). Sediments of river experiencing low stream power are likely hotspots for microplastic deposition (Nizzetto *et al.,* 2016). The reverse is the case for sediments of rivers experiencing high stream power. Settled microplastics are likely to be remobilised at higher stream power either into suspension in the water column or alongside other particulates into biotopes with greater depth.

Stream power impact the movement of high-density, bottom-settling microplastics (i.e., settled microplastics) including bio-fouled microplastics, microplastic homoaggregates, and heteroaggregates that sink to the riverbed. Discharge, flow depth, and channel slope determine the river's stream power and shear stress (Biswas & Chandra Das, 2016). Although, discharge typically increases in the downstream direction, stream power per unit area typically decreases because slopes decrease (Gordon, *et al*., 1992). This may further impact the entrainment of particles upstream and downstream of a river. For instance, the descending order of microplastic abundances from downstream, midstream to upstream observed in a study by Rakib *et al*., (2023) may be attributed to decreasing stream power from upstream to downstream as rivers typically get wider as they flow downstream; this will reduce stream power and result in the accumulation of microplastic particles downstream. Stream power is thus an important variable that needs to be considered in microplastics transport.

### 1.5.3 Biological factors impacting the transport of microplastics.

Microbial colonisation, ingestion by aquatic organisms, and vegetation patchiness are the biological factors reviewed in this section.

***Microbial colonisation***

Microplastics can readily develop surface fouling by accumulating microbial films, followed by colonisation by fungi, algae and invertebrates, which affect their size and thickness and thus their transport and deposition (He *et al*., 2022). McCormick *et al.* (2014) observed extensive microbial colonisation of microplastic pellets and fragments. Microplastics colonised by biofilm-forming microbial communities are known as plastisphere (Tang, 2024). Microplastics that are more rectangular and have more ridges (i.e., fragments, fibres) will have greater biofouling potential than spheres because they possess a large surface-area-to-volume ratio, which provides adequate surface area for microbial colonisation (Fazey & Ryan, 2016), thus, potentially increasing particle density and sedimentation. Microplastics with greater amounts of cracking, and alterations of surface structure, which results from exposure to degradation pathways in the environment, may also provide additional surface area and oxygen containing functional groups that promote the biofouling process (Schorer & Eisele, 1997; Andrady *et al*., 2022). The colonisation of microplastics by microorganisms is an important factor affecting heterogeneous aggregation of microplastics (Yan *et al*., 2021b) with implications for the vertical transport of heteroaggregates.

Recent studies have also shown that nanoplastics and the lower size class of microplastics are increasingly covered by an "environmental- or eco-corona," which alters their physicochemical attributes such as size, stability, surface charge, and other properties (Natarajan *et al*., 2021; Rex *et al*., 2023; Dawson *et al*., 2024). These modifications can significantly influence the transport processes and depositional behaviour of micro- and nanoplastics (Rex *et al*., 2023). The eco-corona on microplastics refers to the initial layer of biomolecular compounds adsorbed onto the surface after environmental exposure (Yao *et al.*, 2023). However, compared to nanoplastics, our understanding of the corona formation on microplastics within the environment remains limited. While insights into microplastic corona formation have been derived largely from nanoparticle research (Cao *et al.*, 2022), there is a crucial need to validate these observations specifically for microplastic particles (Dawson *et al.*, 2024). Both laboratory experiments and in situ environmental studies are essential for this validation, considering that physical properties like surface area to volume ratios and surface roughness can significantly influence corona formation (Dawson *et al*., 2024).

The extrapolation from nanoparticle studies to microplastics provides a foundational understanding, but the unique characteristics of microplastics warrant dedicated investigation.

Laboratory studies allow for controlled conditions to explore corona formation mechanisms, while in situ studies provide insights into real-world environmental interactions and conditions. Addressing these gaps in knowledge is critical for accurately assessing the environmental implications of microplastics, including their behavior, fate, and potential ecological impacts mediated by the corona formation. Future research efforts should prioritize these investigations to enhance our understanding and inform effective mitigation strategies.

***Ingestion by aquatic organisms***

The transport of microplastics is also influenced by the grazing of aquatic organisms such as fish, tadpoles, and macroinvertebrates. Studies have suggested that aquatic organisms may ingest microplastics that resemble their food (Steer *et al.*, 2017; Tien *et al.,*, 2020). The formation of biofilms on microplastics surfaces has been reported particularly to increase the chances of ingestion owing to their taste, smell, and colour, which may be similar to that of plankton (Stabnikova *et al.*, 2021). Following ingestion, microplastic particles pass through the gut system intact. Eventually, these particles are egested alongside other debris, which may lead to the formation of aggregates. The formation of aggregates alters their density and ultimately impacts vertical transport. For example, in a study conducted by Moreschi et al. (2020), the assimilation of microplastics by the freshwater bivalves, *Anodontites trapesialis* resulted in the formation of pseudofaeces. Filter feeders like bivalves can transfer microplastics alongside nutrients from the water column to the benthic zone of rivers through eliminated faeces and pseudofaeces (Moreschi *et al.*, 2020).

Studies on ingestion indicate that microplastics are ingested by a diverse array of aquatic organisms spanning different trophic positions, feeding types, and habitats (Scherer *et al.*, 2017; Franzellitti *et al.*, 2019). Direct ingestion routes include particle uptake by filter-feeders, suspension-feeders, and deposit-feeders, which exhibit relatively indiscriminate feeding behaviors by collecting and sorting particulate matter to trap and ingest particles of suitable size (Franzellitti *et al.*, 2019). Low-density polymers such as polyethylene and polypropylene, which typically float in the water column, can accumulate in sediments after aggregation with other debris following ingestion and subsequent egestion through faeces.

***Vegetation patchiness***

Vegetation (both aquatic and riparian) may act as physical traps of microplastics (Newbould *et al.*, 2021), thus impeding horizontal transport. The impact of vegetation on microplastics transport could be exacerbated when microplastics interact with other barriers such as hydraulic traps, fallen logs, emergent boulders, et cetera, which may act to trap microplastics for a long time (Dahms *et al.*, 2020b; Newbould *et al.*, 2021). Trapped microplastics may either undergo aggregation, forming homoaggregates and heteroaggregates, that could increase their density and cause them to sink to the riverbed (i.e., vertical transport) or are eventually released from the traps during higher flows or flooding, which promote horizontal transport.

In an indoor flume experiments by Gallitelli *et al.* (2023), plant density played a role in the entrapment of plastic particles. The higher the plant density, the higher the entrapment of plastics by plants. This result was true for both micro-, macro-, and mesoplastics used in the experiments. Vegetation, and its patchiness, likely plays a crucial role in influencing riverine microplastic transport and impact (Cesarini & Scalici, 2022; Liro *et al.*, 2022; Gallitelli *et al.*, 2023). Studies have shown that both seagrasses and seaweeds effectively entrap plastics in marine waters (de los Santos *et al.*, 2021; de Smit *et al.*, 2021). While extensive research has been conducted on marine vegetation's ability to intercept plastics in controlled laboratory and in situ environments, there is a noticeable lack of research focused on freshwater systems in this regard (Gallitelli *et al.*, 2023). However, given the sizes of microplastics, entrapment by aquatic plants in riverine systems will depend on factors critical factors, such as plant density, water flow rate, and the water levels. When aquatic plants coexists with natural structures like weirs and fallen logs, it enhances their capacity to entrap microplastics. For example, water hyacinths have been observed to influence plastic transport in freshwater ecosystems, particularly in tropical regions (Schreyers *et al.*, 2021). These plants can form extensive patches on the water surface, spanning several meters, effectively capturing and aggregating large quantities of floating debris, including microplastic particles (Schreyers *et al.*, 2021). Given the pivotal role of hydrological factors in riverine microplastic transport, it is essential to explore how interactions between hydrological variables and vegetation contribute to the entrapment of microplastics and plastics more broadly in riverine systems.

### 1.5.4 Physiochemical factors and microplastic transport

Concerning water physicochemical parameters, microplastics monitoring and evaluation studies seems to differ in their findings. However, some studies have reported definite correlations with some water quality parameters such as turbidity, conductivity, total suspended solid (TSS), biochemical oxygen demand (BOD), pH, temperature, and chlorophyll-a (Huang *et al.*, 2021; Wang *et al.*, 2021; Eamrat *et al.*, 2022; Shekoohiyan & Akbarzadeh, 2022; Rakib *et al.*, 2023). Relevant context is provided in this section for selected physicochemical factors (temperature, dissolved oxygen, nutrients, biochemical oxygen demand, turbidity, and total suspended solids).

The dynamic viscosity of water is strongly temperature dependent (i.e., colder water is more viscous or 'syrupy' than warmer water) (Schlichting, 1979; Gordon, 1992). The implication of this might mean that the translocation of suspended microplastics in the water column would be slower in frigid winter water than in tepid summer water. This variation in temperature will thus impact the distribution of suspended microplastics from a point source and contribute to seasonal variations in abundance and the spread of microplastics in the water column. Rakib *et al*. (2023) reported a positive correlation between high microplastic concentrations and high temperatures at low pH. Yonkos *et al*. (2014) and Buwono *et al*. (2021) further affirmed that temperature has a significant impact on the distribution of microplastics because it influences the hydrodynamic mechanics of water as well as the mechanism of microplastic breakdown. The molecules of cool to moderate water temperatures of winter and early spring move slowly, thus they tend to retain more dissolved oxygen (DO) than the warmer temperatures of summer and autumn (Schlichting, 1979; Gordon, 1992). Turbulent flowing waters also contain more dissolved oxygen because of the churning movement of the water and the water movement at the air-water interface (Allan, 1995; Giller & Malmqvist, 1998). Thus, dissolved oxygen levels change naturally with seasons and flow. As with warm temperatures, high flows usually containing higher amounts of dissolved oxygen will impact the horizontal transport of suspended microplastics as well as the remobilisation of settled microplastics. Buwono *et al*. (2021) reported the presence of fibre-type microplastics in water with high velocity and DO, but at moderate pH. However, fragment types of microplastics were prominent in their study and were predominantly detected at low stream velocity and low DO conditions downstream. This suggests that high dissolved oxygen may co-vary with low abundance of microplastics and higher rate of remobilisation of settled microplastics from upstream. Further, variations in temperature and dissolved oxygen resulting from changes in seasons play a role in the rate of biofilm formation (He *et al*., 2022). Biofilm formation and subsequent colonisation of microplastics by other microorganisms have been reported to impact the deposition and transport of microplastics (Chen *et al*., 2019; Miao *et al*., 2021).

High nutrient and biochemical oxygen demand (BOD) levels generally suggest high organic matter contamination (Buwono *et al*., 2021). The BOD concentration reflects the amount of oxygen required by microorganisms to break down the organic molecules in the water (Yohannes *et al*., 2019). Kataoka *et al*. (2019a) found a significant positive correlation between the numerical and mass concentrations of microplastics and BOD in the river environments of Japan. From the results of their study, Kataoka *et al*. (2019a) deduced that the correlation between microplastics concentration and BOD demonstrated that microplastic pollution in river environments has progressed more (i.e., more severe) in polluted rivers with poor water quality than in rivers with good water quality. However, it is important to note that rivers with higher BOD and nutrients are typical of landscapes with lots of human activities where there is also likely a greater abundance of microplastic loading. Also, biofilm colonization of microplastics has been linked to water environmental conditions and the physicochemical properties of microplastics (McGivney *et al*., 2020; Miao *et al*., 2021). The presence of high organic matter in river water may presumably result in greater diversity and abundance of microorganisms which facilitate a higher rate of biofilm colonization, especially as high organic content might support nutrients (i.e., carbon, nitrogen, and phosphorus sources) required for biofilm formation (He *et al*., 2022). Nutrients such as total nitrogen and total phosphorus have been positively correlated with biofilm growth rate (Li *et al.*, 2019). Therefore, high BOD and nutrient presence in water facilitates biofilm colonisation of microplastics which, in turn, can alter microplastic density and impact on their vertical transport and deposition.

In an assessment of nanoplastic particles and microplastics fibre flux through a pilot wastewater treatment plant using metal-doped plastics, Frehland *et al*. (2020) observed a positive correlation between total suspended solids (TSS) and plastic concentrations in sludge and effluent. In another study, influent wastewater with high concentrations of suspended solids proved to have a low microplastic burden with larger microplastics size (Bayo *et al*., 2020). The low microplastic burden was attributed to heteroaggregation with particulate matter which can influence sinking rates of microplastics. The strong association between particulate plastic and TSS in both studies provides some context with regard to microplastics TSS relationship in rivers. High turbidity and TSS may seem to promote greater heteroaggregation with particulate matter than low turbidity and TSS. For example, Eamrat *et al*. (2022) found a positive relationship between the levels of solids and turbidity in highly polluted water and the concentration of microplastics in an urban canal in Thailand.

The differences in water quality conditions and the influences of seasonal changes on water quality variables may create varying conditions that can influence the interaction and behaviour of microplastics with other aquatic factors. For instance, low dissolved oxygen, high nutrient concentrations which are general indicators of low water quality, and temperature can provide an enabling environment that allows biofilms to form on microplastic surfaces, allowing bacterial to thrive and impacting microplastic transport behaviour. ***Therefore, in this study, the relationship between microplastic abundance and selected physicochemical properties were investigated across the wet and dry seasons to provide a clearer picture of this relationship in the river environment.***

In the next section, we review some characteristics of microplastics that influence their transport in river systems.

### 1.6 Characteristics of microplastics that influence their transport in river systems

Microplastic particles possess intrinsic properties that impact their transport and distribution within river systems. In this section, these properties and how they may impact microplastic transport and distribution in riverine systems were reviewed.

#### 1.6.1 Density

The density of microplastic varies considerably, depending on the polymer types and manufacturing process (Zhang, 2017). Particle density can influence the deposition and mobility of microplastics in riverine ecosystems (Kumar *et al*., 2021). Microplastic in the density range more diminutive than the river water floats or remains in suspension (i.e., suspended microplastics). In contrast, the higher density microplastics tend to deposit over the riverbed (i.e., settled microplastics) (Kumar *et al*., 2021). Because of aggregation, mechanical breakdown, biofouling, photochemical degradation, and flocculation mechanisms, the density of plastic particles might vary with residence time in the riverine ecosystem ( Zhang, 2017; Kumar *et al*., 2021), thus impacting their eventual fate.

Regarding aggregation, Zhang (2017) noted that aggregation with organic and inorganic particles could increase the size and density of microplastics and cause rapid deposition onto benthic sediments. Nguyen *et al.* (2020) also demonstrated the formation of microbial-associated microplastic aggregates possibly caused by cell colonisation. Hoellein *et al*. (2019) observed that the denser fragments could easily be retained and may resist remobilisation, depending on the degree to which they are attached or buried in bed sediments. Many low-density polymers remain in suspension due to buoyancy. Overall, even though density plays an important role in the transport and distribution of microplastics, density is usually altered by several factors such as i) microplastic residence time within the river system, ii) microplastic-microbial interaction, iii) biofouling, iv) microplastic-organic chemical interactions, v) aggregation and breakdown. A combination of these factors implies that predicting the potential distribution and transport of microplastics based on their density is complex and not straightforward. How these factors interact collectively to influence microplastic distribution and transport remains an important area of research.

### 1.6.2 Shape and size of microplastics

Plastic particles in the riverine ecosystem exist in various shapes (e.g., fibres, pellets, fragments, films, foams, microbeads, and granules) and sizes (Kumar *et al*., 2021). Generally, low-density fibres and films have high buoyancy and low settling velocity. Fibres can remain in suspension for longer than fragments and spherical beads in the water column (Kumar *et al*., 2021). Hoellein *et al*. (2019) reported that fragments have high deposition velocity, followed by fibres, and then pellets; therefore, fibres and pellets tend to travel longer than other particle shapes. Deposition or settling velocity is also impacted by particle size of certain microplastic shapes with the effect of particle shape more pronounced for larger particles (Khatmullina & Isachenko, 2017). Generally, insights can be drawn from the results of studies that have investigated how particle size influences deposition and transport of natural particles in streams. For instance, Thomas *et al.* (2001) in a study that investigated how particle size influences deposition and transport of fine particulate organic matter in streams, reported that smaller particles deposited more slowly, and thus travelled farther, than larger size classes. Variation in mean transport distances and field velocity deposition reported from this experiments were strongly correlated across hydrological conditions. Variation in field deposition velocity was poorly associated with physical attributes of the stream while transport distances were positively associated with the cross-sectional area of the transient storage zone and the uptake length of water for the different size classes of particles studied. Thus, it can be deduced from this study that larger size microplastics are likely to deposit faster compared to smaller size particles while smaller size particles will travel faster and for longer distances compared to larger size classes.

## 1.7 Allochthonous factors

The allochthonous factors which impact microplastics transport that are discussed in this section include wind, precipitation, point and non-point discharges.

### 1.7.1 Wind and precipitation

Wind and precipitation impact the lateral transport of microplastics (i.e., movement of particles into rivers from adjacent land sources) (Rezaei *et al*., 2019; Han *et al*., 2022). Microplastics get onto land by several means; i) direct littering and inefficient waste management; ii) plastic use for mulching and wrapping in modern-day agriculture; iii) settled microplastics introduced along with sewage sludge into agricultural lands as fertilisers. Once on land, their characteristic light weight makes it possible for microplastics to be easily transported from adjacent land into surrounding rivers by either wind or rainfall-induced surface runoff. This movement, especially during soil erosion or surface runoff, follows the hydrological and sediment transport pathways (Lwanga *et al.*, 2022) impacting markedly on lateral transport into rivers. For instance, Cheung *et al*. (2019) recorded nearly double the number of microplastics found on coastal sea surfaces as the same area in an urban river following a three-day rainfall event in Hong Kong.

Agricultural drainage and runoff from farmlands and other forms of land-use types can result in input of microplastics and sewage-sludge-derived fibres and microbeads (Horton & Dixon, 2018). Storm drainage and urban runoff can contain microplastics from degraded road paint, plastic litter in gutters, and wear from machinery (Horton & Dixon, 2018), which can impact lateral movement. However, the effect of the interaction of microplastics with soil aggregates, different rainfall scenarios (including rainfall intensity and frequency), the intensity of runoffs and how they may impact lateral transport of microplastics need to be carefully researched. The presence of windbreaks such as trees and shrubs, interception of microplastic obstruction of surface runoff and sediment delivery pathways into rivers by physical barriers such as terrestrial vegetation, depressions, bends, and uneven land surfaces, and their impact on the lateral transport of microplastic is also an interesting area of research.

### 1.7.2 Point and non-point sources

Studies have often linked lateral movement of microplastics as occurring near densely populated or urban areas affected by point and non-point source pollution (McCormick *et al.*, 2014; Kataoka *et al.*, 2019a; Liu *et al.*, 2021). Discharges from point sources (e.g., direct discharge from sewers and drains) and non-point discharges such as runoff from different land-use types (e.g., farmlands and cities) are seen as the major factors impacting the movement of microplastics into rivers, but point discharges such as effluent outfalls, combined sewage overflows and storm drains which are usually isolated from densely populated areas (Horton & Dixon, 2018) markedly impact the lateral transport of microplastics.

As urban areas are the major sources of microplastics from point and non-point discharges, it is expected that the concentration of microplastics in urban rivers would be higher (McCormick *et al.*, 2014; Kataoka *et al.*, 2019a; Koutnik *et al.*, 2021; Liu *et al.*, 2021;Wang *et al.*, 2021). The proximity of rivers to point and non-point microplastic emission sources is a major factor that contributes to a wider variability in microplastic concentrations in rivers (Koutnik *et al.*, 2021). For example, Mani *et al.* (2015) reported a diverse concentration of microplastics along and across the river Rhine (one of Europe's major rivers), reflecting various emission sources. Variations in spatial concentration downstream of a river is impacted by river hydrological characteristics such as flow velocity, depth and bedforms (Koutnik *et al.*, 2021; Kumar *et al.*, 2021). For instance, high flows have been reportedly linked to increased concentration of suspended microplastics and low sedimented concentration (X. Lu et al., 2023). Also, high flow velocity facilitates the transport of more microplastics from source points and for longer distance  (X. Lu et al., 2023).

The flow regime and morphology (e.g., channel width) can affect the distribution of various pollutants, such as heavy metals, pesticides, and polycyclic aromatic hydrocarbons (PAHs) (Zhang, 2017; Wang *et al.*, 2021); but information about whether these factors can affect the *in situ* transport patterns of microplastic in rivers remains scarce. Seasonality and flooding events can also potentially influence the abundances of microplastics in rivers (Kataoka *et al.*, 2019b; Xia *et al.*, 2021). As a result of reinforced hydrodynamic conditions during rainfall, settled microplastics can resuspend and return to the overlying water, which facilitates the horizontal transport of microplastics (Yan *et al.*, 2021a). The patchy concentration of microplastics in rivers from different source points needs further investigation to unravel their fate in different flow compartments or hydraulic biotopes at different flow conditions in other to detect potential sinks. ***This study thus investigates the impact of adjacent land-use types on the spatial and temporal distribution of microplastics in riverine systems.***

### 1.8 Microplastic analytics

Microplastics analytics involve rigorous methods and quality assurance measures to accurately detect, quantify, and characterise microplastic particles in environmental samples. Microplastics analytics is still in evolution and a lot of quality assurance and quality control measures have to be considered during sampling, sample processing, and identification to get reliable data. The steps usually involved in microplastics analysis include sample collection, separation, digestion, and identification (Prata *et al.*, 2019). Effective microplastics analytics and quality assurance are essential for generating reliable data on the presence and impacts of microplastic pollution in aquatic ecosystems, supporting evidence-based decision-making and environmental protection initiatives.

Microplastic sampling strategies can be distinguished into selective, bulk, and volume-reduced samplings ( Hidalgo-Ruz *et al*., 2012; Cutroneo *et al*., 2020). The choice of sampling strategy in rivers is informed by a variety of factors including the objective of the work, available equipment (Prata *et al*., 2019), river morphology, size, and water depth. Sampling is usually differentiated for the aqueous phase (surface water and water column), the sediments, and biota (Campanale *et al*., 2020). Selective sampling consists of direct extraction from the environment of items that are recognisable by the naked eye, usually on the surface of sediments (Hidalgo-Ruz *et al*., 2012). There is a great risk of overlooking microplastics using the selective sampling method, especially when microplastics have no characteristic shapes or when they are mixed with other debris (Hidalgo-Ruz *et al*., 2012). In volume-reduced sampling the volume of the bulk sample is usually reduced during sampling (Campanale *et al*., 2020; Hidalgo-Ruz *et al*., 2012). Only that portion of the sample that is of interest is preserved for further processing. Methods commonly used for volume-reduced sampling include, the use of manta nets towed by a boat (Moses *et al*., 2023) (this method is unsuitable for rivers with low water depth), stationary sampling using plankton nets (Campanale *et al*., 2020), pump systems are also used for water sampling through a filtering system while microplastic traps have been used in small rivers and streams with shallow depths. All these instruments may carry risks such as high degree of contamination of the samples during their manipulation and relocation (Campanale *et al*., 2020). Bulk sampling usually involves the use of discrete sampling devices such as Niskin bottle, rosette, buckets, bottles, steel samplers to collect bulk samples for processing (Cutroneo *et al*., 2020). The bulk sample is usually not reduced during sampling (Hidalgo-Ruz *et al*., 2012). While the method allows for only a limited number of samples to be collected, stored, and processed, it offers several advantages. For instance, it enables sampling of all microplastics present in the environmental matrix without size limitations, thus preventing any loss that may occur with volume-reduced sampling. Additionally, the method is rapid and minimizes the risk of contamination, as the handling and exposure time of the sample to the surrounding environment are shortened (Campanale *et al*., 2020; Cutroneo *et al*., 2020). Considering the advantages of the bulk sampling approach, the available equipment, the objectives of this study, and the morphology and depth of the study rivers, the bulk sampling method was applied in this study. This involved using a bucket to collect comprehensive samples for processing.

During sampling in the field and subsequent laboratory preparations and processing of samples, strict prevention measures against microplastic contamination are normally applied, which during field and laboratory work are accounted for through procedural blanks (Moses *et al.*, 2023). Utilisation of non-plastic items during sampling and laboratory processes, use of ultra-pure or distilled water for rinsing equipment before use, are all aimed to reduce contamination of samples (Apetogbor *et al.*, 2022). Further, the removal of organic fraction from samples is usually performed through chemical or enzymatic digestion in order to extract microplastics from the environmental matrix. Digestion processes are usually coupled with density separation (Masura *et al.*, 2015). The reagents used to digest organic fraction can be acidic, basic, oxidising, or enzymatic. The digestion efficacies differ for different chemicals with some chemicals reported to affect the integrity of particles. For instance, potassium hydroxide as a 10% solution used for 24 h at 60 °C, has a removal efficiency ranging from 99.6% to 99.8% and it seems not to affect the integrity of plastics except for cellulose acetate (CA) (Campanale *et al.*, 2020). Conversely, hot nitric and hydrochloric acids caused the degradation of some polymers such as polystyrene, polyamide, and polyethylene but nitric acid had digestion efficiency between 93% and 98% (Campanale *et al.*, 2020). Acid digestion using a strong acid, such as hydrochloric acid (HCl) or hydrogen fluoride (HF), can compromise the structural integrity of the microplastics, therefore their use is usually not recommended (Cutroneo *et al.*, 2020). The National Oceanic and Atmospheric Administration (NOAA) recommended digestion using 30% hydrogen peroxide ($H_2O_2$) in the presence of 0.05 M Fe (II) solution (Fenton's reaction) (Masura *et al.*, 2015). Avio *et al.* (2015) reported to have used 15% $H_2O_2$ at 50 °C overnight to efficiently remove organic matter. Although, hydrogen peroxide can slightly modify polymer texture by making thinner and smaller at different concentrations (Tirkey & Upadhyay, 2021), it is still the most popular oxidant agent used for sample chemical degradation (Campanale *et al.*, 2020). Enzymatic digestion does not seem to affect the polymer's structure and has excellent removal efficiency of the natural organic fraction, but it takes more time and higher costs ( Prata *et al.*, 2019; Campanale *et al.*, 2020). Choosing a digestion protocol should depend on digestion efficiency, polymer resistance, toxicity of reagents, and costs.

Microplastics density vary according to the type of polymer. Density separation process is especially required for sediments to discriminate the microplastics from the rest of the samples. A common method for flotation of microplastic particles involves a salinity-based density separation using a saturated salt solution (Campanale *et al.*, 2020). This technique utilizes the difference in density between the microplastics and the salt solution to float the microplastic particles on the supernatant of the solution. Sodium chloride (NaCl) and sodium polytungstate solution, are both cost-effective and nontoxic, however, they do not allow separation of heavier polymers from solution (Hidalgo-Ruz *et al*., 2012; Campanale *et al*., 2020). Sodium iodide (NaI) and zinc chloride ($ZnCl_2$) are generally favoured for separating high density polymers (Tirkey & Upadhyay, 2021). However, sodium iodide is quite expensive while zinc chloride which is considered the most effective and least expensive method, is a highly dangerous and corrosive substance. Sodium iodide has been used in low concentrations in support of NaCl solution (Nuelle *et al*., 2014). Sodium iodide can also be recycled, significantly reducing the quantity that is used (Stock *et al*., 2019). The use of NaI in support of NaCl is both cost effective and efficient, hence, it was adopted for the current study.

After extraction and isolation of particles, particles not visible to the naked eye are sorted using a variety of microscopes including fluorescence, dissection, optical, electron, stereo, binocular, inverted and vertical microscopes (Cutroneo *et al*., 2020). In some instances, scanning electron microscopy (SEM) is employed for visualising microplastics (Wang *et al*., 2017). The microscopic analysis of microplastics can be time-consuming and resource-intensive due to the need for researchers to manually count hundreds of particles per sample (Campanale *et al.*, 2020; Cutroneo *et al*., 2020; Tirkey & Upadhyay, 2021). There is also a risk of overestimating data due to false positives. To address these challenges, researchers have recently focused on developing automatic image analysis methods. These approaches aim to achieve time efficiency, accuracy, and standardised data analysis. In addition to image analysis, a wide range of complementary analytical methods are applied for chemical identification of microplastics (Campanale *et al*., 2020; Cutroneo *et al*., 2020).

The spectroscopic methods (FTIR and Raman) are the most common in the chemical identification of microplastics. These techniques are non-destructive, allowing for further processing of samples after acquisition. For instance, larger particles can be subjected to additional techniques such as pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) (Campanale *et al*., 2020. This approach provides additional and complementary information on the composition of plastic polymers. The use of ATR-FTIR is limited in terms of the identification of the lower size range of microplastics (< 500 μm). Micro-FTIR, focal plane array (FPA) detector-based micro-Fourier-transform infrared (FPA-micro- FTIR), and micro-Raman can be used to characterise the sample below 10 μm (Cutroneo *et al.*, 2020; Tirkey & Upadhyay, 2021; Moses *et al*., 2023) . The method adopted will depend on the research goals and the availability of the equipment. This study utilised an optical microscope fitted with an imaging software to visually identify and measure microplastics while a spectroscopic technique (e.g., Fourier-transform infrared spectroscopy - FTIR) was used to identify the polymer composition of microplastics. For further details on microplastic analytics utilised in this study, see Chapter Two of this thesis.

### 1.9 The role of traits on the potential exposure of macroinvertebrates to microplastics in river systems

Traits are measurable attributes, such as morphological, physiological, behavioural and life-history features of a species, which may confer resistance, resilience, vulnerability or adaptability on an individual in an ecosystem and therefore can only be measured at the individual level (Violle *et al*., 2007; Menezes *et al*., 2010; Statzner & Bêche, 2010). The formulation of the habitat template concept (HTC) (Southwood, 1977), its adaptation to streams (Townsend & Hildrew, 1994), and the formulation of the habitat filtering concept (HFC) (Poff, 1997), spawned interest in the use of species traits for biomonitoring. The mechanistic basis for understanding and predicting trait-environment relationships inherent in the habitat template model has been the main driver of the application of traits-based approaches in stream biomonitoring (Schmera *et al*., 2017). The trait-based approach (TBA) in stream ecology is thus theoretically embedded in the HTC of Southwood (1977, 1988) and the HFC of Poff (1997). The HTC and HFC emphasise that species distribution and abundances and local community composition are constrained by the prevailing environmental characteristics so that only species with the right combination of traits are adapted to a particular environment or habitat (Verberk *et al*., 2013). Consequently, habitats with similar characteristics should have a similar composition of species traits, even across different geographical locations where taxonomic composition differs (Southwood, 1977, 1988; Poff *et al*., 2006). In this regard, environmental variables can be considered filters that constrain organisms (Poff *et al*., 2006; Schmera *et al*., 2017) such that organisms thrive in their environment only when they possess the appropriate combination of traits, allowing them to either resist or adapt to external pressure (Abdala-Roberts *et al*., 2018; Odume, 2020).

Macroinvertebrate distribution in riverine systems is associated with a gradient of flow velocity, depth and substratum (Jowett *et al*., 1991; Karaouzas *et al*., 2019; Pastuchová *et al*., 2008; Wegscheider *et al*., 2023). Hydraulic biotope characteristics such as flow velocity and depth may thus act as filters for organisms' traits and shape community composition by selecting well-adapted species with an appropriate combination of traits. Accordingly, macroinvertebrates that prefer high flow velocity such as in rapids, riffles, and fast runs, will have a combination of traits that adapt them to such hydraulic biotopes while excluding species with preference for standing or very slow water velocity, such as in pools and backwaters. The prevailing characteristics of each hydraulic biotope thus constrain each organism such that only species with adaptive traits for each set of hydraulic characteristics can thrive.

In river systems, hydraulic biotopes are characterised by different hydraulic patterns which are a function of the interactions between flow velocity, depth, nature of substratum and the channel morphology (Wadeson & Rowntree, 1998; Thomson *et al*., 2001). The differences in riverine hydrodynamics embodied by hydraulic biotopes may potentially create different spatial distribution and accumulation of microplastics. Higher concentrations of suspended microplastics may characterise fast-flowing hydraulic biotopes and higher concentrations of settled microplastics in standing or slow-flowing hydraulic biotopes (X. Lu et al., 2023). Different concentrations of suspended and settled microplastics may thus characterise hydraulic biotopes potentially creating a situation of 'flush' and 'sink' zones of microplastics in rivers. Depending on the trait possessed, certain macroinvertebrates show a preference for the different types of hydraulic biotopes, potentially being more exposed to microplastics in such biotopes for which they have higher preference and mode of exposure might differ in different biotopes. This preference thus implies that macroinvertebrates may be differentially exposed to suspended and settled microplastics depending on their preferred hydraulic biotopes. ***It is thus possible to draw insights from the field of hydraulics, geomorphology, and trait-based ecology to predict the potential exposure of different macroinvertebrates to microplastics within the different hydraulic biotopes***.

Furthermore, the degree of exposure of macroinvertebrates to microplastics in a hydraulic biotope will depend on the ecological niche selected and the traits of the organism (e.g., body size, feeding group, etc). For example, in freshwater, stagnant zones have been identified as crucial reservoirs for the long-term microplastic sink (Kumar *et al*., 2021). In such reservoirs where there are higher concentrations of settled microplastics than suspended microplastics, gathering collectors, which are deposit feeders that ingest sediments and gather loose particles in depositional areas, would be expected to be more at risk of microplastic ingestion and gut filling than filtering collectors which are mostly suspension feeders. Conversely, in hydraulic biotopes experiencing microplastic flushing (i.e., biotopes with high flow velocity where there is a higher rate of remobilisation and resuspension of settled microplastics), such that there are comparatively more suspended microplastics actively moving in the direction of the water current, filtering collectors, which filter particles from the water column, will be at a higher risk.  Microplastics may clog gills and filtration apparatus of filtering collectors as they attempt to filter particles from the water column. As microplastic particles move with the speed of the water current, microplastic fragments may cause abrasion in soft-bodied macroinvertebrates, while fibres may entangle body parts, such as external gills and limbs.

Another important consideration is that some aquatic macroinvertebrates select different habitats at different stages of their life cycles. For instance, some aquatic invertebrates start life at Reynolds number of about 1-10 in the laminar layer, but when they reach their adult form, they may live in conditions of $Re = 1000$ or higher in the turbulent flow (Rowntree & Wadeson, 1999). The implication is that macroinvertebrates might experience variations in exposure to microplastics at different stages of their lifecycle, depending on the abundance of microplastics and the hydrodynamic condition of the selected hydraulic biotope. The foregoing further suggests that the exposure of macroinvertebrates to microplastics is mediated by the combination of traits possessed and the preferred hydraulic biotopes.

Despite the importance of the TBA, only a few studies have made reference to the role of trait in microplastics research (Berlino *et al*., 2021; McNeish *et al*., 2018). ***Therefore, this study draws on the concepts in river geomorphology, hydraulics, and trait-based ecology to develop an approach to predict the potential exposure of macroinvertebrates to microplastics at the hydraulic biotope scale.***

### 1.10 Rational and significance of the study

Microplastic pollution is currently a global environmental challenge which has impacted almost every natural ecosystem, including riverine systems. Aquatic macroinvertebrates are vital components of riverine ecosystems that provide a link in the transfer of material and energy from producers to top-level consumers and they also act as excellent bioindicators of river health. From the literature review, it is clear that, for a complete assessment of riverine microplastics pollution and the subsequent impacts on aquatic macroinvertebrates, it is important to: i) determine the patchy distribution and transport dynamics of microplastics in riverine systems and develop an approach to predict the potential exposure of macroinvertebrates to microplastic pollution; ii) establish the relationship between microplastics pollution and adjacent land-use types; iii) and establish the relationship between microplastic pollution and physicochemical properties of water.

Given that hydraulic biotopes play a critical role in shaping biological assemblage distribution, and influencing the transport dynamics of microplastics in rivers, an integrated, multi-criteria approach that draws on concepts in river geomorphology, hydraulics and trait-based ecology was employed to better understand riverine pollution and macroinvertebrate disposition to microplastics pollution. The approach involved designing microplastics sampling protocols at the hydraulic biotope scale and incorporating species traits to deepen an understanding of microplastic pollution and its influences on macroinvertebrate community structure and function. Understanding the patchy distribution and transport dynamics of microplastics and how macroinvertebrates are distributed and predisposed to microplastic pollution in riverine systems would provide a solid framework for predicting exposure to microplastics, determining ecological effects and for managing microplastic pollution. This study contributes to this by: i) designing and employing microplastic sampling protocol at the hydraulic biotope scale in two urban rivers, the Swartkops and Buffalo Rivers; ii) determining the relationship between microplastic pollution and anthropogenic land use in the study rivers catchments; iii) determining the relationship between microplastics pollution and physiochemical variables of water in the study rivers; iv) and using the trait-based approach to link hydro-geomorphology of rivers and macroinvertebrate potential exposure to microplastics.

## 1.11 Aim and objectives of the study.

### 1.11.1 Aim

The overall aim of this study was to investigate macroinvertebrates’ distribution and exposure to MPs in riverine systems using a mechanistic and trait-based approach.

The following objectives were set to achieve the overall aim.

### 1.11.2 Objectives

1. To analyse the relationship between land use, selected physicochemical variables and microplastics.
2. To determine the distribution of microplastics in relation to hydraulic biotope types.
3. To use the trait-based approach to examine macroinvertebrate assemblages’ distribution and exposure to microplastics at the hydraulic biotope scale.

## 1.12 Thesis Structure

The structure of the thesis is summarised in Figure 1.2.

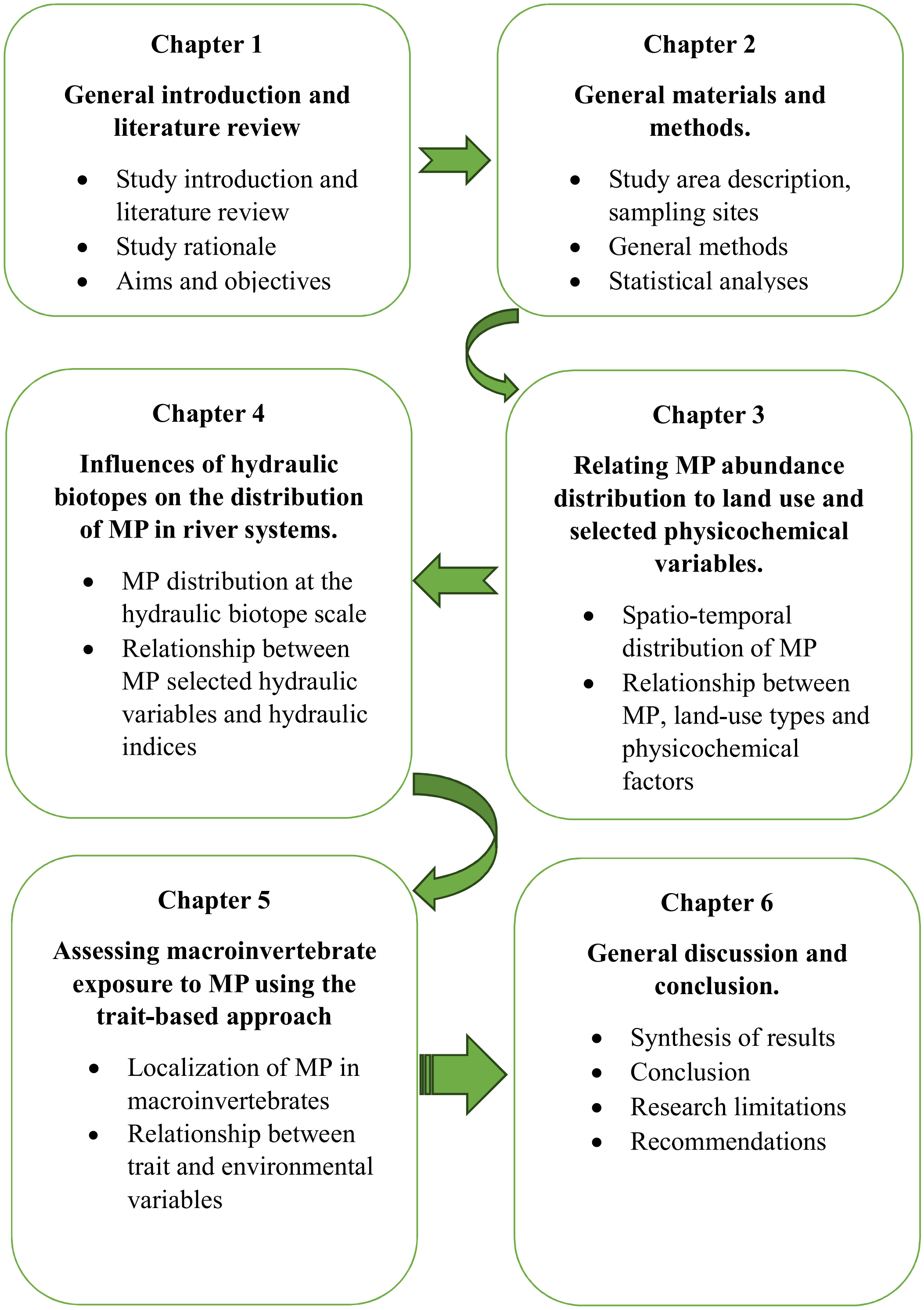


**Figure 1.2.** Flow chart showing the structure of the thesis.

## CHAPTER 2: GENERAL MATERIALS AND METHODS

### 2.1 Introduction

This chapter provides a description of the study area and sampling sites, common methods, procedures, and approaches used in this study. Methods applicable only to specific chapters are described in the relevant chapter(s). The chapter ends with a general description of the statistical methods employed.

### 2.2 Study areas description

The study was conducted in two river systems – the Swartkops and the Buffalo rivers. The Swartkops and Buffalo rivers (Figure 2.1) are both located in the Eastern Cape Province of South Africa and flow through urban catchments.

#### 2.2.1 Biophysical context of the Swartkops River

***General description***

The Swartkops River catchment is about 120 km long and 442 km wide, with a total river length of about 155 km and a total catchment area of 1354 km$^2$ (Quibell & Belcher, 1996; DWS, 1999; Bate *et al.*, 2004). The Swartkops River originates in the foothills of the Groot Winterhoek Mountains and flows across structurally weak and shale-filled synclines in the upper catchment, and through a broad gravel floodplain in the lower catchment (Odume & Muller, 2011; Quibell & Belcher, 1996). The Swartkops River system has two main tributaries, the KwaZunga River to the north and the Elands River to the southwest. The KwaZunga and the Elands rivers form a confluence above Uitenhage in an area called Kruisriver to form the Swartkops River which discharges into Algoa Bay of the Indian Ocean in Bluewater Bay just outside of Port Elizabeth, now called Gqeberha (Quibell & Belcher, 1996; Odume *et al.*, 2012). Two other smaller tributaries, the Brak and Chatty Rivers, which originate in the plains, form a confluence with the Swartkops River below Kruisriver (Odume & Muller, 2011).

***Ecological significance***

The Swartkops River has several ecological and socio-cultural values. The river supports a permanently open, medium-large estuary with moderate aesthetic appeal that is considered the Eastern Cape's most important estuary (Environ-Fish Africa, 2009, 2011). The estuary has the third largest salt marsh in South Africa and is ranked fifteenth in terms of its conservation value (Eviron-Fish Africa, 2009). It is also ranked among the top ten South African estuaries in terms of its bird numbers and species compositions as well as its biogeographical position (Environ-Fish Africa, 2009, 2011). As many as 4000 birds can be present along the estuarine salt marsh during summer, but in winter, owing to the departure of the migrant waders and terns after breeding, the number of birds can drop to less than 1200 (Environ-Fish Africa, 2009, 2011; Odume, 2014). Over 195 bird species have been identified in the Swartkops Estuary, with five species listed as common and nine species listed as being rare, vulnerable, or threatened (Environ-Fish Africa, 2009). Further, the extensive mudflats, shallow creeks and saltmarsh areas in the estuary provide refuge for benthic macrofauna, amphibians, reptiles, and fishes (Nel *et al*., 2015). The estuary serves as a recreational site for swimming, boating, angling, and bait digging while the river supply water for irrigation in the upper catchment. Resources within the Swartkops River catchment are also utilised for church baptism, spiritual rituals and cleansing, and medicinal plant harvesting.

***Topography, geology, and soils***

The dominant topographical features in the catchment are the Groot-Winterhoek, Elands and Zunga Mountains in the west, the Van Stadens Mountains in the south west of the catchment, and in the east, mountain ranges fringed by low-lying coastal plains, which are terraced around an extensive alluvial floodplain and estuary (Quibell & Belcher, 1996). The geology of the Swartkops River catchment is essentially of marine or estuarine origin (Maclear, 1995; Quibell & Belcher, 1996). The upper catchment of the Swartkops River lies in the quartzites of the Table Mountain Group, while its tributary, the Elands River, flows over Bokkeveld Group shales, a region which tends to have well-drained, acidic soils. The Pre-Cretaceous basement, comprising Table Mountain Group quartzites and shales of the Bokkeveld Group, formed a trough into which the Cretaceous shale, mudstones and younger strata, overlaid by marine sedimentary deposits in the upper catchment, and by various alluvial deposits on the flood plains are deposited (Quibell & Belcher, 1996; Odume, 2014). In the upper reaches of the Swartkops, the water is soft, with low mineral content, while below the confluence of the Elands and KwaZunga rivers, the Swartkops has eroded its way into the sedimentary deposits of the Uitenhage Group, which tends to be poorly drained (Quibell & Belcher, 1996; Maclear, 1995). The Cretaceous deposits of the Uitenhage Group underlie the floodplains of the lower region of the KwaZunga, Elands and the Swartkops rivers and comprise coarse, poorly sorted conglomerates interbedded with sandstone and mudstone of the Enon Formation (fluviatile, estuarine, and marine origin), greenish-grey shales and siltstones and sandstones of the Kirkwood Formation (fluviatile origin), and thinly bedded greyish-green mudstones and siltstones of the Sundays River Formation, which are of marine origin (Quibell & Belcher, 1996; Odume, 2014).

The steeply sloping mountainous areas in the upper catchment consist mainly of bare rocks with some shallow soils which have low potential for agriculture (Quibell & Belcher, 1996; Odume, 2014). In the lower catchment, the Uitenhage area is covered by shallow to very shallow soils, which are poorly drained; however, the Swartkops River valley and floodplains are predominantly covered by deep soils suited for agriculture (Quibell & Belcher, 1996; Odume, 2014). Generally, soils in the lower KwaZunga, lower Elands, Brak, Chatty and Swartkops sub-catchments are suited for irrigation. The main soil types in this part of the catchment range from Glenrosa in the lower Elands, Oakleaf and Dundee in the middle Swartkops floodplains and Swartland, Hutton and Mispah in the Upper Swartkops, with soil texture characterized by fine sand, loam and clay of less than thirty percent (Quibell & Belcher, 1996; Odume, 2014).

***Climate***

The climate in the catchment is mostly warm temperate with all months between 6° to 27°C and with at least 60 mm of rain monthly (Bate *et al*., 2004; Odume, 2014). The amount of precipitation varies considerably over the different regions of the catchment area. The average annual rainfall varies between 1000 mm in the upper regions to 500 mm on the dry north-eastern region, while the annual average evaporation for the area is 1700 mm with the highest evaporation rates occurring in the summer months, and mean annual runoff of 84.2 x 106 m$^3$ ( Quibell & Belcher, 1996; Bate *et al*., 2004). The rainfall pattern in the catchment is not strongly seasonal, although the headwaters of the Swartkops River system experience a spring maximum rainfall which decreases towards the coast that falls within a winter rainfall area. Rainfall can be described as evenly distributed throughout the year, although a large proportion of the monthly rainfall often occurs over a few days, which may result in floods (Odume, 2014). The rainfall gradient within the catchment reflects the influences of the topography. The flow pattern in the catchment is characterized by low baseflows, with minor floods and the occasional major floods, while the largest obstruction to river flow in the catchment is the Groendal Dam (Quibell & Belcher, 1996; Bate *et al*., 2004).

***Vegetation***

Mountain Fynbos dominates the upper slopes of the catchment; the lower, northern slopes and plains are covered predominantly by Grassy Fynbos, while Bushveld or Succulent thicket dominates the rest of the lower-lying areas of the catchment (Kleynhans *et al*., 2005). The encroachment of exotic and invader vegetation, such as *Acacia species* (Black wattle) and

*Eucalyptus species* (gum trees) have become prominent in the Swartkops catchment (Quibell & Belcher, 1996; Odume, *et al.*, 2012; Odume, 2014) impacting the integrity of the catchment. Invader aquatic vegetation such as *Eichhomia crassipes* (water hyacinth), *Salvinia molesta* (Kariba weed), and *Phragmites australis* (reed) occur extensively between Uitenhage and Perseverance (Odume *et al.*, 2012; Odume, 2014). The extensive and excessive presence of invader aquatic vegetation impacts streamflow, compromises instream physical habitat integrity, affects recreational activities, and reduces the aesthetic value of the river.

### 2.2.2 Biophysical context of the Buffalo River

***General description***

The Buffalo River has its source in the seeps and sponges of the forested Amatole Mountains at an altitude of 1200 m above sea level (River Health Programme [RHP], 2004). The river is 126 km long and drains a catchment of 1287 $km^2$ (RHP, 2005; Yahaya *et al.*, 2019). It flows eastwards, meandering through Maden Dam, Izele, King William's town, Zwelitsha, Mdantsane and finally discharges into the Indian Ocean at East London harbour (RHP, 2005; Yahaya *et al.*, 2019). The river can be divided into three reaches and contains four conspicuous dams built as an impoundment on the river and sited across the reaches: the upper reaches to King William's Town, which comprise the mountain stream in montane forest down to Maden Dam, and the foothill zone flowing through agricultural land downstream of Rooikrans Dam; the middle reaches, comprising the urban/industrial complex and an area of agricultural land downstream of Laing Dam, and the lower reaches downstream of Bridle Drift Dam (O'Keeffe *et al.*, 1996). The major tributaries of the Buffalo River are Mgqakwebe, Nqokweni and Yellowwoods Rivers; all three join the mainstream above Laing Dam (Carter *et al.,* 2016).

***Ecological significance***

The Buffalo River, like the Swartkops River, is of critical importance in the Eastern Cape Province. It is considered a major water resource in the Eastern Cape Province as it feeds dams such as Maden, Rooikrans, Laing and Bridle Drift (RHP, 2005; Yahaya *et al.*, 2019). The Buffalo River and its dams are a major source of surface water for communities within the river's catchment, including urban, rural, industrial and irrigation consumers (RHP, 2005; Chigor *et al.*, 2013; Yahaya *et al.*, 2019). The Buffalo River also supports one of the essential river estuaries found in the Buffalo City Metropolitan Municipality in East London (Ohoro *et al.*, 2021). The Buffalo River estuary is permanently open and is the only river port in South Africa, also known as the Port of East London (DEA, 2018). Resources within the Buffalo

River catchment, just like in the Swartkops, are also utilised for activities such as church baptism, spiritual rituals, and cleansing. Various recreational activities also take place in the estuary, for example, rowing, yachting, ski boat launching and recreational fishing. Small-scale subsistence fishing and bait collection is practised in the estuary, while the estuary also provides a nursery for fish and invertebrates that require estuaries as part of their life cycle (Department of Environmental Affairs [DEA], 2018). Although subsistence farming predominates, the river provides water for local areas of intensive irrigation which provide fresh produce and other crops (Chigor *et al*., 2013).

***Topography, geology, and soils***

Topographical features in the catchment include the Amatole Mountains, rocky cliffs, and undulating plains. Geologically, the Buffalo River catchment sediment is mainly of marine origin (O'Keeffe *et al.*, 1996; Owolabi *et al*., 2021). Three-quarters of the catchment comprises Tatarian red and grey mudstone of the Balfour Formation while the lower quarter of the catchment grades into the older Kazarian silty-sandstone dominated arenaceous strata of the Middleton Formation (Owolabi *et al*., 2021). The two formations belong to the Beaufort Group and the Adelaide subgroup of the Karoo supergroup (Baiyegunhi *et al.*, 2017). These sedimentary rocks have been intruded by dykes, sills and inclined sheets of Jurassic-age dolerite (Carter *et al*., 2016).

Soils are a grey sandy loam derived from the Beaufort sediments, and red and black clays from the dolerite (Carter *et al.*, 2016; Owolabi *et al.*, 2021). Most of the catchment consists of soils with minimal development owing to the shallowness of the underlying sedimentary bedrock materials and crystalline basement (Carter *et al.*, 2016; Owolabi *et al.*, 2021). Soils with a marked clay accumulation such as Luvisols, Planosols, Solonetz, occur closer to the estuary dolerite (Carter *et al.*, 2016; Owolabi *et al.*, 2021). Most of the catchment has a high concentration of dissolved salts as a consequence of the sediment being of marine-origin dolerite (O'Keeffe *et al*., 1996; Carter *et al.*, 2016; Owolabi *et al.*, 2021).

***Climate***

The Buffalo catchment can be divided into three climatic zones: the mountainous, high-rainfall upper catchment, the lower rainfall middle reaches, and the coastal rainfall zone (O'Keeffe *et al.,* 1996). The catchment has a warm and temperate climate. Temperatures are moderate in the coastal zone, between 8 – 39 $^{o}$C, with a warm mean annual value of 21 $^{o}$C. Inland temperatures vary between -2 and 42 $^{o}$C with a mean annual value of 18 $^{o}$C (RHP, 2005; Chigor *et al*., 2013).

The catchment receives mainly summer rainfall which is approximately double that of winter. Mean annual rainfall for the whole catchment is 736 mm, ranging from 500 to 1000 mm (O'Keeffe *et al.*, 1996; Chigor *et al.*, 2013). The upper zones provide 40% of the runoff for the whole catchment. Evaporation rates are 160-170 mm per month in December and January and reduce to 70 mm during June and July (O'Keeffe *et al.*, 1996).

***Vegetation***

The natural vegetation consists of False Macchia (Fynbos) at the top of the Amatole Mountains, Afro-montane Forest on the slopes of the mountains, False Thornveld are found in the middle catchment, valley Bushveld in the immediate river valley, while coastal forest and Thornveld occur the lower reaches (O'Keeffe *et al.*, 1996; Department of Water Affairs and Forestry [DWAF], 2004). However, a large proportion of the Buffalo River Catchment has been transformed (almost 17% is degraded thicket and grassland) and there is now little of the natural vegetation remaining except in the upper catchment, and in the protected coastal forests (DWAF, 2004; RHP, 2005). The lower reaches downstream of Bridle Drift Dam comprise coastal forest and the East London harbour (Carter *et al.,* 2016). At the headwater of the river, alien plantations reduce the runoff and impact river flow (DWAF, 2004; RHP, 2005).

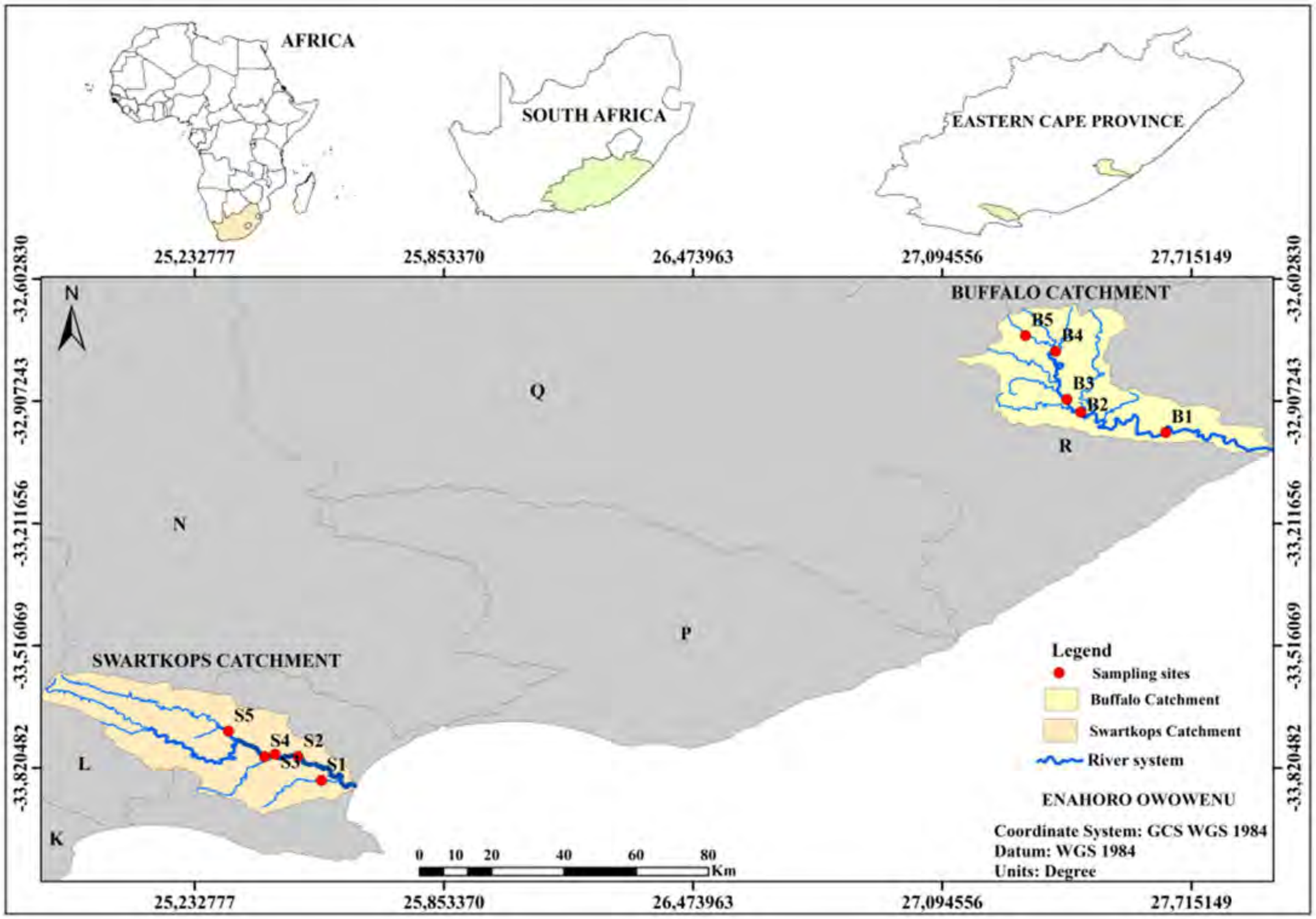


**Figure 2.1.** Map of the study areas showing the location of the Swartkops and Buffalo rivers

### 2.2.3 Social-economic contexts of the Swartkops and Buffalo river catchments

***Population and demography***

The Swartkops River catchment has a population of about 1.2 million people and it includes the most populous city in the Eastern Cape, Port Elizabeth (now called Gqeberha), the industrial city of Uitenhage, residential towns of Despatch and Perseverance, Motherwell, Zwide, and KwaNobuhle (Odume *et al.*, 2022). Within the catchment, poverty is a major challenge and is spatially distributed as some households in rural areas of the catchment depend on less than USD 300 per month compared to households in the Gqeberha area with much higher household income (Odume *et al.*, 2022). Population growth within the catchment has been implicated as an important contributor to ecological degradation in the catchment (Odume *et al.*, 2012)

The Buffalo River catchment has one of the highest population densities in the Eastern Cape with approximately 642,000 inhabitants (Carter *et al.*, 2016). The catchment has the second largest township (Mdantsane) in the country and the population density is highest in the middle and lower reaches of the catchment (Carter *et al.*, 2016). The largest towns within the catchment are East London, Bhisho, King William's Town, Zwelitsha and Mdantsane. Expanding populations and townships put pressure on water and waste systems, which cannot cope with the demand, resulting in an overloaded WWTW which can spill effluent that is not properly treated into the river. The upper catchment upstream of Laing Dam is faced with pressures from the dense rural population which can lead to increased solid waste dumps on riverbanks, increased mining activities in the riverbed that can destroy natural habitats, cattle crossings and overgrazing which destroy the riparian zone, and eroded riverbanks that form gullies.

***Industrial and urban activities***

In the Swartkops River, the upper catchment area of the Swartkops is a predominantly natural area, with irrigated agriculture, becoming highly urbanized in the lower catchment area of Gqeberha, Despatch and highly industrialized at Uitenhage. The economy in the catchment is primarily oriented towards automotive assembly, manufacturing and export industries including textile and clothing works, food and beverage, chemicals, metal and metal products, machinery, and electrical machinery. Situated around the Swartkops Estuary is the Coega industrial area which includes tanneries, freight and container transportation facilities, foundries, metal recyclers, material testing laboratories, automotive repair facilities and manufacturers of different plastic polymers such as polystyrene, rigid polyurethane, foam, an pellets (Mmachaka *et al*., 2023). The lower catchment is known for many blue flag beaches along the urban coastline of Gqeberha which serves as a popular international and local holiday destination.

In the Buffalo River catchment, the coastal zone is commercially important for tourism, fishing, and related activities. Agriculture along the coastal plain is extensive and overgrazing is common which leads to erosion and increased sediment loads. Community, social and personal services are the main source of employment, followed closely by manufacturing, trade, private households, financial, construction, transport, and communication and then agriculture, forestry, and fishing. Extensive urban development dominates the Buffalo catchment upstream of Laing Dam. A textile factory which discharges its waste into the river and releases from WWTW aggravated by the high population densities put increasing pressure on the river. The Buffalo Estuary also houses a harbour port. Agriculture is widespread in the middle reaches of the catchment, predominantly in the form of subsistence farming of goat, cattle and sheep (Carter *et al*., 2016).

***Anthropogenic influences on the Swartkops and Buffalo rivers***

The Swartkops and Buffalo river catchments experience varying levels of anthropogenic pressures such as flow modifications, urban and agricultural pollution, and alien invasive plants (Omarjee *et al*., 2021). In the Swartkops River, the upper catchment lies within the pristine, inaccessible area of the Groot Winterhoek Mountain. The lower catchment is subject to different sources of pollution including discharges from wastewater treatment works (WWTW), effluents from tanneries, wool processing factories, run-off from informal settlements, surrounding road networks, industrial sites and stormwater canals (Odume, *et al*., 2012; Odume & Muller, 2011). Waste water treatment works, industrial effluents and run-offs from informal settlements have all been reported as potential sources of microplastics pollution in rivers (Apetogbor et al., 2022; T. Wang et al., 2021). Near Uitenhage and Despatch there are considerable residential and industrial developments, related predominantly to the wool and motor industries, and industries concerned with extractive processes. Below Despatch are privately owned farms and land where irrigation farming, cattle and dairy farming are carried out.

Poor waste management in informal settlements leads to the dumping of solid and other waste materials close to the river. Increasing human activities in the Swartkops catchment have resulted in the discharge of pollutants into the river (Odume *et al.*, 2012). Discharges from the Kelvin Jones, KwaNobuhle and Despatch wastewater treatment works are a major source of faecal contamination in the lower catchment. The wool-washing factory upstream of Uitenhage, the sewage outfall near Nivens Bridge, the Uitenhage sewage works outfall near De Mist Bridge, and the tannery at Perseverance are major sources of pollution that can introduce microplastics and other contaminants into the system. Diffuse sources of pollution come mainly from agricultural activities and run-off from informal settlements, surrounding roads and stormwater canals carrying untreated wastewater. Other diffuse sources come from litter from urban settlements and inputs from tributaries, such as the Chatty River, which drains a highly congested informal settlement in its lower catchment just before it joins the Swartkops.

In the Buffalo River catchment, the four dams on the river have no mechanisms for releasing water in a controlled pattern, thus obstructing natural river flow to maintain functioning aquatic ecosystems (RHP, 2005). River flow is mostly augmented by inflows from tributaries. The river is almost pristine at its source but owing to its proximity to the urban-industrial complex of East London and King Williams Town, there is extensive reliance on the river for various purposes; consequently, the quality and quantity of the river water is impacted. The greatest anthropogenic input into the river is the discharge of effluent from WWTW and industries around Zwelitsha, King Williams Town and East London. Inputs from a textile factory around Zwelitsha, overflow from sewers of Mdantsane and solid waste dumps resulting from inadequate disposal sites in some parts of the catchment further contribute to the pollution of the river (Yahaya *et al.*, 2019). The lower catchment of the river receives treated domestic and industrial effluent, while the immediate catchment of Bridle Drift Dam receives domestic effluents from four small tributaries (RHP, 2005).

There are at least nine different WWTW in the Buffalo River catchment discharging effluents directly or indirectly (via major tributaries like Ngqokweni and Yellowwoods rivers). Consequently, major water quality concerns such as eutrophication in Laing and Bridle Drift Dams, and faecal contamination persist. Both municipal and industrial effluents are major sources of microplastics inputs into river systems (Bayo *et al.*, 2020; Shekoohiyan & Akbarzadeh, 2022). Other activities that impact the river include excessive use of riparian trees for firewood, sand mining in the riverbeds, cattle crossings and overgrazing, poor management practices associated with subsistence farming, and solid waste dumps on riverbanks. High populations and expanding townships put further pressure on water and waste systems which can result in blockages in the sewerage systems, inadequate treatment capacity and poor management, all of which ultimately result in the discharge of partially treated and untreated sewage into the river and dams (RHP, 2005).

Generally, across the Swartkops and Buffalo river catchments, several man-made obstructions modify the natural flow of the rivers. The Groendal Dam on the Swartkops River with a storage capacity of about 12 x $10^6$ $m^3$ and the four dams on the Buffalo River, are the largest obstructions in terms of size and potential effects on downstream flow (Bate *et al.*, 2004; RHP, 2005; Odume, 2014). Anthropogenic activities (e.g., industrial, domestic, and agricultural) within the Swartkops and Buffalo river catchments are sources of microplastics and other wastes that can impact the river quality and quantity with consequences for both the aquatic organisms and human population that depend on these rivers.

### 2.2.4 Sampling sites

The study was conducted seasonally at ten selected sampling sites over a period of one year, beginning in spring, October 2021 and ending in winter, July 2022. The selected sites are situated in the Buffalo River, the Swartkops River and the Swartkops tributary, the Chatty River. Five sites were selected in the Buffalo River, four in the Swartkops River and one downstream of the Chatty River before it joins the Swartkops. In the Swartkops River (Figure 2.2), the sites were coded S1 to S5 (i.e., Site S1 - S5) to include the single site in the Chatty River, while in the Buffalo River (Figure 2.3), the sites were coded B1 to B5 (i.e., Sites B1 – B5). Sites were situated in the upper, middle, and lower reaches of the Swartkops and Buffalo rivers to monitor the dynamics of microplastic transport and the potential impacts of the range of anthropogenic activities within the catchments. The site located in the Chatty was to monitor inputs into the Swartkops as the Chatty drains a highly clustered informal settlement, with reports indicating high levels of pollution because of direct dumping on the riverbank and improperly treated effluent outflow. Other considerations for site selection were accessibility and safety concerns.

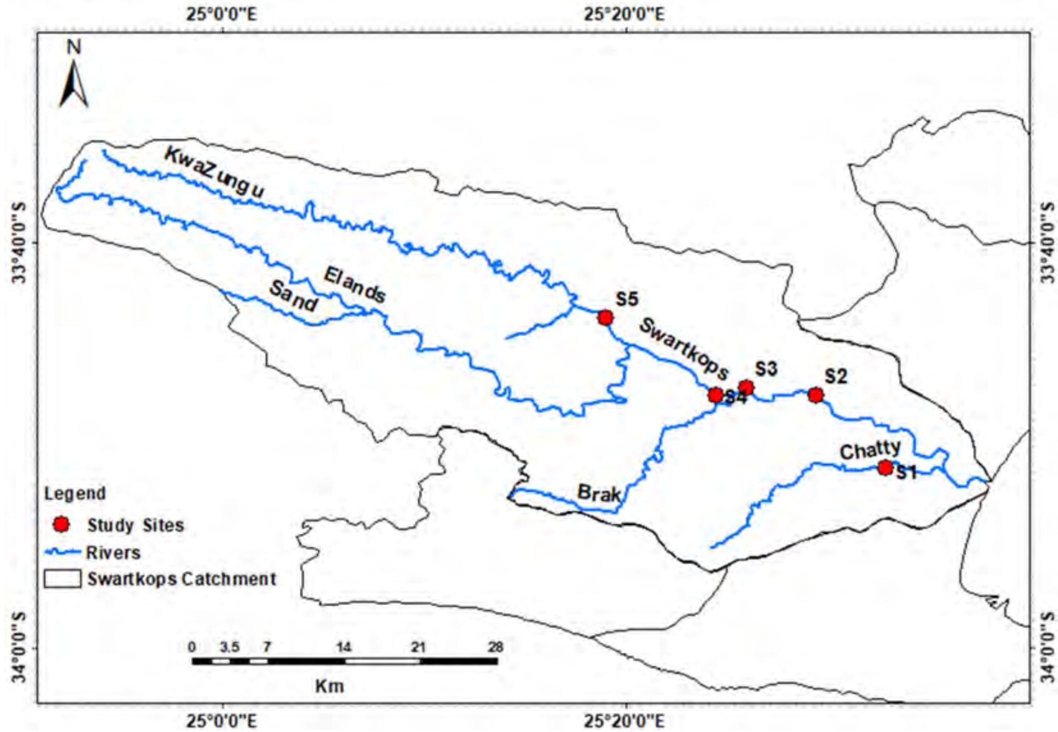


**Figure 2.2.** The Swartkops River Catchment showing the study sites, the Swartkops and its tributaries.

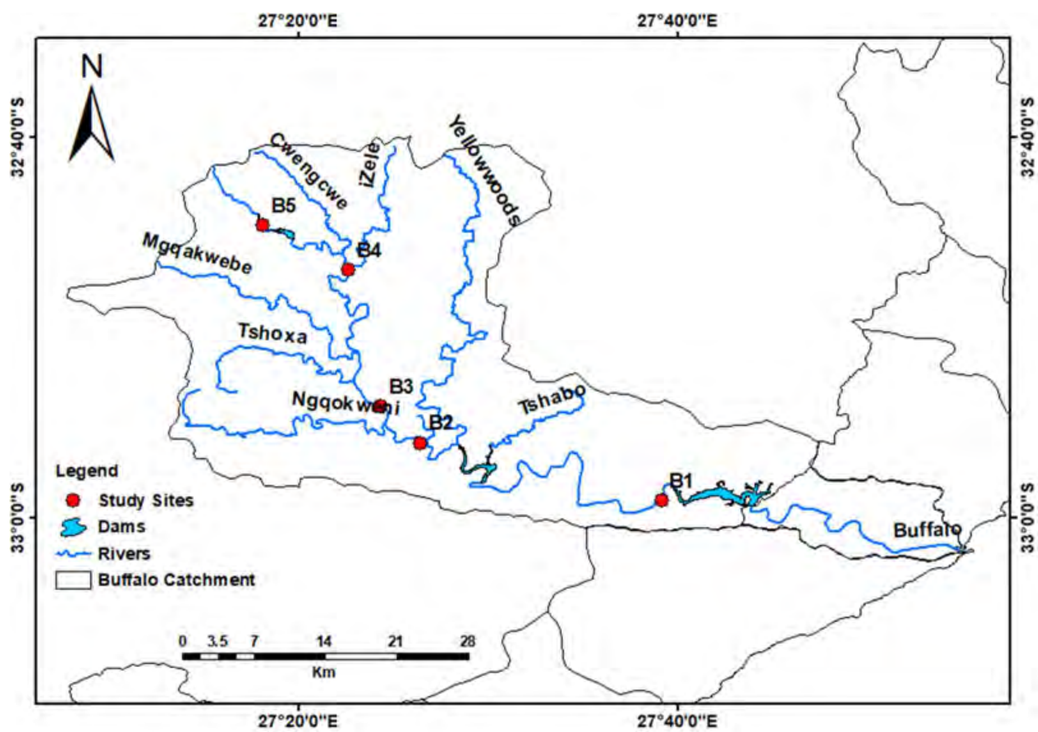


**Figure 2.3.** The Buffalo River Catchment showing the study sites, the Buffalo, and its tributaries.

Site S1 (33°51′04.88″S, 25°32′54.79″E) is in the lower reaches of the Chatty River (Figure 2.4), in an area surrounded by a densely clustered informal settlement close to Chelsea Road and Soweto-on-Sea. There is evidence of improper waste disposal and waste management practices in the area as solid waste materials, including plastic litter, are visible in the riparian areas and the vicinity of the site. Other impacts include run-off from surrounding roads and human settlements. There is evidence of habitat modification on the riparian zone and adjacent land resulting from sand mining. Both marginal and aquatic weeds were also visible at this site.

**Figure 2.4.** Site S1 in the Chatty River at Soweto-on-Sea showing the different hydraulic biotopes.

Site S2 (33°47′32.28″S, 25°29′26.88″E) is located close to Despatch and downstream of site S3 (Figure 2.5). Surrounding impacts include discharge from the Kelvin Jones WWTW, run-off from roads, livestock farming and other agricultural activities as well as sand mining in its riparian zone. The site was covered with water hyacinth and other aquatic weeds during the sampling period. Normal flow at this site is modified by the presence of dense instream vegetation.

**Figure 2.5.** Site S2 in the Swartkops River located close to Despatch showing the different hydraulic biotopes.

Site S3 (33°47′11.40″S, 25°26′00.06″E) is located within the industrial city of Uitenhage, where surrounding impacts include industrial and wastewater discharges, run-off from road and an abandoned rail network. The site is downstream of the effluent discharge point from the Kelvin Jones WWTW at Uitenhage. This site is also covered by water hyacinth and other aquatic weeds with evidence of riparian zone modification (Figure 2.6).

**Figure 2.6.** Site S3 in the Swartkops River located at Uitenhage, downstream of the Kelvin Jones WWTW, and showing the different hydraulic biotopes.

Site S4 (33°47′31.52″S, 25°24′27.29″E) is also located in the industrial city of Uitenhage. This site is subjected to impacts from livestock farming and other agricultural practices, run-off from roads and informal settlements. There is evidence of moderate habitat modification on the riparian zone and overgrazing. The site is located upstream of the discharge point of the Kelvin Jones WWTW. There is evidence of riparian zone modification (Figure 2.7).

**Figure 2.7.** Site S4 in the Swartkops River located at Uitenhage showing the different hydraulic biotopes.

Site S5 (33°43′44.26″S, 25°19′02.33″E) is situated upstream of Uitenhage. The water is cleaner than at other sites, but there is serious and extensive habitat modification and degradation of the riparian zone due to gravel mining activities. Trucks and other heavy machinery used for lifting and transferring gravel from the quarry have been used to form a pathway across the river with excessive obstruction of the natural flow of the river caused by mounds of gravel (Figure 2.8).

**Figure 2.8.** Site S5 in the Swartkops River located upstream showing the different hydraulic biotopes.

Site B1 (32°59′05.70″S, 27°39′04.84″E) is located at Postdam, upstream of the Bridle Drift Dam. The Bridle Drift Dam is the largest dam and obstruction to the natural flow of the river. The Laing Dam is located some kilometres upstream of this site. Surrounding impacts at this site include run-off from an informal settlement, road networks, agricultural practices including livestock farming. Livestock can be seen at this site grazing freely on the riparian zone and the surrounding bushes. Riparian zone habitat has been modified by sand and gravel mining (Figure 2.9).

**Figure 2.9.** Site B1 in the Buffalo River located at Postdam, showing the different hydraulic biotopes.

Site B2 (32°56′03.86″S, 27°26′25.16″E) is situated at Zwelitsha. A causeway across the river width at this site modifies the river flow (Figure 2.10). Surrounding impacts include run-off from human settlements, road networks and livestock farming. The site is situated upstream of the discharge point of the WWTW at Zwelitsha. Both the confluence of the Buffalo and the Yellowwoods River and the Liang Dam are located downstream of this site.

**Figure 2.10.** Site B2 in the Buffalo River located at Zwelitsha showing the different hydraulic biotopes.

Site B3 (32°54′08.60″S, 27°24′18.47″E) is situated downstream of the discharge point of the WWTW at Schornville. Surrounding impacts include industrial and wastewater discharges, run-off from road and human settlements (Figure 2.11).

**Figure 2.11.** Site B3 in the Buffalo River located at Schornville showing the different hydraulic biotopes.

Site B4 (32°46′57.83″S, 27°22′37.82″E) is located downstream of the Rooikrans Dam. Surrounding impacts include run-off from informal settlements, roads, livestock farms and other agricultural activities. The riparian zone is slightly modified by overgrazing of free-roaming livestock and human settlers. There are two causeways at this site which greatly impede the flow of water and obstruct the natural course of the river water flow. Habitat on the riparian zone is slightly modified because of sand mining and overgrazing by free-roaming livestock (Figure 2.12).

**Figure 2.12.** Site B4 in the Buffalo River located downstream of Rooikrans Dam and showing the different hydraulic biotopes.

Site B5 (32°44′41.10″S, 27°18′02.05″E) is situated upstream of Rooikrans Dam and downstream of Maden Dam and the nature reserve area. Surrounding impacts include run-off from road and footpaths created by farmers and other surrounding settlers. There is evidence of erosion washing off sediments into a portion of the site. The riparian zones seem undisturbed except for free-grazing livestock constantly trampling the vegetation around the riverbank and settlers cutting down trees for various purposes (Figure 2.13).

**Figure 2.13.** Site B5 in the Buffalo River located upstream of Rooikrans dam and showing the different hydraulic biotopes.

### 2.3 Hydraulic biotopes delineation

At each of the selected study sites, two hydraulic biotopes, herein referred to as 'Flush' and 'Sink' hydraulic zones were visually identified and later characterized using appropriate hydraulic and flow measures and indices (e.g., flow types and substrate compositions) (Tables 2.1, 2.2 and 2.3). The flow type is believed to reflect the complex hydraulic conditions in the water column while the substrate type determines bed roughness which directly impacts on flow type and determines the amount and type of cover available to aquatic fauna (Wadeson & Rowntree, 1998; Thomson *et al.*, 2001). However, because the focus of this study was to understand how ecohydraulics impact microplastic distribution and mediate organismal exposure to microplastics in their preferred hydraulic environment, it was important to collapse the varieties of hydraulic biotopes (e.g., riffles, runs, rapids, pools, backwaters etc.) into two distinct conceptualized forms as indicated earlier in this section as 'Flush' and 'Sink' hydraulic zones.

In this study, flush hydraulic zones typically represent fast flowing hydraulic biotopes such as rapids, runs, riffles, while the sink hydraulic zones represent slow flowing to still biotopes such as pools and backwaters. Physical measurements of water depth and water velocity along predetermined cross sections were further used to effectively classify the hydraulic biotopes into the two distinct ecologically relevant hydraulic sampling zones per site (Jowett, 1993). These hydraulic zones were then characterized by relevant hydraulic indices such as Froude number (Fr), Reynolds number (Re), and shear velocity (U*) that describe the flow conditions at the water surface, water column and stream bottom conditions, respectively (Statzner *et al.*, 1988; Newson & Newson, 2000). The roughness Reynolds number (Re*) and a fifth index, the velocity depth ratio (VDR), were also calculated. The flush zones were conceptualized as hydraulic biotopes with potential for high MP remobilization and suspension within the water column, while the sink zones were conceptualized as hydraulic biotopes with potential for high particle deposition and settling. In this study, the conceptualized flush and sink zones were collectively referred to as hydraulic zones. The macroinvertebrate and MP samplings were accordingly designed following the flush and sink zone logic per site.

**Table 2.1.** Classification of flow types used to define hydraulic zones in this study (after Wadeson and Rowntree, 1998).

| **Code** | **Flow types** | **Definition** |
|---|---|---|
| FT1 | No flow / standing water | No water movement except during high flows. |
| FT2 | Barely perceptible flow | Smooth surface appears stationary, flow only visible though the movement of suspended materials. |
| FT3 | Smooth surface flow | The water surface remains smooth; streaming flow takes place throughout the water profile with very small turbulent flow cells visible as the upward movement of fine suspended matter. |
| FT4 | Upwelling / boil | The direction of flow is predominantly vertical, with secondary flow cells visible at the surface as boils or strong circular horizontal eddies. |
| FT5 | Rippled flow | Surface water has regular disturbances which form low symmetrical ripples that move across the direction of flow. |
| FT6 | Undulating standing waves | Undular standing waves form at the surface facing upstream without breaking. |
| FT7 | Broken standing waves | Standing waves present which break at the crest facing an upstream direction. |
| FT8 | Chaotic flow | Complex mixture of uninterrupted fluctuating flow types connected with unsteady, energetic flow common at high flows. |

Hydraulic variables measured along each cross-section were averaged for general habitat comparisons between the hydraulic zones. To provide more information about each hydraulic zone, other parameters such as presence of macrophytes and woody debris, organic matter, et cetera were visually assessed.

**Table 2.2.** Parameters used to measure the characteristics of each hydraulic zone along a reach (based on Thomson *et al*., 2001)

| Parameters measured | Method of measurement |
|---|---|
| ***Dimensions*** | |
| Maximum width and length | Maximum width and length of each hydraulic zone obtained using a standard measuring tape. |
| ***Hydraulic variables*** | |
| Flow type | Surface flow type based on Table 2.1 |
| Velocity (depth average) | Measured with a Flo-mate flowmeter at 0.6, 0.4 or 0.2 depth for 20 seconds. |
| Depth | Average water depth measured using a calibrated depth stick |
| Roughness height | Mean height of roughness elements above riverbed. Averaged for all clast within a $0.5m^2$ grid of each point of velocity mesurement. |
| ***Substrate*** | |
| Substrate class | Dominant substrate based on Table 2.3 |
| Substrate sorting | Visually estimated using standard grain size analyses |
| Substrate packing | Categorised: 1= packed and armoured; 2 = packed but not armoured; 3= moderate compaction; 4 = low compaction; 5 = no packing |
| ***Aquatic vegetation*** | Percentage cover of emergent, floating, submerged, algae and moss types using visual estimates |
| ***Organic matter*** | |
| Multiple and single logs | Number of pieces per hydraulic zone |
| Twigs and leaves | Percentage cover per hydraulic zone |
| ***Derived indices*** | |
| Froude number (Fr) | Calculated for each hydraulic zone, using: $Fr = u /(gD)^{0.5}$ |
| Reynolds number (Re) | Calculated for each hydraulic zone, using: $Re = uR/V$ |
| Shear velocity (U*) | Calculated for each hydraulic zone, using: $U^* = u / 5.75 \log(12D/k)$ |
| Roughness Reynolds (Re*) | Calculated for each hydraulic zone, using: $Re^* = U^* k /v$ |
| Velocity depth ratio (VDR) | Calculated for each hydraulic zone, using: $VDR = u / D$ |

S, grain size (mm); D, water depth (m); g, gravitational constant ($m\ s^{-2}$); k, roughness height; R, hydraulic radius; u, depth average velocity ($m\ s^{-1}$), V, kinematic viscosity of water; v, kinematic viscosity of water at 20ºC

## 2.4 Hydraulic biotopes characterisation

To characterise each sampling zone (i.e., flush and sink hydraulic zones), a tape measure was strung between two fixed points in each hydraulic zone along the river channel. At two metre intervals, velocity measurements were taken along the cross-section at 0.6 m depth below the water surface, but in some cases at 0.4 m and 0.2 m depth. At each point of velocity measurement, water depth above roughness elements (substratum) was recorded (Wadeson & Rowntree, 1998). The variations in the depth at which velocity measurements were taken was due mainly to the problems of shallow water and highly variable bed topography in the study rivers. A description was made of substratum type based on Wentworth (1922) and Blott & Pye

(2001) (Table 2.3). An estimation of percentage cover of substratum within a 0.5 $m^2$ grid of each point of velocity measurement in the cross-section was carried out (Thomson *et al.*, 2001). Substrate composition was based on a visual assessment of the distribution of substrate particle size classes, that is, the proportion of silt, sand, pebbles, cobbles, boulders, bedrock (Thomson *et al.*, 2001). The b axis length of each clast found within the 0.5 $m^2$ grid was measured. Roughness height (k) was equal to the mean height of clearly defined substrate elements within the 0.5 $m^2$ grid and was taken from a datum equal to the lowest point that protruded above the riverbed (Thomson *et al.*, 2001; Wadeson & Rowntree, 1998). These data together with the temperature readings were used to calculate the Froude number (Fr), Reynolds number (Re), roughness Reynolds number (Re*), Shear velocity (U*), and the velocity depth ratio (VDR) for each hydraulic zone. The type and range of data derived from field assessments for each hydraulic zone are present in Chapter 4.

**Table 2.3.** Substrate classification according to grain size identified for the hydraulic zones (Wenthworth, 1922; Blott & Pye, 2001)

| Code | Substrate class | Particle diameter (mm, b-axis) |
|---|---|---|
| S1 | Clay and silt | < 0.06 |
| S2 | Coarse sand | 0.50 – 1.00 |
| S3 | Fine granules, granules | 2.00 – 4.00 |
| S4 | Large granules, small pebbles | 4.00 – 8.00 |
| S5 | Small pebbles, pebbles | 8.00 – 32.00 |
| S6 | Pebbles, small cobbles | 32.00 – 64.00 |
| S7 | Small cobbles, medium cobbles | 64.00 – 128.00 |
| S8 | Large cobbles | 128.00 – 256.00 |
| S9 | Small boulders | 256.00 – 512.00 |
| S10 | Medium boulders, | 512.00 – 1024.00 |
| S11 | Large boulders | 1024.00 – 4096.00 |

It is important to note that the collection of several velocity profiles within the water column is preferred to the six-tenths depth method. Unfortunately, limited depth at many points (less than 0.75 m) together with numerous sub-surface flow obstructions did not allow the collection of velocity profiles in this study. To standardize the data collection technique, a single velocity reading was taken at each point of velocity measurement. Flow velocities were measured using the Flo-mate flowmeter (Marsh-McBirney Model 2000); water depth was measured using a calibrated depth pole.

## 2.5 Measuring water physicochemical variables

Before MP and macroinvertebrate sampling, water physicochemical variables were measured at all sites at each hydraulic zone for each sampling event. Sub-surface dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature and pH were measured *in situ* using the Hanna multiparameter probe, model HI 9828.

To analyse for nutrients, nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), ammonium-nitrogen ($NH_4$-N), orthophosphate-phosphorus ($PO_4$-P), and total suspended solids (TSS), water samples were collected from the midpoint of each hydraulic zone in acid-washed 250 ml plastic bottles. Water samples were collected facing the flow direction. Bottles were capped under water to avoid air spaces. Water samples were transported to the laboratory in an ice-filled cooler box and preserved at 4 $^{o}$C until chemical analysis. Nutrient and TSS analyses were undertaken within 24 hours of sample collection.

Nutrients, nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), ammonium-nitrogen ($NH_4$-N), orthophosphate-phosphorus ($PO_4$-P) were analysed using the Palintest Photometer 7500 Bluetooth, in accordance with the manufacturer's recommendations and instructions. Total suspended solids (TSS) were analysed according to standard method 2540D (APHA 2005) and method 160.2 (residue, non-filterable) (USEPA, 1983). All chemical analyses (e.g., nutrient and TSS), were undertaken in triplicate and averaged. This was done to obtain representative samples and reduce sample variability.

## 2.6 Suspended and settled microplastics sampling.

To sample for suspended microplastics, 12 L water samples were collected in three replicates of 4 L at each hydraulic zone per sampling site. Surface water samples were collected prior to settled microplastic sampling to avoid collecting settled MP (Osorio *et al.*, 2021; Yano *et al.*, 2021).

To sample settled microplastics from the riverbed, the disturbance technique used for sampling sediments in lotic freshwaters was adapted for sampling settled microplastics (Collins *et al.*, 2007; Jones *et al.*, 2012; Duerdoth *et al.*, 2015). Here, an open-ended, cylindrical bucket with known dimensions (diameter 0.48 m; height 0.75 m) was carefully inserted into an undisturbed patch of the water column to reach the riverbed. A wooden pole 0.70 m long was then used to agitate the stream bed vigorously for approximately one minute to remobilize microplastics that had been deposited, settled, or embedded on the riverbed. A 4 L mixture of remobilised sediment and water was then immediately collected from within the bucket while the water was still vigorously in motion. This procedure was repeated across the cross-section for each hydraulic zone to obtain three replicate samples per hydraulic zone. Water samples were then transported to the laboratory for further processing.

The method applied to sample settled microplastics was best suited for the objective of this study as it affords a comparison of both settled and suspended microplastics across the different flow regime. The method also enabled a wider area of the site to be sampled which is advantageous since the distribution of microplastics in sediments could be very low or heterogeneous in some areas (Nuelle *et al.*, 2014). It is important to note that the water level in most of the sites was less than the height of the open-ended bucket used for sampling. Additionally, a relatively small amount of sediment was contained in sample, thus, it was both easier to process and cost effective, compared to grabbing of sediments. Settled microplastics (i.e., microplastics that settle on sediments) were thus reported as microplastics per litre. This method of reporting settled microplastics was to ensure that comparisons can be made between results obtained for suspended particles and settled particles. Overall, the sampling strategy employed in this study was designed to provide perspectives on the occurrence of microplastics across different hydraulic biotopes.

**2.7 Sample preparation and processing**

In the laboratory, the extraction of suspended microplastics from the water samples followed a method adapted from Masura *et al.* (2015). Replicate samples were pulled and analysed as composite samples. Each 4 L sample brought into the laboratory was filtered through a 63 µm stainless steel sieve. The filtered residues from the metallic sieve were then transferred into a 500 ml glass beaker using a spatula and minimal rinsing with a squirt bottle containing milli-Q water. The glass beakers containing residues were oven-dried at 50°C for 24 hours or longer in some instances for sample dryness. Prior to drying the samples, any visible MP were transferred using precleaned forceps, into a well-labelled petri dish covered with aluminium foil. 20 mL each of aqueous 0.05 M Fe (II) solution and 30% hydrogen peroxide solution ($H_2O_2$) were added to the beakers containing residues to digest the organic matter. Density separation was undertaken using NaCl (6.0 g per 20 mL of sample). The supernatant collected was subsequently filtered through a vacuum pump system with a 0.45 µm pore size glass microfibre filter paper (GF/F, 47 mm Ø, Whatman). Where the digestion of a sample was deemed sufficient (i.e., where very little or no visible organic matter remained after digestion), the density separation step was skipped to reduce the amount of NaCl used. The filter paper was immediately transferred into a glass Petri dish and placed in a drying chamber (T. Wang et al., 2021). The dried sample was preserved at 4 ºC until microscopic analysis. The use of NaCl for the density separation of suspended microplastics in this study was deemed sufficient as most microplastics found in suspension would have a density lower than that of NaCl, while denser particles found in suspension must have undergone some degradation process which could have altered their densities, enabling them to remain suspended in the river water.

The extraction of settled microplastics from the water mixture followed a modified method used by Nuelle *et al*. (2014)**.** The method utilized a combination of a lower density salt (NaCl) and a higher density salt (NaI) in a two-extraction step to recover microplastics. An advantage of using the combination of two different density separating salts was to ensure that high density microplastics that usually settle in sediments are recovered while using relatively smaller quantities of the high-density salt since the sample mass is usually reduced in the first extraction step where the lower density salt is applied. The 4 L samples were allowed to stand undisturbed for 1 hour to allow sediment and other higher density particles to settle to the bottom. The top liquid part of sample was decanted and filtered through a stainless-steel sieve with 63 µm mesh, leaving behind the solid portion. The residual particles on the sieve were transferred into a well-labelled glass beaker and treated as described for suspended microplastics. The solid portion that remained after the top liquid portion had been decanted was covered with aluminium foil and oven-dried at 50ºC for 48–72 hours. The sample was treated with 30% $H_2O_2$ solution for 24 hours at 50 ºC before density separation.

In the first extraction step, 500 ml saturated NaCl solution (26% weight/weight) with a density of 1.2 g/cm$^3$ (Nuelle *et al*., 2014) was added to samples and the solution stirred continuously with a glass rod for at least 1 minute and allowed to stand undisturbed for 2 hours. The supernatant containing floating particles was transferred into another beaker. The sample was then refilled with more saturated NaCl solution. This process of refilling, stirring, sedimentation and decantation was repeated thrice. In the second extraction step, 60% (weight/weight) NaI solution was added to the contents and stirred continuously for 1 minute and allowed to stand for 2 hours. Floating particles were drained off and all supernatant containing floating plastics were vacuum filtered through a 0.45 µm glass fibre mesh. Filter papers were dried and preserved as described earlier in this section. All safety precautions were adhered to in the use and disposal of NaI as specified by the manufacturer (Minema Chemicals, Johannesburg, South Africa).

**Figure 2.14.** Samples prepared for digestion (left) and digestion on magnetic stirrer (right)

To avoid background contamination of samples, the following preventive measures were taken during sampling and extraction procedures: before sampling, all tools were washed with pre-filtered milli-Q water to avoid cross-contamination between sampling zones and sites. All materials and vessels used for the first time and between samples in the laboratory were thoroughly washed with a phosphate-free soap and thoroughly rinsed in pre-filtered milli-Q water to ensure all soap was removed. All vessels were covered with aluminium foil immediately after washing. All materials and vessels were covered with aluminium foil after each individual step in the laboratory. The work surfaces for microplastic extraction and microscopic analysis were regularly wiped with milli-Q water and 70% ethanol. Only glass or stainless-steel tools were used during sample handling in the laboratory (Nuelle *et al*., 2014; Crew *et al*., 2020).

To reduce errors due to contamination of samples during microplastic processing in the laboratory, both procedural and contamination blanks were set up and underwent the full laboratory procedure (McCormick *et al*., 2016). The contamination blanks, which quantified environmental microplastics present within the laboratory, where obtained by placing wet glass microfibre filters on the work benches while procedural blanks were run in parallel to environmental samples processing after every 10 samples to account for any potential cross-contamination during extraction procedures. The total amount of  microplastic extracted from environmental samples was adjusted by subtracting the number of microplastics observed in contamination blanks (Crew *et al*., 2020; Apetogbor *et al*., 2022).

### 2.8 Quantification and characterisation of microplastics

The dried filter papers were examined for MP under a light microscope followings keys in Hidalgo-Ruz *et al*. (2012) and Wang *et al*. (2021), for example, particles possessing unnatural colouration and shape, perfectly spherical particles, et cetera. The suspected microplastics were identified, captured, and processed using a light microscope (Olympus SZX16) fitted with a digital camera (Olympus DP72) and connected to image-processing software (Stream motion; XV Image processing) for further morphology description. Microplastics were categorised based on their morphological characteristics (size, shape, and colour) as previously described by Hidalgo-Ruz *et al*. (2012), Masura *et al*. (2015) and Zhang *et al*. (2019). The size, shape, colour, and amount of the microplastic at each sampling site were recorded for each sampling zone. The microplastics size, measured along the longest axis (i.e., the longest side of the MP particle) was divided into four size classes: 0.063 < 0.5 mm, 0.5 < 1.0 mm, 1.0 < 2.0 mm and 2.0 < 5.0 mm (Apetogbor *et al*., 2022). Particles were classified based on their shapes, whether they were fibre, fragment, film, pellet/spheres, and foam (T. Wang et al., 2021). Their respective colours were simultaneously recorded during sample screening and categorized into seven groups based on the dominant colours: white, red, blue, green, transparent, black, and yellow. The white category included silver particles while the transparent category included colourless particles. The black category included transparent black, grey, and white-striped black particles; the red category included all shades of red and pink; the blue category incorporated all shades of blue and purple particles while the yellow category included all shades of yellow, orange and brown particles (Zhang *et al*., 2019).

Further, samples from the higher size fractions 0.5 < 5.0 mm were subjected to polymer type verification using a Perkin Elmer Attenuated Total Reflectance - Fourier Transformed Infrared spectrometer (ATR-FTIR). With a resolution of 4 $cm^{-1}$, spectra in the wavenumber range of 4000 to 600 $cm^{-1}$ were captured. Before sample analysis, background scans were performed, and the ATR crystal was cleaned with 70% methanol. Absorption bands identified using a peak height algorithm within the Perkin Elmer software were compared to absorption bands reported in the literature (Chércoles Asensio *et al*., 2009; Asefnejad *et al*., 2011; Guidelli *et al*., 2011; Jung *et al*., 2018) and a communal spectral library ([www.openspecy.org](www.openspecy.org)) with comparable accuracy to popular software (OMNIC Picta and KnowItAll) (Cowger *et al*., 2021). Visual checking of the wavenumber (or wavelength) of the IR spectrum peaks captured by the FTIR was done to avoid errors associated with cross-referencing with a spectral library (Kataoka *et al*., 2019). All non-plastic particles were expunged from the data.

The numerical concentrations of microplastics were obtained by dividing the number of microplastics obtained per water sample by the filtered water volume described in Section 2.7. Results were then reported as the number of items per litre (simply written as items/L).

### 2.9 Macroinvertebrate sampling

Concurrent with the seasonal microplastic sampling, macroinvertebrates were taken at each point of the cross section of each hydraulic zone per site based on available biotopes (i.e., stones, vegetation, and sediments). Macroinvertebrates were collected using a kick net (300 x 300 mm frame, 1000 µm mesh size) as prescribed in the South African Scoring System (SASS) version 5 rapid bioassessment method for rivers (Dickens & Graham, 2002). One sample was collected from each distinct physical biotope found within every hydraulic zone, resulting in a maximum of three samples per hydraulic biotope. Collected macroinvertebrates were fixed using 70% ethanol and transported to the laboratory for further processing (Odume, 2020). Details regarding macroinvertebrate sorting, identification and dissection are provided in Chapter 5.

### 2.10 Statistical analyses

The purpose of this section is to give a brief overview of the major statistical analyses applied in this study. Specific statistics used are discussed in the relevant chapters. Where relevant, alpha was set at 0.05 and p-values significant at $p \leq 0.05$. All statistical analysis was carried out using the R-statistical programming environment, R version 4.3.2 (R Core Team, 2023). Specific R-packages used are also stated in the relevant chapters.

#### 2.10.1 Box plots

Box plots are a useful statistical technique for exploratory data analysis as they provide a visual display of data distribution and summary statistics, including quartiles, medians, and outliers and allow a quick analysis of symmetry (Tukey, 1977; Baptista *et al*., 2007; Francis, 2008). Box plots were used to display microplastic abundances across the hydraulic zones, sampling sites and season (Chapters 3 and 4).

#### 2.10.2 Kruskal-Wallis test

The Kruskal-Wallis is a rank-based non-parametric test and an extension of the Mann-Whitney U test that allow the comparison of more than two independent groups. It is the non-parametric equivalent of the analysis of variance (ANOVA) and it is applicable where the parametric single ANOVA is applicable as well as in instances where ANOVA is not applicable (i.e., when the underlying assumptions for ANOVA are not met) (Ogbeibu 2014). The Kruskal-Wallis test was used to ascertain significant differences in physicochemical parameters between sites

(Chapter 3). The Kruskal-Wallis test was automatically followed by a Dunn's post-hoc multiple comparison test using the ggstatsplot package and the ggbetweenstats function in R-statistical programming environment (Patil, 2021; R Core Team, 2023). The Dunn's test is a non-parametric pairwise multiple comparisons procedure based on rank sums, often used as a post-hoc procedure following a Kruskal-Wallis test. P-values for multiple comparison were automatically adjusted on the ggstatsplot R-package using the Holm-Bonferroni method.

### 2.10.3 Mann-Whitney U test

The non-parametric Mann-Whitney U test, also called the Wilcoxon rank-sum test, is the analogue of the parametric unpaired t-test. As in the Kruskal-Wallis test, it is employed when the samples are not drawn from a normal distribution and when the independent variables are just two (Ogbeibu, 2014). In this test, values from all samples are combined into a single overall ranking. The rankings from each sample are summed, and the mean ranks of each sample are then calculated. The test examines whether the mean rank values between two groups are significantly different (Ogbeibu, 2014). The Mann-Whitney test was used to examine whether there was a significant difference in hydraulic indices derived for the hydraulic zones (Chapter 4). The Mann-Whitney U test was also carried out using the ggstatsplot package and the ggbetweenstats function in R-statistical programming environment (Patil, 2021; R Core Team, 2023).

### 2.10.4 Multivariate analysis of variance (MANOVA), Permutational multivariate analysis of variance (PERMANOVA) and analysis of variance (ANOVA)

The global multivariate analysis of variance (MANOVA) is a parametric statistic that compares the means of two or more independent variables simultaneously using multiple dependent variables. MANOVA was used to compare the hydraulic zones and seasons (independent variables) in terms of the abundance of total suspended and settled microplastics (dependent variables) and to understand whether there was any significant interaction effect between the hydraulic zones and seasons on the dependent variables (Chapter 4). Prior to MANOVA analysis, the assumptions of parametric analysis for MANOVA were investigated, including multivariate normality using the Shapiro-Wilk multivariate normality test, equality of variance – covariance matrices of the groups using the Box M test, and the confirmation test that indicates whether the determinant of variance-covariance matrix is positive. The Shapiro-Wilk multivariate normality test was undertaken using the mvnormtest R-package version 0.1-9 (Jarek, 2012) while the Box M test was conducted using the Biotools R-package version 4.2 (da Silva, 2021). When it appeared that the assumptions of normality were violated, data were log (x + a) transformed. If MANOVA indicated a significant result, the effect size of the significant result was determined using the “effectsize” package in R-statistical programming environment. A two-way ANOVA was then used to determine how settled and suspended microplastics responded according to the levels of the independent variables that were significant with the MANOVA analysis. Two-way ANOVA was also conducted to determine microplastics spatial and temporal patterns (Chapter 3).

Permutational multivariate analysis of variance (PERMANOVA) is a non-parametric multivariate statistical test used to compare the groups of objects and test whether the centroids and dispersion of samples for one group differ from another group. PERMANOVA is a non-parametric alternative where the assumptions of MANOVA are not met. PERMANOVA was undertaken to compare the hydraulic zones and seasons (independent variables) in terms of the microplastics’ morphological characteristics such as the individual shape (fibre, fragments, films, foams, and pellet/sphere), size (size classes as categorised in Section 2.8 of the current chapter), colour (white, red, blue, yellow, green, transparent and black), and polymer types that were common to both hydraulic zones (PE, PP, PET, PS), and to understand whether there was any significant interaction effect between the hydraulic zones and seasons on the dependent variables (Chapter 4). PERMANOVA was undertaken using the ‘vegan’ R package version 2.6-4 (Oksanen, 2022) and ‘pairwiseAdonis’ R package version 0.4.1 (Martinez, 2017) in R-statistical programming environment (R Core Team, 2023).

### 2.10.5 Generalised linear models (GLMs) and generalized additive models (GAMs)

A generalized linear model (the binomial logistic model) was used to explore the relationship between the hydraulic zones and macroinvertebrate exposure to microplastics based on a binomial distribution data computed for microplastic localisation in gills and guts of macroinvertebrates (Chapter 5). The relationship between selected traits of macroinvertebrates and exposure was also modelled using the binomial logistic model. Generalised linear models generalise regression to multiple explanatory variables. Generalised linear models are a flexible generalisation of ordinary linear regression that allows for response variables that have error distribution models other than a normal distribution. It allows the linear model to be related to the response variable via a link function and by allowing the magnitude of the variance of each measurement to be a function of its predicted value (Legendre & Legendre, 2012). A binomial logistic model predicts the probability that an observation falls into one of two categories of a dichotomous dependent variable based on one or more independent variables. The independent variables in a binomial logistic analysis can be either continuous or categorical.

Generalized additive models (GAMs) were used to describe the relationships between microplastics abundance data and survey-derived hydraulic indices for the hydraulic zones (Chapter 4). A GAM uses a link function to establish a relationship between the mean of the response variable and a smooth function of the explanatory variables (Guisan & Hastie, 2002; Pedersen *et al*., 2019). The strength of GAMs is their ability to deal with highly non-linear and non-monotonic relationships between the response and the set of explanatory variables. The GAMs are more flexible and better suited for analysing complex ecological relationships which can lead to the development of ecological models that can better represent the underlying data, and hence increase our understanding of ecological systems (Guisan & Hastie, 2002). Generalized additive models (GAMs) were thus used to examine the relationship between microplastic distribution and the hydraulic indices derived for the hydraulic zones. The approach assumed a negative binomial distribution for the microplastic data with a log link function, while the restricted maximum likelihood (REML) method was used to estimate model coefficients and smoothing parameters. The REML method was chosen because of its numerical stability compared to the minimised generalised cross-validation (GCV) (Wood, 2011), while the negative binomial distribution was utilised to account for over-dispersion in the microplastic data. Deviance results were used to identify the hydraulic zone and hydraulic indices that explained the most variability in the microplastic data. The GAMs were performed using the mgcv and gratia packages (Simpson, 2023; Wood 2017) in the R-statistical programming environment (R Core Team, 2023).

### 2.10.6 Canonical analysis

Redundancy analysis (RDA) was used to examine the associations between MP and water quality parameters and to understand the role of land use in MP spatial patterns (Chapter 3). The test of significance for the RDA was done using the 999 permutations by testing the global RDA significance, the RDA axis, and terms of significance while the adjusted R squared was calculated to measure the unbiased amount of explained variation in the dataset. The variance inflation factor was used to inspect for multicollinearity among the water quality parameters to create a simple model. Redundancy analysis (RDA) is a multivariate canonical or constrained form of principal component analysis (Legendre & Legendre, 2012). Redundancy analysis is both asymmetric and predictive and is the appropriate canonical model when both data sets are linear, and when an asymmetric analysis is required (ter Braak & Smilauer, 2002; Dray *et al*., 2003; Legendre & Legendre, 2012). Prior to RDA analysis, a detrended correspondence analysis (DCA) (Hill & Gauch, 1980) was carried out on the MP data to determine the gradient length of the dataset. Determination of the gradient length was necessary to choose either applying a linear method (e.g., redundancy analysis [RDA]) or a unimodal method (e.g., canonical correspondence analysis [CCA]). If DCA returns a gradient length less than 3.0 standard deviation, an RDA is deemed more appropriate as the data is linear, but if DCA returns a gradient length greater than or equal to 4.0 standard deviation, CCA is recommended as the data is unimodal in distribution (Šmilauer & Lepš, 2014). The DCA and RDA were undertaken using the Vegan R-package version 2.6-4 (Oksanen *et al.*, 2022; R Core Team, 2023).

# CHAPTER 3: RELATING THE DISTRIBUTION OF MICROPLASTICS TO LAND USE AND SELECTED PHYSICOCHEMICAL VARIABLES IN THE SWARTKOPS AND BUFFALO RIVERS

## 3.1 Introduction

River systems are prone to contamination by microplastics from a wide range of terrestrial sources (Kunz *et al.*, 2023). Most microplastic particles are generated by land-based anthropogenic activities and are transported into rivers and other lotic freshwater ecosystems through various processes including surface runoff, wind, and atmospheric deposition (Horton & Dixon, 2018; Abbasi, 2021; Talbot & Chang, 2022). Land cover and land-use types can influence the influx of microplastics into river systems (Grbić *et al.*, 2020; Talbot & Chang, 2022). Studies have shown the links between microplastic pollution in riverine systems and specific land cover categories and land-use types (Su *et al.*, 2020; de Carvalho *et al.*, 2021). For example, Kunz *et al.* (2023), in a study conducted in the Wu River network located in the Taichung City in central Taiwan, recorded the lowest abundances of microplastics in sites located in mountainous areas, and the highest abundances in sites located in the city centre of Taichung. The authors found that microplastic abundance positively correlated with the size of industrial, residential and traffic areas, and negatively correlated with the size of forest areas.

The relationship between the abundance of microplastics in rivers and the surrounding land cover and land-use patterns remain unresolved and ambiguous, and thus necessitating further research (Dikareva & Simon, 2019; Kunz *et al.*, 2023). For instance, He *et al.* (2020) and Grbić *et al.* (2020) found negative correlations between microplastic abundance and urban land cover and agricultural land cover respectively, while no relationships were found by others (e.g., Stanton *et al.*, 2020; Mai *et al.*, 2021). Although these conflictual results have been reported in the literature, it is believed that relating aquatic microplastic occurrence and abundance distribution to surrounding catchment land uses is useful for tracking transport pathways of microplastics and identifying their potential land-based sources (Talbot & Chang, 2022). For this reason, He *et al.* (2020) suggested the incorporation of a focus on relationships between land use and the abundances of specific microplastic types (e.g., the abundances of microplastic shapes such as fibre, fragment, or size class such as > 3 mm, < 3 mm etc.), as correlations between microplastic type and land use could potentially be stronger than those between microplastic concentration and land use.

The spatial dispersion of microplastics may also be influenced by water physicochemical variables (Kataoka *et al*., 2019a; Rakib *et al*., 2023). Water physicochemical variables such as temperature, pH, and nutrient supply shape microbial diversity, activity, and community structure in rivers (Jordaan & Bezuidenhout, 2013; Ibekwe *et al*., 2016; Niu *et al*., 2019). For instance, high temperatures, long illumination time, nutrients (e.g., total nitrogen and total phosphorus), and low dissolved oxygen impact microbial cell metabolism and enzyme activity (Chen *et al.*, 2019; Li *et al*., 2019) resulting in faster growth rates and greater biomass of biofilms growing on the surfaces of microplastics (He *et al.*, 2022). Microplastic surface transformation, thickness, crystallinity, mass, hydrophobicity and molecular weight have all been indicated to change with microbial colonisation (McGivney *et al.*, 2020), which can alter microplastic transport behaviour and distribution characteristics (Miao *et al*., 2021; He *et al*., 2022). Chen *et al.* (2019) demonstrated that variations in the sinking rate of biofouled microplastics across different seasons occasioned by differences in biofilm growth rate and algae composition are related to the difference in temperature and nutrient levels in different seasons. Thus, low dissolved oxygen, high nutrient concentrations, which are general indicators of low water quality, and temperature can provide an enabling environment that allows biofilms to form on microplastic surfaces, allowing bacterial to thrive and impacting microplastics' transport behaviour.

Yonkos *et al*., (2014) and Buwono *et al*., (2021) noted that water temperature has a significant impact on the distribution of microplastics because it influences the hydrodynamic mechanics of water and the mechanism of microplastic breakdown. The dynamic viscosity of water is strongly temperature dependent (Gordon, 1992), which might mean that the rate of microplastics translocation and settling would vary depending on the temperature and season. For instance, Rakib *et al*. (2023) reported a positive correlation between high microplastic concentrations and high temperatures at low pH, indicating the influences of temperature on microplastics' transport behaviour. The translocation of suspended microplastics might be slower in frigid winter water than in tepid summer water. Further, biodegradation of plastics is influenced by water pH and temperature (Oliveira *et al*., 2020). In this regard, the physicochemical properties of water may thus contribute to the processes that drives the generation of microplastic fragments and indirectly contribute to the presence of microplastics in rivers. However, in certain cases, the relationship between microplastics and water quality in rivers can be characterised by strong co-dependence, particularly influenced by land use. This means that poor water quality and the presence of microplastics may co-occur, indicating a correlation rather than a direct cause-effect relationship where water quality drives microplastic presence. Land use activities such as urbanisation, industrialisation, and agricultural practices can contribute pollutants and microplastics to river systems simultaneously. For example, runoff from urban areas or agricultural fields can carry both pollutants and microplastics into water bodies, leading to a correlation between degraded water quality and increased microplastic concentrations. In such instances, while water quality indicators can signal the presence of microplastics in a river, they may not necessarily be the primary driver of microplastic pollution. Instead, both factors, microplastic presence and poor water quality, could be driven by shared sources and mechanisms related to human activities and land use practices.

The relationship and interdependencies between physicochemical qualities of water and microplastic occurrence is mostly unexplored (Buwono *et al*., 2021; Eamrat *et al*., 2022; Rakib *et al*., 2023), yet, this understanding would shed light on microplastics abundance distribution and allow for more accurate estimates of the percentage of microplastics transported to marine systems via rivers (Rakib *et al*., 2023). Understanding the relationship and interdependencies between microplastic concentrations and water quality could be used to identify microplastics sources and inflow processes (Kataoka *et al*., 2019a), and is crucial for effectively managing and mitigating microplastic pollution in riverine environments, as it requires comprehensive approaches that address both water quality improvement and microplastic reduction strategies (Lebreton *et al*., 2017). This chapter thus focusses on exploring the relationship between the distribution of microplastic and land uses on the one hand, and the association and interdependencies between microplastic pollution and selected water quality variables on the other. First, the microplastic contamination burden of the Study Rivers was established in relation to their abundance, morphological and polymer compositions. The spatial-temporal variabilities were also established, elucidating the role of seasonality and land uses on the distribution of microplastic (Stanton *et al*., 2020; Xia *et al*., 2021; Talbot & Chang, 2022). Second, microplastic distribution was correlated with selected physicochemical variables to elucidate any observable pattern(s).

### 3.2 Materials and methods

#### 3.2.1 Measurement of water physicochemical variables

Water physicochemical variables including dissolved oxygen (DO), electrical conductivity (EC), turbidity, temperature, and pH, were measured *in situ* at each hydraulic zone and averaged for each site. In addition, total suspended solids (TSS), nitrate-nitrogen ($NO_3$-N), nitrite-nitrogen ($NO_2$-N), ammonium-nitrogen ($NH_4$-N), orthophosphate-phosphorus ($PO_4$-P), and total inorganic nitrogen (TIN) were computed for each site from results derived for each hydraulic zone (see details in Chapter 2, Section 2.5). Average values obtained from results recorded for each hydraulic zone were used as the site level values for each sampling event. Physicochemical data for the two rivers were analysed together and are presented in Tables 3.3a and 3.3b in Section 3.5.1.

### 3.2.2 Suspended and settled microplastics sampling.

Suspended and settled microplastics were sampled as described in Chapter 2, Section 2.6.

### 3.2.3 Sample preparation and processing

Processing of water samples for microplastics examination followed the procedure described in Chapter 2, Section 2.7.

### 3.2.4 Quantification and characterisation of microplastics

Microplastics were examined and quantified into various shapes, sizes and colour as discussed in Chapter 2, Section 2.8.

### 3.2.5 Land-use pre-processing and mapping

The catchments of the study locations span a broad range of land-use types (Figure 3.1 and 3.2). The most recent South Africa National Land Cover data (SANLC, 2021) was acquired from the South African Department of Forestry, Fisheries, and the Environment (DFFE, 2021) (https://egis.environment.gov.za/data_egis/data_download/current) and used to classify the land-use types within the catchments.

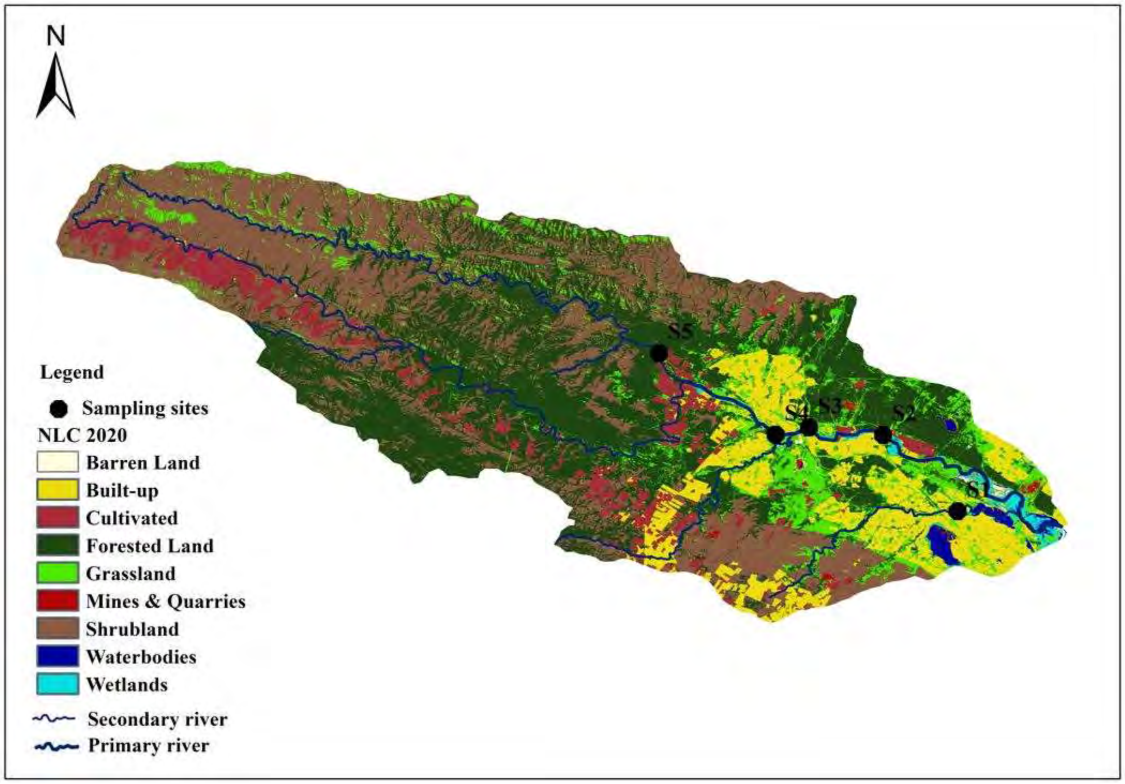


**Figure 3.1.** Swartkops River catchment landcover map

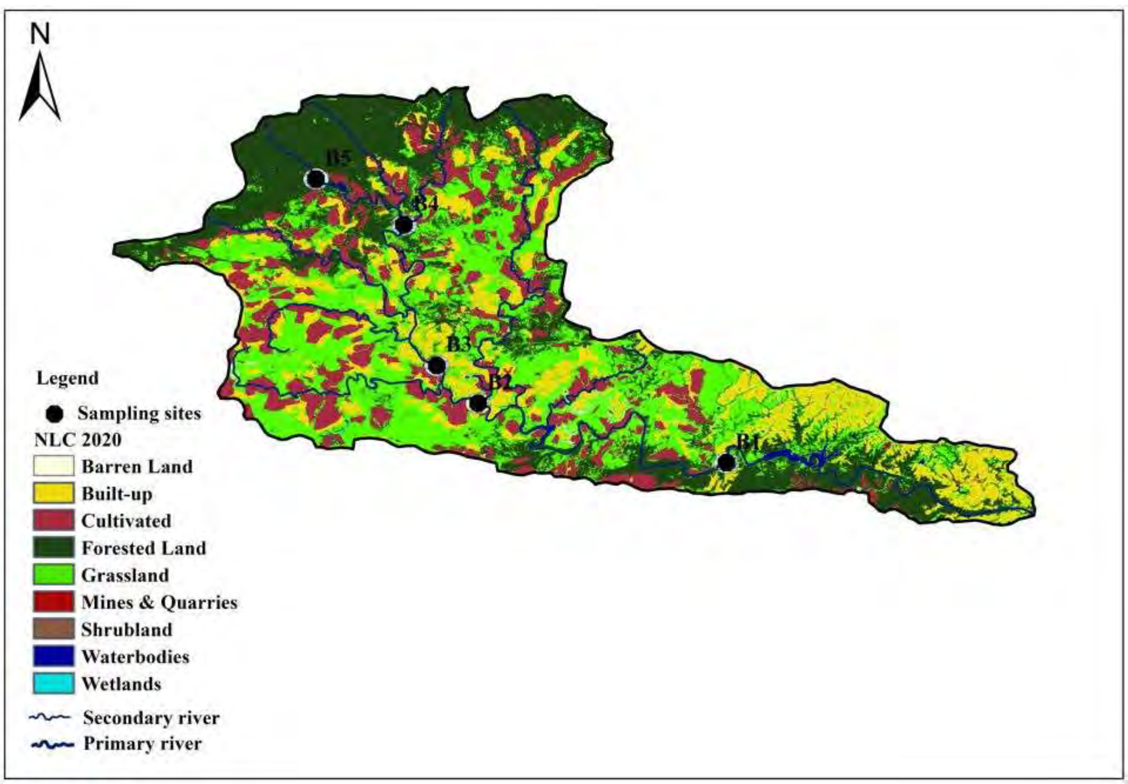


**Figure 3.2.** The Buffalo River catchment landcover map

Additionally, in ArcGIS 10.3.1 software, the buffer tool was used to create a radius of 3 km around each sampling location with the sampling point as the geographical centre (Chen *et al.*, 2020; Wang *et al.,* 2021). The land-use types within the boundary were analysed and classified. The choice of a 3 km radius was thought to be large enough to capture in a meaningful way the majority of the relevant processes that contribute microplastics to a site from land-based sources. Based on the South African Land Cover Classification Classes (SALCC2), the land within the 3 km radius of each sampling site was classified as natural land, agricultural land, industrial land, urban land, and rural land, or as a combination of these types' (Table 3.1) (Crew *et al*., 2020). The sizes (area and proportion) of the various land-use types were calculated (Table 3.2). Total percentage for each radius was not 100 percent as water bodies, both natural and artificial, and wetlands were excluded. The extent of anthropogenic land cover within each radius was determined by subtracting the proportion of natural land cover.

**Table 3.1.** Classification of land use types within the 3 km radius of each sampling location based on the South Africa National Land Cover dataset.

| Land use category | SALCC1 & SALCC2 |
|---|---|
| Natural land | Natural wooded land, shrubs, natural grassland |
| Industrial land | Industrial, extraction sites (i.e., mines and quarries) |
| Urban land | Built-up (i.e., residential – formal and informal), commercial, transport (i.e., roads and rail) |
| Agricultural land | Temporary and permanent crops, fallow lands, old fields (cultivated), small holdings |
| Rural land | Built-up (village) |

A hierarchical cluster analysis was used to classify the study sites into groups in terms of similarity in the extent of anthropogenic land cover within each 3 km radius. A cluster analysis returns a hierarchical agglomerative diagram, with the x-axis indicating sites and the y-axis the distance measure of similarity or dissimilarity (Figure 3.3). The classification of sites into groups from the cluster analysis was based on the Euclidean distance measure between the sites. The Euclidean distance measure was used because it was deemed a more appropriate measure for non-biological variables. The sites that were closely clustered together were categorised into one group (i.e., site groups). Land use, together with measured physicochemical variables were analysed to explain the variation of distribution of microplastics between the different sites. The extent of anthropogenic land cover was also analysed to explain the variation in microplastic distribution between the site groups.

### 3.2.6 Statistical analysis

To elucidate the spatial and temporal variability of microplastics in the two river systems, a two-way analysis of variance (ANOVA) was conducted. Prior to ANOVA, the data were subjected to homoscedasticity and a normality test using the Levene's and Shapiro-Wilk test respectively. If the two-way ANOVA returned a significant result, the Tukey's Honest Significant Difference post-hoc test was undertaken to uncover significant differences between and within sites using a pairwise comparison of the two-way ANOVA. The effect size (Eta-squared, $\eta^2p$) for each significant result returned in the ANOVA test was conducted to determine the percentage of the variance associated with each site using the "eta_squared" function and the "effectsize" package in R (Ben-Shachar, 2020). To use ANOVA seasonal summer, winter, spring and autumn and were allocated as wet (two independent sampling season) and dry season (two independent sampling season) for temporal replication while Microplastic results obtained during the four sampling events were used as spatial replicates.

Given the distribution of physicochemical data for the study sites, the Kruskal-Wallis test was applied to explore any significant differences among sites, which was followed by a pairwise multiple comparison procedure using the Dunn's test. Both the Kruskal-Wallis and Dunn's tests were performed using the ggstatsplot package in R (Patil, 2021). Redundancy analysis (RDA) was undertaken to investigate the relationship between microplastic concentrations and the water quality parameters (DO, EC, turbidity, pH, TSS, $NO_3$-N, $NO_2$-N, $NH_4$-N, $PO_4$-P, and TIN). Prior to RDA analysis, a detrended correspondence analysis (DCA) was conducted to determine the gradient length of microplastics abundance data, so that the right analysis could be chosen as explained in Chapter 2, Section 2.10, to choose the right ordination procedure as explained in Chapter 2, Section 2.10.6. Redundancy analysis (RDA) was also conducted to understand the role of land use in microplastic spatial patterns.

The Spearman's rank correlation coefficient analysis was further used to assess the relationship between land use and microplastic concentrations in different sites. Spearman's rank correlation coefficient examines whether two variables are co-related with one another by measuring the strength and direction of association between the two ranked variables. The Spearman's test is the non-parametric equivalent of the Pearson correlation. Spearman's rank correlation analyses ordinal level, as well as continuous level data and uses ranks instead of the assumptions of normality. Because of the distribution of the microplastics and land use data, the Spearman's rank correlation coefficient was deemed more suitable for this analysis.

### 3.3 Results

### 3.3.1 Water physicochemical results for the study sites

The means, standard deviations, and ranges of the physicochemical variables analysed for all ten sampling sites during the study period are presented in Table 3.2. In the Buffalo River, physicochemical properties showed statistically significant differences between the sites for $NO_2$-N, $NH_4$-N, $PO_4$-P, EC, TIN, and turbidity. The Dunn's post-hoc test revealed that the mean $NO_2$-N and EC values for site B5 were significantly different from the $NO_2$-N and EC values at sites B2 and B3. For $NH_4$-N, the mean values of site B5 were significantly different from those recorded at sites B2 and B3, while those of site B3 differ significantly from the mean values of site B4. The mean $PO_4$-P values for site B5 were significantly different from those of site B3, while those of site B3 differ significantly from site B4. Also, the mean TIN and turbidity values for site B5 differ significantly from those of sites B3 and B1, respectively. The values for pH, temperature, and $NO_3$-N were not different statistically. After adjusting for multiple comparisons using the false discovery rate (FDR) method and subsequently applying Dunn's test, no statistically significant differences were found in the mean dissolved oxygen (DO) values between the different sites. This suggests that any observed variations in DO levels across sites are likely due to random fluctuations or factors other than site location. Results at sites B3 located downstream of the wastewater effluent discharge point of the Buffalo River seems to suggest lower water quality in comparison to other sites located in the Buffalo River.

In the Swartkops River, physicochemical properties indicated statistically significant differences between the sites for several variables, including $NO_2$-N, $NH_4$-N, $PO_4$-P, EC, turbidity and TSS. The Dunn's post-hoc test revealed that the mean $NO_2$-N and EC values for site S5 were significantly different from the $NO_2$-N and EC values at site S4 and S1. For $NH_4$-N and TIN, the mean values of site S5 were statistically significantly different from those recorded at sites S2 and S3, while the mean $PO_4$-P values for site S5 were significantly different from those of site S2. The mean turbidity and TSS values for site S5 were statistically significantly different from the mean turbidity and TSS values at site S3. The values for pH and temperature were similar across all the sites.

The results seem to suggest that site B3 located downstream of the wastewater discharge of the Buffalo River and sites S1, S2 and S3 located downstream of the Swartkops are the most polluted sites. Site S1 is in the centre of a highly populated residential hub, while sites S2 and S3 are located downstream of the Kelvin Jones wastewater discharge point in the Swartkops River and are impacted by effluent from the WWTW. The two most upstream sites in both rivers, site S5 located in the Swartkops River and site B5 located in the Buffalo River were the least impacted sites, having very low levels of nutrients. The results also seem to suggest that sites located in the Swartkops were more impacted in terms of their water quality than sites in the Buffalo River. The Kruskal-Wallis and Dunn's test statistics are presented in (Appendix A, Figures A1 – A11).

**Table 3.2a.** Means, standard deviations, and ranges (in brackets) of the measured physicochemical variables at the five sites in the Buffalo River during the four sampling events (October 2021 – July 2022). P-value is indicated for all variables based on the Kruskal-Wallis test and significant p-values in bold established by the Dunn's test.

| **Variables** | **Site** | | | | | ***P*-value** |
|---|---|---|---|---|---|---|
| | **B1** | **B2** | **B3** | **B4** | **B5** | |
| Temp (°C) | 20.3 ± 5.6 | 20.0 ± 6.0 | 18.8 ± 4.9 | 20.0 ± 7.2 | 17.4 ± 5.3 | 0.96 |
| | (14.8 – 27.7) | (13.4 – 27.7) | (14.7 – 25.8) | (12.1 – 29) | (11.0 – 21.8) | |
| pH | 7.39 ± 0.9 | 7.9 ± 0.7 | 7.5 ± 0.9 | 7.5 ± 1.1 | 7.6 ± 0.8 | 0.83 |
| | (6.2 – 8.4) | (7.01 – 8.4) | (6.3 – 8.4) | (6.0 – 8.4) | (6.9 – 8.5) | |
| DO (mg/l) | 8.9 ± 1.3 | 7.2 ± 1.0 | 4.5 ± 2.6 | 8.4 ± 2.3 | 9.1 ± 0.9 | 0.05 |
| | (7.4 – 10.2) | (6.3 – 8.1) | (2.0 – 7.9) | (5.6 – 10.6) | (8.3 – 10.3) | |
| EC (mS/m) | 43.6 ± 3.8 | 55.2 ± 9.7 | 51.4 ± 11.6 | 26.8 ± 12.2 | 66.6 ± 5.7 | **0.007** |
| | (39.5 – 48.6) | (43.2 – 65.9) | (35.9 – 60.7) | (16.6 – 44.5) | (62.2 – 74.4) | |
| Turbidity | 35.4 ± 17.2 | 28.1 ± 16.5 | 20.3 ± 6.8 | 16.4 ± 2.0 | 4.5 ± 0.8 | **0.01** |
| (NTU) | (24.2 – 60.8) | (8.8 – 46.1) | (10.1 – 24.3) | (14.2 – 18.7) | (3.8 – 5.2) | |
| TSS (mg/l) | 63.6 ± 38.0 | 61.4 ± 54.7 | 45.3 ± 26.4 | 28.4 ± 14.5 | 4.8 ± 2.7 | **0.003** |
| | (25.0 – 98.3) | (7.3 – 137.7) | (15.3 – 79.7) | (7.0 – 38.1) | (2.0 – 7.8) | |
| ($NO_3$-N) (mg/l) | 3.56 ± 2.02 | 2.54 ± 1.32 | 9.99 ± 12.79 | 1.84 ± 0.56 | 1.04 ± 0.10 | 0.06 |
| | (1.02 – 5.70) | (1.15 – 4.25) | (1.31 – 29.00) | (1.04 – 2.35) | (0.90 – 1.13) | |
| ($NO_2$-N) (mg/l) | 0.22 ± 0.08 | 0.48 ± 0.17 | 0.50 ± 0.13 | 0.08 ± 0.02 | 0.04 ± 0.01 | **0.002** |
| | (0.15 – 0.34) | (0.27 – 0.69) | (0.34 – 0.65) | (0.06 – 0.10) | (0.03 – 0.06) | |
| ($NH_4$-N) (mg/l) | 0.35 ± 0.07 | 1.65 ± 1.99 | 7.34 ± 0.61 | 0.16 ± 0.04 | 0.06 ± 0.02 | **0.001** |
| | (0.24 – 0.41) | (0.56 – 4.62) | (6.60 – 7.92) | (0.13 – 0.15) | (0.04 – 0.09) | |
| ($PO_4$-P) (mg/l) | 0.97 ± 0.10 | 1.56 ± 0.76 | 8.13 ± 6.14 | 0.21 ± 0.07 | 0.18 ± 0.13 | **0.002** |
| | (0.87 – 1.10) | (0.47 – 2.20) | (1.90 – 16.50) | (0.11 – 0.28) | (0.05 – 0.33) | |
| TIN (mg/l) | 4.12 ± 1.94 | 4.66 ± 3.36 | 17.83 ± 12.19 | 2.08 ± 0.56 | 1.14 ± 0.09 | **0.003** |
| | (1.72 – 6.25) | (2.20 – 9.56) | (9.70 – 35.94) | (1.29 – 2.28) | (1.00 – 1.19) | |

**Table 3.2b:** Means, standard deviations and ranges (in brackets) of measured physicochemical variables at the five sites in the Swartkops River and tributary during the four sampling events (October 2021 – July 2022). P-value is indicated for all variables based on the Kruskal-Wallis test and significant p-values in bold established by the Dunn's test.

| **Variables** | **Site** | | | | | ***P*-value** |
|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 | |
| Temp (°C) | 21.6 ± 6.3<br>(15.1 – 28.3) | 20.4 ± 4.0<br>(15.3 – 24.7) | 22.7 ± 4.3<br>(18.1 – 26.9) | 20.9 ± 3.7<br>(17.0 – 25.2) | 21.3 ± 44.5<br>(16.4 – 26.8) | 0.90 |
| pH | 7.7 ± 0.7<br>(7.2 – 8.5) | 6.9 ± 1.1<br>(5.5 – 8.0) | 7.4 ± 0.7<br>(6.8 – 8.0) | 7.7 ± 0.8<br>(6.7 – 8.6) | 7.2 ± 0.6<br>(6.7 – 8.0) | 0.50 |
| DO (mg/l) | 2.8 ± 1.1<br>(1.7 – 4.1) | 4.2 ± 2.9<br>(1.4 – 7.4) | 3.2 ± 1.3<br>(2.0 – 4.5) | 5.6 ± 3.6<br>(1.2 – 9.6) | 8.4 ± 0.8<br>(7.6 – 9.3) | 0.07 |
| EC (mS/m) | 417.3 ± 215.9<br>(230.9 – 726.6) | 295.1 ± 26.0<br>(268.0 – 309.5) | 228.2 ± 36.9<br>(183.7 – 271.3) | 317.0 ± 21.8<br>(294.2 – 346.7) | 25.6 ± 1.4<br>(24.3 – 27.7) | **0.007** |
| Turbidity (NTU) | 15.4 ± 23.2<br>(2.1 – 50.1) | 8.0 ± 3.4<br>(4.3 – 12.5) | 51.1 ± 19.4<br>(32.6 – 74.0) | 20.1 ± 13.0<br>(4.4 – 31.2) | 3.5 ± 1.6<br>(1.8 – 5.5) | **0.02** |
| TSS (mg/l) | 17.2 ± 16.8<br>(4.3 – 40.5 ) | 20.9 ± 28.6<br>(2.8 – 63.5) | 116.0 ± 56.0<br>(41.7 – 153.3) | 18.7 ± 10.0<br>(4.7 – 28.2) | 2.8 ± 1.4<br>(1.5 – 4.8) | **0.01** |
| ($NO_3$-N) (mg/l) | 6.53 ± 4.19<br>(1.76 – 11.60) | 10.86 ± 7.53<br>(3.20 – 21.22) | 19.66 ± 17.97<br>(1.48 – 35.1) | 7.66 ± 5.73<br>(1.28 – 15.16) | 1.92 ± 2.70<br>(0.33 – 5.97) | 0.11 |
| ($NO_2$-N) (mg/l) | 1.13 ± 0.54<br>(0.58 – 1.86) | 0.29 ± 0.18<br>(0.12 – 0.45) | 0.43 ± 0.21<br>(0.22 – 0.67) | 1.31 ± 1.12<br>(0.04 – 2.72) | 0.02 ± 0.02<br>(0.01 – 0.05) | **0.01** |
| ($NH_4$-N) (mg/l) | 7.32 ± 3.40<br>(3.20 – 10.80) | 21.73 ± 6.31<br>(15.31 – 30.40) | 23.66 ± 9.33<br>(10.07 – 29.95) | 8.47 ± 10.25<br>(0.25 – 23.4) | 0.07 ± 0.08<br>(0.00 – 0.17) | **0.006** |
| ($PO_4$-P) (mg/l) | 8.03 ± 2.05<br>(6.25 – 10.65) | 14.42 ± 4.01<br>(12.25 – 18.50) | 13.34 ± 9.31<br>(3.07 – 25.50) | 5.06 ± 2.19<br>(3.25 – 8.15) | 0.82 ± 1.52<br>(0.06 – 3.10) | **0.01** |
| TIN (mg/l) | 14.98 ± 5.97<br>(9.63 – 23.35) | 32.88 ± 6.92<br>(25.48 – 41.83) | 43.74 ± 22.98<br>(17.23 – 65.01) | 17.44 ± 12.20<br>(2.51 – 31.24) | 2.01 ± 2.77<br>(0.36 – 0.77) | **0.007** |

### 3.3.2 Spatial and temporal distribution of suspended microplastics

The two-way ANOVA revealed that the average suspended microplastics abundance was spatially statistically significant (ANOVA; $p < 0.01$) across the sites but was not temporally statistically significant (ANOVA; $p > 0.05$) across the wet and dry seasons for both river systems (Table 3.3). The result also indicated a large effect size for the significant result (Eta-squared, $\eta^2 p = 0.62$) at the 95% confidence level. Tukey's post-hoc test using the pairwise comparison of the two-way ANOVA revealed that the average microplastics abundance was significantly different in a limited number of sites (Figure 3.3). The post-hoc test indicated that the average microplastics abundance values for site S5 (located in the upper reaches of the Swartkops River, downstream of the nature reserve area) was significantly different (i.e., lesser in comparison) from those of site S1 (located in the Swartkops tributary, the Chatty River, in an area surrounded by a highly clustered human settlement) and site S2 (located in the lower reaches of the Swartkops further downstream of the Kelvin Jones WWTW effluent discharge point). Sites located in the Buffalo River were not significantly different. The mean abundance of suspended microplastics (mean ± standard deviation) was 1.83 ± 1.31 items/L and 1.47 ± 1.06 items/L in the Swartkops and Buffalo Rivers, respectively. Overall, across the ten sites, suspended microplastics had a range of 0.00 – 4.67 items/L and a mean abundance (mean ± standard deviation) of 1.65 ± 1.19 items/L. The highest mean occurrence of microplastics (2.92 ± 1.56 items/L) over the ten sampling sites was recorded at site S2 (located in the lower reaches of the Swartkops further downstream of the Kelvin Jones WWTW effluent discharge point) and the lowest (0.13 ± 0.08 items/L) was recorded at site S5 (located in the upper reaches of the Swartkops River downstream of the nature reserve area).

**Table 3.3.** Two-way ANOVA results indicating that the abundance of suspended microplastics was significantly different spatially but not temporally (Bold face values are significant).

| **Source of variation** | **Multivariate test of significance** | | | | | |
|---|---|---|---|---|---|---|
| | **Df** | **Sum Sq** | **Mean Sq** | **F -value** | **Pr(>F)** | Partial Eta$^2$ (95% CI) |
| Sites | 9 | 29.808 | 3.312 | 3.697 | **0.007** | 0.62 |
| Season | 1 | 1.469 | 1.469 | 1.640 | 0.215 | 0.08 |
| Site: Season interaction | 9 | 5.961 | 0.662 | 0.739 | 0.670 | 0.25 |

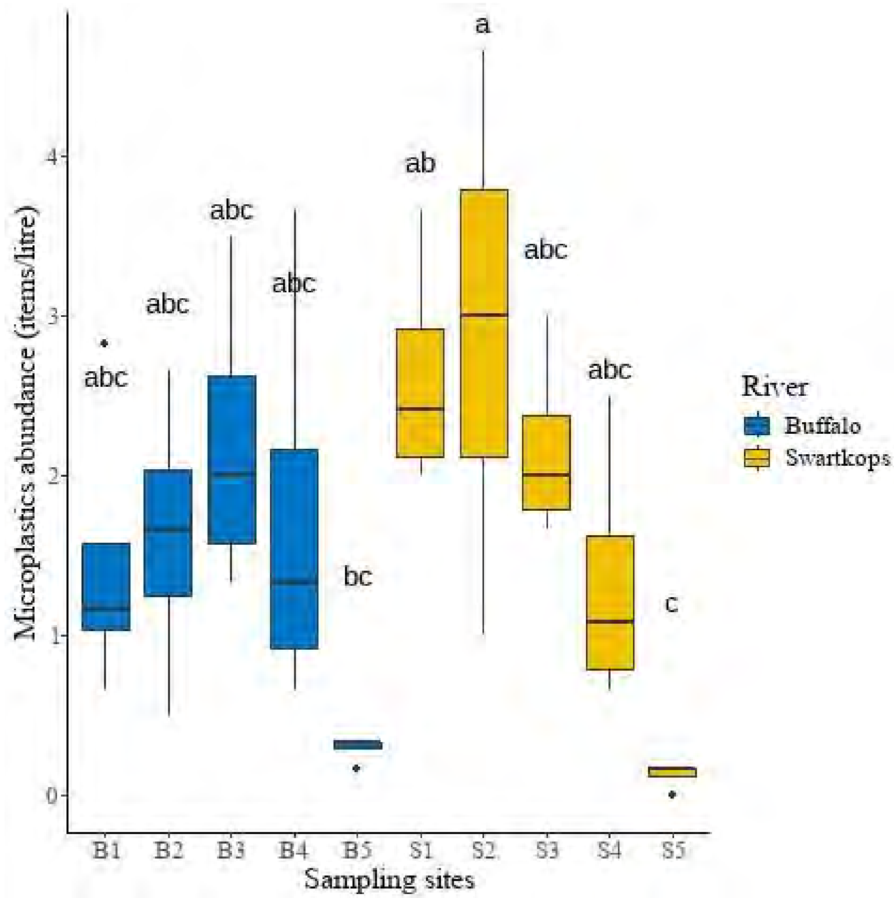


**Figure 3.3.** Spatial distribution of suspended microplastics abundance for the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5. Sites with superscript letter in common are not statistically significantly different, whereas sites with no common superscript letter(s) are statistically significantly different, established using Tukey's HSD. Microplastic results obtained during the four sampling events were used as replicates for each site.

The results of temporal microplastic abundance variability are presented in Figure 3.4. Seasonally, microplastics were more in abundant (mean ± standard deviation) during the wet season (1.84 ± 1.25 items/L), as the combined results for both river systems showed. Statistically, the differences in microplastic abundance between the wet and dry seasons was not significant. In the Buffalo River, suspended microplastics were more in abundant during the wet season (wet: 1.85 ± 1.07). In the Swartkops, there was no difference in the mean suspended microplastics distribution across the wet and dry season. The highest mean microplastic occurrence (3.00 ± 0.71) across the ten sampling sites during the dry season was recorded at site S2 (located in the lower reaches of the Swartkops further downstream of the Kelvin Jones WWTW effluent discharge point) and the lowest occurrence (0.08 ± 0.12) was recorded at site S5 (located in the upper reaches of the Swartkops River downstream of the nature reserve area). Conversely, the highest mean occurrence (2.83 ± 2.59) during the wet season was recorded at site S2 (located in the lower reaches of the Swartkops further downstream of the Kelvin Jones WWTW) and the lowest (0.17 ± 0.00) at site B5 (located in the upper reaches of the Buffalo River upstream of Rooikrans dam).

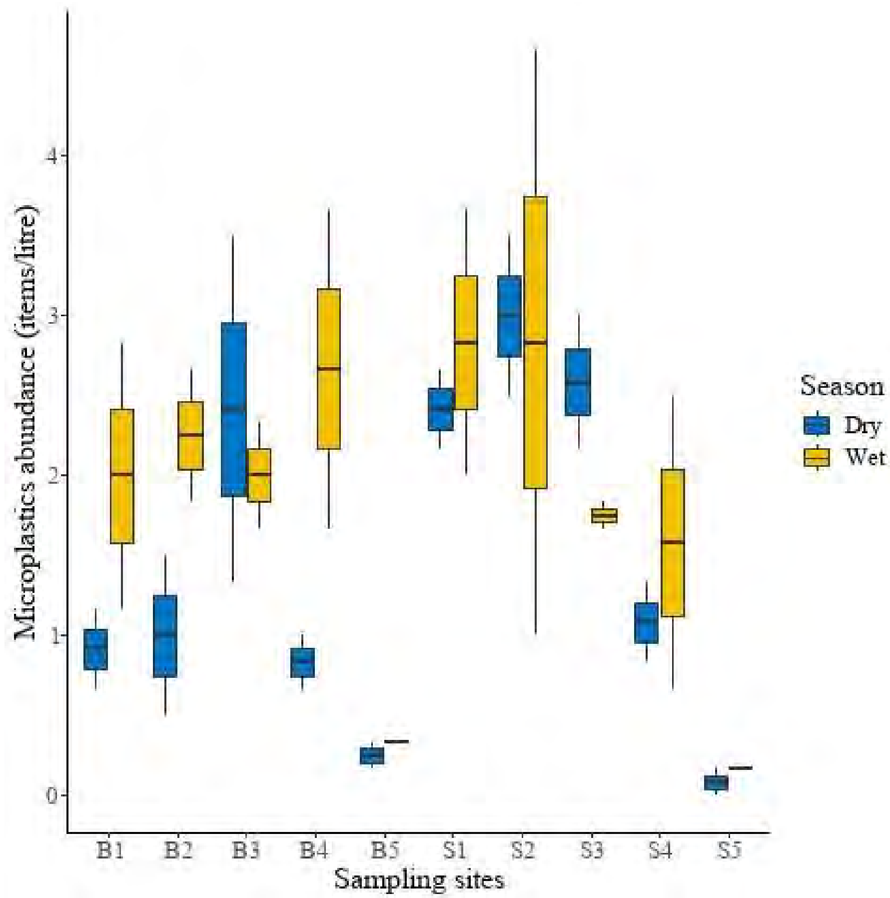


**Figure 3.4.** Temporal distribution of suspended microplastics abundance for the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

### 3.3.3 Spatial and temporal distribution of settled microplastics

The analysis of spatial and temporal variations in microplastic abundances using a two-way ANOVA showed that the mean distribution of settled microplastics was statistically significantly different (ANOVA; $p < 0.05$) both spatially across the sites and temporally across the seasons for both river systems. The result also indicated large effect sizes for the significant results at the 95% confidence level (Table 3.4). The Tukey HSD test further revealed that spatially, the average settled microplastics abundance values for sites located in the upper reaches of both rivers (i.e., sites S5 and B5 in the Swartkops and Buffalo Rivers, respectively) were statistically significantly different (i.e., values were lesser in comparison) from their counterparts located in the middle and lower reaches of each of the study rivers, except for site B1 (Figure 3.5). Site B1 (located in the lower reaches of the Buffalo River and upstream of the Bridle Drift dam) was not statistically significantly different from site B5 in the composition of the mean settled microplastic abundances. The abundances of settled microplastics across the ten sites ranged between 0.17 – 5.67 items/L, with a mean abundance of 1.58 ± 1.36 items/L (mean ± standard deviation). In the Swartkops, the mean abundance (mean ± standard deviation) was 1.61 ± 1.27 items/L and 1.54 ± 1.48 items/L in the Buffalo River. The highest mean occurrence of settled microplastics (2.79 ± 0.44 items/L) over the ten sampling sites was recorded at site S3 (located downstream of the Kelvin Jones WWTW effluent discharge point) and the lowest (0.29 ± 0.08 items/L) occurred at sites S5 and B5 located in the upper reaches of the Swartkops and Buffalo rivers, respectively.

**Table 3.4**. Two-way ANOVA results indicating that the abundance of settled microplastics was significantly different spatially and temporally (Bold face values are significant).

| **Source of variation** | **Multivariate test of significance** | | | | | |
|---|---|---|---|---|---|---|
| | **Df** | **Sum Sq** | **Mean Sq** | **F -value** | **Pr(>F)** | Partial Eta$^2$ (95% CI) |
| Sites | 9 | 21.453 | 2.384 | 9.539 | **0.000** | 0.81 |
| Season | 1 | 3.153 | 3.153 | 12.619 | **0.002** | 0.39 |
| Site: Season interaction | 9 | 4.209 | 0.468 | 1.872 | 0.117 | 0.46 |

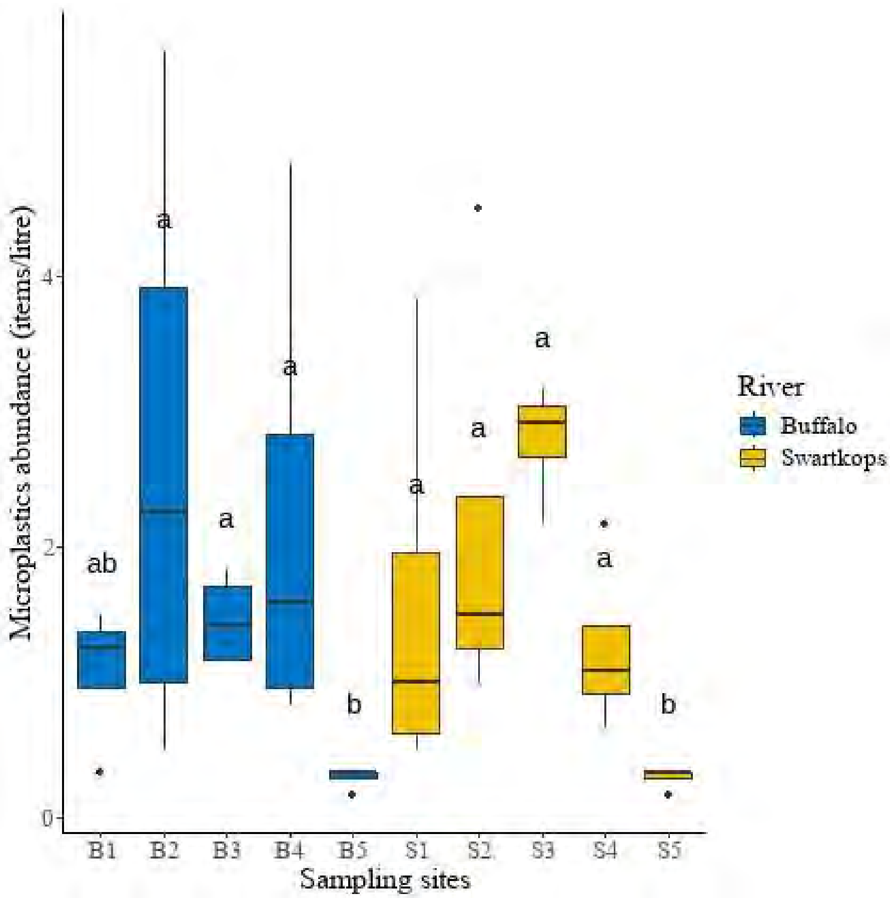


**Figure 3.5.** Spatial distribution of settled microplastics abundance for the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5. Sites with superscript letter in common are not statistically significant, whereas sites with no common superscript letter(s) are statistically significantly different, established using Tukey's HSD.

Temporally, the pairwise comparison between and within sites using Tukey HSD test revealed that the significant differences in settled microplastic abundances occurred only between sites but no significant differences within sites (Figure 3.6). The Tukey test also established significant differences between the mean abundance values of the wet and dry seasons ($p < 0.01$). Seasonally, settled microplastics were more in abundance during the wet season (1.99 ± 1.53; mean ± standard deviation), evidenced by results combined for the two river systems. In the two river systems, the mean abundances of settled microplastics (mean ± standard deviation) during the wet season (Swartkops: 1.77 ± 1.21; Buffalo: 2.22 ± 1.83) was higher than that obtained for the dry season (Swartkops:1.45 ± 1.38; Buffalo: 0.87 ± 0.53). The highest mean abundance (2.75 ± 2.48) across the ten sampling sites during the dry season occurred at site S2 (located in the lower reaches of the Swartkops further downstream of the Kelvin Jones

WWTW effluent discharge point) and the lowest occurrence (0.25 ± 0.12) was recorded at sites B5 and S5 (located in the upper reaches of the Buffalo and Swartkops Rivers, respectively). During the wet season, the highest mean occurrence (4.50 ± 1.64) was recorded at site B2 (located in the middle reaches of the Buffalo River) and the lowest at sites S5 and B5 (0.33 ± 0.00). The highest single concentration of settled microplastics across the four sampling events was recorded during the wet season (summer) at site B2 (5.67 items/L) (Figure 3.6). Sites located in the upper reaches in both river systems (i.e., sites B5 and S5) had the lowest abundances of microplastics across all seasons.

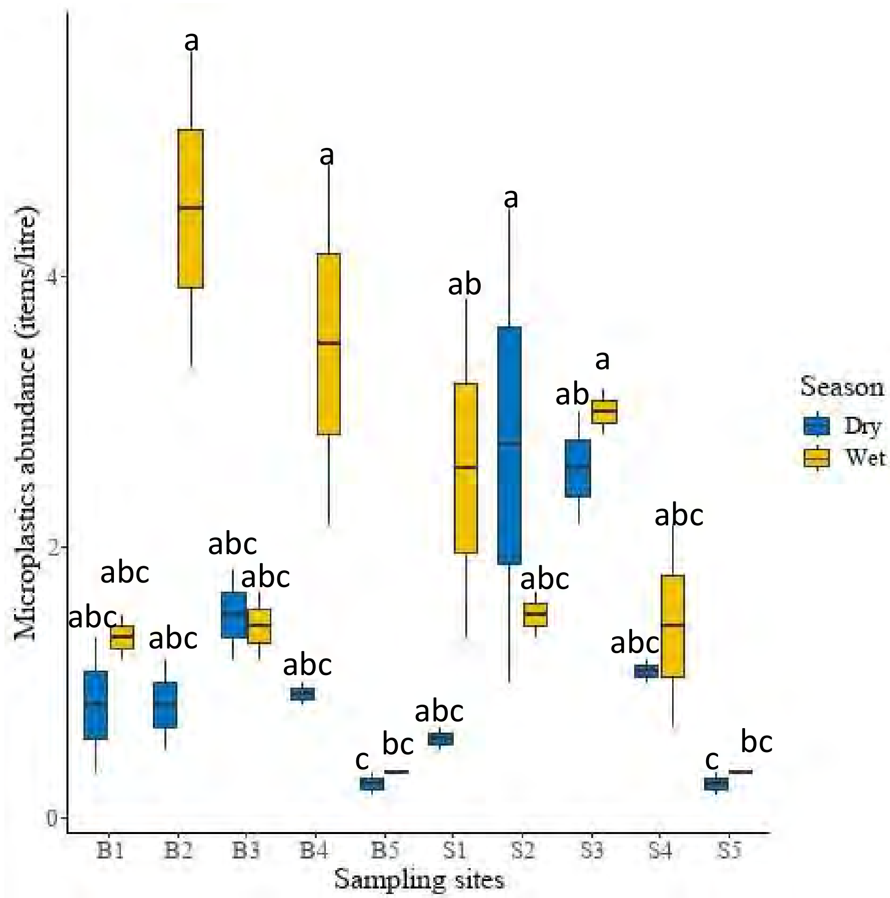


**Figure 3.6.** Temporal distribution of settled microplastics abundance for the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5. Sites with a superscript letter in common are not statistically significantly different, whereas sites with no common superscript letter(s) are statistically significantly different, established using Tukey's HSD.

### 3.3.4 Morphological characteristics of microplastics

Identified microplastic particles were categorised based on their morphological characteristics (size, shape, and colour) as described by Hidalgo-Ruz *et al.* (2012), Masura *et al.* (2015), and Zhang *et al.* (2019). In terms of shape, suspended and settled microplastic particles were categorised into five morphological group based on their shape: fibre, fragment, film, foam, and pellet/sphere (Figure 3.7). Fibres and fragments were the dominant forms of microplastic particles in the Swartkops and Buffalo River systems. The combination of fibres and fragments had the highest proportion, about 77% of the total microplastics while a combination of foam and pellet/sphere had the lowest proportions, 6.1%.

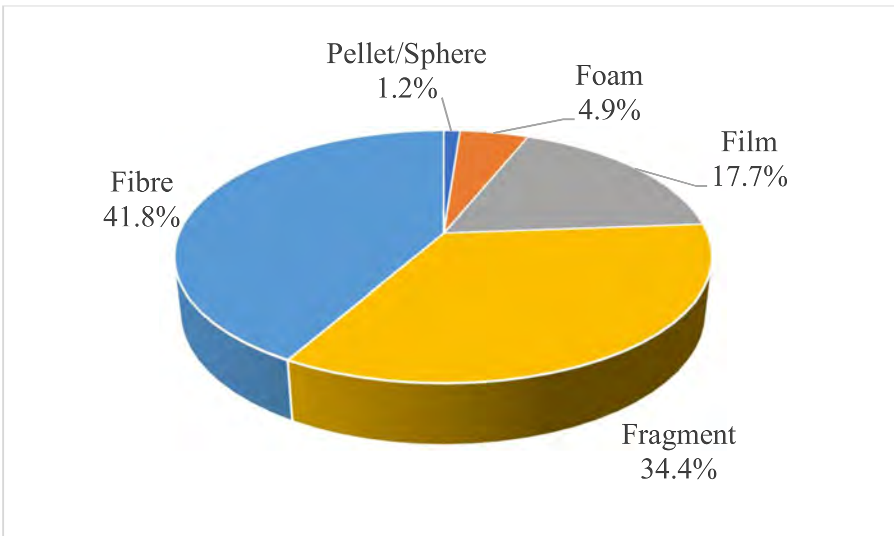


**Figure 3.7.** Percentage composition of the shapes of microplastic for suspended and settled microplastics in the Swartkops and Buffalo rivers during the study period (October 2021–July 2022).

With the exception of sites S1 (located in the Swartkops tributary, the Chatty River) and S5 (located in the upper reaches of the Swartkops River), fibres and fragments dominated all sites (Figure 3.8). Films were the highest proportion at site S1, while films and fragments were the highest proportion at site S5. Also, fibres were the highest proportion of suspended microplastic particles (43.9%), while fragments were the highest proportion among settled microplastics (36.2%) (Figure 3.9). With the exception of site S5 (located in the upper reaches of the Swartkops River), fibres, fragments and films were present at all sites (Figure 3.8). Fragments, foams, and pellet/spheres had a more uniform distribution across settled and suspended microplastics than the distribution of films and fibres (Figure 3.9). For instance, the proportion of films found in settled microplastics was almost twice the proportion found in suspended microplastics.

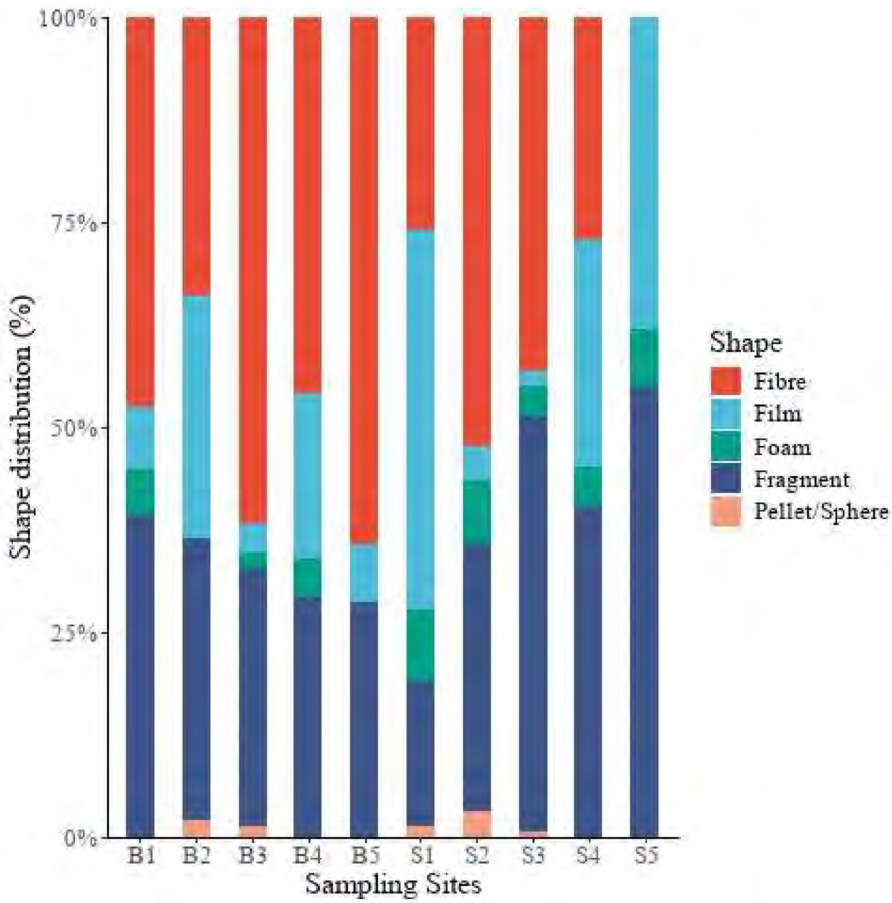


**Figure 3.8.** Percentage distribution of the shapes of microplastic particles (settled and suspended particles combined) found in the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

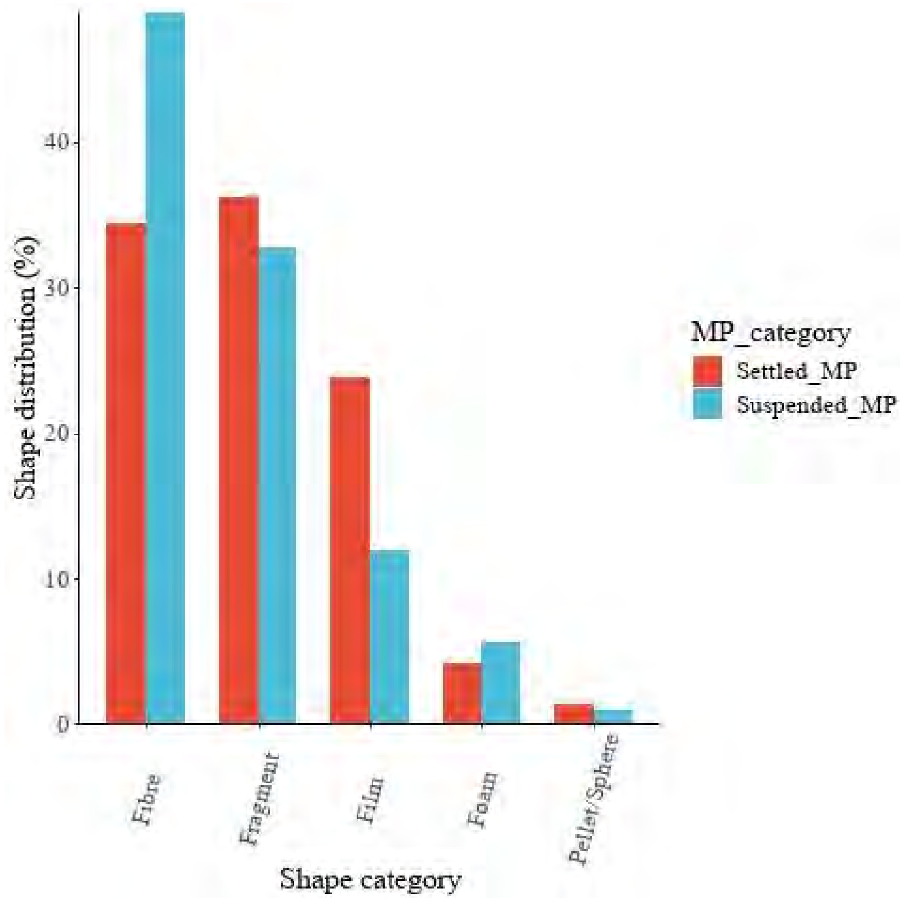


**Figure 3.9.** Comparison of the distribution of shapes for suspended and settled microplastic particles in the Buffalo and Swartkops river systems during the study period (October 2021–July 2022).

Microplastic particles were categorised into four size classes: 0.063 < 0.5 mm; 0.5 < 1 mm; 1 < 2 mm, and 2 < 5 mm (Figure 3.10). Overall, the most abundant microplastic size class was 0.063 < 0.5 mm (37.5%), while size class 2 < 5 mm (16.4%) had the lowest proportion of microplastic particles (Figure 3.10). Microplastic particles less than 2 mm make up about 84% of total particle sizes (Figure 3.10). In terms of settled microplastics, size class 0.063 < 0.5 mm was the most abundant (34.7%) as it was for suspended microplastics (40.2%), while size class 2 < 5 mm had the lowest proportions in settled (20.1%) and suspended microplastic particles (12.9%) (Figure 3.11). All microplastics size classes were found in every site except, in site S5 (located in the upper reaches of the Swartkops), where only two size classes (0.063 < 0.5 mm; 0.5 < 1 mm) were found (Figure 3.12).

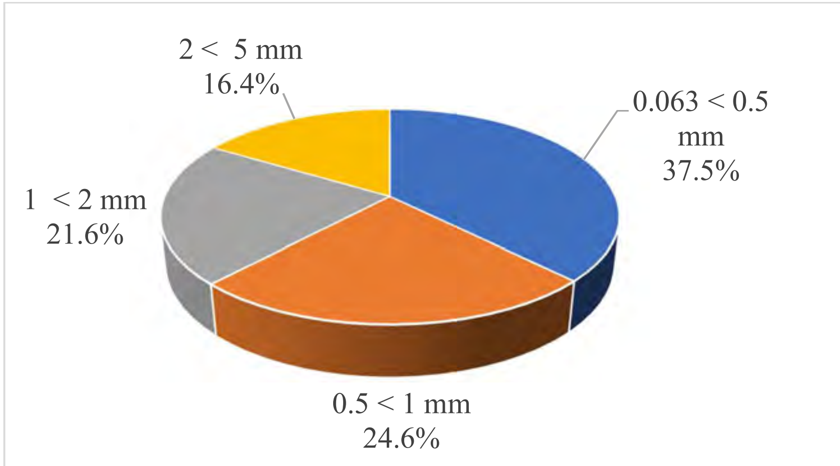


**Figure 3.10.** Percentage composition of microplastic size classes (settled and suspended samples combined) in the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

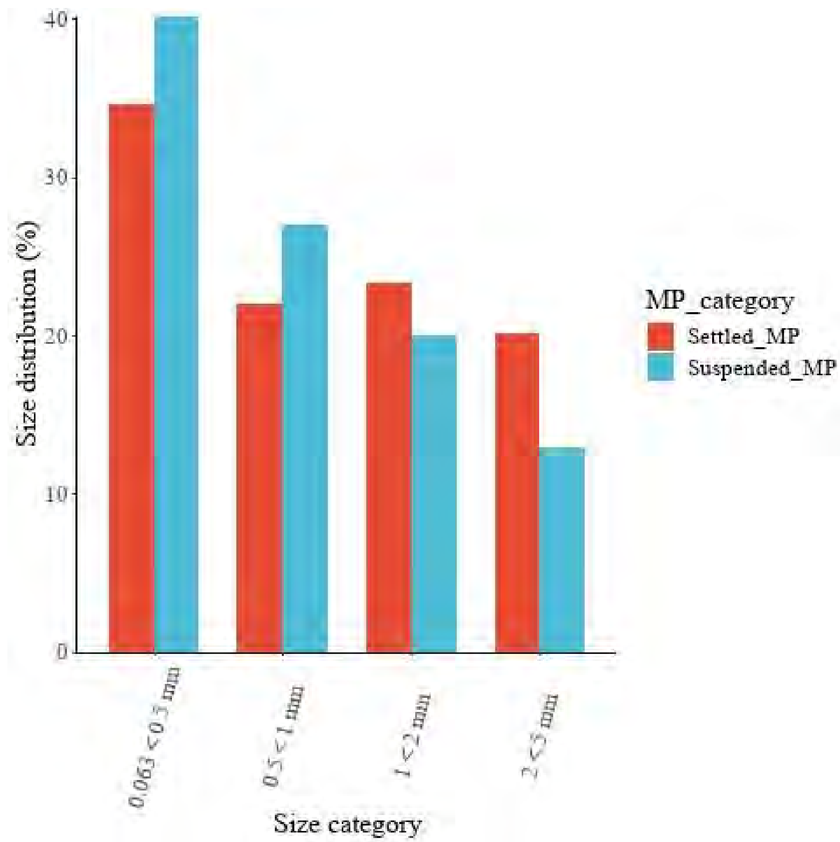


**Figure 3.11.** Comparison of the distribution of size classes for suspended and settled microplastic particles in the Buffalo and Swartkops River systems during the sampling period (October 2021–July 2022).

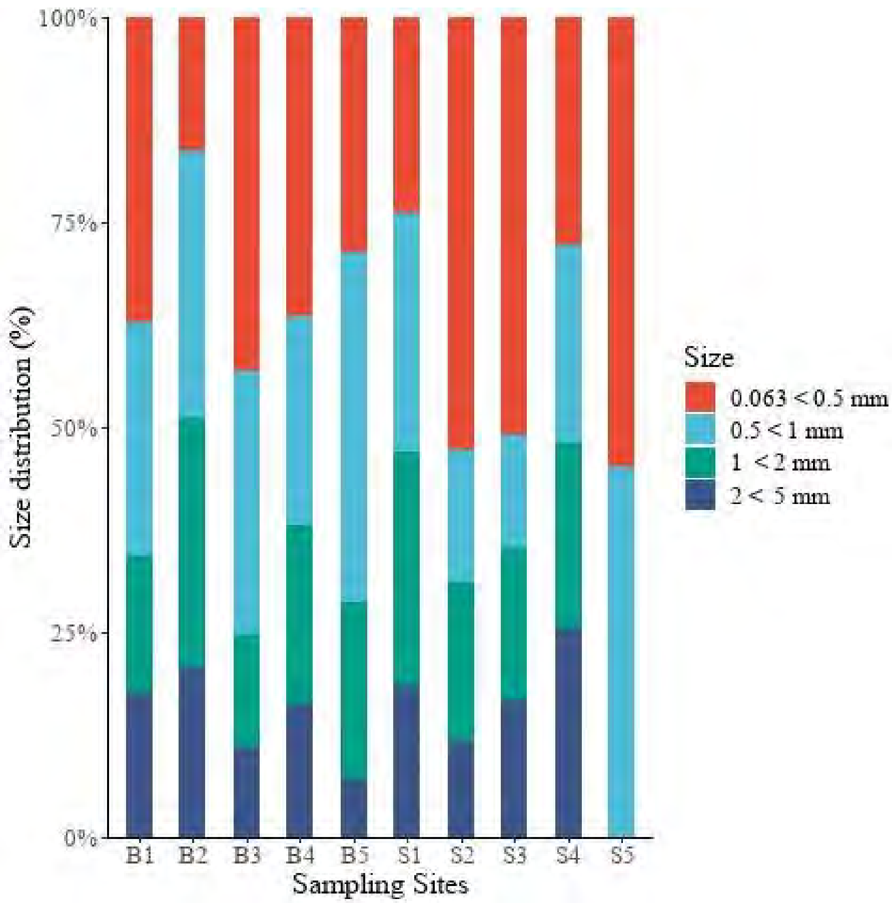


**Figure 3.12.** The distribution of microplastic size classes at each sampling site (suspended and settled samples combined) for the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

The plastic material was categorised into seven groups based on the dominant colours: white, red, blue, green, transparent, black, and yellow (Figure 3.13). In increasing order of dominance, the white (11.0%), green (13.8%), black (19.8%), blue (20.4%), and red (25.3%) accounted for 90% of the plastic material (Figure 3.13). Transparent and yellow colours had the lowest proportions, 8.0% and 1.7%, respectively. The red, blue, black, and green particles dominated settled and suspended forms of plastics (Figure 3.14).

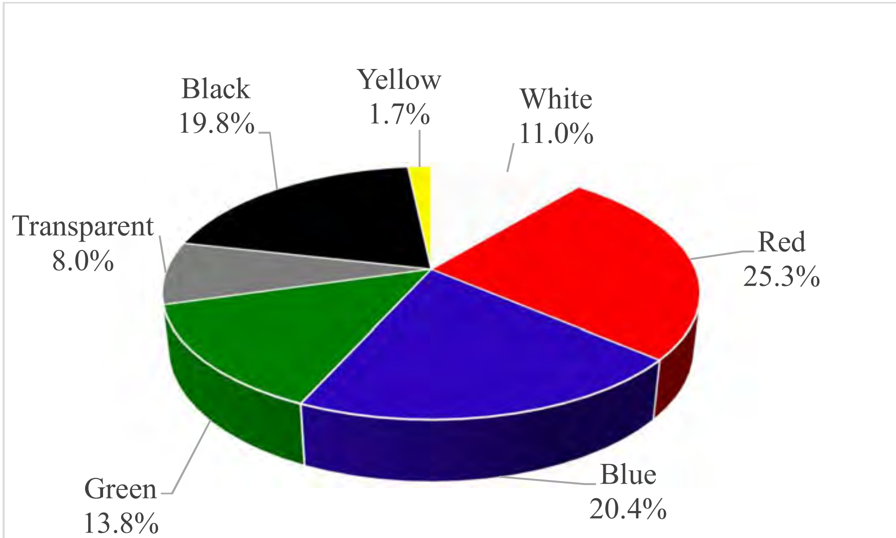


**Figure 3.13.** Percentage composition of the microplastic colours for suspended and settled microplastics in the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

In terms of settled microplastics, the blue particles were the most abundant (23.6%) and for suspended microplastics, the red particles were the most abundant (28.8%), while yellow had the lowest proportions in settled (2.1%) and suspended microplastic particles (1.3%) (Figure 3.15).

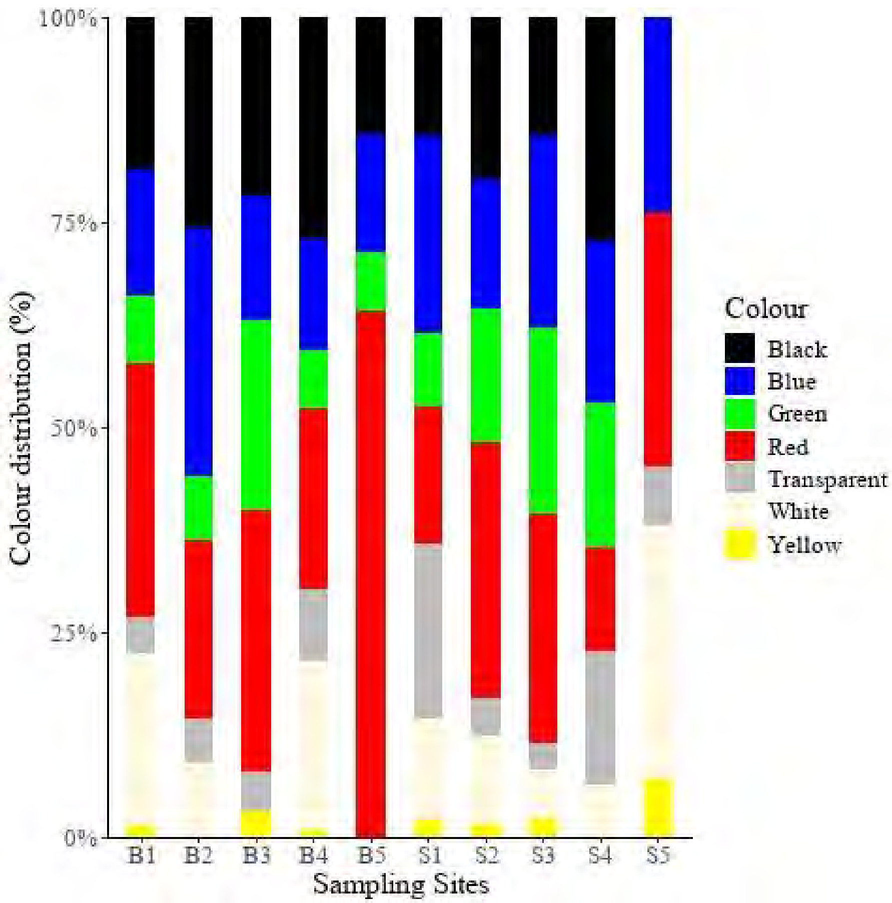


**Figure 3.14.** Percentage distribution of the colours of microplastic particles (settled and suspended particles combined) found in the Swartkops and Buffalo rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

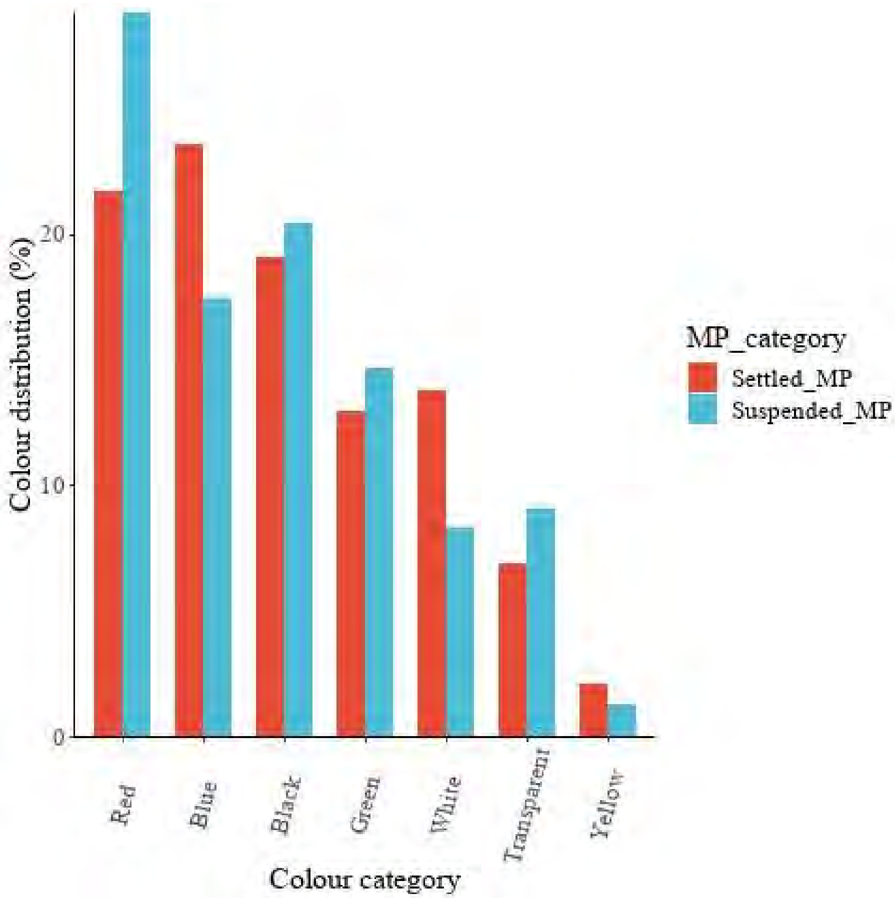


**Figure 3.15.** Comparison of the distribution of colours for suspended and settled microplastic particles in the Buffalo and Swartkops river systems during the study period (October 2021–July 2022).

### 3.3.5 Polymer identification

A Perkin Elmer Attenuated Total Reflectance - Fourier Transform Infrared spectrometer (ATR-FTIR) was used to identify the polymer types. The polymers detected in samples were polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyethylene-vinyl acetate (PEVA), polyurethane (PU), polycarbonate (PC), polyvinyl chloride (PVC), polyamide (PA), and Latex (Figure 3.16). Polyethylene (PE), PP, and PET were dominant. Polyethylene had the highest percentage occurrence (43.8%) while PC and PVC had the lowest (0.4%). Based on the proportion of the density of each type of microplastic, different percentages were recorded among settled and suspended forms of microplastics (Figure 3.17). Settled microplastics had a wider variety of polymer types of microplastics (ten polymers identified) than suspended forms (eight polymers identified) (Figure 3.17). Polyethylene (PE) had the highest percentage occurrence among settled and suspended microplastics (Figure 3.17). High density polymers such as PVC, PU and PA were only found among settled microplastics, while PET had a higher occurrence in settled microplastics than in suspended microplastics.

It is important to note that this study had some limitations regarding the confirmation of microplastics. The FTIR method used for confirmation was only capable of identifying particles ranging from 0.5 mm to less than 5 mm. Consequently, smaller particles recorded in this study were not chemically confirmed, potentially leading to over or underestimation of their presence. Nevertheless, the accuracy of identifying larger particles through FTIR exceeded 95%. This suggests that even the smaller fractions estimated using image processing software coupled with an optical microscope are likely to have very high accuracy

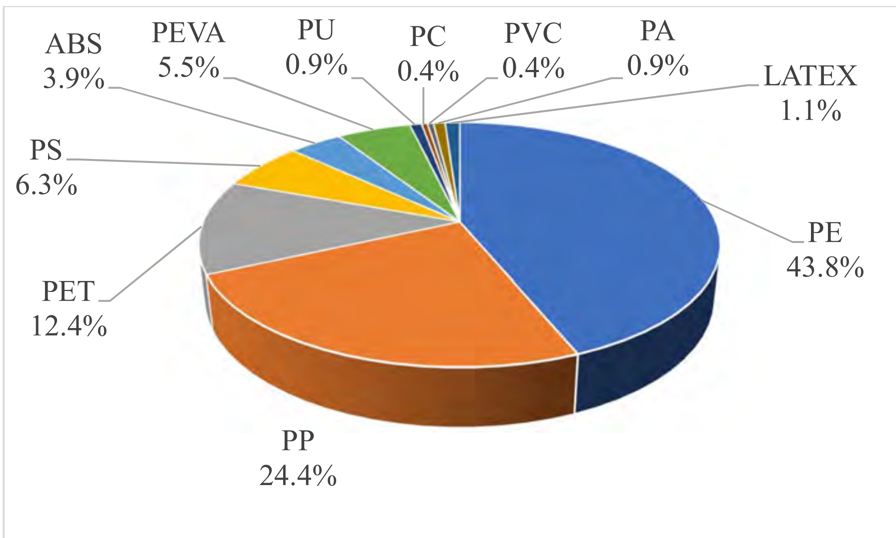


**Figure 3.16.** Percentage composition of the polymers of microplastic for suspended and settled microplastics in the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

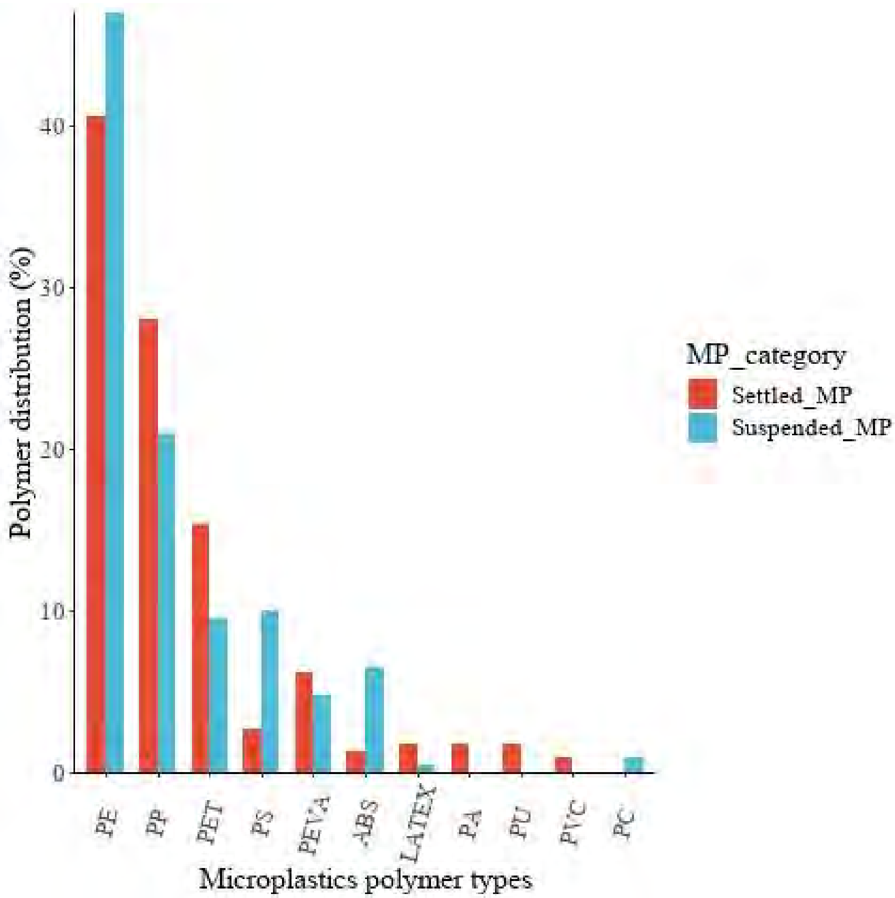


**Figure 3.17.** Comparison of the distribution of polymers for suspended and settled microplastic particles in the Buffalo and Swartkops river systems during the study period (October 2021–July 2022).

Typical spectra peaks used to identify polymers in this study were: PE characteristics spectra showed peaks around 717, 730, 1377, 1462, 1467, 2845, and 2915; PP showed peaks around 972, 1166, 1377, 1455, 2838, 2915, and 2950; PET showed peaks around 720, 1094, 141 and 1713; PS showed peaks around 808, 840, 972, 997, 1166, 1377, 1455, 2838, 2915, and 2950. Polymer spectra of selected microplastics are shown in Appendix B. Plates of some microplastics subjected to polymer confirmation test are shown in Appendix C.

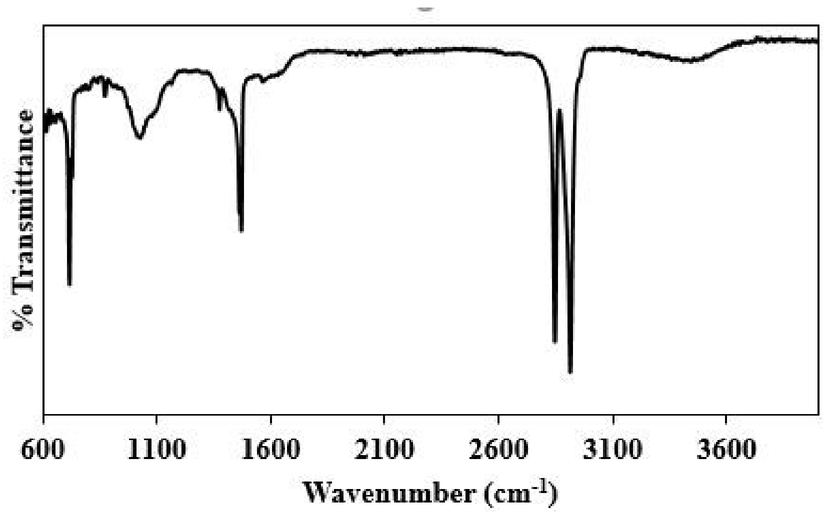


**Figure 3.18.** Typical polyethylene spectra acquired through FTIR analysis for this study.

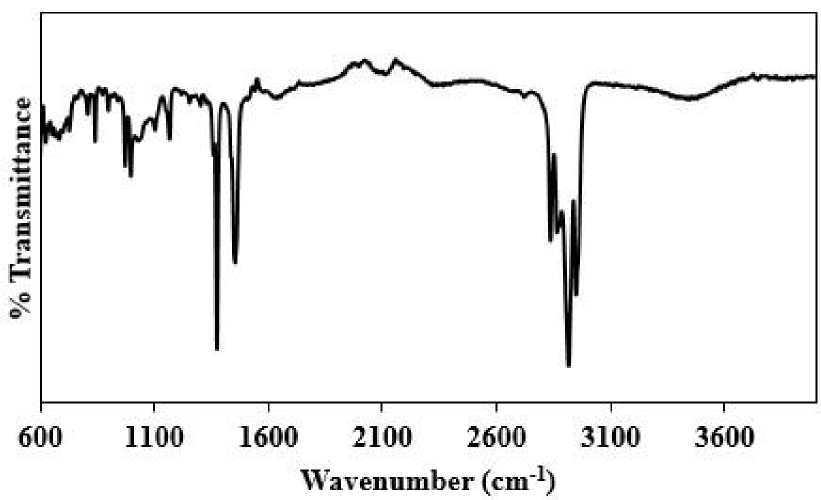


**Figure 3.19.** Typical polypropylene spectra acquired through FTIR analysis for this study.

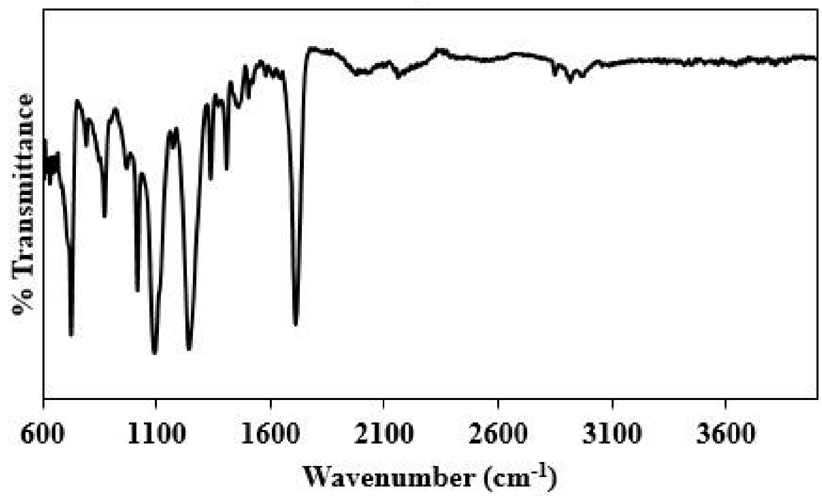


**Figure 3.20.** Typical polyethylene terephthalate spectra acquired through FTIR analysis for this study.

### 3.3.6 Classification of land-use types

The land-use types within the 3 km radius of each sampling location are provided in Table 3.5. In decreasing order of magnitude, natural land, urban land, agricultural land, industrial land, and rural land accounted for 61.98%, 22.74%, 11.82%, 2.77% and 0.69%, respectively.

**Table 3.5.** The proportion of land use within 3 km radius at each sampling location

| Site | Natural land (%) | Industrial land (%) | Urban land (%) | Agricultural land (%) | Rural land (%) |
|---|---|---|---|---|---|
| S1 | 44.67 | 4.12 | 56.20 | 0.00 | 0.36 |
| S2 | 60.34 | 0.38 | 18.07 | 11.88 | 0.44 |
| S3 | 62.10 | 7.61 | 21.13 | 2.79 | 0.79 |
| S4 | 48.95 | 9.76 | 30.96 | 0.75 | 0.88 |
| S5 | 86.35 | 0.58 | 0.00 | 11.83 | 0.18 |
| B1 | 70.51 | 0.04 | 18.39 | 7.17 | 1.20 |
| B2 | 39.66 | 0.79 | 26.76 | 29.82 | 1.30 |
| B3 | 40.87 | 1.74 | 35.70 | 19.65 | 1.16 |
| B4 | 75.43 | 0.07 | 6.01 | 17.44 | 0.91 |
| B5 | 83.53 | 0.00 | 3.06 | 10.43 | 0.08 |

Given the number of study sites, three site groups, were deduced from the cluster analysis of the sites based on their land use types (Figure 3.21). From left to right, an urban group (Sites S4, S1, B2, and B3), a natural group (Sites S5 and B5) and a mixed land use group (Sites S2, S3, B1, and B4).

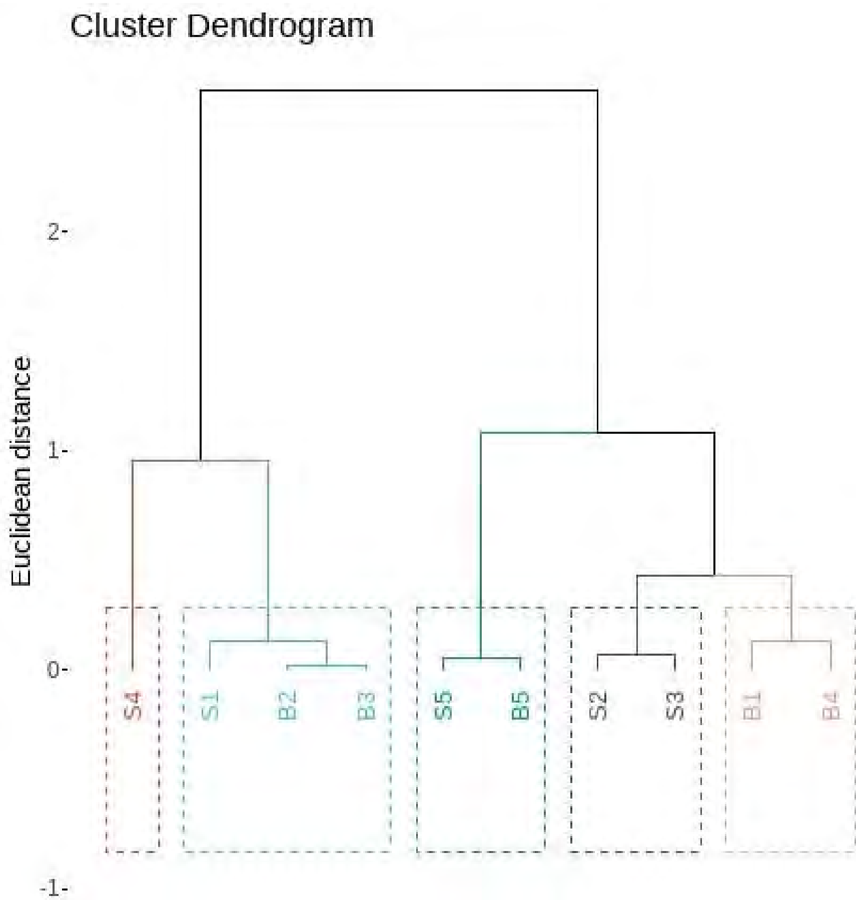


**Figure 3.21.** Dendrogram showing the clustering of the sites based on the land-use types within the 3 km radius of each site in the Swartkops and Buffalo river systems.

### 3.3.7 Relating microplastics distribution to land-use types

The results of the Spearman's rank correlation between microplastic abundance and land use at each site showed microplastics were positively correlated with industrial, urban, and rural land-use types (Figure 3.22). Settled microplastics showed very low positive and non-significant correlations with industrial and urban land-use types while suspended microplastics showed significantly high positive correlations with urban land-use type (suspended microplastics: $r = 0.7$; $p = 0.03$).

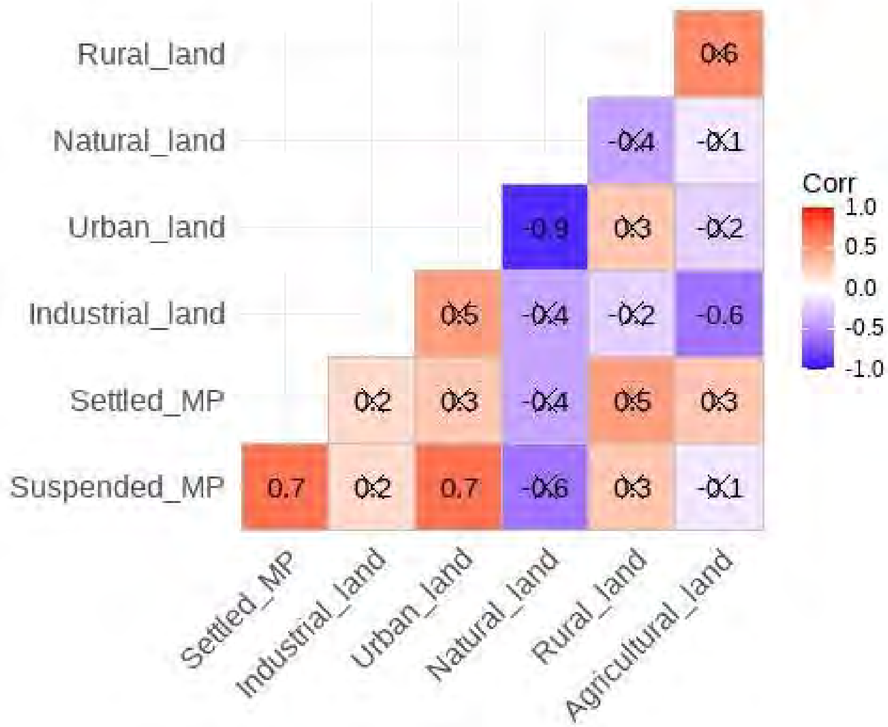


**Figure 3.22.** Spearman's rank correlation coefficient between land-use data and microplastics abundance distribution at each sampling site. Correlation coefficient values marked 'X' are not significant.

The results of the microplastic abundance distribution in relation to the site groups indicated that the mixed and urban land use types had similar percentage distribution of microplastics while the natural land use type had very low percentage distribution of microplastics. More than 95 % of the total microplastics were distributed in sites located in areas other natural land (Figure 3.23).

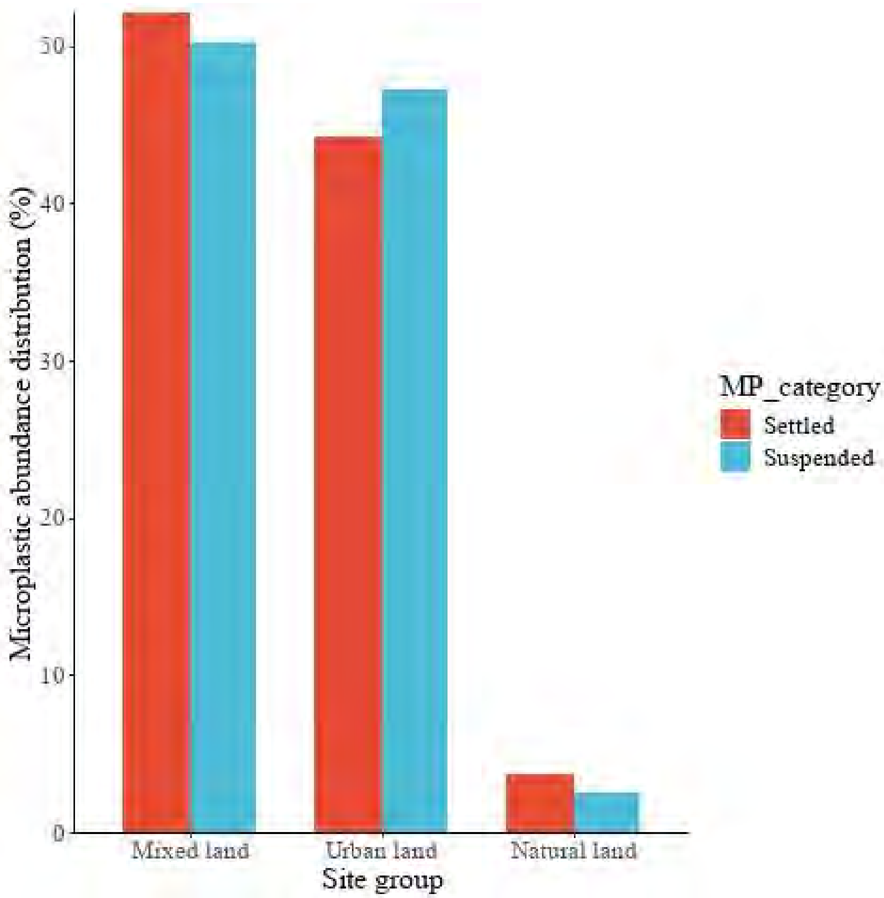


**Figure 3.23.** Land-use site groups and microplastic abundance distribution.

The first two axes of the RDA analysis explained a cumulative variance of 73.8% of the variation between the land-use types and microplastics morphological characteristics datasets, indicating a good ordination result (Figure 3.24). The first axis with an Eigen value 0.050 explained a variance of 48.8% (Table 3.6). Based on the adjusted $R^2$, the RDA analysis explained an unbiased variation of 54% of the variation between the land-use types and microplastics morphological characteristics datasets, indicating a significant model ($p = 0.017$).

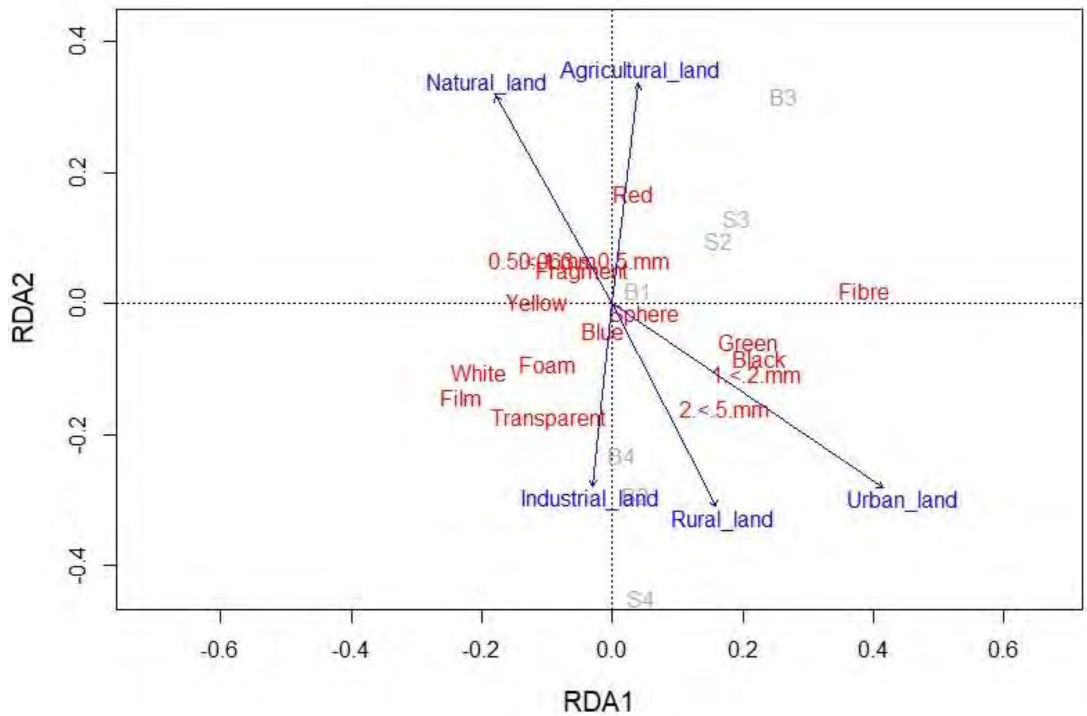


**Figure 3.24.** RDA ordination showing the correlation between land-use types and microplastic morphological characteristics for the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

In terms of axis significance, the first axis of the RDA model was significant ($p = 0.014$) while urban land and agricultural land were indicated as the most significant land-use types for this study (Urban land: $p = 0.002$; Agricultural land: $p = 0.046$), by the order of significance test for the different land-use types following 999 permutations. The RDA model terms of significance test indicated natural land (p = 0.040) and urban land (p = 0.021) to be significant with increasing natural land negatively correlated with decreasing urban land. The higher microplastic size class (i.e., sizes > 1 mm), green and black colour microplastic particles were closely associated with urban land use.

**Table 3.6.** Properties of the RDA analysis for land-use types and microplastics morphological characteristics datasets collected for the Swartkops and Buffalo river systems during the study period (October 2021–July 2022). Bold face value is significant (based on 999 permutation)

| **RDA properties** | **Axis** | | |
|---|---|---|---|
| | **RDA 1** | **RDA 2** | **RDA 3** |
| Eigenvalues | 0.050 | 0.017 | 0.009 |
| % variance explained | 48.8 | 16.6 | 08.4 |
| % cumulative variance explained | 48.8 | 65.4 | 73.8 |
| Test of axis significance | *p* -value = **0.014**<br>*F*-score = 9.591 | *p* -value = 0.245<br>*F*-score = 3.258 | *p* -value = 0.555<br>*F*-score = 1.639 |

| | |
|---|---|
| Test of global RDA significance | *p* -value = **0.017** *F*-score = 3.128 |
| Adjusted $R^2$ | 0.54 |

### 3.3.8 Relating microplastics and physicochemical variables

The two axes of the RDA model for the dry season explained a cumulative variance of 98.7% of the variation between the physicochemical variables and microplastics, while the RDA model for the wet season explained a cumulative variance of 89.5% (Table 3.7). From this study, the relationship between physiochemical variables and microplastics was more profound during the dry season. Based on the adjusted $R^2$ value of the RDA model for the dry season, the RDA analysis explained an unbiased variation of 88% of the variation between the physicochemical variables and microplastics, while the RDA model for the wet season explained an unbiased variation of only 6%. The results indicate that the RDA model for the dry season explained a greater proportion of the variability embedded in the interactions between microplastics and the analysed physicochemical variables.

**Table 3.7.** Properties of the RDA analysis for microplastics abundance distribution and water physicochemical variables datasets collected for the Swartkops and Buffalo river systems during the study period (October 2021–July 2022)

| **RDA properties** | **Axis** | | | |
|---|---|---|---|---|
| | **Dry season** | | **Wet season** | |
| | **RDA 1** | **RDA 2** | **RDA 1** | **RDA 2** |
| Eigenvalues | 0.421 | 0.039 | 0.537 | 0.079 |
| % variance explained | 90.3 | 08.4 | 77.9 | 11.5 |
| % cumulative variance explained | 90.3 | 98.7 | 77.9 | 89.5 |
| Test of axis significance | *p* -value = 0.165 *F*-score = 471.845 | *p* -value = 0.687 *F*-score = 43.717 | *p* -value = 0.628 *F*-score = 51.997 | *p* -value = 0.985 *F*-score = 7.690 |
| Test of global RDA significance | *p* -value = 0.165 *F*-score = 9.207 | | *p* -value = 0.589 *F*-score = 1.066 | |
| Adjusted $R^2$ | 0.88 | | 0.06 | |

During the dry season, settled microplastics were associated with high levels of $PO4^-P$ and TIN, increasing levels of turbidity, TSS, temperature and EC, while suspended microplastics were associated with high EC, TIN, $PO4^-P$, increasing levels of TSS, and temperature (Figure 3.25). In the wet season, settled microplastics were associated with high TSS, increasing levels of turbidity, pH, temperature, and low EC, while suspended microplastics were associated with low levels of turbidity, pH and increasing levels of TIN, EC, PO4-P, TSS and temperature (Figure 3.26).

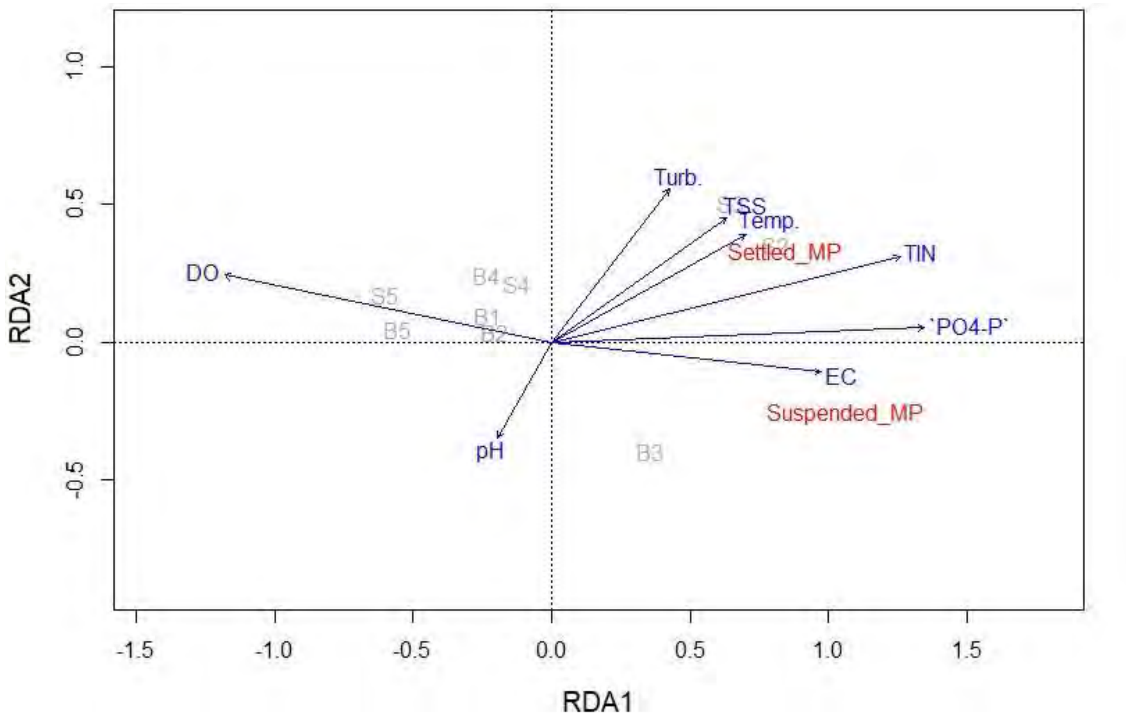


**Figure 3.25.** An RDA ordination showing the correlation between microplastics and physicochemical variables for the dry season in the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

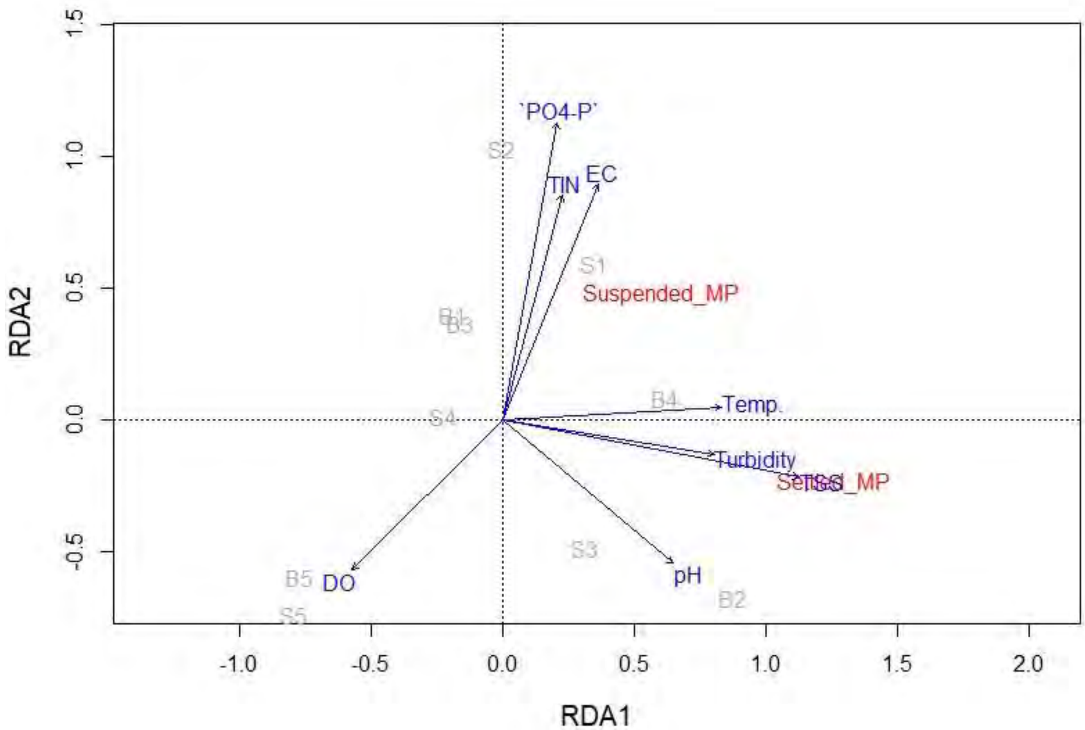


**Figure 3.26**. An RDA ordination showing the correlation between microplastics and physicochemical variables for the wet season in the Swartkops and Buffalo river systems during the study period (October 2021–July 2022).

### 3.4 Discussion

The study reveals significant spatial variability in microplastic abundance within rivers, attributing these variations to a combination of factors both within the watercourse (instream) and stemming from surrounding land use. It is critical to stress that given that microplastics and water quality effects are both strongly linked to land use, rivers with poorer water quality are also likely to have greater amounts of microplastics, thus, designating such instream factors (e.g., water quality variables) as drivers of microplastics should be approached cautiously. For instance, sites directly impacted by point source pollution, such as wastewater treatment works (WWTW), exhibited significantly elevated abundances of suspended and settled microplastics**.** This result is corroborated by other studies that have reported the influences of WWTW on microplastic abundances (McCormick *et al.*, 2014; McCormick *et al.*, 2016; Chen, Jia, *et al.*, 2020; Grbić *et al.*, 2020). The high concentrations observed suggest a direct link between the discharge of wastewater effluents and the presence of microplastics in the aquatic environment. These sites also showed very poor water quality as a result of discharges from the WWTW, indicating a co-dependence and shared relationships between poor water quality and microplastic pollution with the land use (i.e., WWTW) that impact both sets of variables. In these instances the poor water quality could be considered indicators or modulators of microplastic abundance. It is essential to recognise that in the absence of an input source, these instream parameters may not hold significant importance. Thus, it is vital to approach instream factors like water quality variables cautiously when attributing them as drivers of microplastics, given their complex interplay. Effective management strategies for mitigating microplastic pollution in rivers should address both land-based sources and in-stream conditions comprehensively.

The mean concentrations of suspended microplastic measured in this study (1.65 ± 1.19 MP/L) contrasts notably with findings from other investigations. For instance, it exceeded the mean abundance reported by Weideman *et al*. (2019) for the Orange-Vaal River in South Africa (0.23 ± 0.27 items/L), yet falls below values documented in studies conducted in distant countries by Leterme *et al*. (2023) and Wang *et al.* (2021). These disparities in microplastic levels across studies can be attributed to several factors: 1.) site-specific characteristics - variations in microplastic concentrations are influenced by unique attributes of study sites, including local sources, hydrological patterns, and geological features; 2) survey area scope - the extent of survey coverage plays a crucial role, as larger survey areas encompass a broader array of potential microplastic sources and environmental influences; and 3) regional disparities - differences in human activities, waste management practices, and environmental conditions contribute to regional variations in microplastic pollution. The results of this study are more closely comparable to the observed abundance of 1.72 ± 0.67 MP/L obtained with a 250 µm mesh size, reported for the Plankenburg River system in South Africa by Apetogbor *et al.* (2022).

Settled microplastics showed significant temporal variations in abundance distribution. The mean settled microplastics abundance for the wet season differed significantly from that of the dry season. This temporal variation in settled microplastic distribution suggests that microplastic distribution on the riverbed may be influenced more by instream factors than land-based factors. Changes in hydrological conditions such as flow velocity may regulate the mobilisation of settled microplastics and cause an increase in the concentration of suspended microplastics during high flows. Riverine microplastic hotspots may thus be only temporary as high flows and flooding events can potentially re-mobilise settled microplastics into suspension and carry them for longer distances from the initial point of deposition and settling. This observation is supported by Hurley *et al.* (2018) who reported that severe flooding events significantly reduced microplastic contamination of a riverbed. However, precipitation and flooding can mobilise particles previously settled on land into rivers, potentially causing an increase in microplastic contamination of the river environment after the precipitation and flooding events have subsided. Eo *et al*. (2019) and Kataoka *et al*. (2019a) made similar observations regarding the contamination of the river environment after precipitation and flooding events. Thus, riverine microplastic hotspots may change temporally because of changes in hydrological and meteorological conditions but may not translate to overall reduction in the contamination of rivers. Measures aimed at reducing and mitigating microplastic pollution in rivers should be focussed on reducing leakages into the terrestrial environments.

Microplastic particles (< 2 mm) dominated the samples in this study. The small-size classes of microplastic particles presents a large surface area for microbial colonisation and for sorption of toxic chemicals present in the water. The colonisation of microplastic surfaces by bacterial and other organisms impact the sinking rates of microplastics (Miao *et al*., 2021), potentially making them available in sediments where they can be ingested alongside food by benthonic feeding organisms. Variation in microplastic shapes and sizes is a unique factor influencing the vector capacity of microplastics for toxic chemicals and microbial communities, the latter may be introduced to new portions of the river via the biofilm formed on the plastic material. Microplastics may also absorb toxic chemicals to levels that are detrimental to both aquatic and human health. The interaction of microplastics with toxic chemicals in the river water can facilitate the transfer of such chemicals into other aquatic organisms. Concerns have been raised regarding microplastics potentially acting as drivers for antibiotic-resistant genes as a result of the interaction between microplastics and antibiotics (Atugoda *et al*., 2021). The exposure of antibiotics to microbial communities may induce mutagenic transformation to develop genetic resistance to a particular antibiotic. Such resistant genes can be propagated across other bacterial strains.

Urban land-use type was significantly positively correlated with suspended microplastics, and microplastic pollution increased with increasing proportions of anthropogenic land cover relative to natural land cover within the 3 km radius of a sampled site. The lowest concentration of microplastics were found in the site groups with the highest percentage of natural land cover relative to anthropogenic land cover. Industrial and agricultural land-use types showed low positive correlation with settled microplastic, while rural land showed moderate positive correlation with settled microplastics. The increasing trend recorded for microplastic pollution with increasing anthropogenic land cover relative to natural land cover underscores the need for proper management of anthropogenic activities, including industrial, agricultural, and urban related activities, especially in catchments drained by rivers. The results of this study regarding the positive correlation of urban land with the concentration of microplastics is corroborated by the results of previous studies ( Kataoka *et al*., 2019b; Chen, Jia, *et al*., 2020; Su *et al*., 2020). The negative correlation of natural land with microplastics in this study further strengthens the results, indicating a reduction in microplastics concentrations with increasing natural land cover relative to anthropogenic land cover within the 3 km radius of a site. In this study, sites located downstream of a WWTW effluent discharge point were heavily impacted in terms of the water quality and the abundance of microplastics. The implication is that aquatic macroinvertebrates with potentially tolerant traits of urban pollution are at a greater risk of exposure to microplastics than are urban-pollution-sensitive taxa. Accordingly, proper urban land planning, effective waste management, proper maintenance, and the upgrade of WWTWs may help to reduce the influx of microplastics from land-based sources. In the current study, land use was associated with microplastic concentrations and microplastic morphological characters.

The relationship between water physicochemical properties and microplastics was more profound during the dry season, as indicated by the proportion of the unbiased variability explained by the RDA model for the interactions between microplastics and the analysed physicochemical variables. During the dry season, microplastic concentrations were generally associated with high nutrient levels indicated by high levels of TIN and $PO_4^-$P, moderate temperature, low levels of dissolved oxygen and a low pH. He *et al.* (2020) noted a comparable positive correlation between nutrients and microplastic abundance. This finding is not surprising, given the shared sources of both nutrients and microplastics, particularly through land use and wastewater discharge. The impact of wastewater on nutrient levels in various sites is evident in this study. Moreover, during low flows (e.g., during the dry season), the dilution of wastewater is reduced, potentially exacerbating the presence of water chemicals. Therefore, it's plausible that microplastic concentrations are more influenced by their original sources rather than by instream chemical concentrations. Also, water physicochemical variables such as pH, nutrients, and temperature play a role in shaping microbial activity and diversity (Jordaan & Bezuidenhout, 2013; Ibekwe *et al*., 2016; Niu *et al*., 2019; Moyal *et al*., 2023). Nutrients such as TIN and $PO_4^-$P at high temperatures and low dissolved oxygen create conditions suitable for development of biofilms on microplastics surfaces. Biofilm development usually alters microplastic density and impacts their sinking rates which can make them available for aquatic organisms on the riverbed. Microplastic with biofilm may also act as a vector of pathogenic microbes in river systems. The differences in the physicochemical variables during the wet and dry seasons may thus impact microbial activities and regulate conditions suitable for the formation of biofilms on microplastic surfaces. Further, the presence of biofilms on microplastic surfaces may influence microplastic aging and capacity for bioaccumulation and toxicity of additives and adsorbed pollutants. Biofilms develop and occupy microplastic surfaces, consequently reducing the surface available for chemical sorption and bioaccumulation of toxic chemicals.

Suspended microplastics were generally associated with low turbidity and low to moderate levels of TSS across the wet and dry seasons, while settled microplastics were associated with increasing levels of TSS in the wet and dry seasons. The concentration of microplastics in riverine water can diminish with high levels of turbidity and TSS. Microplastic heteroaggregation with particulate matter can be facilitated by high levels of TSS and turbidity, potentially impacting settling and sinking rates of previously suspended microplastics. Bayo *et al*. (2020) reported that effluent wastewater with high concentrations of suspended solids proved to have a low microplastic burden with larger microplastic sizes. The low microplastic burden was attributed to heteroaggregation with particulate matter. Heteroaggregation of microplastic particles with particulate matter can also alter microplastic density, potentially impacting their sinking rates and making them unavailable for microbial colonisation. Thus, suspended microplastics may prevail with low turbidity and TSS, as observed in this study, and could facilitate microbial colonisation of microplastics and the development of microplastic vectors for pathogenic microbes.

### 3.5. Conclusion

In this study, suspended microplastics showed significant spatial variations in abundance distribution. Sites affected by point sources of pollution and sites located downstream of WWTW recorded high abundances of microplastics. Settled microplastics showed significant spatial and temporal variations in abundance distribution. Riverine microplastic hotspots may change temporally because of changes in hydrological and meteorological conditions, but these changes may not translate to overall reduction in the contamination of rivers. Measures aimed at reducing and mitigating microplastic pollution in rivers should be focussed on reducing leakages into the terrestrial environments. Microplastic particles (< 2 mm) dominated the samples in this study. The small-size classes of microplastic particles present a large surface area for microbial colonisation and for sorption of toxic chemicals present in the water. Urban land-use type was significantly positively correlated with suspended microplastics, and microplastic pollution increased with increasing proportions of anthropogenic land cover relative to natural land cover. The relationship between water physicochemical properties and microplastics was more profound during the dry season, as indicated by the proportion of the unbiased variability explained by the RDA model for the interactions between microplastics and the analysed physicochemical variables. Suspended microplastics were generally associated with low turbidity and low to moderate levels of TSS across the wet and dry seasons, while settled microplastics were associated with increasing levels of TSS in the wet and dry seasons.

The study underscores the close relationship between microplastic pollution in rivers and land use activities. It highlights that microplastic abundance in polluted sites is intricately linked to the quality of water in those areas. Specifically, the research identifies a co-dependence where poor water quality tends to correlate with higher levels of microplastics. This co-dependence suggests that certain land use practices, such as urbanisation, industrial activities, and agricultural runoff, can contribute to both deteriorating water quality and increased microplastic contamination in rivers. The study's findings imply that efforts to improve water quality in polluted river sites could also have a positive impact on reducing microplastic abundance. Conversely, addressing microplastic pollution may require mitigating its sources through better land use management practices and pollution control measures. Overall, the results of this study underscore the need for context-specific assessments of microplastic pollution, recognising the diverse influences shaping its distribution in river systems. By understanding these complexities, policymakers and environmental managers can develop targeted interventions to reduce microplastic contamination effectively.

## CHAPTER 4: INFLUENCES OF HYDRAULIC BIOTOPES ON THE DISTRIBUTION OF MICROPLASTICS IN RIVER SYSTEMS

### 4.1 Introduction

The transport and distribution of microplastics (MPs) in riverine systems at the reach-scale are mediated by river geomorphology and hydraulic characteristics such as depth, flow velocity, and substrate roughness (de Carvalho *et al*., 2021; Kiss *et al*., 2021; Kumar *et al*., 2021). These factors interact with microplastic characteristics such as density, size, shape, and polymer type to influence the aggregation, adsorption, advection, dispersion, diffusion, sedimentation, re-mobilisation, and settling of microplastics (Kumar *et al*., 2021; Yan *et al*., 2021b). At the reach scale, pools, riffles, and runs are hydraulic biotopes, reflecting a combination of substrate type, depth, and flow velocity (Wadeson & Rowntree, 1998; Newson & Newson, 2000; Thomson *et al*., 2001). These hydraulic biotopes may vary from shallow fast-flowing-, shallow slow-flowing-, to deep fast-flowing- and deep slow-flowing waters, so that microplastic in rivers tends to settle and potentially re-mobilise in the slow-flowing hydraulic biotopes but are transported speedily in the fast-flowing hydraulic biotopes. For instance, Lu *et al*. (2023) demonstrated the variations in microplastic dispersal and transport behaviour under different flow conditions in riverine ecosystems. The authors used an approach that combined particle tracking and hydrodynamic modelling and reported that high water velocity facilitated microplastic particle migration, which tends to correspond with higher concentrations of suspended microplastics and lower concentrations of sedimented microplastics. Conversely, slow-flow velocity facilitates the sedimentation of microplastics and decreases the potential for resuspension of settled microplastics (Ding *et al*., 2019; Hübner *et al*., 2020).

Dahms *et al*. (2020) reported that patches of flow with increased depth and decreased flow allowed microplastics to settle down to the sediments where benthic macroinvertebrates could ingest the particles. Thus, in relation to microplastic transport at the reach-scale, the slow flowing hydraulic biotopes can be likened to ‘sink zones’ for microplastics (i.e., hydraulic biotopes that facilitate microplastic settling on the riverbed), whereas the fast-flowing hydraulic biotopes, are the ‘flush zones’ (i.e., hydraulic biotopes where microplastic particles are more in suspension, and tend to be constantly re-mobilised). Viewed this way, rivers are made up of patchy distribution of sink and flush hydraulic zones for microplastics. These processes that facilitate microplastic particle transport, instream behaviour, deposition, and retention in river systems are quite complex (Horton & Dixon, 2018) and require further investigation. Different hydraulic biotopes are characterized by distinct hydrodynamic conditions which can be measured by certain hydraulic variables and quantified by hydraulic indices such as the Froude and Reynolds numbers (Newson & Newson, 2000; Thomson *et al.*, 2001; Wadeson & Rowntree, 1998). Microplastic monitoring studies can thus integrate these indices for interpreting microplastic results in river systems and standardize sampling and collecting techniques for inter-laboratory calibration and study comparison.

Studies that have reported on the influences of flow variability and river geomorphology on microplastics transport behaviour are on the increase (Defontaine *et al.*, 2020; Miller & Orbock Miller, 2020; de Carvalho *et al.*, 2021; Kiss *et al.*, 2021; Yan *et al.*, 2021a ; Lu *et al.*, 2023). Yet, microplastic monitoring and evaluation studies are not designed to reflect the role of hydraulic biotopes in the distribution of microplastics in river systems. Microplastic sampling designs that incorporate riverine hydro-geomorphology considerations might be useful for detecting microplastics hotspots located farther from point sources that may otherwise be missed by other sampling protocols (Owowenu *et al.*, 2023). Results obtained from sampling designs that incorporate hydro-geomorphological considerations might be more representative of the microplastic pollution level in a river and would be useful for evaluating the effectiveness of mitigation measures aimed at preventing the continuous influx of microplastics from land-based sources. The influences of hydraulic biotopes may also be partly responsible for the heterogeneous distribution of microplastics reported for riverine systems by some authors (Wagner & Lambert, 2018; de Carvalho *et al.*, 2021; Xia *et al.*, 2021; Apetogbor *et al.*, 2022). He *et al.* (2020) also noted that the nature of the substratum, which is a fundamental characteristic of hydraulic biotopes, influences the retention capacity and the rising velocities of settled microplastics. Thus, it is critical to evaluate the role played by hydraulic biotopes in determining the transport dynamics and fate of microplastics in riverine systems. Besides, a complete assessment of riverine microplastics requires a comprehensive assessment of factors that impact the behaviour and fate of microplastics in river systems (Xia *et al.*, 2021).

In this chapter, the influences of hydraulic biotopes on microplastic distribution are explored. The chapter poses the questions whether sampling and monitoring at the hydraulic biotope scale can: i) improve our understanding of the transport dynamics and fate of microplastics; ii) enable the detection of riverine microplastics hotspots; and iii) be a useful approach for microplastic sampling and evaluation in riverine and other lotic freshwater systems.

Given the diversity of hydraulic biotopes / geomorphological units in riverine systems, this study conceptualised and grouped hydraulic biotopes (e.g., riffles, runs, pools) which infer geomorphology and hydrologic condition into two distinct functional groups / names or hydraulic zones viz; ‘flush’ and ‘sink’. The designation of functional groups / names was done to test the hypothesised functions. The sampling approach undertaken in this study was designed to facilitate the comparison of the abundance and composition of suspended and settled microplastics across the designated functional groups or hydraulic zones. The body of knowledge regarding factors impacting microplastic occurrence, transport and distribution in rivers has been extensively reviewed in Chapter 1, Sections 1.5–1.7. This current chapter addresses objective two stated in Chapter 1: *to determine the distribution of microplastics in relation to hydraulic biotope types* through the following sub-objectives: i) to provide a hydraulic characterization of the two functional groups or hydraulic zones; ii) to analyse the impact of hydraulic biotopes on the distribution, abundance, and composition of microplastics; iii) to elucidate the relationship between hydraulic indices and microplastic. Two hypotheses were tested in this study to determine the functional group or hydraulic zone that is a potential microplastic hotspot in the study rivers.

Hypothesis I: The differences in suspended microplastics abundances between the two functional groups or hydraulic zones are significantly different.

Hypothesis II: The differences in settled microplastics abundances between the two functional groups or hydraulic zones are significantly different.

## 4.2 Materials and methods

### 4.2.1 Hydraulic zone delineation

In each of the study sites (described in Chapter 2, Subsection 2.1.2), hydraulic zones (i.e., flushes and sinks) were visually delineated in each site, based on the flow appearance, the dominant substrate class and measurable hydraulic attributes (i.e., flow velocity, depth) (Jowett, 1993; Wadeson & Rowntree, 1998). However, it is important to note that owing to relatively low flow velocities and low water depth experienced in the study rivers, which was exacerbated in some cases by excessive growth of hydrophytes and high sedimentation, some flush zones (i.e., runs, riffles) were delineated by lower velocities than anticipated. Flushes were mostly runs and riffles, while sinks were mostly pools. In this study, the hydraulic biotope classification was largely determined by the geomorphological classification of morphological units and there was no attempt to reclassify hydraulic biotopes as discharge changed. This classification approach was used to maximise the stability of the hydraulic zones at the time of sampling as morphological units are more stable with respect to changes in discharge.

### 4.2.2 Hydraulic data collection and computation of hydraulic indices

At each site, a rope was strung between two fixed points in each delineated hydraulic zone along the river channel and a cross-sectional area was mapped. Measurements of velocities along the cross-section and water depth above roughness elements (substratum) was recorded for every two-metre cell and results averaged for each zone. Roughness height was estimated from the mean height of all identifiable roughness elements within a 0.5 $m^2$ grid of each point of velocity measurement. These data, together with temperature readings at each hydraulic zone, were used to estimate velocity depth ratios (*VDR*), Shear velocity ($U^*$), Reynolds number (*Re*), Froude number (*Fr*), and roughness Reynolds numbers, for each hydraulic zone. Detailed explanations on hydraulic data measurements are stated in Chapter 2, Section 2.3.

Flow velocity and water depth act independently or in combination through the different hydraulic indices to describe the average conditions in the water profile and near-bed conditions (Rowntree & Wadeson, 1999). The velocity/depth ratio, Reynolds number (*Re*), and the Froude number (*Fr*) describe the mean flow in the water column while shear velocity ($U^*$) and the roughness Reynolds number ($Re^*$) describe near-bed hydraulics (Gordon *et al.*,1992; Newson & Newson, 2000). The Reynolds number gives an indication as to whether the flow is laminar, transitional or turbulent by representing the ratio of inertial forces to viscous forces (Wadeson & Rowntree, 1998). A large value of *Re* indicates turbulence, and a small value, laminar flow. From experimental data, the transitional range for open channels is usually considered to be from *Re* values 500 to 2000 (Gordon *et al.*,1992). For this study *Re < 500 = laminar; 500 < Re < 2000 = transitional; and Re > 2000 = turbulent.* The Reynolds number is given by:

$$Re = {uR}/{V}$$

Where u = mean velocity in (m/s), R = hydraulic radius which is equivalent to the average depth and V= kinematic viscosity of water ($m^2\ s^{-1}$). V is a function of the temperature.

The Froude number (*Fr*) describes the effect of gravity upon the state of flow and is represented by a ratio of inertial forces to gravity forces. The Fr values describes three classes of flow; *Fr < 1 = subcritical (or slow or tranquil) flow; Fr = 1 is critical flow; Fr > 1 is supercritical (or* *rapid) flow* (Gordon *et al*., 1992; Rowntree & Wadeson, 1999). The Froude number was computed using the formula:

$$Fr = u \,/\, (gD)^{0.5}$$

Where u = mean velocity (m $s^{-1}$), g = gravitational constant (m $s^{-2}$), D = water depth (m).

Shear velocity ($U$*) is a measure of the shear stress expressed in velocity units (m/s) (Wadeson & Rowntree, 1998). Riverbed shear stress is a force per unit area of the bed (in N $m^{-2}$) and increases with flow depth and steepness of the channel (Biswas & Chandra Das, 2016). A critical shear stress is required to set a particle in motion (Gordon *et al*., 1992). The coarse particles lying on riverbeds are set in motion when the critical shear stress is passed (Bravard & Petit, 2009a). Shear velocity ($U$*) is given by:

$$U* = {}^{u}/_{5.75} < \log {}^{12D}/_{k}$$

Where u = depth average velocity ($ms^{-1}$), D = water depth (m), k = roughness height

The roughness Reynolds numbers (*Re**) is used to describe riverbed roughness and combines the effect of velocity and substrate type. The height of the roughness elements relative to the thickness of the laminar sub-layer (i.e., the region close to the bed where the flow is entirely laminar) is an essential determinant of flow conditions near the bed (Gordon *et al*., 1992; Rowntree & Wadeson, 1999). Flow is impacted depending on the height of the roughness elements that protrude above the laminal sublayer. A surface is considered hydraulically smooth if *Re* < 5, hydraulically rough if Re* >70, and transition at 5 < Re* < 70* (Schlichting, 1979). The roughness Reynolds number is given by:

$$Re* = {}^{U*k}/_{V}$$

Where U* = shear velocity, k is roughness height, and V = is kinematic viscosity (1 x $10^{-6}$ $m^2$ $s^{-1}$ for water at 20ºC).

Velocity depth ratio (VDR) is a function of flow velocity and depth. Areas with low velocity and increased depth have been reported to facilitate vertical transport or settling of microplastics (Dahms *et al*., 2020b). Depending on the *Re*,* the velocity depth ratio will have a considerable impact on microplastics transport. Velocity depth ratio is given by:

$$VDR = {}^{u}/_{D}$$

Where u = depth average velocity ($ms^{-1}$), D = water depth (m)

The range of data derived for the hydraulic zones are shown in Appendix E (Table E1–E8)

### 4.2.3 Sampling suspended and settled microplastics.

At each hydraulic zone, 12 L water samples were collected in three replicates of 4 L for suspended and settled microplastics. Water samples were transported to the laboratory and filtered through a 63 µm stainless steel sieve. Replicate samples were pulled and treated as composite samples. Details on sampling methodology are stated in Chapter 2, Section 2.6.

### 4.2.4 Sample preparation and processing

Samples were prepared and processed separately for the hydraulic zones. Details of sample preparation and processing methods are explicitly described in Chapter 2, Section 2.7.

### 4.2.5 Quantification and characterisation of microplastics

Quantification and characterisation of microplastics as well as quality assurance measures have been detailed in Chapter 2, Section 2.8.

## 4.3 Statistical analysis

Different techniques for exploratory data analysis were carried out. Box plots were used to show the distribution patterns of the hydraulic indices across the hydraulic zones. The non-parametric Mann-Whitney U test was used to examine whether there was a significant difference between the hydraulic indices derived for the flush and sink hydraulic zones with a < 0.05 alpha level. The Mann-Whitney U test is the non-parametric alternative of the parametric unpaired t-test. It is used when the samples are not drawn from a normal distribution and when the independent variables are just two (Ogbeibu 2014). The microplastic abundance, in mean and standard deviation (SD) from the samples, was expressed as the number of items per litre (written mostly as items/L, for this study). To test the effects of hydraulic biotopes (i.e., hydraulic zones), seasons, and sampling sites on the variability of aquatic microplastics, multi-group comparisons were made using the global multivariate analysis of variance (MANOVA). If MANOVA returned a significant result, the effect size of the significant result was calculated using the "effectsize" package in R- statistical programming environment. A two-way ANOVA was then used to determine how settled and suspended microplastics responded according to the levels of the independent variables that were significant with the MANOVA analysis. The assumptions of MANOVA and how they were tested prior to the analysis are detailed in Chapter 2, Section 2.10, Sub-section 2.10.4. The independent two sample t-test was undertaken to test the two hypotheses stated for this study. Bar charts were used to show microplastics morphological distribution and polymer compositions in each hydraulic zone.

PERMANOVA was undertaken to compare the hydraulic zones and seasons (independent variables) in terms of the microplastics morphological characteristics such as the individual shape (fibre, fragments, films, foams, and pellet/sphere), size (size classes as categorised in Section 2.8 of the current study), colour (red, blue, green, and black), and polymer types that were common to both hydraulic zones (PE, PP, PET, PS, ABS and PEVA) and to understand whether there was any significant interaction effect between the hydraulic zones and seasons on the dependent variables. Pairwise comparison of significant results from PERMANOVA was carried out using the 'pairwise.Adonis' R package and the 'pairwise.adonis' function in R statistical programming environment. The generalized additive models (GAMs) were used to describe the relationships between microplastics abundance data and survey-derived hydraulic indices for the hydraulic zones. Given the distribution of the microplastic data, the Wilcoxon rank sum test also called the Mann Whitney U test was used to test the two hypotheses.

## 4.4 Results

### 4.4.1 Hydraulic characterisation of sampling sites

The first level of analysis grouped all hydraulic data according to the hydraulic zones (i.e., flush and sink). The variability of the hydraulic characteristics between the hydraulic zones is shown in Figures 4.1 to 4.5. As evident in Figure 4.1, there was a large variability of the Froude number between the flush and sink hydraulic zones. The Froude number (*Fr*) ranged between 0.12 to 1.09 and 0.00 to 0.09 for the flush and sink hydraulic zones, respectively. The mean *Fr* (mean ± SD) was 0.39 ± 0.21 and 0.05 ± 0.02 for the flush and sink zones in the Buffalo River and 0.29 ± 0.18 and 0.03 ± 0.02 for the flush and sink zones, respectively, in the Swartkops River. A Mann Whitney U analysis of the hydraulic characteristics (Appendix D, Figures D1 – D7) confirms that, for each hydraulic characteristic (mean depth, *MD*; mean velocity, *MV*; velocity depth ratio, *VDR*; Froude number, *Fr*; Reynolds number, *Re*; roughness Reynolds number, *Re**; and shear velocity, U*), the mean values significantly differentiated between the delineated flush and sink zones at the 99% confidence level, ($p < 0.0001$). The highest Froude number derived for the flush zone (*Fr* = 1.09) in the supercritical flow regime was recorded during the wet season at site B1 (located in the lower reaches of the Buffalo River and upstream of Bridle Drift dam) in the Buffalo River. By comparison, in the Swartkops River, the highest Froude number (*Fr* = 0.91) in the subcritical regime was recorded at site S1 (located in the Swartkops tributary, the Chatty River) in the flush hydraulic zone during the wet season.

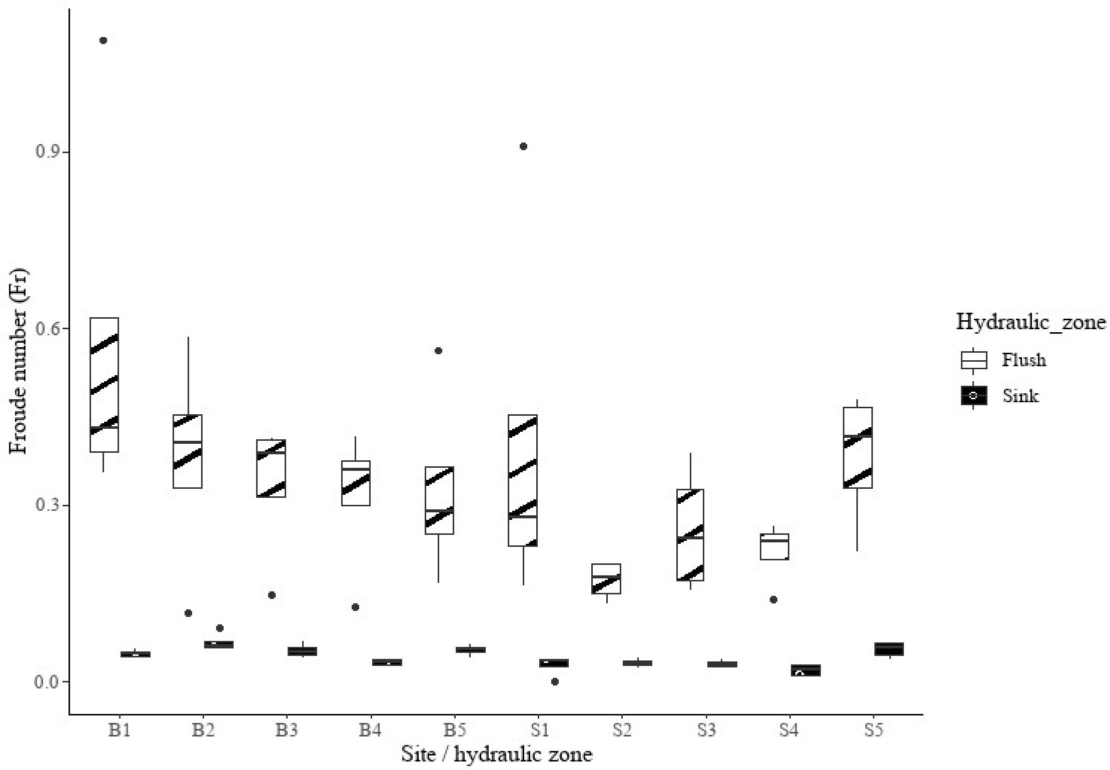


**Figure 4.1.** Box plot showing the variability of the Froude number (Fr) between the hydraulic zones in the Buffalo and Swartkops rivers during the period (October 2021–July 2022)**.** Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

As expected, Reynolds numbers were generally higher in flushes than in sinks, reflecting a higher degree of turbulent flow conditions in flushes than in sinks. In this study, *Re* ranged between 19418 and 403401 and between 0 and 59521 in the flush and sink hydraulic zones, respectively. In the Swartkops River, *Re* ranged between 19418 to 403401 with a mean value of 72414 ± 81209 (mean + SD ) and between 0 to 46262 with a mean value of 18246 ± 12631 (mean + SD) for the flush and sink hydraulic zones, respectively, while in the Buffalo River, *Re* was between 35257 and 306990 with a mean value of 110059 ± 65565 (mean ± SD) and between 9510 and 59521 with a mean value of 26357 ± 12996 (mean ± SD) for the flush and sink hydraulic zones, respectively (Figure 4.2). The regime of flow in flushes and sinks was classified as either subcritical-turbulent or subcritical-laminar, depending on the mean *Fr* and *Re* values of each hydraulic zone. Flushes showed significantly higher levels of turbulence and flow nearer the critical and supercritical flow regimes compared to sinks which had *Re* values relatively nearer laminar conditions and *Fr* values in the subcritical flow regime. Average velocities were generally not as high in flushes as anticipated; hence, *Fr* values for flushes were mostly farther from the supercritical flow conditions.

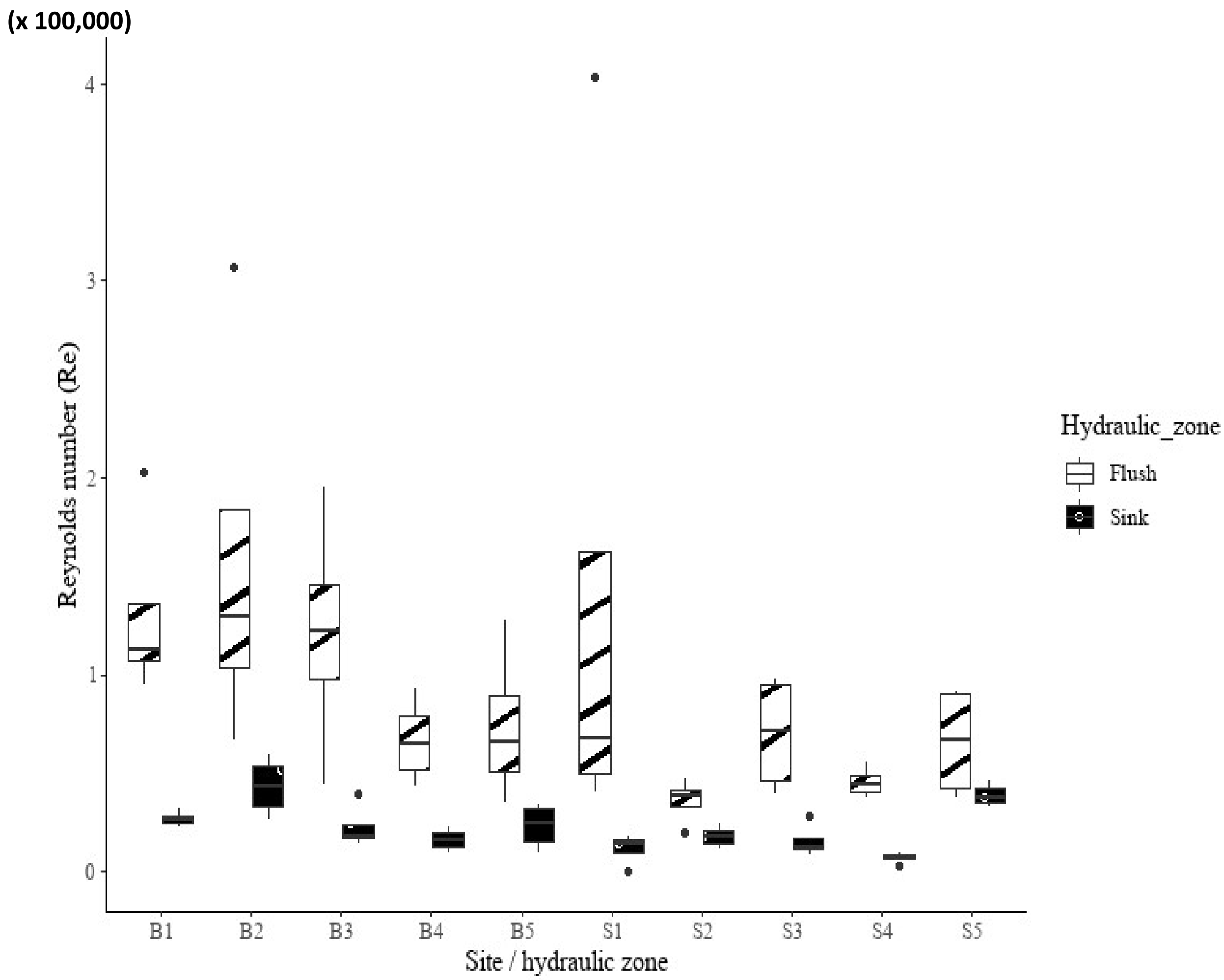


**Figure 4.2.** Box plot showing the variability of the Reynolds number (Re) between the hydraulic zones in the Buffalo and Swartkops rivers during the period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

The roughness Reynolds numbers (*Re**) and shear velocities (*U**) recorded higher values in the flush zones than in the sinks (Figures 4.3 & 4.4). Based on the *Re** values, the riverbed and flow near the riverbed in each hydraulic zones were classified as hydraulically smooth, transition and hydraulically rough. Flush zones were also associated with *U** values ranging from 0.018 to 0.29 and *Re** values which ranged between 2040 and 69500 in the hydraulically rough category. In the sinks, *U** values ranged between 0.00 and 0.042 while *Re** values ranged between 0 and 4460 in the hydraulically smooth and rough categories. Sites S4 and S5 with sinks comprising Re* values less than 5 were categorised as hydraulically smooth, while site B1 (Sink) was classified as rough, transition and smooth depending on the *Re** value derived for the sink hydraulic zone at each sampling event.

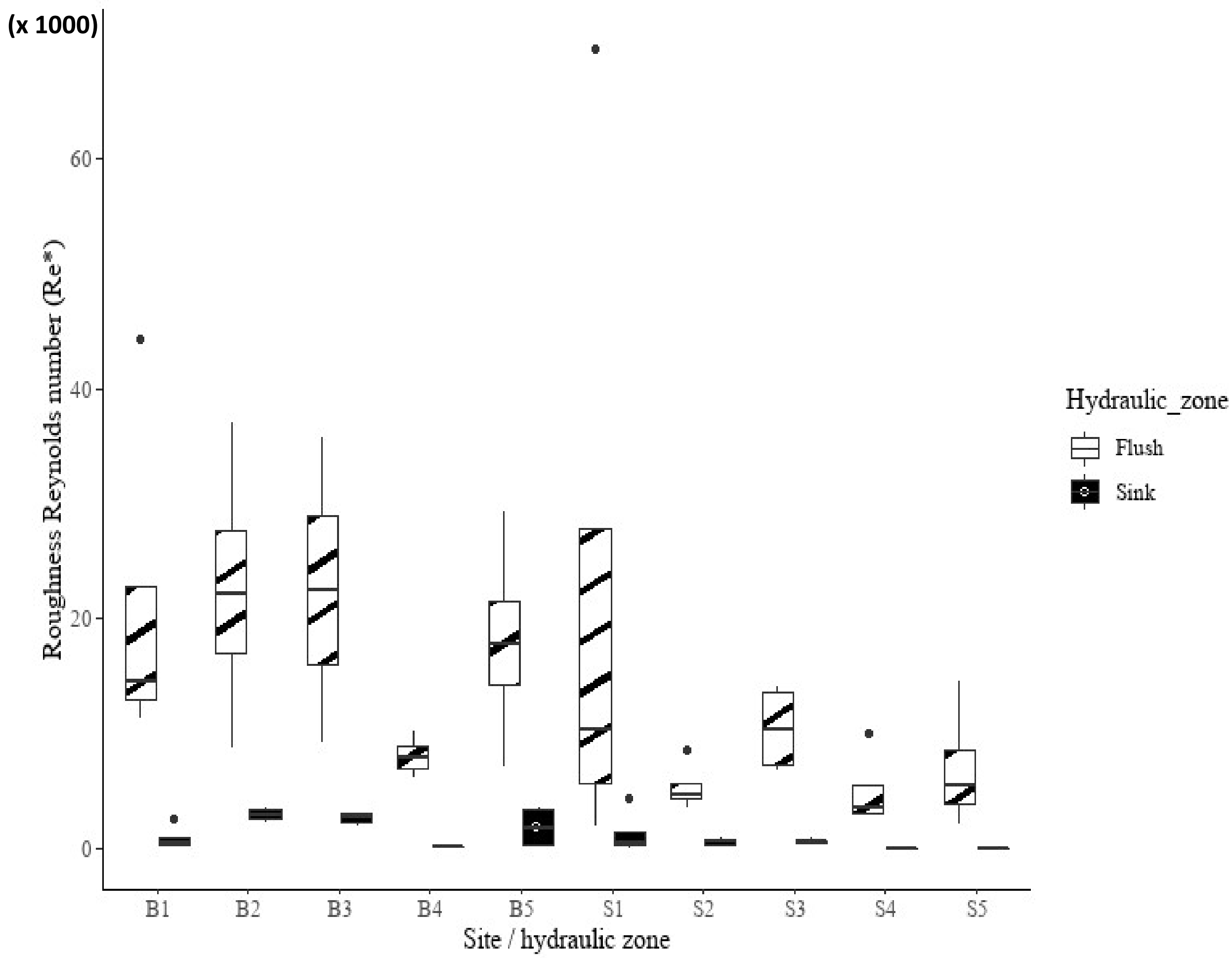


**Figure 4.3.** Box plot showing the variability of the roughness Reynolds number (Re*) between the hydraulic zones in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

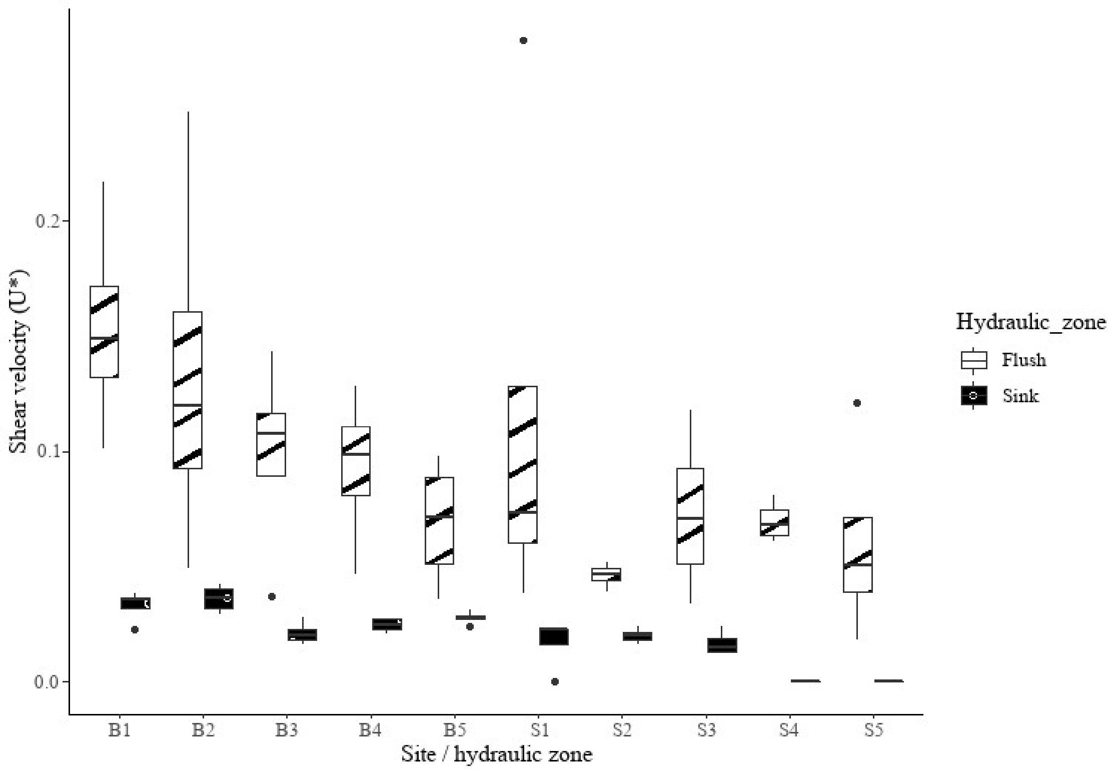


**Figure 4.4.** Box plot showing the variability of the shear velocity (U*) between the hydraulic zones in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

Velocity depth ratios (*VDR*) ranged between 0.66 and 8.80 with a mean value of 2.49 ± 1.52 in the flush zone and between 0.00 and 0.54 with a mean value of 0.25 ± 0.11 in sinks. In the flush hydraulic zones, *VDR* ranged between 0.66 and 8.80 and 0.87 and 5.62, while in sinks the range was between 0.14 and 0.54 and 0.00 and 0.36 for the Buffalo and Swartkops rivers, respectively (Figure 4.5). Just as the *VDR*, mean velocities (*MV*), were comparatively higher in the flush zones, as expected, but mean depth (*MD*) values were higher in sink zones (Figure 4.6). Across the study sites, *MV* ranged between 0.12 to 1.09 and 0.03 to 0.09 in the flush and sink zones (Figure 4.7), while *MD* ranged between 0.10 and 0.32 and 0.18 and 0.44 in the flush and sink zones, respectively. Both *MV* and *MD* were incorporated in the derivation of the Froude numbers in Figure 4.1.

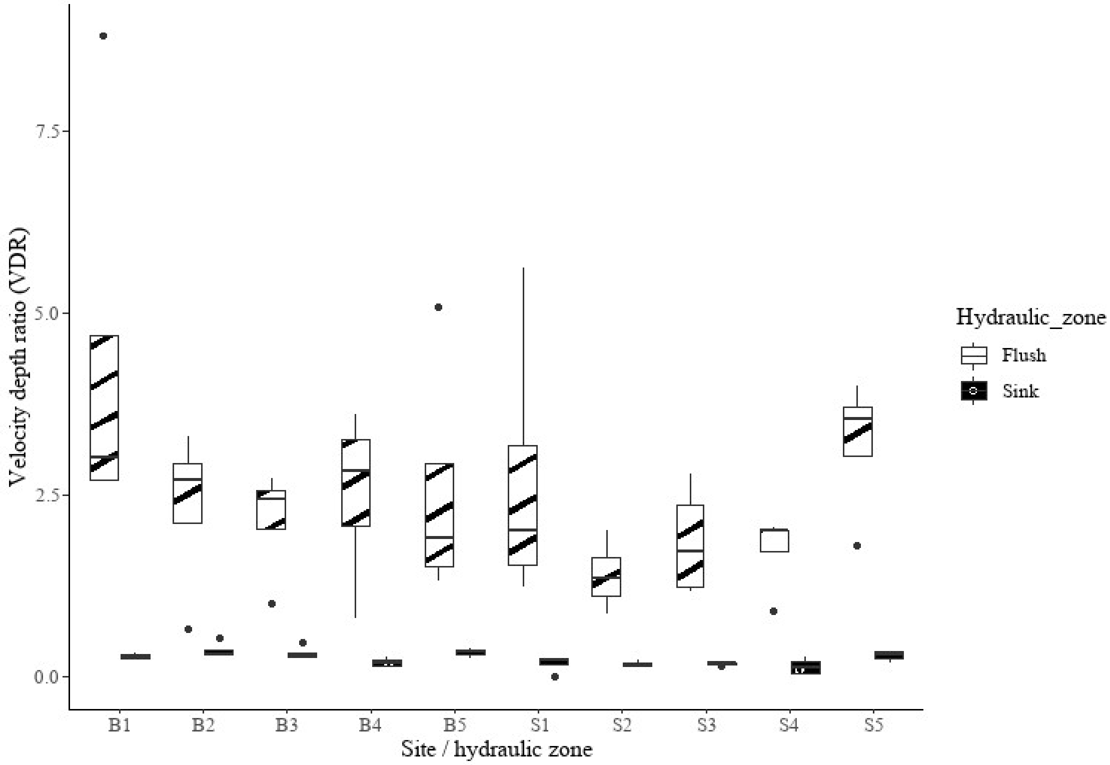


**Figure 4.5.** Box plot showing the variability of the velocity depth ratios (VDR) between the hydraulic zones in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

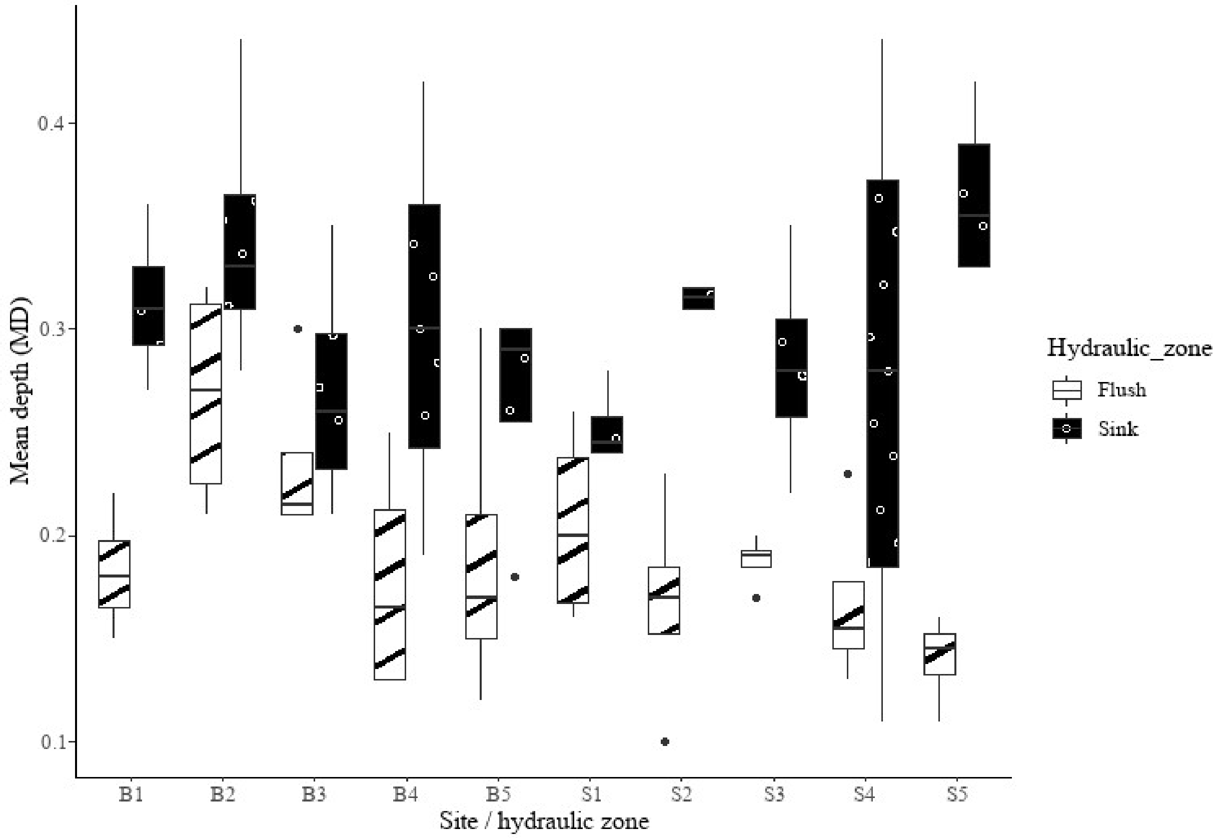


**Figure 4.6.** Box plot showing the variability of the mean depth (MD) between the hydraulic zones in the Buffalo and Swartkops ivers during the study period (October 2021–July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

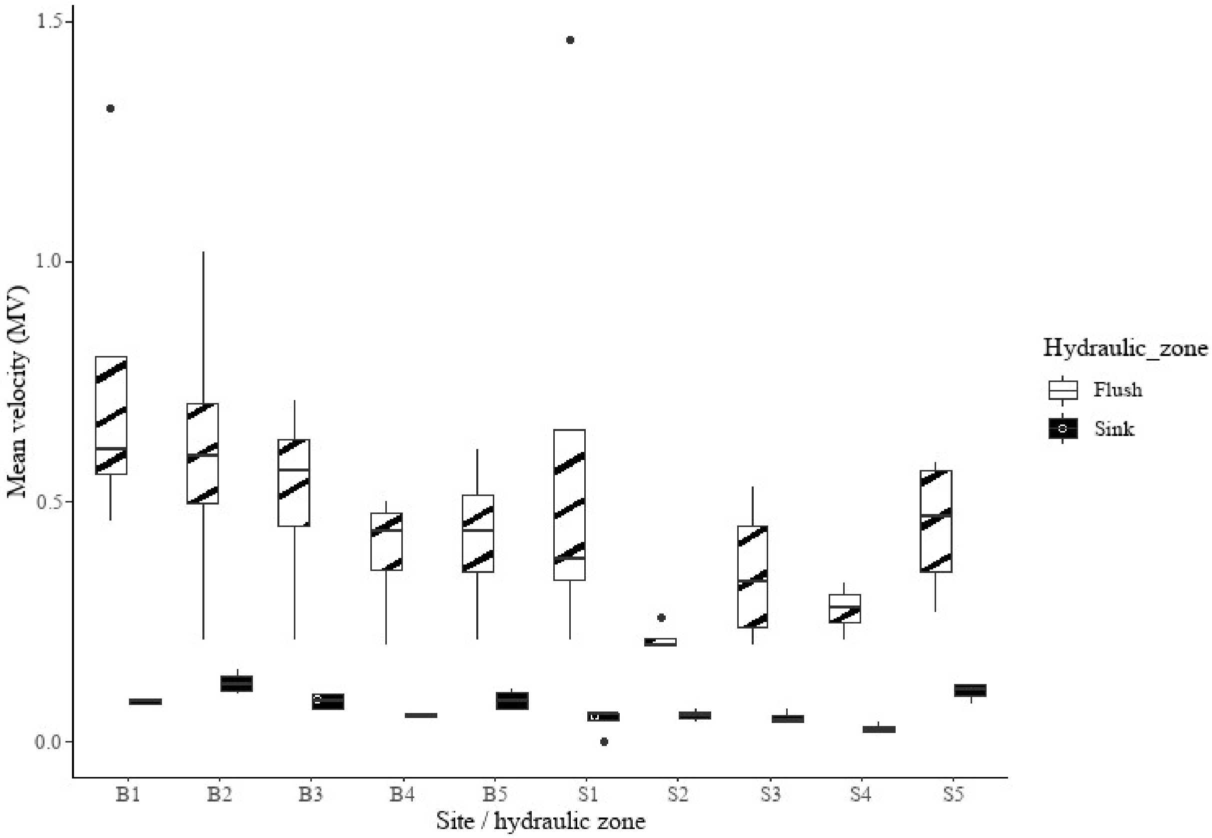


**Figure 4.7.** Box plot showing the variability of the mean velocity (MV) between the hydraulic zones in the Buffalo and Swartkops rivers during the study period (October 2021– July 2022). Site codes: B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkop tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5.

### 4.4.2 Microplastics abundance and distribution in the hydraulic zones

The microplastic burden of the Buffalo and Swartkops rivers was assessed spatially and temporally at the hydraulic biotope scale to provide insight into the impact of river hydro-geomorphology (encapsulated by hydraulic biotopes) on microplastic transport and concentration in river systems. Four sampling events were carried out, two sampling events during the wet season and two during the dry season. Two conceptualized hydraulic biotopes, flush and sink hydraulic biotopes, were sampled. The results of microplastic distribution across the hydraulic zones are presented in Figures 4.8 and 4.9.

The two-way MANOVA undertaken to simultaneously compare the hydraulic zones and seasons in terms of suspended and settled microplastic distribution, indicated statistically significant results for the hydraulic zones, but there were no significant interactions between the hydraulic zones and seasons (Table 4.1). The results of the two-way ANOVA undertaken to determine how suspended and settled microplastics responded according to the levels of the independent variable that was significant (i.e., the hydraulic zones) with the MANOVA analysis revealed that settled microplastics varied significantly between the hydraulic zones ($P < 0.05$), while suspended microplastics were not statistically significantly different between the hydraulic zones ($P > 0.05$). Neither settled nor suspended microplastics were statistically significantly different between the seasons.

**Table 4.1.** Global two-way MANOVA output indicating statistically significant results for the hydraulic zone and season in relation to suspended and settled microplastics abundance distribution (Bold face values are significant).

| Effect | Multivariate test of significance | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Test** | **Value** | **Approx. F** | **Model (df)** | **Error (df)** | **Signf.** | **Partial Eta$^2$** |
| Intercept | Wilks Lambda | 0.90590 | 3.8952 | 2 | 75 | **0.0246** | |
| | Pillai's Trace | 0.09410 | 3.8952 | 2 | 75 | **0.0246** | |
| Hydraulic zone | Wilks Lambda | 0.88351 | 4.9444 | 2 | 75 | **0.0096** | 0.12 |
| | Pillai's Trace | 0.11649 | 4.9444 | 2 | 75 | **0.0096** | |
| Season | Wilks Lambda | 0.95587 | 1.7312 | 2 | 75 | 0.1841 | 0.04 |
| | Pillai's Trace | 0.04413 | 1.7312 | 2 | 75 | 0.1841 | |
| Hydraulic zone: season interaction | Wilks Lambda | 0.97889 | 0.8087 | 2 | 75 | 0.4493 | 0.02 |
| | Pillai's Trace | 0.02111 | 0.8087 | 2 | 75 | 0.4493 | |

The Tukey HSD post-hoc test using the pairwise comparison of the two-way ANOVA further established that settled microplastics were statistically significantly different between the hydraulic zones, but there were no statistically significant differences in settled microplastic distribution between the wet and dry seasons. In the interaction between the hydraulic zones and seasons, Tukey HSD revealed that settled microplastics were not significantly different between the wet and dry seasons within a hydraulic zone; however, a statistically significant difference was found in a cross-comparison between the sink zone during the wet season and the flush zone during the dry season ($P < 0.05$). Tukey HSD post-hoc tests were undertaken at the 95% family-wise confidence level.

The Wilcoxon rank-sum test indicated that the median difference in suspended microplastics between the flush and sink hydraulic zone was not significantly different from zero (W = 712.5, *P* = 0.399). Therefore, there was not enough evidence to reject the null hypothesis that the median difference in suspended microplastics between the flush and sink zones equals zero. For settled microplastics, the test revealed sufficient evidence to reject the null hypothesis in favour of the alternative. The median differences in settled microplastics between the flush and sink zone was not equal to zero (W = 594.5, *P* = 0.047). Test were conducted at alpha = 0.05.

Generally, across the hydraulic zones, settled microplastics abundance ranged between 0.00 to 6.67 items/L and between 0.00 to 11.00 items/L in the flush and sink hydraulic zones, respectively. In the Swartkops River, settled microplastics ranged between 0.00 to 4.00 items/L and 0.33 to 6.33 items/L in the flush and sink hydraulic zones, while in the Buffalo River, settled microplastics ranged between 0.33 to 6.67 items/L and 0.00 to 11.00 items/L in the flush and sink hydraulic zones, respectively. The highest occurrences of settled microplastics were recorded during the wet season in the Buffalo River at site B2 (11.00 items/L, summer) and B4 (6.67 items/L, spring) for the sink and flush hydraulic zones, respectively. Spatially, settled microplastics were more abundant in the sink zone (1.83 ± 1.98; mean + SD) (Figure 4.8), and temporally, they were more abundant during the wet season in the sink zone (2.20 ± 2.50; mean + SD) (Figure 4.8).

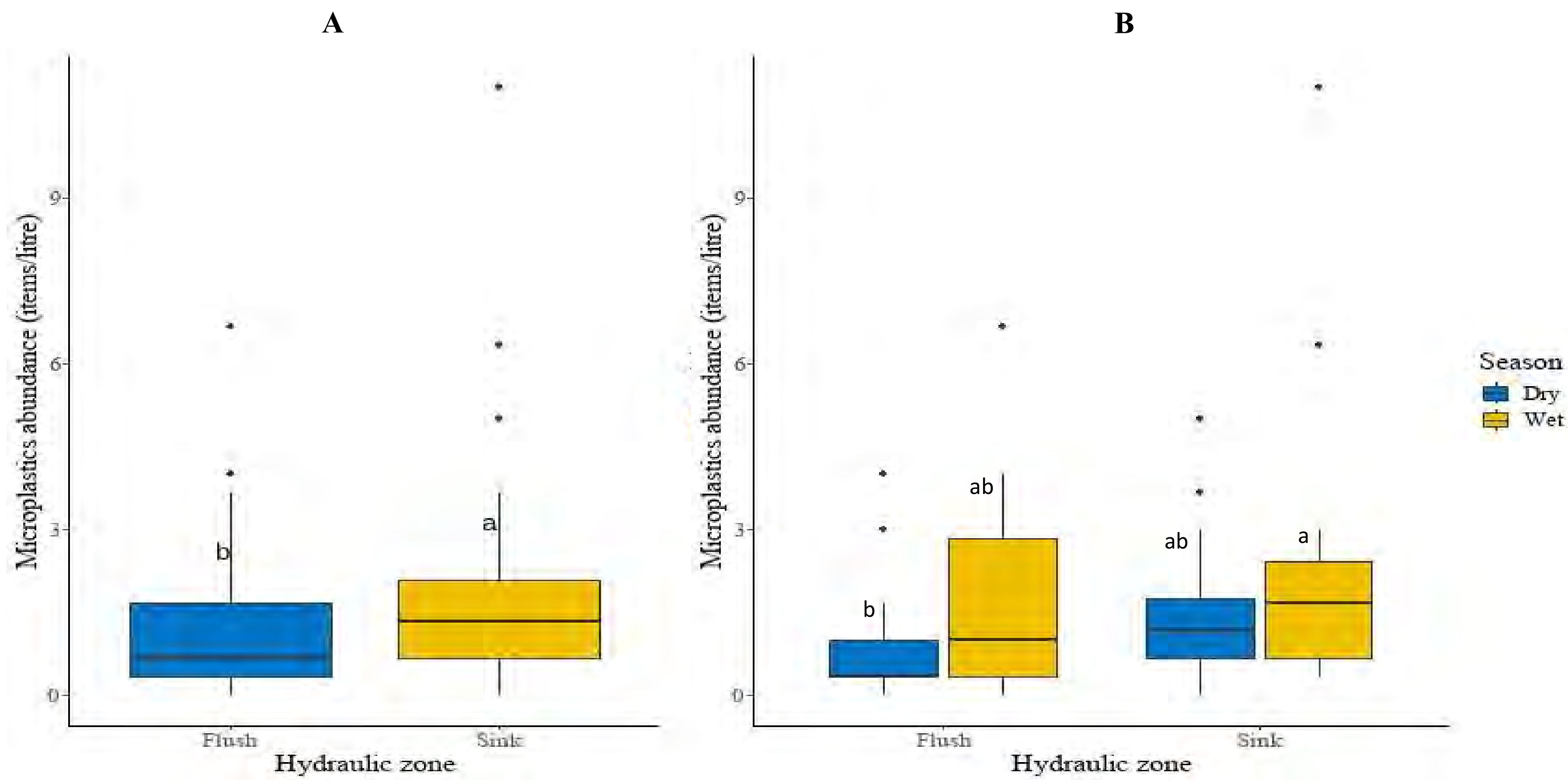


**Figure 4.8.** Box plots showing the comparison of **settled** microplastics between the hydraulic zones **(A)** and the interaction of **settled** microplastics between the hydraulic zones and seasons **(B)** in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Different superscript letters indicate statistically significant differences between the hydraulic zones and between the seasons, established using Tukey HSD.

Suspended microplastics, on the other hand, had an abundance that ranged between 0.00 and 6.67 items/L in the flush zone and between 0.00 and 7.00 items/L in the sink zone. The highest occurrences of suspended microplastics were recorded during the wet season (spring) at site B4 (6.67 items/L) for the flush zone and site S2 (7.00 items/L) for the sink hydraulic zones. In the Swartkops River, the concentration of suspended microplastics varied from 0.00 to 4.67 items/L and between 0.00 - 7.00 items/L for the flush and sink hydraulic zones, respectively (Figure 4.8). Suspended microplastics also ranged between 0.00 to 6.67 items/L and 0.33 to 3.33 items/L in the flush and sink hydraulic zones of the Buffalo River, respectively. Suspended microplastics were more abundant in the flush hydraulic zone (1.76 ± 1.44 items/L; mean + SD), and temporally, they were more abundant during the wet season in the sink zone (1.88 ± 1.76) (Figure 4.9).

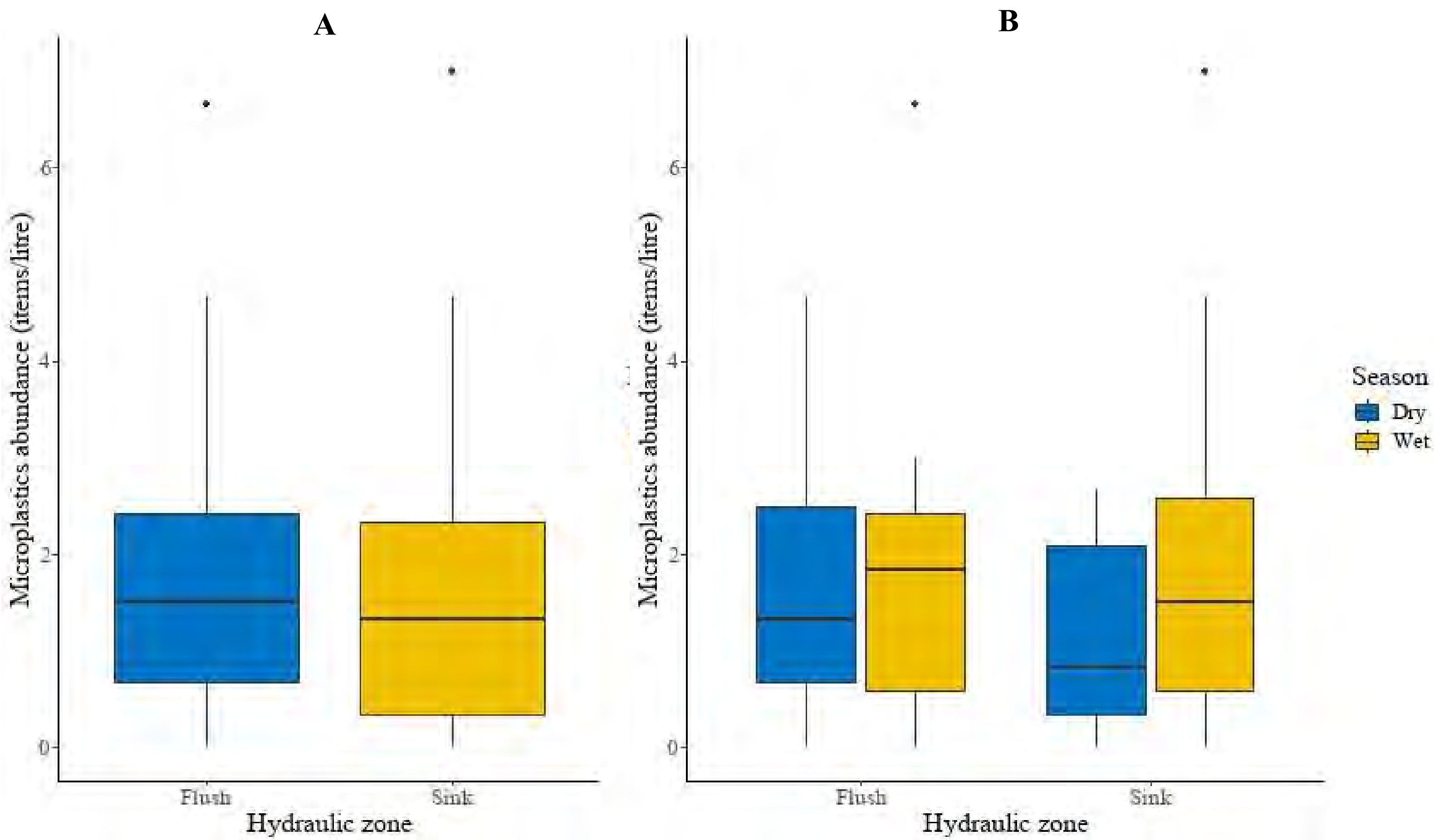


**Figure 4.9.** Box plots showing the comparison of **suspended** microplastics between the hydraulic zones **(A)** and the interaction of **suspended** microplastics between the hydraulic zones and seasons **(B)** in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Results were not statistically significantly different, established using Tukey HSD

### 4.4.3 Microplastic morphological distribution in hydraulic zones

Particles were categorised based on their morphological characteristics (size, shape, and colour) as described in Chapter 2, Section 2.8. The shape composition of the total microplastics recovered from samples collected from the flush and sink hydraulic zones is presented in Figure 4.10. Fibres and fragments were the most abundant shapes. In combination, fibres and fragments accounted for about 80% of the total microplastics in the flush zone and 73.50% of total microplastics in the sink zone. Pellets/spheres had the lowest distribution of microplastic shapes, accounting for 1.10% and 1.20% of microplastics in the flush and sink zones, respectively.

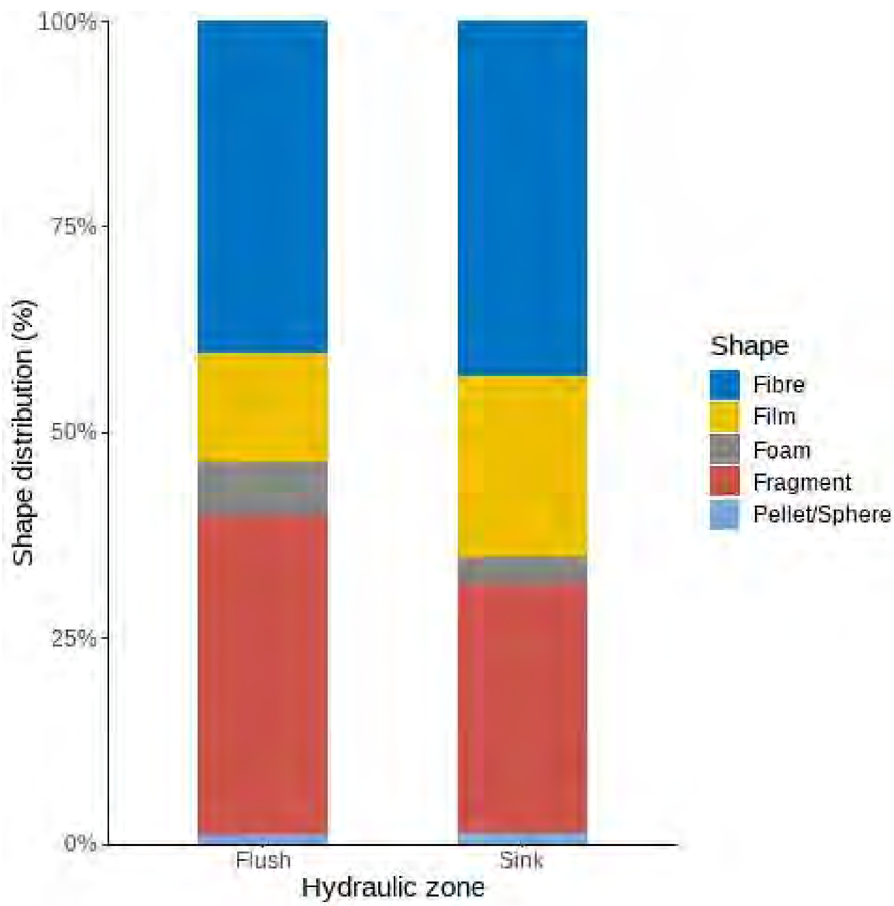


**Figure 4.10.** Percentage distribution of microplastic particle shapes (settled and suspended particles combined) found in the flush and sink hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Results from each site were used as replicates.

The fibre abundance in the flush hydraulic zone was not significantly higher than that in the sink hydraulic zone, which had a higher percentage distribution of fibres. PERMANOVA results indicated no statistically significant difference in the distribution of the individual shapes of microplastics between the flush and sink zones (Figure 4.11). In terms of seasonal distribution of the individual microplastic shapes, there was no significant difference. PERMANOVA also indicated no significant difference in the interaction between hydraulic zones and seasons with respect to individual microplastic shape distribution (Figure 4.12).

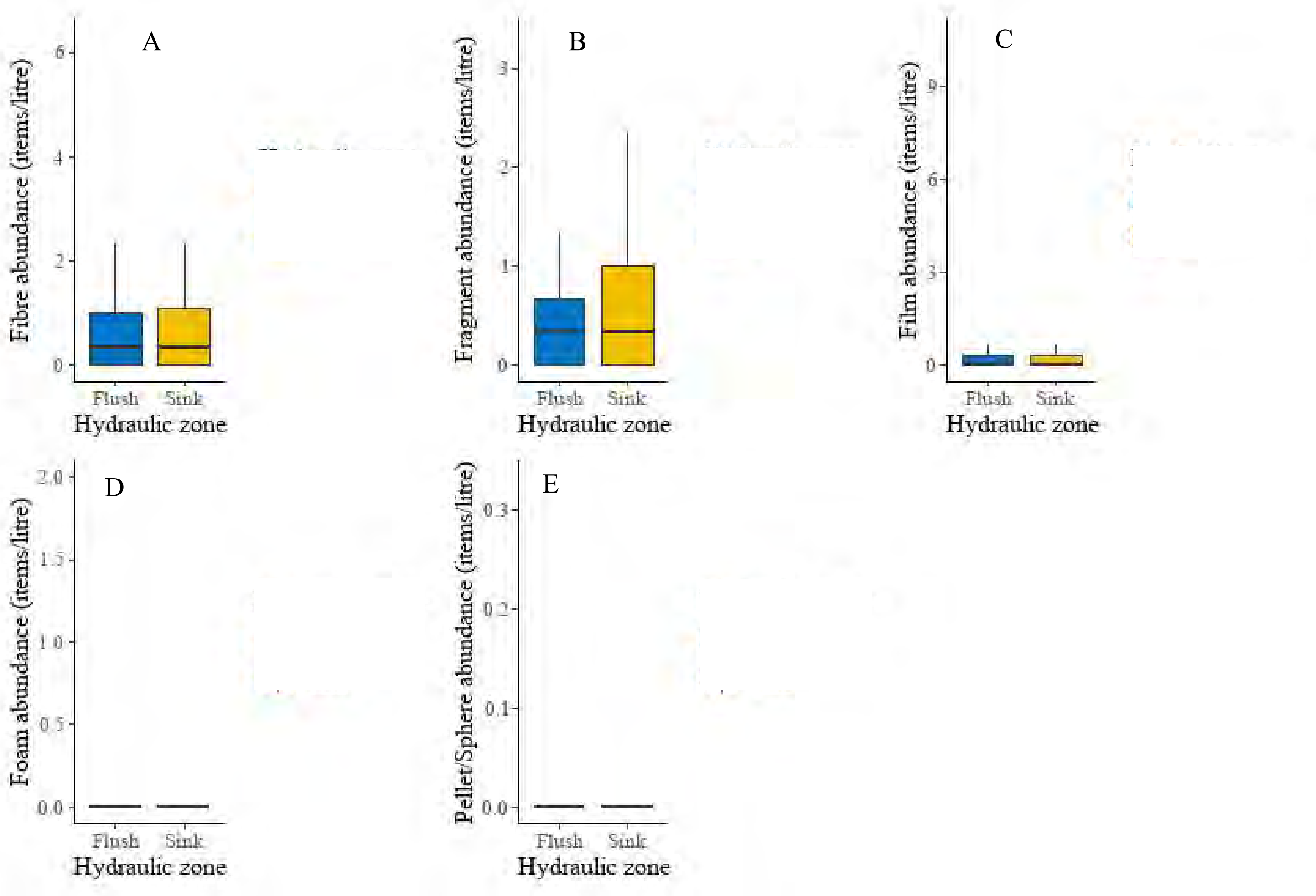


**Figure 4.11.** Boxplots showing the abundance of different shapes of total microplastics found in the hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = Fibre, B= Fragment, C = Film, D = Foam, E = Pellet/Sphere.

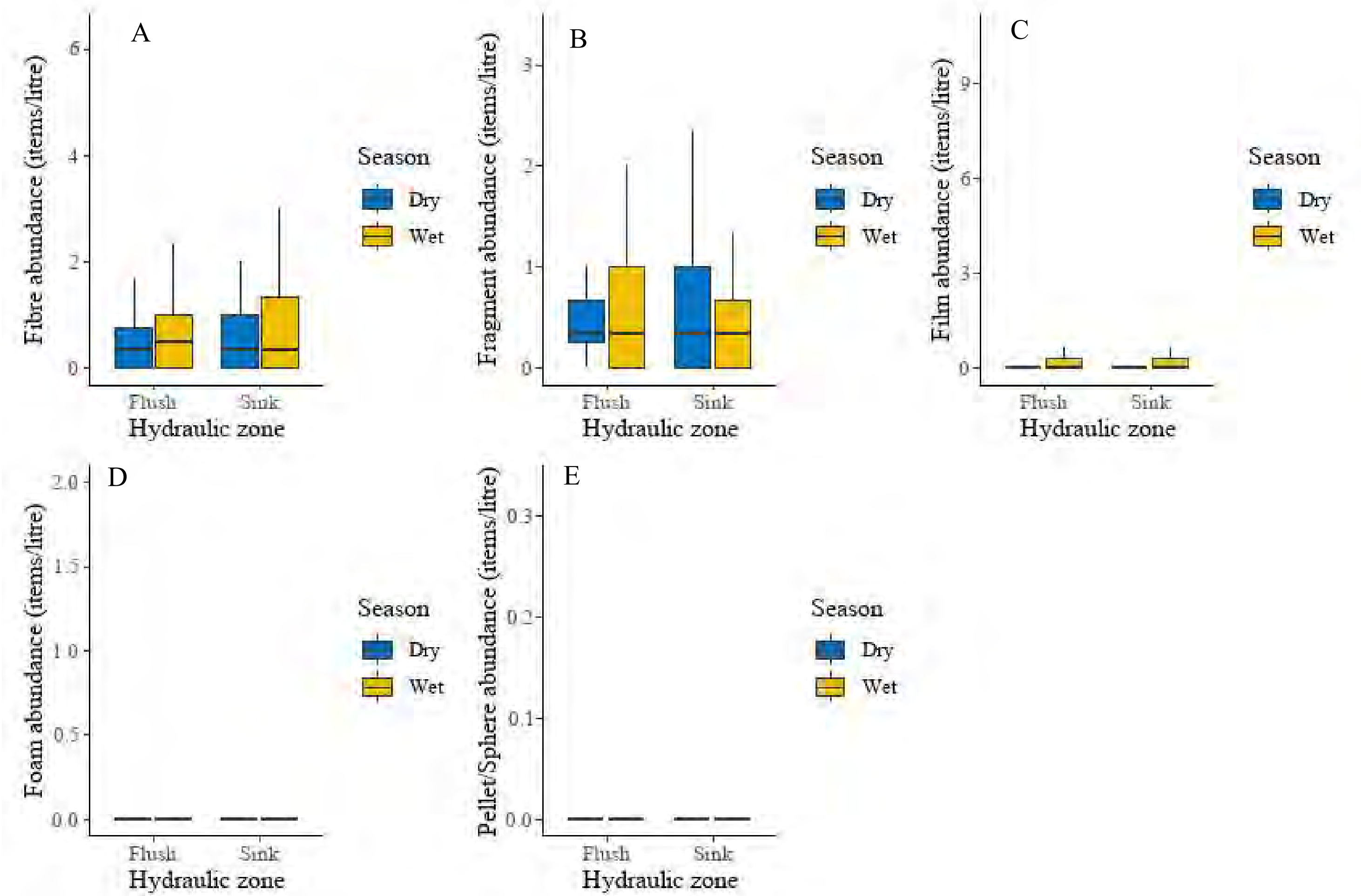


**Figure 4.12.** Box plots showing the interaction of different shapes of total microplastics between the hydraulic zones and seasons in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = Fibre, B= Fragment, C = Film, D = Foam, E = Pellet/Sphere.

Regarding the colours of microplastics, red, blue, and black colour particles dominated the flush and sink hydraulic zones. In combination, red, blue, and black particles accounted for 67.7% of total microplastics in the sink hydraulic zone, and 63% of total microplastics in the flush hydraulic zone. Yellow microplastics had the lowest percentage distribution across the flush and sink zones, accounting for only 0.7% and 2.7% of the total microplastics found in the sink and flush zones, respectively (Figure 4.13).

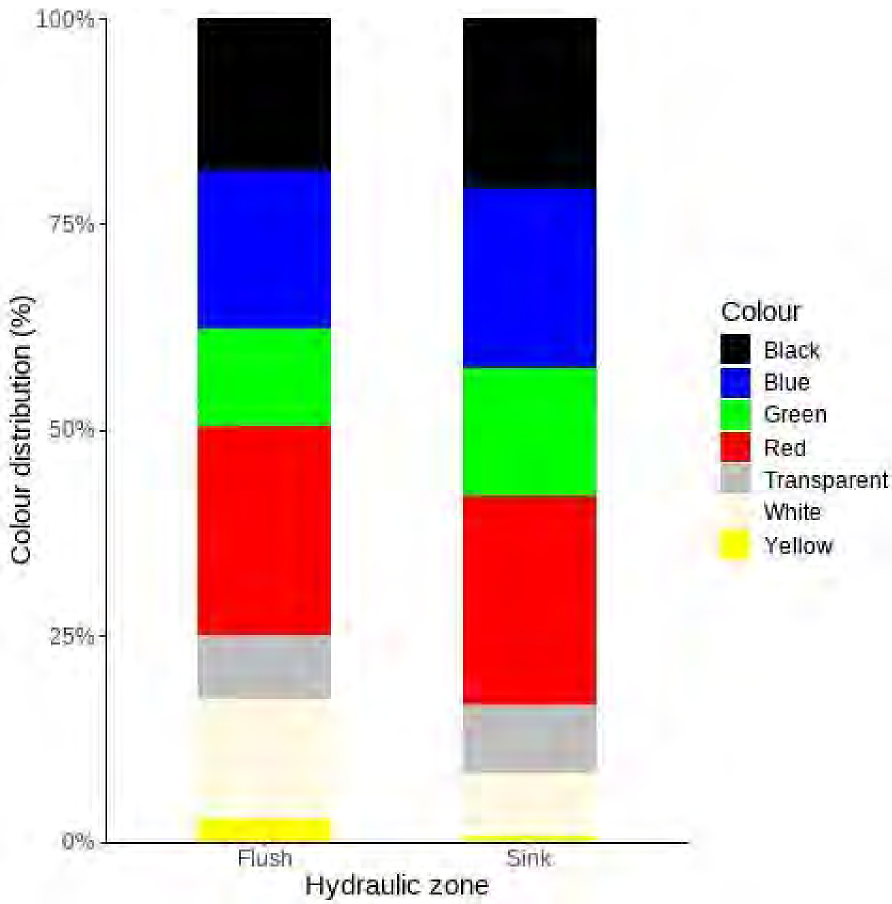


**Figure 4.13.** Percentage distribution of microplastics particle colour (settled and suspended particles combined) found in the flush and sink hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Results from each site were used as replicates.

PERMANOVA indicated no significant differences in the distribution of the different colours of microplastics between the flush and sink zones (Figure 4.14) and no significant interaction between the hydraulic zones and seasons (Figure 4.15). Only the top four colours of microplastics with the highest percentage distribution are shown in Figures 4.14 and 4.15.

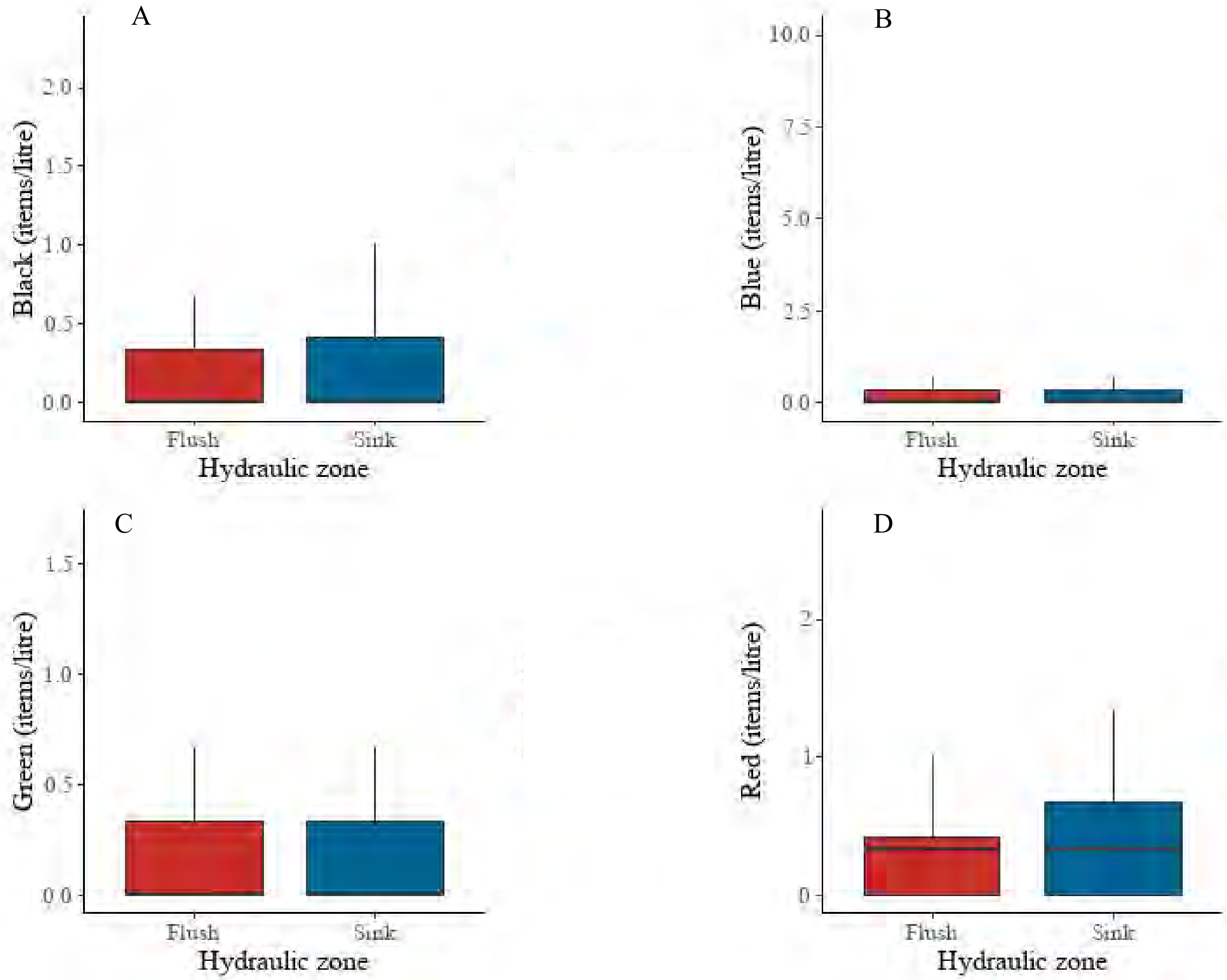


**Figure 4.14.** Boxplots showing the abundance of different colours of total microplastics found in the hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = Black, B= Blue, C = Green, D = Red.

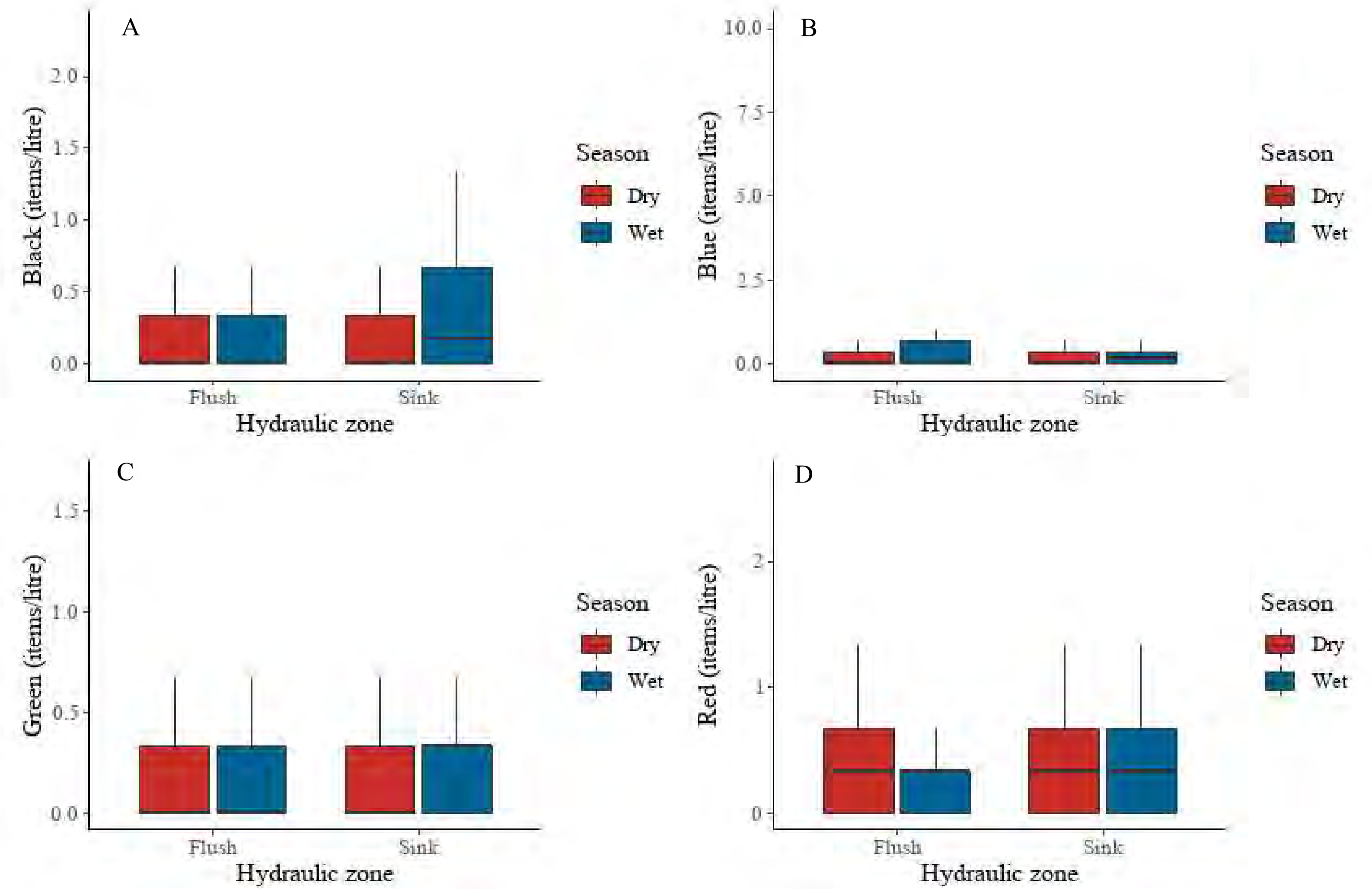


**Figure 4.15.** Box plots showing the interaction of different colours of total microplastics between the hydraulic zones and seasons in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = Black, B= Blue, C = Green, D = Red.

In terms of size, 0.063 < 0.5 mm was the most abundant microplastic size class, accounting for 41% and 34.2% of the total microplastics in the flush and sink hydraulic zones, respectively. The smallest size class was followed by size class 0.5 < 1 mm in the flush zone and size class 1< 2 mm in the sink zone. Size class 2 < 5 mm had the lowest distribution in both hydraulic zones, accounting for 16.4 % of total microplastics in the flush zone and 16.4% in the sink zone (Figure 4.16).

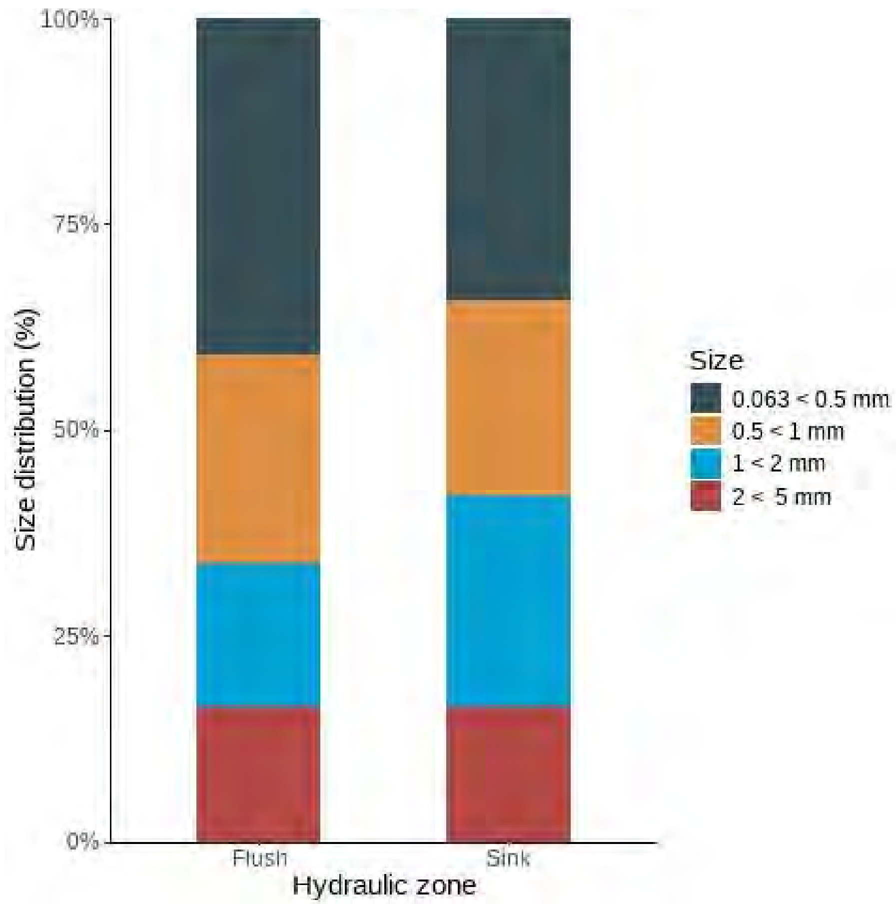


**Figure 4.16.** Percentage distribution of microplastics particle size (settled and suspended particles combined) found in the flush and sink hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). Results from each site were used as replicates.

Microplastic size classes showed no significant differences in abundance between the flush and sink hydraulic zones (Figure 4.17). Seasonally, microplastic size classes showed significant differences in distribution. Size classes 1 < 2 mm and 2 < 5 mm showed significantly higher abundances during the wet season (PERMANOVA, $P < 0.05$). However, the interaction between hydraulic zones and seasons was not significant, although size class 1 < 2 mm showed statistically significant differences in abundance between the sink zone during the wet season and the flush zone during the dry season (Figure 4.18). Size class 1 < 2 mm showed significant higher abundances in the sink zone during the wet season than the flush zone during the dry season.

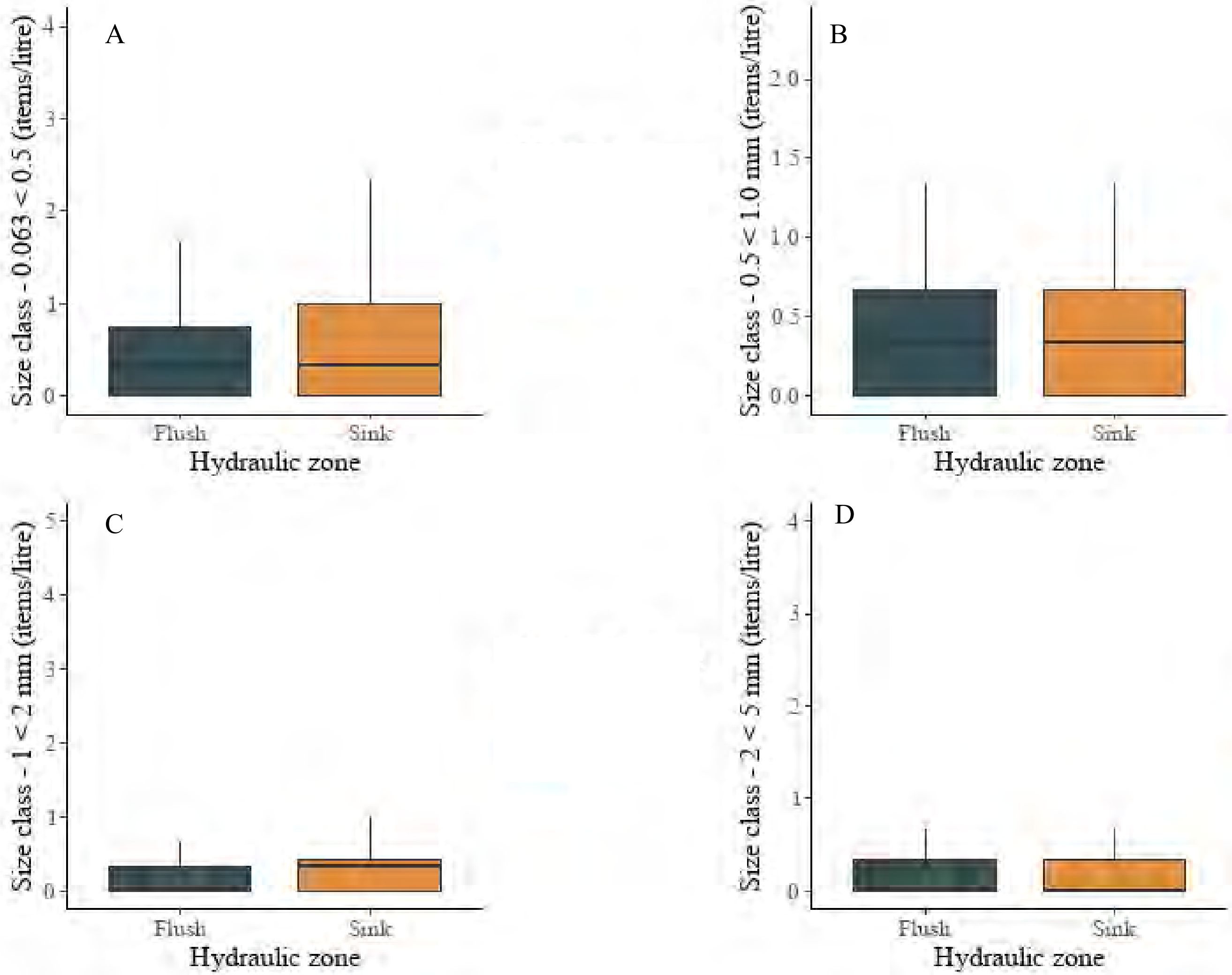


**Figure 4.17.** Boxplots showing the abundance of different size classes of microplastics found in the hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = 0.063 < 0.5 mm, B= 0.5 < 1 mm, C = 1 < 2 mm, D = 2 < 5 mm.

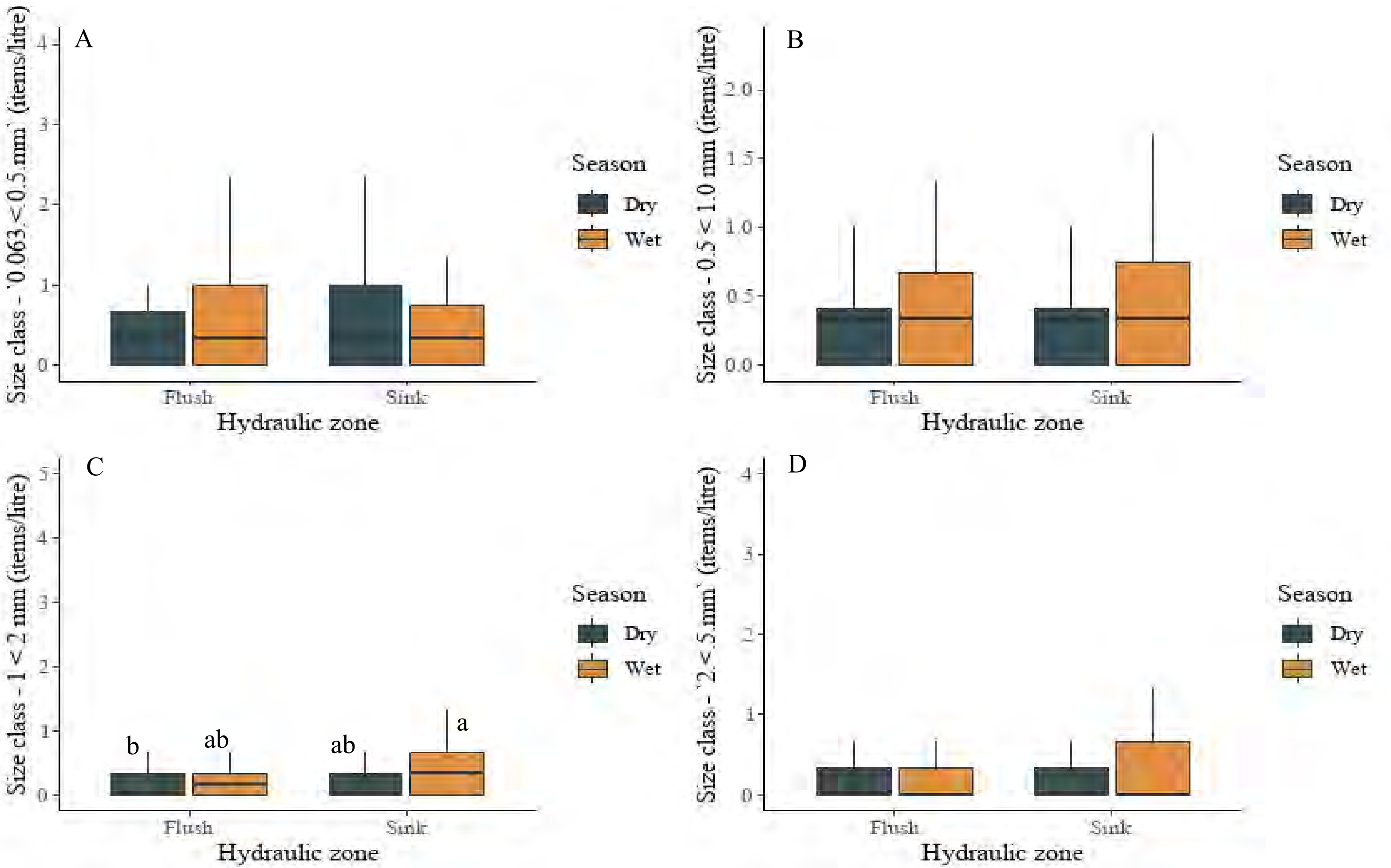


**Figure 4.18.** Box plots showing the interaction of different size classes of microplastics between the hydraulic zones and seasons in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = 0.063 < 0.5 mm, B= 0.5 < 1 mm, C = 1 < 2 mm, D = 2 < 5 mm. Superscript letter in common are not statistically significantly different, different superscript letter(s) are statistically significantly different, established using "pairwise.adonis".

With regard to polymer types, a total of 11 polymers were identified. All 11 polymers identified were associated with the sink hydraulic zone, while eight were identified in the flush zone. Polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) were the dominant polymers among the samples subjected to FTIR (Figure 4.19). Polyethylene, PP and PET, when combined, accounted for 79.7% and 81.5% of the microplastics in the flush and sink hydraulic zones. Polyvinyl chloride (PVC), Polyurethane (PU) and Polycarbonate (PC) with individual percentages of 0.8% each had the lowest distribution in the sink zone, while polyamide (PA - 0.5%) had the lowest distribution in the flush zone. Latex, PC, and PVC were only found in the sink zone. Polystyrene (PS), acrylonitrile butadiene styrene (ABS) and polyethylene vinyl acetate (PEVA) were the other polymers detected.

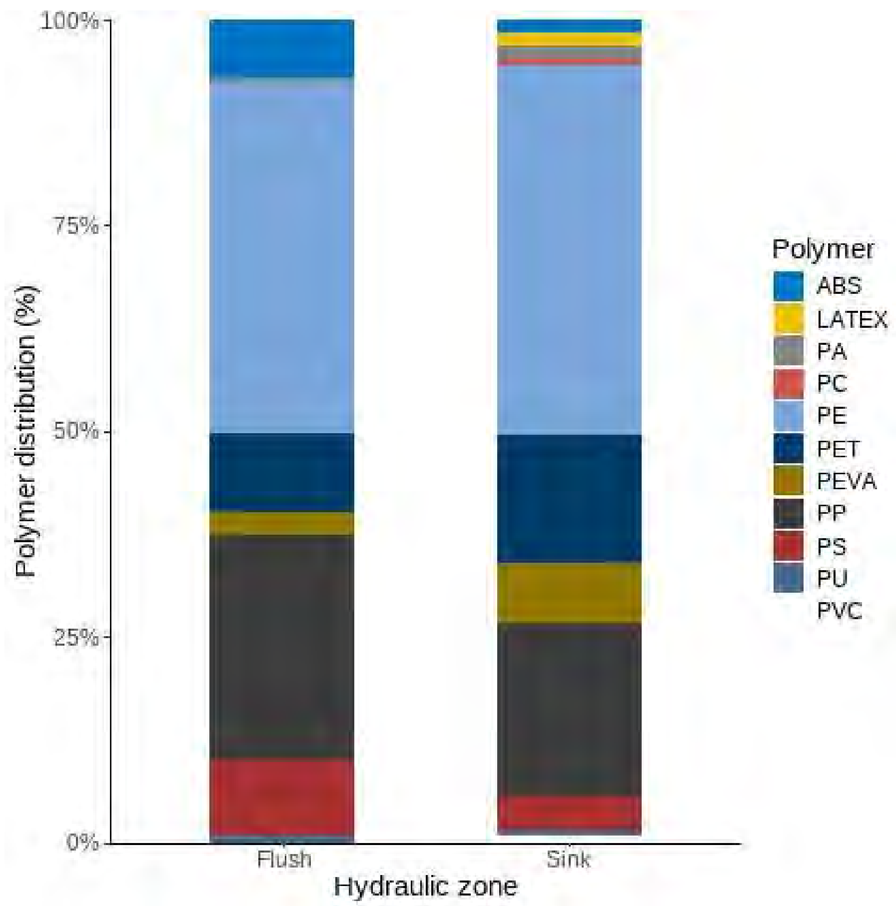


**Figure 4.19.** Percentage distribution of microplastic polymer type (settled and suspended particles combined) found in the hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022).

The distribution of individual polymer types was not significantly different between the hydraulic zones (Figure 4.20), but there were statistically significant differences in seasonal distribution between the wet and dry season. Polypropylene had a significantly higher distribution during the wet season. The seasonal differences in distribution did not translate into a significant interaction between the seasons and the hydraulic zone (Figure 4.21). The abundances of the top six most abundant polymers are presented in Figure 4.21.

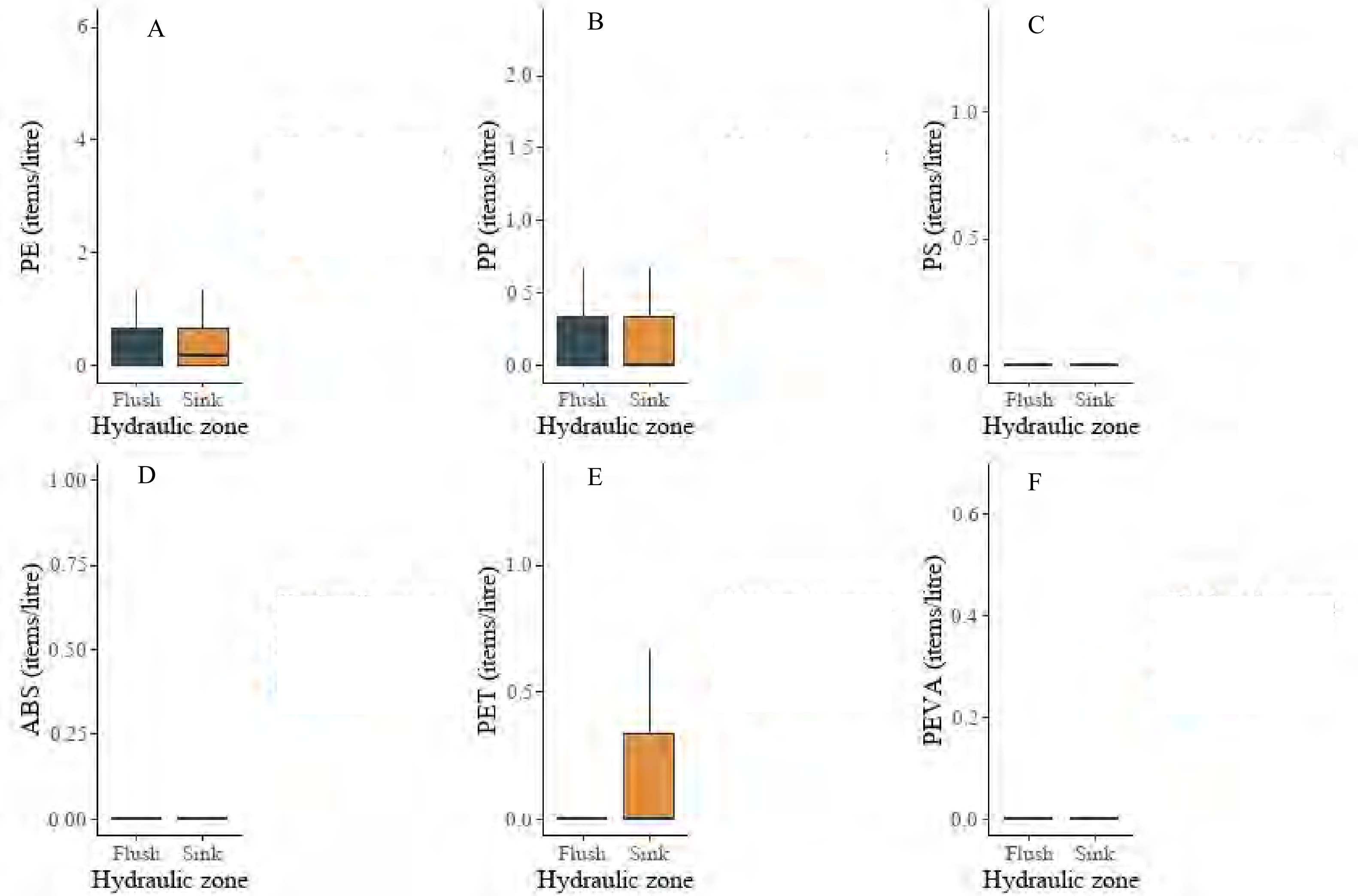


**Figure 4.20.** Boxplots showing the abundance of different microplastics polymer type found in the hydraulic zones of the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = **PE**, B= **PP**, C = **PS**, D = **ABS**, E = **PET**, F = **PEVA**.

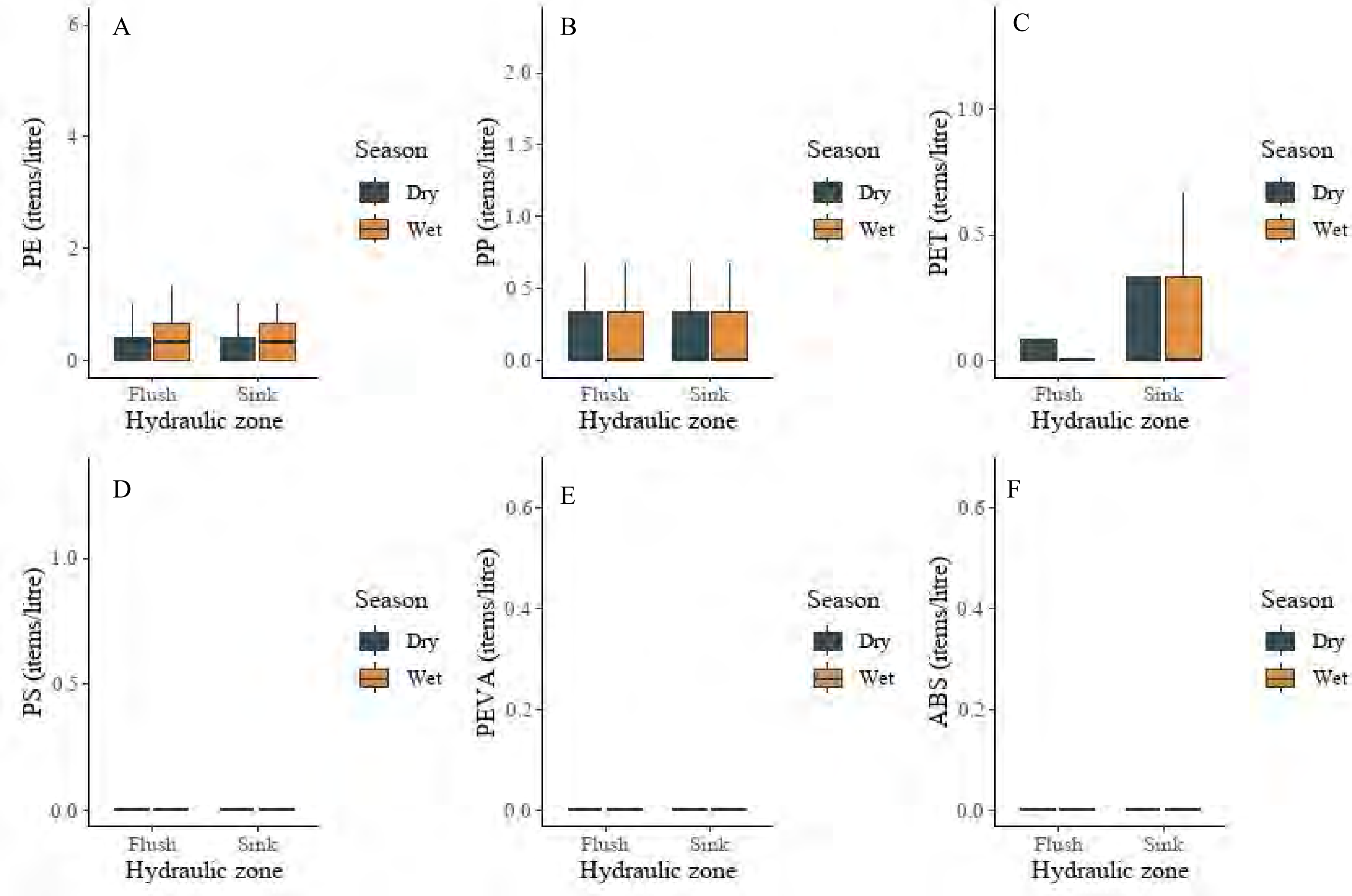


**Figure 4.21.** Box plots showing the interaction of different microplastics polymer types between the hydraulic zones and seasons in the Buffalo and Swartkops rivers during the study period (October 2021–July 2022). A = **PE**, B= **PP**, C = **PET**, D = **PS**, E = **PEVA**, F = **ABS**

### 4.4.4 Relating hydraulic indices to microplastics distribution in the hydraulic zones.

The GAMs, performed to examine the relationship between the different hydraulic indices (i.e., Fr, Re, Re*, U*) and microplastic abundance distribution across the flush and sink hydraulic zones, indicated that the negative binomial model explained 27.0% of the variance in suspended microplastics and 45.7% of the variance in settled microplastics distribution across the hydraulic zones (Table 4.2). The results suggest that the relationship between microplastic distribution and the hydraulic indices was more profound for settled microplastics than for suspended microplastics. Given the parametric coefficient estimate for the hydraulic zones in relation to microplastics, the sink hydraulic zone has a higher likelihood for explaining variations in settled microplastics and a lesser likelihood for explaining variations in suspended microplastics than the flush hydraulic zones. The model also indicated that only the relationship between Froude number (*Fr*) and microplastics was significant for suspended microplastics ($P = 0.030$) and settled microplastics ($P = 0.026$).

It is important to note from the modelled data (Figures 4.22 and 4.23), that when there are no data points, the confidence intervals become considerably larger because of the uncertainty of the relationship at such points. Generally, the modelled data also revealed that the relationship between the hydraulic index type and microplastic abundance distribution does not have much flexibility (i.e., wiggliness of the curves) as indicated by the effective degrees of freedom (edf) in each case (Table 4.2). However, some level of "wiggliness" is clearly visible in Figures 4.22E, 4.23C and 4.23G.

For settled microplastics, the modelled data predicted no significant relationship between Reynolds number (*Re*) and microplastic distribution in the flush and sink hydraulic zones (Figure 4.22A and 4.22B). The smoothing term for *Re* in both the sink and flush hydraulic zones were not significantly different from zero ($P > 0.05$) for the settled microplastics (Table 4.2). However, the *Fr* indicated a smoothing term significantly different from zero in the flush hydraulic zone ($P = 0.026$) for settled microplastic distribution. Therefore, the *Fr* is a relevant variable to the model fit (Table 4.2). As shown in Figure 4.22C, there is a decreasing trend in settled microplastic abundance with increasing *Fr* values. In the sink hydraulic zone, the smoothing term for *Fr* was not significant ($p > 0.05$) for settled microplastics (Table 4.4; Figure 4.17D). Both shear velocity (*U**) and 'roughness' Reynolds number (*Re**) indicated a smoothing term not significantly different from zero ($p>0.05$). Although the *U** in the flush and sink hydraulic zone seems to suggest a linear relationship with settled microplastic abundance (Figure 4.22G and 4.23H), however, *U** showed the least flexibility compared to other indices fitted to the model for settled microplastics (Table 4.2). Figure 4.22I shows the estimated parametric effects with a 95% confidence interval. The sink hydraulic zone was predicted to hold higher quantities of settled microplastics then the flush zone; however, the estimated effect also included the point of comparison (i.e., zero) which suggests that the sink zone was not significantly different from the flush zone in this regard.

**Table 4.2.** Detailed summary of the generalized additive model (GAM) results for the microplastic abundances derived from the surveys (October 2021–July 2022) in the Swartkops and Buffalo river systems. Hydraulic indices: Reynolds number (Re), Froude number (Fr), roughness Reynolds number (Re*), and Shear velocity (U*).

| **Family** | **Link Function** | **Formula (Prototype)** | **Adjusted $R^2$** | **Deviance Explained** |
|---|---|---|---|---|
| Negative binomial | Log | MP(suspended or settled) ~ hydraulic.zone + s(Fr, by = hydraulic.zone, bs = “tp”, k = 10) + s(Re, by = hydraulic zone, bs = “tp”, k = 10) + s(Re*, by = hydraulic.zone, bs = “tp”, k =10) + s(U*, by = hydraulic.zone, bs = “tp”, k =10) | 0.162 (suspended)<br>0.562 (settled MP) | 27.0%<br>45.7% |

| **Parametric coefficient** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Suspended MP** | | | | | **Settled MP** | | | |
| | **Estimate** | **Standard Error** | **z Value** | **Pr(>\|z\|)** | | **Estimate** | **Standard Error** | **z Value** | **Pr(>\|z\|)** |
| (Intercept) | 0.922 | 0.283 | 3.252 | 0.001 | (Intercept) | 0.605 | 0.246 | 2.457 | 0.014 |
| Hydraulic.zoneSink | -0.381 | 10.549 | -0.036 | 0.971 | Hydraulic.zoneSink | 11.44 | 25.200 | 0.454 | 0.650 |

| **Approximate Significance of Smooth Terms** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **edf** | **Ref.df** | **Chi.sq** | **p-Value** | | **edf** | **Ref.df** | **Chi.sq** | **p-Value** |
| s(Re):Hydraulic.zoneFlush | 1.000 | 1.000 | 1.191 | 0.275 | s(Re):Hydraulic.zoneFlush | 1.000 | 1.000 | 0.033 | 0.856 |
| s(Re):Hydraulic.zoneSink | 1.000 | 1.000 | 0.445 | 0.505 | s(Re):Hydraulic.zoneSink | 1.599 | 1.958 | 0.221 | 0.905 |
| s(Fr):Hydraulic.zoneFlush | 2.436 | 2.932 | 8.199 | **0.030** | s(Fr):Hydraulic.zoneFlush | 1.491 | 1.809 | 5.883 | **0.026** |
| s(Fr):Hydraulic.zoneSink | 1.576 | 1.829 | 3.692 | 0.129 | s(Fr):Hydraulic.zoneSink | 2.036 | 2.140 | 1.925 | 0.235 |
| s(Re*):Hydraulic.zoneFlush | 1.000 | 1.000 | 0.073 | 0.787 | s(Re*):Hydraulic.zoneFlush | 2.568 | 3.096 | 2.590 | 0.475 |
| s(Re*):Hydraulic.zoneSink | 1.389 | 1.628 | 2.395 | 0.214 | s(Re*):Hydraulic.zoneSink | 1.685 | 1.905 | 0.768 | 0.699 |
| s(U*):Hydraulic.zoneFlush | 2.093 | 2.618 | 1.723 | 0.424 | s(U*):Hydraulic.zoneFlush | 1.000 | 1.000 | 0.724 | 0.395 |
| s(U*):Hydraulic.zoneSink | 1.000 | 1.000 | 1.513 | 0.2186 | S(U*):Hydraulic.zoneSink | 1.000 | 1.000 | 3.714 | 0.054 |

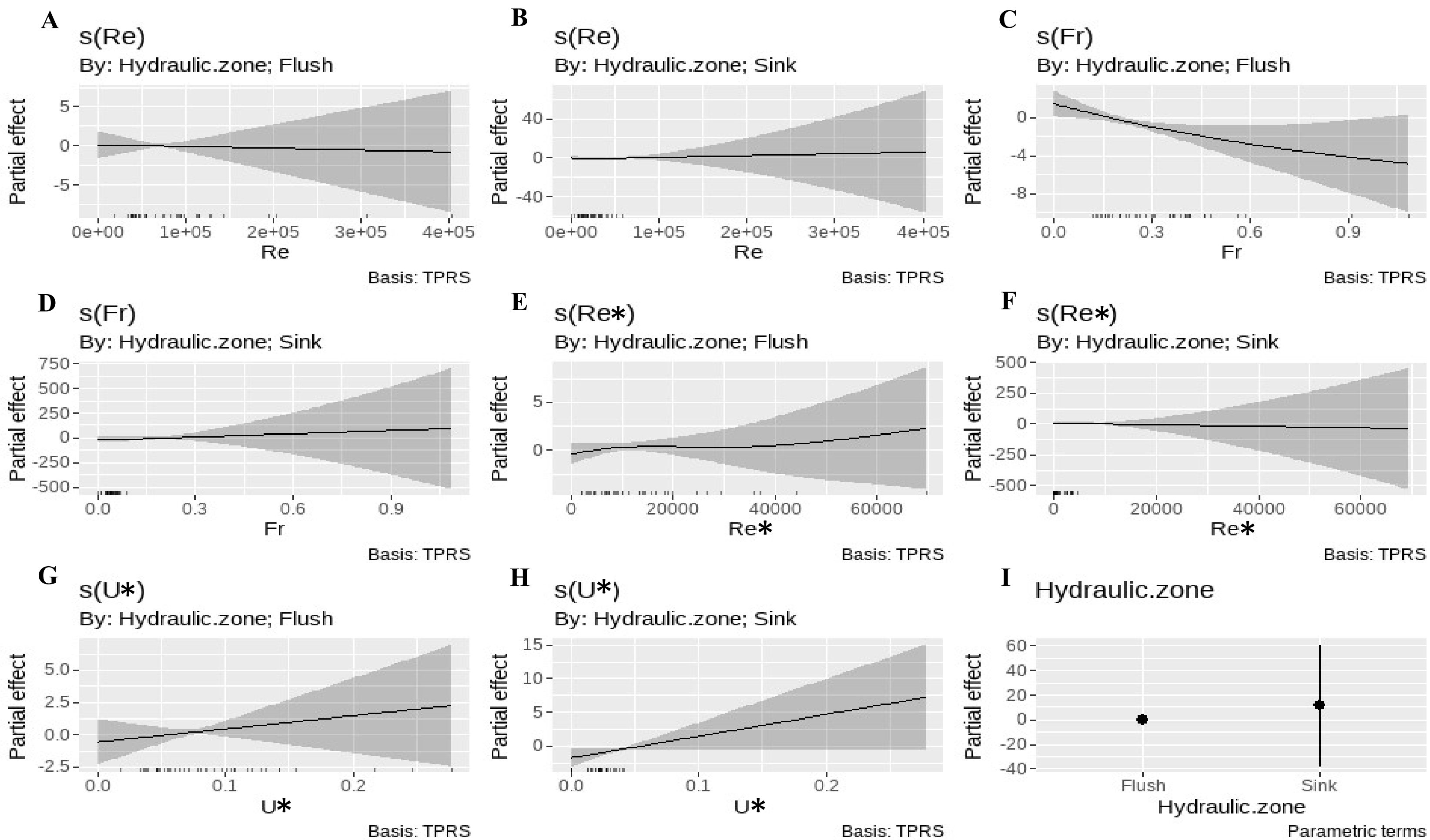


**Figure 4.22.** Residual plots for predictors obtained by the negative binomial generalized additive models (GAMs) for **settled microplastics** abundance distribution recovered during the surveys (October 2021–July 2022) from the delineated hydraulic zones of the Buffalo and Swartkops river systems. Hydraulic index predictors / hydraulic zones: A = Reynolds number (flush), B = Reynolds number (sink), C = Froude number (flush), D = Froude number (sink), E = Roughness Reynolds number (flush), F = Roughness Reynolds number (sink), G = Shear velocity (U*), H = Shear velocity (sink), I = parametric terms. Solid lines and the grey shaded portions represent the smoother fit and the approximate 95% confidence level, respectively. Observed data points are indicated as tick marks (i.e., rug plot) on the x-axis.

For suspended microplastics, *Fr* had a smoothing term significantly different from zero in the flush hydraulic zone ($P$ = 0.030) with a comparatively higher flexibility, as indicated by the edf (Table 4.2). Figure 4.23C shows a decreasing trend in suspended microplastics abundance at low *Fr* values, and an increasing trend from higher Fr value of about 0.75. Smoothing terms for *Re*, *Re** and *U** were indicated as not significant across the flush and sink hydraulic zones for suspended microplastics (Table 4.2). However, the modelled data showed a marginal linear relationship between microplastics distribution and *Re* in the flush zone (Figure 4.23A) and between microplastics distribution and *U** in the sink zone (Figure 4.23H), respectively. The estimated parametric effect at the 95% confidence level predicted no significant relationship between the hydraulic zones and suspended microplastics.

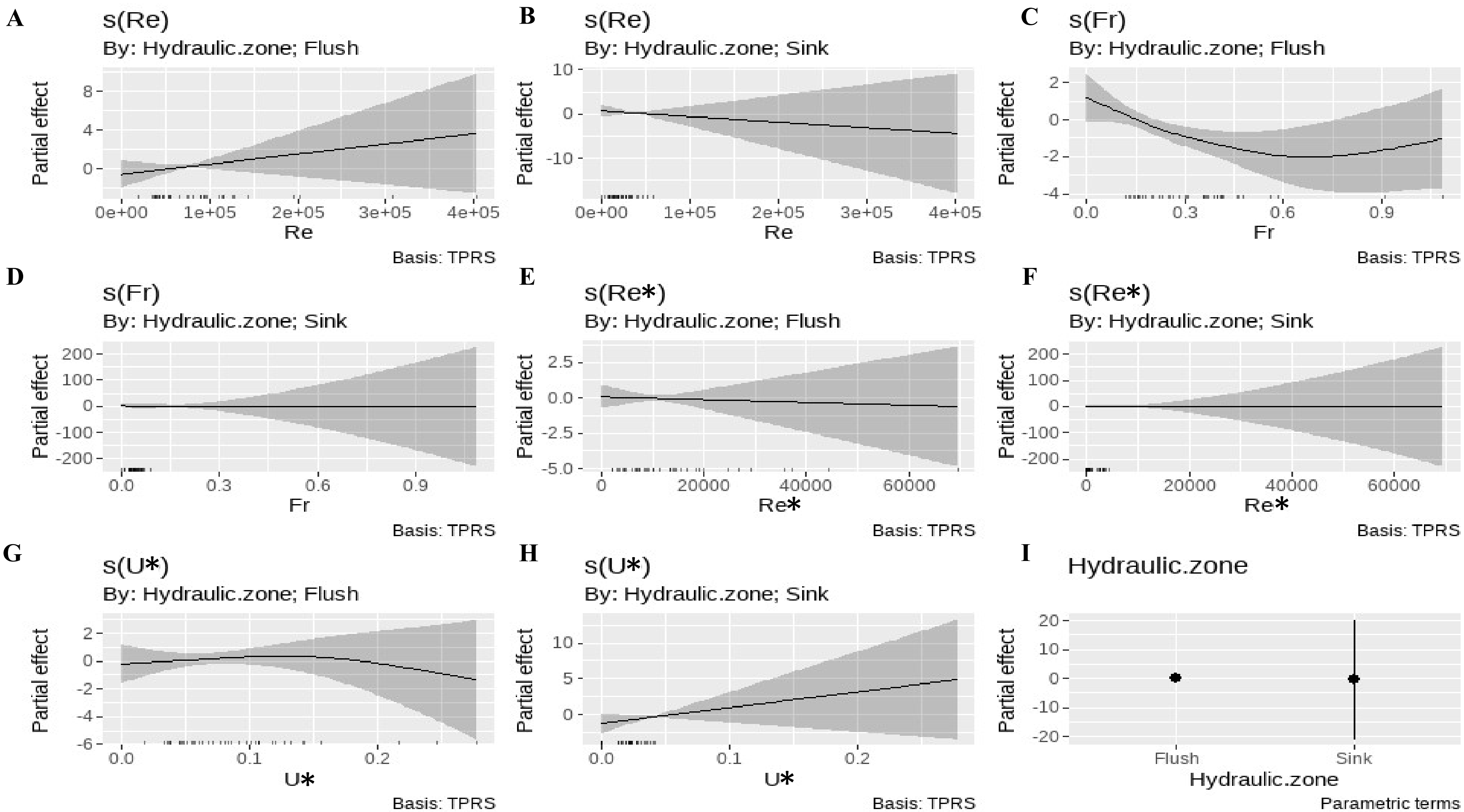


**Figure 4.23.** Residual plots for predictors obtained by the negative binomial generalized additive models (GAMs) for **suspended microplastics** abundance distribution recovered during the surveys (0ctober 2021–July 2022) from the delineated hydraulic zones of the Buffalo and Swartkops river systems. Hydraulic index predictors / hydraulic zones: A = Reynolds number (flush), B = Reynolds number (sink), C = Froude number (flush), D = Froude number (sink), E = Roughness Reynolds number (flush), F = Roughness Reynolds number (sink), G = Shear velocity (U*), H = Shear velocity (sink), I = parametric terms. Solid lines and the grey shaded portions represent the smoother fit and the approximate 95% confidence interval, respectively. Observed data points are indicated as tick marks on the x-axis.

### 4.5 Discussion

The differences in settled microplastic abundances between the different hydraulic zones and across the seasons is indicative of the impact of spatial and seasonal variations in riverine hydrodynamics on the remobilisation of settled microplastics. Settled microplastics are likely to be remobilised into suspension as sink zones transform into flushes when flow velocities approach critical to supercritical-turbulent conditions, giving rise to a higher abundance of suspended microplastics in flushes, as observed in this study. Suspended microplastics had the highest counts in flushes (1.76 ± 1.44 items/L) compared to the sink zones (1.54 ± 1.46 items/L). The presence of settled microplastics in flushes in this study, though in significantly lower quantities than in the sinks, could be related to the role of other factors in determining microplastics remobilisation, such as microplastic density, the nature of the substratum, and shear stress. For instance, in a hydrodynamic modelling study, Lu *et al*. (2023) observed that microplastic particles with high density had the worst mobility in water and were more prone to deposition. Bravard & Petit (2009b) noted that the coarse particles lying on riverbeds are set in motion when the critical shear stress is passed. In this study, high density microplastics such as PU, PVC and PA were only found among settled microplastics, although they had very low distribution. The implication is that while river systems are known pathways for microplastics to other aquatic systems such as the ocean (Meijer *et al*., 2021), some proportion of high density, homo- and heteroaggregated particles are likely to be retained on the riverbed where benthic macroinvertebrates such as collector-gatherers and other benthonic feeders can pick them up.

The results of the hydraulic indices derived for this study show that the sink hydraulic zones are characterised by very low flows and a low level of turbulence, as indicated by the *Re* and *Fr* values (Figures 4.1 and 4.2). Low flows and high depth, which characterise the sink zones, are known to favour sedimentation and aggregation of microplastics (Ding *et al*., 2019; Dahms *et al*., 2020b; Hübner *et al*., 2020). Accordingly, fragment microplastics had the highest distribution among settled microplastics in the sink hydraulic zone while fibres dominated suspended microplastics. Owing to the low level of flow and turbulence in sinks, water mixing is usually impeded, and nutrients, chemicals and other organic matter accumulate, favouring the sorption of toxic chemicals onto microplastics and microbial activities such as biofilm formation. During high flows, both suspended and settled microplastics from the sink zones can be transported to other portions of the river, along with toxic chemicals and pathogenic microbes that may have colonised the microplastic materials.

The highest occurrence of microplastics in the sink and flush zones were detected at sites B2 and B4, respectively, during the wet season in the Buffalo River. Both sites were not directly impacted by point sources of pollution such as WWTW, which may suggest that sampling microplastic at the hydraulic biotope scale may be appropriate for detecting seasonal changes in microplastic hotspots as the hydraulic biotopes transform due to changes in flow. This assumption is further supported by the consistently high microplastic abundance results obtained at sites B3 and S3 which were directly impacted by effluent discharges from the WWTW in the Buffalo and Swartkops rivers, respectively. The differences between the hydraulic zones in terms of the concentration of settled microplastics suggest that the transport of settled microplastics is much slower in sinks than in the flush zone. This observation is in line with the initial prediction for this study. Microplastic particles are likely to accumulate in sinks that are directly affected by point sources of pollution as longitudinal movement of particles is slowed as result of low flow that characterise the sinks.

Among the hydraulic indices explored in this study, the Froude number (*Fr*), which incorporates flow velocity, depth, and gravity in describing river flow as either tranquil, critical, or supercritical, showed a significant relationship with settled and suspended microplastics in the flush zone (Figure 4.22C; 4.23C); hence, the *Fr* was a good fit for the model. The significant relationship of the *Fr* with microplastics in the flush hydraulic zone suggests that riverine hydrodynamics such as flow variability and flow depth may have a more profound influence on microplastic transport in fast-flowing hydraulic biotopes (Tibbetts *et al.*, 2018). The modelled data (Figure 4.23C) clearly indicate an increasing trend in suspended microplastics as flow approach the critical and supercritical regime ($Fr$ = > 0.75). The predicted increase in suspended microplastics as *Fr* approaches critical flow conditions in the model reinforces the assumption that settled microplastics are increasingly remobilised into suspension at higher *Fr* values, which is a vital characteristic of fast-flowing hydraulic biotopes such as fast runs, riffles, and rapids. The rate of settled microplastic remobilisation will also depend on microplastic morphology (i.e., size and shape) and microplastic density (i.e., polymer type). Smaller sized, streamlined particles that offer little resistance and drag, as well as low density microplastic polymers that settled because of aggregation with other microplastics, or other particulates will be easily remobilised as *Fr* values increase in the positive direction. The implication is that smaller-sized, low density microplastics such PE and PP dominate suspended microplastics, as revealed in the current study. However, at supercritical flow regimes such as flooding events, rivers will experience microplastic flushing from riverbeds

(Hurley *et al.*, 2018). Therefore, the *Fr* may be a useful index that should be incorporated in future microplastics modelling studies.

Both the *Re* and *Re** showed some useful trends with microplastic abundances that could be further explored. For instance, the roughness Reynolds number (*Re**) showed an increasing trend in settled microplastic abundance distribution at very high hydraulically substrate roughness (*Re** > 40000) in the flush zone (Figure 4.22E). This may indicate that, depending on the height and nature of the substrate element, microplastic remobilisation from sediments may be impeded. However, limited data pointing in the region *Re** > 4000 means that the assumption regarding *Re** needs to be pursued further to draw any useful conclusions. The Reynolds number (*Re*), which defines turbulent and laminar flow conditions, also seem to suggest an almost linear relationship with suspended microplastics at *Re* values greater than 100,000 in the flush zone (Figure 4.23A). However, the trends showed by both *Re* and *Re** need further investigation as they were not significant in this study.

### 4.6 Conclusion

This study revealed that the distribution of microplastics on the riverbed differs significantly between different hydraulic zones. The significant differences were attributed to the influences of the distinct riverine hydrodynamics that characterise hydraulic biotopes. Hydraulic biotopes were conceptualised as flushes and sinks, defining two different hydraulic zones characterised by distinct hydrodynamic conditions. Suspended microplastics, which were defined as microplastic particles floating in the river water, and settled microplastics which were regarded as microplastic particles on the riverbed, were both found to be more in abundance during the wet season across the hydraulic biotopes. The distribution of higher levels of microplastics during the wet season was attributed to higher rate of microplastic remobilisation and increased influxes from land-based sources, which reflects the ineffectiveness of waste management and land-based mitigation measures. The sink hydraulic zones, which were hydraulic biotopes characterised by very slow flow with little or no turbulence, had the highest distribution of settled microplastics, while flushes, which were characterised by subcritical-supercritical-turbulent flow, had the highest distribution of suspended microplastics. Microplastic sampling at the hydraulic biotope scale has the potential for unravelling microplastic hotspots and should be integrated into future microplastics monitoring and evaluation studies. The Froude number showed a significant relationship with suspended and settled microplastic distribution in the flush hydraulic zone, hence it would be a useful tool in modelling studies.

# CHAPTER 5: ASSESSING MACROINVERTEBRATE EXPOSURE TO MICROPLASTICS USING THE TRAIT-BASED APPROACH

## 5.1 Introduction

Macroinvertebrate distribution in rivers is strongly associated with a gradient of flow velocity and depth (Pastuchová *et al.*, 2008; Wegscheider *et al.*, 2023). In the context of the River Geomorphological Hierarchical Classification framework (RHMC) by Lehotský (2004), spatial organisation of geomorphological units and hydraulic biotopes form a medley of different types of physical habitats changing longitudinally along the river length (Pastuchová *et al.*, 2008), and provide distinct habitats for macroinvertebrates and other aquatic organisms (Thomson *et al.*, 2001). Rapids, riffles, runs, and pools are examples of spatial organisation at the morphological and hydraulic biotope scale that reflect distinct hydraulic patterns that result from the interaction between flow, substrate and channel morphology (Wadeson & Rowntree, 1998). Different hydraulic patterns such as high flows, low flows, high depths associated with hydraulic biotopes have been reported to interact with microplastics properties such as shape, density, and size to influence the distribution and fate of microplastics in riverine systems (Kumar et al., 2021; X. Lu et al., 2023).

Macroinvertebrates, through their traits are adapted to different hydraulic biotopes which may impose on them different levels of exposure to microplastics. A trait is a morphological, physiological or phenological feature measurable at the individual level, without reference to the environment or any other level of organisation (Violle *et al.*, 2007). The identification of organismal traits and ecological preferences that mediate macroinvertebrate exposure to microplastics in different hydraulic biotopes could be an important step towards the development of trait-based indices for biomonitoring microplastic pollution in riverine systems. Different authors have used this trait-based approach for the identification of sensitive and tolerant traits of various environmental disturbances. For instance, the trait-based approach was applied by Odume (2020) in identifying macroinvertebrates signature and sensitive traits of urban pollution in a river in the Eastern Cape of South Africa. Akamagwuna *et al.* (2019) applied the trait-based approach in detecting the responses of the EPT taxa to sediment stress in the Tsitsa River and its tributaries in the Eastern Cape of South Africa. The trait-based approach has also been used to characterise the functional strategies of European taxa of freshwater macroinvertebrates (Schmera *et al.*, 2022).

The trait-based approach is premised on the understanding that organisms possess traits that enable them to adapt to local environmental conditions, that is, organisms thrive in their environment only when they have the appropriate combination of traits, allowing them to either resist or adapt to local environmental conditions or pressures (Verberk *et al.*, 2013; Abdala-Roberts *et al.*, 2018; Kiørboe *et al.*, 2018). In this regard, hydraulic biotope characteristics, such as flow velocity, depth, and substrate act as filters for traits of organisms and shape community composition by selecting well-adapted species with an appropriate combination of traits. Each hydraulic biotope, run, pools, backwater, etc., will provide physical habitats for different compositions of macroinvertebrates, depending on the prevailing hydraulic conditions. Accordingly, macroinvertebrates that prefer high-flow velocity (e.g., rapids and fast runs) will have a combination of traits (i.e., attachment structures, external gills for respiration, filtration apparatus, etc.) that adapt them to such hydraulic biotopes while excluding species with a preference for standing or very slow water velocity such as pools and backwater. The prevailing characteristics of each hydraulic biotope, thus, constrain each organism such that only species with adaptive traits for each set of hydraulic characteristics can thrive. Therefore, depending on the trait possessed, certain macroinvertebrates may show a preference for the different hydraulic biotopes, potentially being more exposed to microplastics in such biotopes for which they have higher preferences. Showing preference for a particular biotope thus implies that macroinvertebrates may be differentially exposed to microplastics, depending on their preferred hydraulic biotopes and ecological niche within such biotopes. For instance, macroinvertebrates that select the water column in moderate to fast-flowing biotopes are likely to be more exposed to suspended microplastics than benthonic macroinvertebrates in the same hydraulic biotope, as fast-flowing waters have been demonstrated to contain greater concentrations of suspended microplastics than settled microplastics (X. Lu et al., 2023). Organs such as external gills and filtration apparatus may become clogged as macroinvertebrates interact with microplastics in the water column.

Theoretically, the trait-based approach emanated from the habitat template concept of Southwood (1977, 1988) and the habitat filtering concept of Poff (1997). According to the habitat filtering concept, environmental drivers of change acts as trait filters, selecting appropriate combinations of traits while eliminating others (Poff, 1997). Verberk *et al*. (2013) noted that the use of traits could increase the mechanistic understanding of biological responses to environmental stressors, such that it is possible to transform descriptive field studies in community ecology into a predictive science. One of the potential strengths of the trait-based approach is that theoretical ecology provides a foundation for understanding how functional trait characteristics are expected to respond to environmental gradients (Pollard & Yuan, 2010).

In other words, species trait interaction with microplastics could provide a clear mechanistic understanding of the risk posed by microplastics to macroinvertebrates in their preferred hydraulic biotopes. For example, the uptake of microplastics during respiration (Welden & Cowie, 2016b) can be mechanistically linked to gill clogging and gut obstruction. Soft-bodied macroinvertebrates may suffer from external abrasion as they interact with the particulates (e.g., fragments) in transport at high speed, while sessile organisms could suffer entanglement with fibres. Thus, the mechanistic trait-based approach provides useful mechanistic links by paying attention to traits and focussing on the underlying processes and interactions between the organism and the external environment.

Thus, this chapter focuses on assessing potential macroinvertebrate exposure to microplastics using a mechanistic trait-based approach. The current chapter addresses Objective three stated in Chapter One: *to use the trait-based approach to examine macroinvertebrate distribution and exposure to microplastics at the hydraulic biotope scale* through the following sub-objectives: i) to identify macroinvertebrate traits associated with the different hydraulic zones; ii) to identify potentially sensitive and tolerant traits of microplastic pollution; iii) to identify species at risk of exposure to microplastics at the hydraulic biotope scale. As presented in Chapter Four of this study, hydraulic biotopes were categorized into two unique hydraulic zones: flush and sink hydraulic zones.

## 5.2 Materials and methods

### 5.2.1 Macroinvertebrate sampling and identification

Macroinvertebrates were collected from available physical biotopes (i.e. stones, vegetation, gravel, sand, and mud) in the two hydraulic zones for each site during the four sampling events. Details on macroinvertebrate sampling are provided in Chapter Two, Section 2.8. In the laboratory, macroinvertebrate specimens were sorted to the family level for each hydraulic zone per site and preserved in 70% alcohol for further analysis. Preserved specimens were identified with identification keys ( Hodkinson *et al.*, 1981; Day *et al.*, 2001; Gerber & Gabriel, 2002; Picker *et al.*, 2002; Vlok, 2002; de Moor *et al.*, 2003).

### 5.2.2 Selected macroinvertebrate traits and ecological preferences

The traits used in this study consisted of biological features identified in the literature as reflecting organismal performance and adaptations to environmental stressors. In the context of this study, the traits selected can provide a mechanistic connection between the organism and its preferred hydraulic zone as well as the organismal exposure to microplastics. In selecting traits for this study, several factors were considered, and these include i) ease of measurement; ii) mechanistic relationship between the trait, hydraulic zones, and organismal exposure to microplastics; iii) adaptive value of the trait, and iv) trait role in the ecological function of the organism. Two traits (gills and body size) and two ecological preferences (feeding habit and hydraulic (velocity) preferences) that can easily be described at the family-level were selected and resolved into 17 attributes (Table 5.1).

**Table 5.1.** Selected biological traits and ecological preferences of macroinvertebrates (with codes) examined in this study.

| Trait / ecological preference category | Trait attribute | Code |
|---|---|---|
| Gill type | Operculate gill | GO |
| | Plate-like gill | GP |
| | Lamellate gill | GL |
| | Filamentous gill | GF |
| Body size (mm) | Very small (≤5) | SZ1 |
| | Small (>5-10) | SZ2 |
| | Medium (>10-20) | SZ3 |
| | Large (>20) | SZ4 |
| Feeding habit | Shredder | SH |
| | Collector-gatherer | CG |
| | Collector-filterer | CF |
| | Scraper (grazers, brushes) | SC |
| | Predator | PR |
| Velocity preference (velocity m/s) | Very low (< 0.1) | V1 |
| | Low (0.1 - 0.3) | V2 |
| | Medium (>0.3 – 0.6) | V3 |
| | High (>0.6) | V4 |

A fuzzy coding system was then used to describe the link between a taxon and each trait class or ecological preference category (Chevene *et al.*, 1994; Appendix F). As noted in Odume (2020), the fuzzy coding system uses affinity scores to account for potential functional variation between species within a taxon in relation to a particular trait attribute or ecological preference. Affinity scores ranging from 0 to 5 were assigned each taxon per trait attribute and ecological preference. A 0 score indicates no affinity/no preference of a taxon to a given trait attribute/ecological preference, 1 = low affinity/low preference to a trait/ecological preference attribute, 3 = moderate affinity/moderate preference, and a score of 5 indicates a high affinity/high preference to a trait/ecological preference attribute. The selected traits and ecological preferences and the rationale for their selection are discussed.

The first trait class selected is the gill type. Macroinvertebrates respire using different structures (e.g., trachea teguments, plastron, and gills) but the commonest respiratory structure is the gills. Gills may either be internal or external and may be of different shapes such as filamentous, lamellate, plate-like or operculate. Depending on the hydraulic preferences of the organisms, the risk of gills encountering microplastics will differ. Fast-flowing conditions favour longitudinal transport of microplastics and will also increase the rate of settled microplastic remobilisation into the water column, creating a relatively higher abundances of microplastics in the water column than in sediments (X. Lu et al., 2023). As a result, taxa that filter food within fast-flowing turbulent conditions are likely to be more exposed to microplastics and face a higher risk of encountering microplastics in their gills compared to organisms preferring slow or still conditions, where the risk of microplastic remobilisation from sediment is lower and the concentration of microplastics in the water column is relatively reduced. Consequently, species inhabiting turbulent flow zones with exposed gills are more likely to encounter greater quantities of microplastics in their gills than those inhabiting slow-flowing waters with covered, operculated gills. The type of gill, either external or internal and gill shape (e.g., operculate, lamellate, filamentous, plate-like) mediate the level of risks to taxa, that is, external gills are in more contact with microplastics than internal gills. To analyse how gill types responded to microplastics occurrence in the different hydraulic regime, gill trait was resolved into filamentous, lamellate, plate-like and operculate gills. Macroinvertebrate family traits information for this category of trait was obtained through observation, expert opinion and relevant literature (Picker *et al*., 2002; de Moor *et al*., 2003; Vieira *et al*., 2006; Akamagwuna *et al*., 2019; Odume *et al*., 2023).

The second trait class selected is body size. Body size is an important feature that constrains ecological and life-history traits of species as is strongly correlated with many physiological and fitness characteristics (Hui & McGeoch, 2006). Body size is a form of a 'taxon-free' classification of individuals, which may be a useful metric for describing variation within and between ecological communities. It provide clues to understanding species' interaction with their environment, and the underlying processes affecting community assemblages (Robson *et al*., 2005). Thus, body-size distributions may reflect changes in habitat complexity, productivity, species diversity and abundance, habitat architecture, biological interactions and effects of anthropogenic disturbances (Robson *et al*., 2005; Odume, 2020). Body size may also be related to species diet and niche (Hui & McGeoch, 2006). Macroinvertebrates can be exposed to microplastics in their preferred hydraulic zone through external contact and food ingestion. Body size will, therefore, play a critical role in determining the amount of microplastics ingested with food. Euryphagic species are likely to consume more microplastics in their diet than are stenophagic species. Body size also regulates the size of ingested microplastics, in terms of morphology and quantity.

Robson *et al.* (2005) argued that body length is the most appropriate measure of body size for most aquatic macroinvertebrates as it is functionally connected to locomotion, predation, and habitat architectures. The body size of the macroinvertebrate families was resolved into four categories: ≤ 5mm, > 5–10 mm, > 10–20 mm, and > 20 mm. The body length of individuals of each macroinvertebrate family at each sampling zone during each sampling season was measured from the tip of the head to the abdominal tip, exclusive of terminal appendages. Measurements were undertaken for the juvenile, that is, nymph, where the adult is non-aquatic. The carapace length was used for the family Potamonautidae. Macroinvertebrate family trait information for this category was obtained through observation and relevant literature (Day *et al.*, 2001; Vieira *et al.*, 2006; Odume, 2020; Odume *et al.*, 2023).

Feeding habit, which refers to the way in which macroinvertebrates obtain their food (Cummins & Klug, 1979) was selected in this study. Freshwater macroinvertebrates consume various food sources, e.g., algae, detritus, and other animals (Schmera *et al.*, 2022). The mechanisms by which these food materials are obtained are an essential ecological feature related to macroinvertebrate ecological function and ecosystem processes (Wallace & Webster, 1996). Macroinvertebrates were grouped into feeding groups based on how food material is obtained (Cummins & Klug, 1979). Scrapers graze upon resources that grow over substrates (e.g., algae on rock surfaces and stream plants, benthic biofilms composed of bacteria, fungi etc) by scraping them with their mouthparts (Ramírez & Gutiérrez-Fonseca, 2014; Serna *et al.*, 2022). Shredders feed mainly by cutting, chewing, or mining pieces of living or dead plant material such as leaves, buds, and wood. Shredders convert coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM). Collector-gatherers have generalised adaptations to collect fine particulate organic matter (less than 1 mm) from crevices, depositional areas or the stream bottom (Ramírez & Gutiérrez-Fonseca, 2014). Collector-filterers have adaptations to capture particles from the water column, while predators feed on other animals (Ramírez & Gutiérrez-Fonseca, 2014). It has been suggested that predators pick microplastics that resembles their prey from the water column (Welden & Cowie, 2016a). Both collector-gatherers and grazers are more likely to consume settled microplastic particles deposited on substrate surfaces. At the same time, filterers are hypothesized to consume more light-density microplastics found in suspension in the water column. Macroinvertebrate family traits information for this category was obtained from relevant literature (Picker *et al*., 2002; Vieira *et al*., 2006; Odume, 2020; Odume *et al*., 2023).

Regarding velocity preferences, macroinvertebrates are strongly associated with a gradient of flow velocity (Pastuchová *et al*., 2008; Wegscheider *et al*., 2023). Hydraulic biotopes with high flow velocity are characterised by high dissolved oxygen owing to the high turbulence and the churning movement of water in contact with the air, shallow depth, and the presence of stones. Macroinvertebrates preferring this environment tend to possess external gills for respiration, attachment structures and apparatus for filtering food particles in transport. Macroinvertebrates may be prone to entanglement of attachment structures by fibre microplastics, and to clogging of gills and filtration apparatus. Conversely, slow to still velocity environments favour microplastic settling and deposition (Tibbetts *et al*., 2018; Dahms *et al*., 2020a), potentially increasing the interaction of biota with microplastics and magnifying the risk of ingestion. Another important consideration is that some aquatic macroinvertebrates select different habitats at different stages of their life cycles. For instance, some aquatic macroinvertebrates start life at Reynolds number of about 1-10 in the laminar layer, but when they reach their adult form, they may live in conditions of $Re$ = 1000 or higher in the turbulent flow (Rowntree & Wadeson, 1999). The implication is that macroinvertebrates preferring different flow conditions will likely experience different exposure patterns to microplastics at different stages of their life cycle because of their preference for different flow conditions. In this study, macroinvertebrate velocity preferences were resolved into four categories: very low flow velocity (< 0.1)**,** low flow velocity (0.1– 0.3), medium flow velocity (>0.3– 0.6), and high flow velocity (>0.6). Macroinvertebrate family traits information for this category was obtained from relevant literature (Picker *et al*., 2002; de Moor *et al*., 2003a; de Moor *et al*., 2003b; Odume, 2020; Odume *et al*., 2023).

### 5.2.3 Macroinvertebrates dissection and processing

For each family of macroinvertebrates where at least ten individuals had been collected from a hydraulic zone, five members of the same family with similar body lengths were dissected for microplastic analysis (Table 5.2). The five individuals were taken as composite samples and were weighed together (mg / wet weight) and their gills removed and examined for microplastics under a dissecting microscope. Each composite sample is representative of each hydraulic zone per site. Henceforth, composite samples are referred to as 'samples'. Where necessary, portions of the samples containing gills were carefully extracted using a dissecting blade and a pair of forceps and digested before being examined under a light microscope. Samples were also dissected to extract portions containing the gut system. Prior to extraction of portions containing the guts, samples were dipped in milli-Q water to remove any externally adhered plastics. All dissection was carried out with the aid of a dissecting microscope in the laboratory.

**Table 5.2**. Macroinvertebrate families selected for examination for microplastic ingestion and/or gill entanglement/clogging.

| Macroinvertebrate order/family | Life stage | Average body length (mm) | Sample wet weight (mg) |
|---|---|---|---|
| **Odonata: Aeshnidae** | Nymph | 23 ± 3 | 622.7 |
| **Ephemeroptera: Baetidae** | Nymph | 5 ± 0 | 1.6 |
| **Hemiptera: Belostomatidae** | Adult | 12 ± 2 | 450.0 |
| **Ephemeroptera: Caenidae** | Nymph | 5 + 1 | 1.9 |
| **Diptera: Chironomidae** | Larvae | 18 ± 2 | 63.0 |
| **Odonata: Coenagrionidae** | Nymph | 19 ± 3 | 143.3 |
| **Odonata: Gomphidae** | Nymph | 18 ± 2 | 624.6 |
| **Ephemeroptera: Heptageniidae** | Nymph | 8 ± 2 | 51.3 |
| **Coleoptera: Hydrophilidae** | Larvae | 11 ± 2 | 122.5 |
| **Trichoptera: Hydropsychidae** | Larvae | 12 ± 3 | 119.4 |
| **Ephemeroptera: Leptophlebiidae** | Nymph | 7 ± 2 | 39.3 |
| **Odonata: Libellulidae** | Nymph | 17 ± 3 | 512.6 |
| **Hemiptera: Naucoridae** | Adult | 11 ± 2 | 398.2 |
| **Plecoptera: Perlidae** | Nymph | 11 ± 3 | 60.2 |
| **Decapoda: Potamonautidae** | Adult/Nymph | 19 ± 3 | 7280.0 |
| **Diptera: Simuliidae** | Larvae | 5 ± 0 | 1.3 |

| | | | |
|---|---|---|---|
| **Ephemeroptera: Tricorythidae** | Nymph | 8 ± 2 | 51.0 |

Digestion of biological samples followed a protocol by Dehaut *et al.* (2016) with slight modification. Dissected portions of samples were first homogenised using a ceramic mortar and pestle (Avio *et al*., 2015). Each homogenised sample was then carefully transferred into a 250 ml glass beaker and filled with 10% potassium hydroxide (KOH) solution to 3 x the volume of the tissue. A beaker containing the mixture of KOH and sample was then incubated for 24 hours at 60 $^{o}$C to digest biogenic materials before vacuum filtration onto a 1.6 µm Whatman glass microfiber filter paper. The choice of KOH in digesting samples is because of the proven results in removing biogenic material (Lusher *et al*., 2017). Most polymers are also resistant to KOH and are able to retain their form and mass, except for cellulose acetate (Dehaut *et al*., 2016; Kühn *et al*., 2017).

Where debris and other recalcitrant indigestible materials remained after digestion, a density-based step using sodium iodide (NaI) was incorporated. A saturated NaI solution, density = 1.8g/cm$^3$, (Nuelle *et al*., 2014) was added to the mixture in the beaker (2:1, v/v). The mixture was then thoroughly stirred for 5 minutes before being left to settle for 2 hours. The supernatant containing the floating plastic particles was subsequently collected and vacuum-filtered as previously described. The filter was flushed thoroughly with pre-filtered Milli-Q water to remove all traces of NaI. Filtered particles were then oven-dried on the filter paper at 60 $^{o}$C (Horton *et al*., 2017) and stored in a glass petri-dish for further analysis. The choice of NaI for density separation was owing to its high density, which is capable of floating high-density plastics ( Nuelle *et al*., 2014; Lusher *et al*., 2017).

Steps were taken to avoid cross-contamination and procedural contamination during sample processing in the laboratory. To avoid cross-contamination, all tools and glassware were washed with phosphate-free soap, rinsed thoroughly with milli-Q water, and dried. Tools and glassware were also rinsed thoroughly with milli-Q water between samples. To account for procedural contamination, three control samples were run by passing approximately 200-300 mL of the NaI solution through the vacuum filter onto a 1.6 µm pore size glass microfiber filter paper (GF/F, 47 mm Ø, Whatman) and analysed under a light microscope for contamination.

### 5.2.4 Quantification and characterisation of microplastics

Ingested microplastics were examined and identified using a light microscope (Olympus SZX16) fitted with a digital camera (Olympus DP72) and connected to image-processing software (Stream motion; XV Image processing) which was used for microplastic examination, counting and morphological identification. Criteria used for selecting microplastic particles conformed to the following outlined by Horton *et al* (2017) and Nor and Obbard (2014): no visible cellular or organic structures; particles not segmented; fibres equally thick throughout their entire length and not tapered at the end; unnaturally coloured and homogenous texture; unnatural, brightly coloured coating on another particle unnatural shape; fibre that remained intact with a firm poke with tweezers; flexible, that is, not brittle when compressed. The shapes and colours of microplastics were recorded as stated in Chapter 2, Section 2.8. Only suspected microplastic particles in the size range 0.5 mm and higher were subjected to polymer confirmation using an ATR-FTIR. Because of the limitation of the FTIR equipment and the unavailability of a micro-FTIR, particles in the size range lower than 0.5 mm were not subjected to chemical identification. Nevertheless, as highlighted in Chapter Three of this study, the FTIR method demonstrated excellent accuracy in identifying larger particles. This suggests that the smaller fractions estimated using image processing software coupled with an optical microscope are also likely to achieve high accuracy.

## 5.3 Statistical analysis

Three distinct statistical analyses were explored; a generalized linear model (GLM), RLQ and the fourth-corner analysis. Data regarding the localization of microplastics in the gills and guts of macroinvertebrates were aggregated and translated into a presence-absence or binomial distribution data (i.e., absence of microplastic particles in the gills and guts was coded zero, '0' while the presence of microplastic particles in the gill and gut was coded one, '1'). The binomial distribution data was used to model the likelihood of gill clogging/entanglement in macroinvertebrates using the hydraulic zones as predictor, then the different gill shapes were also used as predictors. This first level of analysis was undertaken using a binary logistic regression. A binomial logistic model is used to predict a dichotomous dependent variable based on one or more independent variables that can either be continuous or nominal. The binomial logistic model does not predict a specific value for the dependent variable, instead it predicts the probability of an observation falling into a particular category based on the independent variables. The use of the absence-presence data was thought to eliminate or reduce the bias that may have been created by the differences in the size of macroinvertebrates used for the study. The binomial logistic regression analysis was undertaken to determine (i) the hydraulic zone with a higher likelihood to influence gill clogging/entanglement by microplastics; (ii) to determine whether gills of macroinvertebrates were a good fit for studying and understanding macroinvertebrate exposure to microplastics in riverine systems, and (iii) to determine which gill type has a higher likelihood of clogging/entanglement, based on the presence-absence data computed for the gills.

In the second level of analysis, the presence-absence data computed for ingested microplastics (guts) was used to model the likelihood of exposure to microplastics among the different attributes of each trait class (i.e., the likelihood of microplastic ingestion among macroinvertebrate size classes – SZ1, SZ2, SZ3, SZ3; feeding habit – SC, PR, SH, CF, CG) and the hydraulic preferences (i.e., flow velocity preference). In each case of the binomial logistic regression analysis, a null model was also computed to compare model fitness. The overall model fit of each original model was then tested against each null model by conducting the likelihood ratio Chi-square test using the ANOVA function in R.

In the third level of analysis, a three-table ordination technique, RLQ, was computed to evaluate the relationship between macroinvertebrate trait attributes and environmental variables such as hydraulic indices (e.g., Froude number, Fr; Reynolds number, Re; roughness Reynolds number, Re*; shear velocity, U*), hydraulic variables (e.g., mean water depth, MD; mean velocity MV), and selected physicochemical attributes: temperature, dissolved oxygen, DO; total suspended solids, TSS; and total inorganic nitrogen, TIN. RLQ is a three-step ordination procedure developed by Dolédec *et al*. (1996) in which a matrix of environmental variables (R) is related to a matrix of associated traits (Q) using a species abundances matrix (L). To perform the RLQ analysis, taxa at each hydraulic zone per site were pooled seasonally resulting in a taxon-hydraulic zone/site matrix for each season. A second matrix was then created containing trait-taxon with trait data fuzzy-coded and the abundance of taxa was log (x+1) transformed. The first ordination of the RLQ analysis was then performed on the taxa dataset L-table, using correspondence analysis (CA). Then a second ordination, principal component analysis (PCA) was conducted on the environmental variables' dataset R-table, with row weights corresponding to the previous CA. The third ordination, principal component analysis (PCA) was carried out on the trait dataset Q-table, with the row weights corresponding to the column weight of the previous CA. The choice of a multivariate method in the second and third ordination was strongly linked with the data type (i.e., R and Q tables were continuous data, hence, the choice of PCA). The final step of the RLQ analysis simultaneously performs ordination on the separate ordinations by searching for a linear combination of traits (taxon scores in Q-PCA ordination) and environmental characteristics (sample scores in R-PCA ordination), maximizing covariance between Q and R through L (Dolédec *et al*., 1996). The Monte Carlo permutation test with 4999 permutations at alpha = 0.05 was used to test the statistical significance of the RLQ analysis. Only macroinvertebrates examined for microplastics (Table 5.2) were incorporated into the RLQ procedure. The RLQ was performed using the 'ade4' statistical package in R (Dray & Dufour, 2007).

The fourth-corner analysis was further used to test the association between individual traits and environmental variables. To conduct the fourth-corner analysis, results of model 2 (i.e., permutations of sites/hydraulic zones, rows) and model 4 (i.e., permutations of species, columns) were combined by specifying the model type argument to 6 during analysis using the ade4 package in R. *P*-values were adjusted for multiple comparisons using the FDR method which controls the false discovery rate (Jafari & Ansari-Pour, 2019). All statistical analyses in this chapter were performed in R version 4.3.2 environment (R Core Team, 2023).

## 5.4 Results

### 5.4.1 Localization of microplastics in macroinvertebrate gills and guts

Given that each sample was made up of five individuals, microplastics count were reported as number of items per sample. This was expressed as (items/sample). Of the 15 macroinvertebrate families examined (excluding the Hemipterans) for the presence of microplastics in gills, microplastics were observed in only six families across the four sampling events (Figure 5.1). The highest count of microplastics found was two items/sample. The morphology of ingested microplastics is shown in Appendix G. Samples of ingested microplastic particles are presented in Appendix H (Plates H1 – H10).

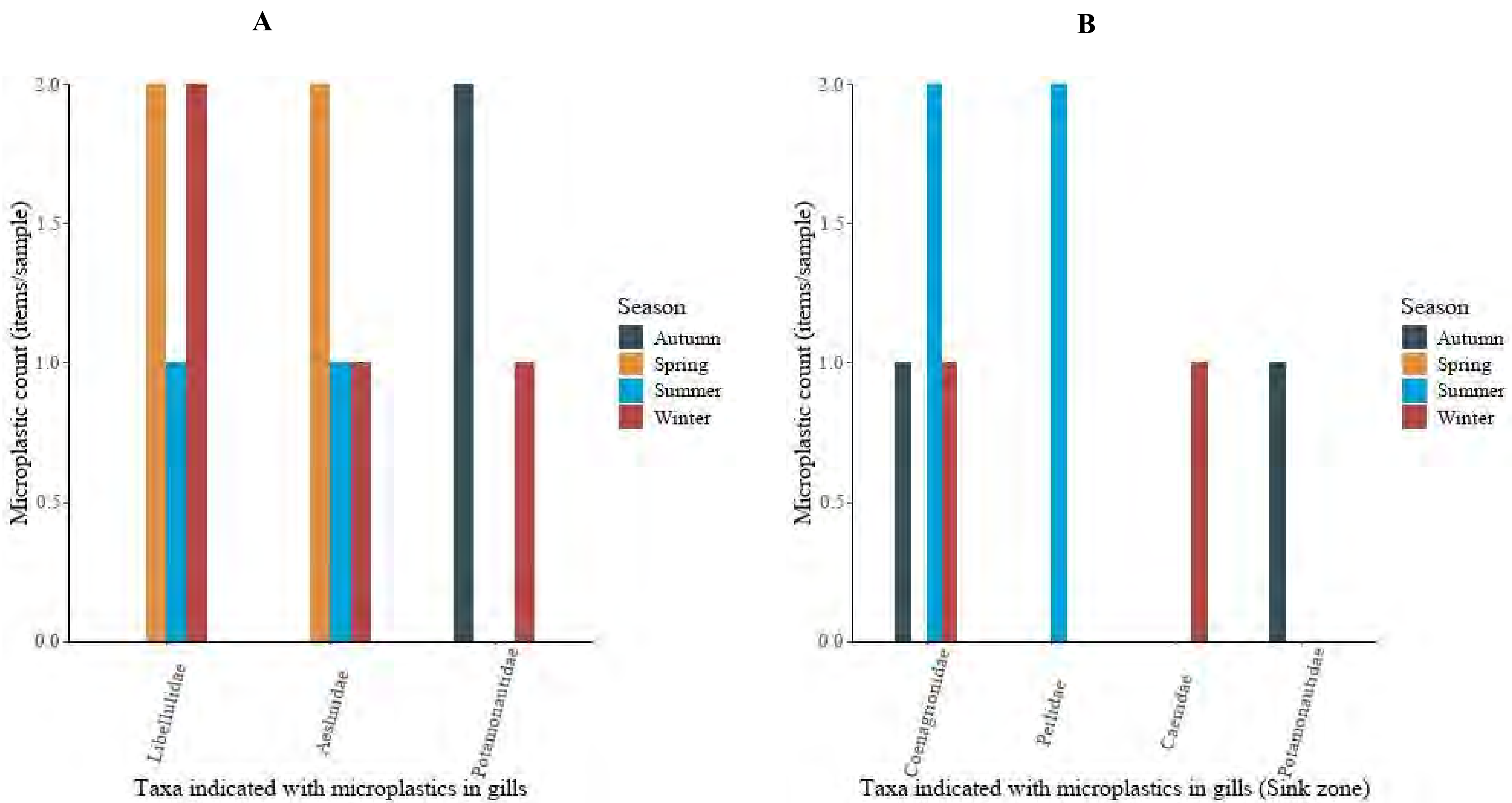


**Figure 5.1.** Microplastics count in the gills of the selected taxa collected from the hydraulic zones (A – Flush zone; B – Sink zone)

The binomial logistic regressions performed to assess the likelihood of gill clogging/entanglement mediated by hydraulic zones and gill shape, indicated a positive coefficient estimate for the sink zone (Table 5.3). A positive coefficient estimate indicates the likelihood of finding more microplastics in the gills in the sink hydraulic zone. However, at the 95% confidence interval, this result was not significant ($p > 0.05$). Operculate gill shape was indicated with a higher likelihood to have microplastics than other gill shapes. The overall model fit for the gill shape model was highly significant at the 95% confidence level with a Chi-square p-value, Pr (>Chi = 0.00028) (Table 5.3).

**Table 5.3.** Results of binomial logistic model showing the likelihood of gill clogging in the flush and sink hydraulic zones and among the different gill shapes. The presence of microplastics in gills was coded "Yes = 1", and the absence of microplastics in gills was coded "No = 0". A bold face is significant.

| Coefficients | Estimate | Standard Error | z value | Pr (> \|z\|) | Confint Lower 2.5% | Confint Upper 97.5% | Odd ratios |
|---|---|---|---|---|---|---|---|
| Intercept | -1.7750 | 0.3420 | -5.190 | 0.000 | -2.4452 | -1.1047 | 0.1695 |
| Hydraulic zone (sink) | 0.6400 | 0.5136 | 1.246 | 0.213 | -0.3667 | 1.6466 | 1.8964 |
| **Deviance statistics** | 1.5324 | | | | | | |
| **Pr (>Chi)** | 0.2158 | | | | | | |
| Intercept | 0.6978 | 3.5798 | 0.195 | 0.845 | -6.3183 | 7.7140 | 2.0094 |
| GO (Operculate gill) | 0.1283 | 0.4219 | 0.304 | 0.761 | -0.6987 | 0.9553 | 1.1369 |
| GP (Plate-like gill) | -0.0261 | 1.0775 | -0.024 | 0.981 | -2.1379 | 2.0856 | 0.9742 |
| GL (Lamellate gill) | -0.2323 | 0.5433 | -0.427 | 0.669 | -1.2972 | 0.8327 | 0.7927 |
| GF (Filamentous gill) | -0.9143 | 0.6365 | -1.436 | 0.151 | -2.1619 | 0.3333 | 0.4008 |
| **Deviance statistics** | 21.233 | | | | | | |
| **Pr (>Chi)** | **0.0003***** | | | | | | |

Regarding ingested microplastics, out of the 17 macroinvertebrate families examined for the presence of microplastics in the gut system (Table 5.2), Regarding ingested microplastics, out of the 17 macroinvertebrate families examined for the presence of microplastics in the gut system (Table 5.2), 13 families were found to have ingested microplastics during one or more sampling events (Figure 5.2). The highest occurrence of microplastics (6 items/sample) was found in the family Chironomidae collected from the sink hydraulic zone at site S3 (located in the middle reaches of the Swartkops, downstream of the Kelvin Jones WWTW effluent discharge point). Ingested microplastics were made up of black, blue, red and green colours. Fibres were the dominant microplastic shape ingested (Appendices L; Figures L1 – L2).

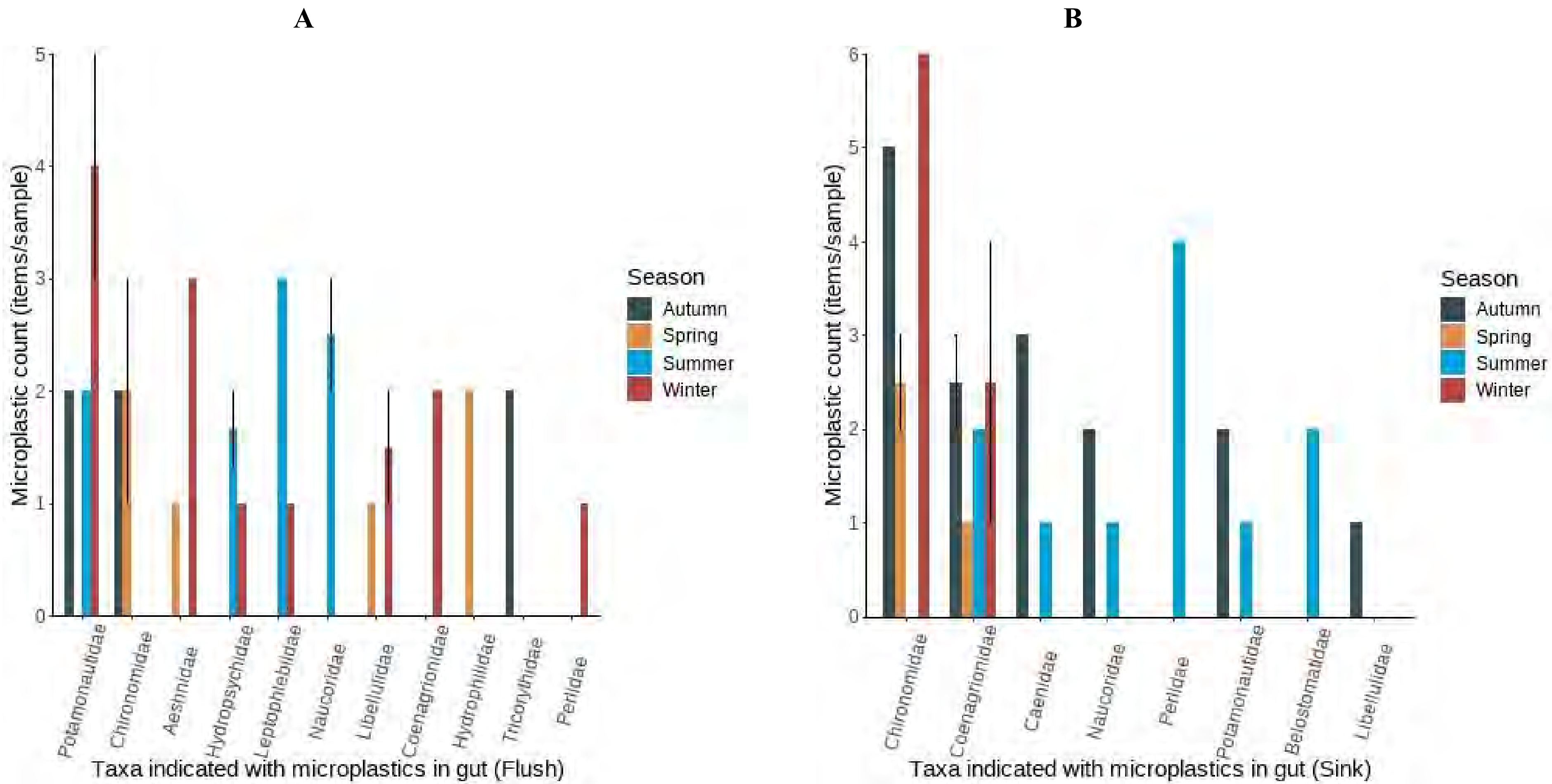


**Figure 5.2.** Microplastics counts found in the guts of the examined taxa collected from the flush hydraulic zone (A) and the sink hydraulic zone (B). Average count and standard deviation are presented for taxa that occurred and ingested microplastics in more than one site in the same sampling season

The binomial logistic regression performed to assess the influences of hydraulic zones on the likelihood of macroinvertebrates ingesting microplastics indicated a higher likelihood of ingestion of microplastics in the sink hydraulic zone. The positive coefficient estimate (0.1054) indicates the likelihood of microplastic ingestion in the sinks compared to the flush zone (Table 5.4). The lower and upper confidence intervals did not include one, signifying that the odds of microplastic ingestion for the flush and sink hydraulic zones were different. Regarding the sizes of macroinvertebrates, small, medium, and large sizes were less likely to be exposed to microplastic than the very small size. Accordingly, the lower and upper confidence limits also excluded one, which indicates that the odd ratios for the very small body size and other body sizes were different.

Collector-filterers, predators, and scrapers were less likely to ingest microplastics than were shredders and collector-gatherers. The lower and upper confidence limits also indicated that the odd ratios were different, except for shredders. The confidence intervals for shredders included one at the upper limit. Hence, the results seem to suggest that the data was insufficient to distinguish shredders from the feeding group indicated with a lesser likelihood for exposure to microplastics.

Regarding velocity preferences, both low and high velocities indicated a higher likelihood of exposure to microplastic than the very low and medium flow velocities. Although the result was not significant, the lower and upper confidence limits seem to suggest that there were sufficient data to support the assertion regarding the influences of different velocity preferences (Table 5.4).

**Table 5.4.** Results of binomial logistic regression showing the likelihood of exposure to microplastics (e.g., using the presence or absence of microplastics in the gut) across the flush and sink hydraulic zones and among the different trait categories. The presence of microplastics in guts was coded Yes = 1', and the absence of microplastics was coded 'No = 0'. Code: Body size (mm) SZ1 (≤ 5), SZ2 (> 5 – 10), SZ3 (> 10 – 20), SZ4 (> 20); Feeding habit SH (shredder), CG (collector-gatherer), CF (collector-filterer), PR (predator), SC (scraper); Velocity preference (m/s) V1 (< 0.1), V2 (0.1 – 0.3), V3 (> 0.3 – 0.6), V4 (> 0.6).

| Coefficients | Estimate | Standard Error | z value | Pr (> \|z\|) | Confint. Lower 2.5% | Confint. Upper 97.5% | Odd ratios |
|---|---|---|---|---|---|---|---|
| Intercept | -0.4055 | 0.2357 | -1.720 | 0.0854 | -0.8674 | 0.0565 | 0.6667 |
| Hydraulic zone (sink) | 0.1054 | 0.3776 | 0.279 | 0.7802 | -0.6348 | 0.8455 | 1.1111 |
| Intercept | 0.4538 | 0.9217 | 0.492 | 0.622 | -1.3526 | 2.2603 | 1.5743 |
| SZ1 | 0.0211 | 0.2316 | 0.091 | 0.928 | -0.4328 | 0.4749 | 1.0213 |
| SZ2 | -0.1238 | 0.1624 | -0.762 | 0.446 | -0.4420 | 0.1945 | 0.8836 |
| SZ3 | -0.0683 | 0.1535 | -0.445 | 0.656 | -0.3691 | 0.2325 | 0.9340 |
| SZ4 | -0.1399 | 0.1270 | -1.102 | 0.271 | -0.3888 | 0.1090 | 0.8694 |
| Intercept | 0.9293 | 1.0534 | 0.882 | 0.3777 | -1.1353 | 2.9939 | 2.5328 |
| SH | 0.5653 | 0.5443 | 1.039 | 0.2990 | -0.5015 | 1.6321 | 1.7600 |
| CG | 0.1348 | 0.1714 | 0.787 | 0.4314 | -0.2011 | 0.4707 | 1.1444 |
| CF | -0.3238 | 0.2010 | -1.611 | 0.1072 | -0.7177 | 0.0701 | 0.7234 |
| PR | -0.2039 | 0.1824 | -1.118 | 0.2637 | -0.5614 | 0.1536 | 0.8156 |
| SC | -0.6461 | 0.3781 | -1.709 | 0.0875 | -1.3872 | 0.0950 | 0.5241 |
| Intercept | -0.0149 | 0.8508 | -0.018 | 0.986 | -1.6825 | 1.6527 | 0.9852 |
| V1 | -0.1933 | 0.1767 | -1.094 | 0.274 | -0.5396 | 0.1530 | 0.8242 |
| V2 | 0.2860 | 0.2500 | 1.144 | 0.253 | -0.2041 | 0.7760 | 1.3310 |
| V3 | -0.3676 | 0.2757 | -1.333 | 0.182 | -0.9079 | 0.1727 | 0.6924 |
| V4 | 0.2293 | 0.2163 | 1.060 | 0.289 | -0.1947 | 0.6532 | 1.2577 |

### 5.4.2 Relationship between macroinvertebrate trait and environmental variables

The RLQ analysis indicated distinct clustering of the hydraulic zones, with the sink hydraulic zones mostly clustered together (Figure 5.3). The flush hydraulic zones were clustered together depending on the hydraulic conditions. For instance, Site B1 (located in the lower reaches of the Buffalo River and upstream of Bridle Drift Dam) and site S1 in the Swartkops River tributary, the Chatty River, were more closely clustered together during spring as they both had

Froude number values in the supercritical flow regime. Slow runs classified as flushes during one or more sampling event because of low base flow at the time of sampling, were generally clustered together. The flush hydraulic zones were associated with high values of hydraulic indices such as the Froude numbers, Reynolds numbers, shear velocity, roughness Reynolds number, velocity depth ratio as well as high mean velocities and decreasing water depth (Figure 5.4). Conversely, the sink hydraulic zones were associated with high water depth, decreased values of the Froude numbers, Reynolds numbers, shear velocity, roughness Reynolds number, velocity depth ratio as well as low mean velocities.

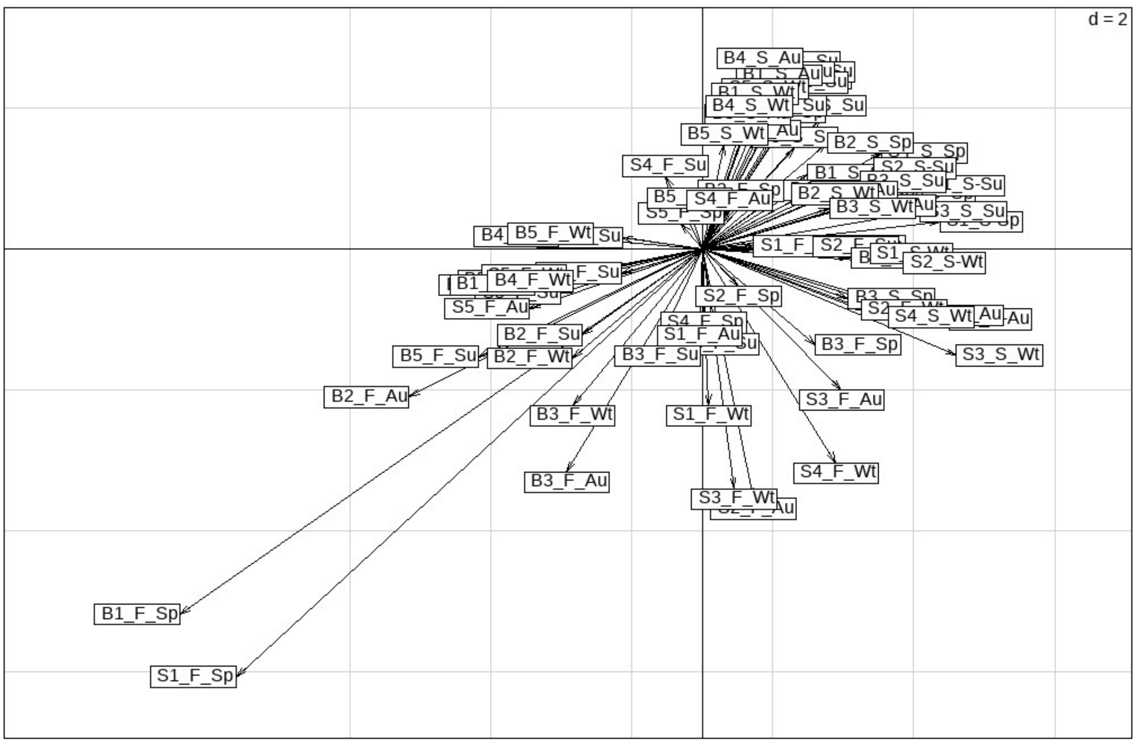


**Figure 5.3.** RLQ analysis showing the site/hydraulic zone grouping during the sampling seasons based on the analysed environmental variables. Site/hydraulic zone/season. B1 = Buffalo River site 1, B2 = Buffalo River site 2, B3 = Buffalo River site 3, B4 = Buffalo River site 4, B5 = Buffalo River site 5; S1 = Swartkops tributary (Chatty River) site 1, S2 = Swartkops River site 2, S3 = Swartkops River site 3, S4 = Swartkops River site 4, S5 = Swartkops River site 5; Hydraulic zone: F = flush hydraulic zone, S = sink hydraulic zone; Seasons: Au (autumn), Su (summer), Sp (spring) and Wi (winter).

The RLQ analysis showed that collector-filterers and shredders were closely associated with flush hydraulic zone, while predators were associated with the sink zone. Taxa with preferences for medium and high flow velocities were associated with the flush hydraulic zone while preferences for very low velocity were associated with the sinks. In terms of gill shape, taxa with operculated gills were associated with the sink hydraulic zone, while filamentous gills were associated with the flush hydraulic zones (Figure 5.4). The association of operculate gill shape with sinks is further strengthened by the results of the binomial regression model which indicated a higher likelihood of the operculate gill shape and the sink zones to influence exposure to microplastics. The RLQ results seems to suggest that traits associated with flushes may have reduced risks of exposure to microplastics, while traits associated with sinks may be more predisposed to microplastics. The risk factor may be further heightened in the presence of other pollutants, as indicated by the association of increasing levels of TIN with microplastic ingestion.

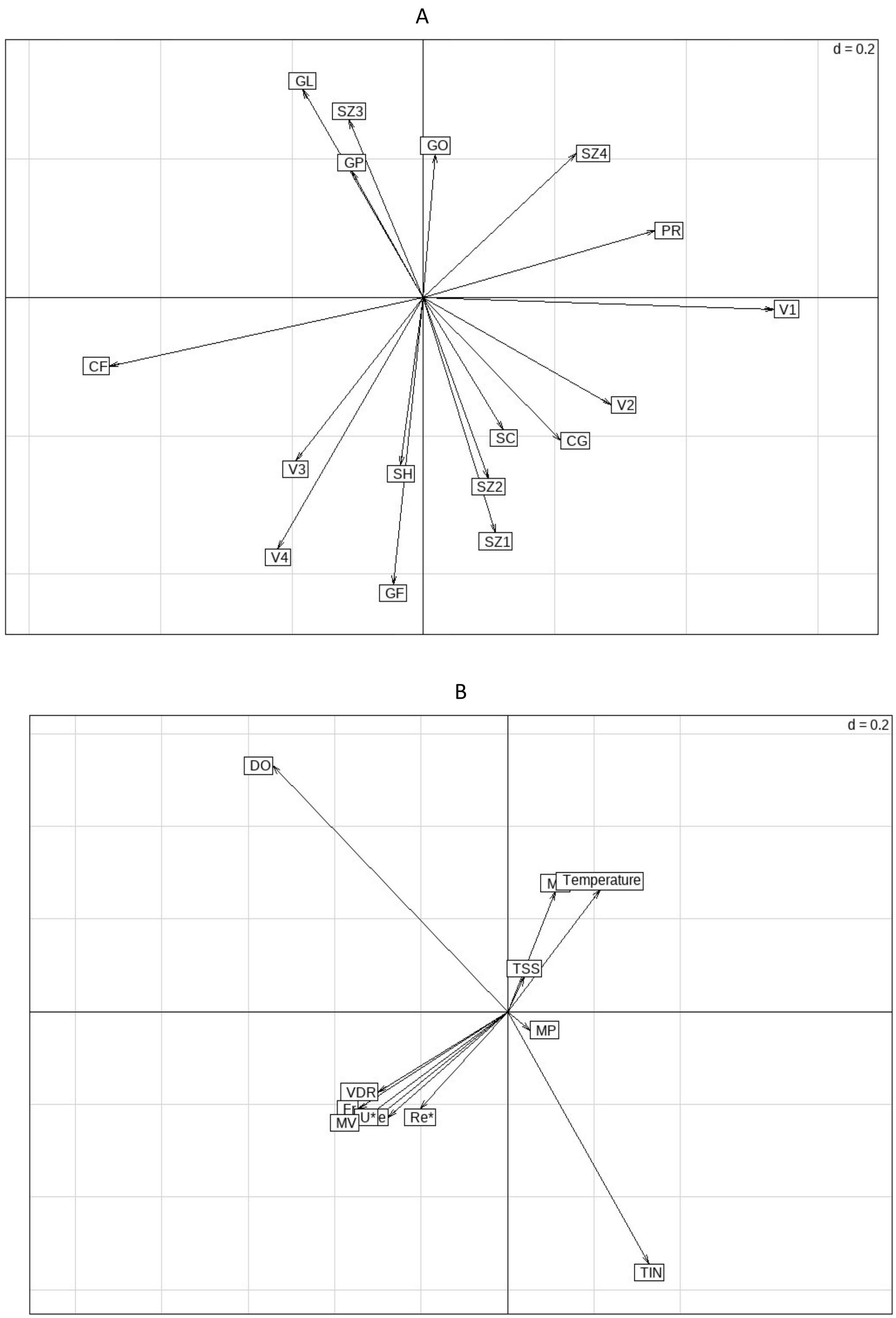


**Figure 5.4.** RLQ analysis showing the distribution of macroinvertebrate traits (A) and environmental variables (B) in relation to the hydraulic zones.

The first axis of the RLQ analysis with an Eigen value of 2.045, explained a variance of 65.96%, while the second axis, with an Eigen value of 0.945, explained a variance of 30.48% (Table 5.5). The third axis, with an Eigen value of 0.064 explained a variance of 2.07%. Overall, the first three axes of the RLQ analysis explained a cumulative variance of 98.50% of the variation between the traits, environment, and taxa dataset, indicating a very good ordination result.

**Table 5.5.** Properties of the RLQ analysis for the traits, environment and taxa datasets collected in the Swartkops and Buffalo rivers during the study period (October 2021–June 2022)

| | **Axis** | | |
|---|---|---|---|
| **Properties** | **1** | **2** | **3** |
| % Variance explained (RLQ) | 65.96 | 30.48 | 2.07 |
| % Cumulative variance explained | 65.96 | 96.43 | 98.50 |
| Eigenvalue | 2.045 | 0.945 | 0.064 |

Of the 17 trait attributes and ecological preferences examined in this study, only one was significantly positively correlated with one or more environmental variables, and one was significantly negatively correlated with one environmental variable (Table 5.6). The trait attribute, filtering collection as a feeding habit, showed a significantly positive association with increasing Froude numbers and Reynolds numbers, high shear velocities, increasing velocity depth ratios, and high mean velocities. The results of the fourth-corner analysis further indicated that one trait attribute, a preference for very low flow velocity, was significantly negatively associated with high velocity depth ratio while filtering collection was significantly positively assoicated with Froude number, Reynolds number, velocity depth ratio, and mean velocity. The fourth-corner statistics showed that filtering collection as a feeding habit and mean flow velocity had the strongest positive correlation ($r = 0.27$; $p = 0.027$), followed by the correlation between filtering collection and the Froude number (Table 5.5). It thus suggests that filtering collection as a feeding habit is associated with the flush zone or fast-flowing water. The RLQ analysis biplot showing significant associations revealed by the fourth-corner approach is also presented in the appendix (Appendix I, Figure I 1). Based on the total inertia of the RLQ analysis, an evaluation of the global significance of the traits-environment relationships showed highly significant results for model two, that is, permutations of sites/hydraulic zones, rows ($p = 0.0002$). The link between the RLQ axes and trait (Q-axes) or environmental variables (R-axes) was also directly tested by the fourth-corner approach at alpha = 0.05 and $p$-values adjusted by the FDR method. The results are presented in the appendix (Appendix I, Figure I 2).

**Table 5.6.** Fourth-corner statistics after 4999 permutations showing correlation coefficient and level of probability of statistical significance for correlation between traits and environmental variables *(p = 0.05). $P$-values were adjusted for multiple comparison using the false discovery rate (FDR) method. Only significant correlations are shown.

| | Environmental variable | | | | |
|---|---|---|---|---|---|
| **Trait** | **Fr** | **Re** | **U*** | **VDR** | **MV** |
| CF | 0.027* | 0.048* | 0.027* | 0.041* | 0.027* |
| V1 | | | | 0.048* | |

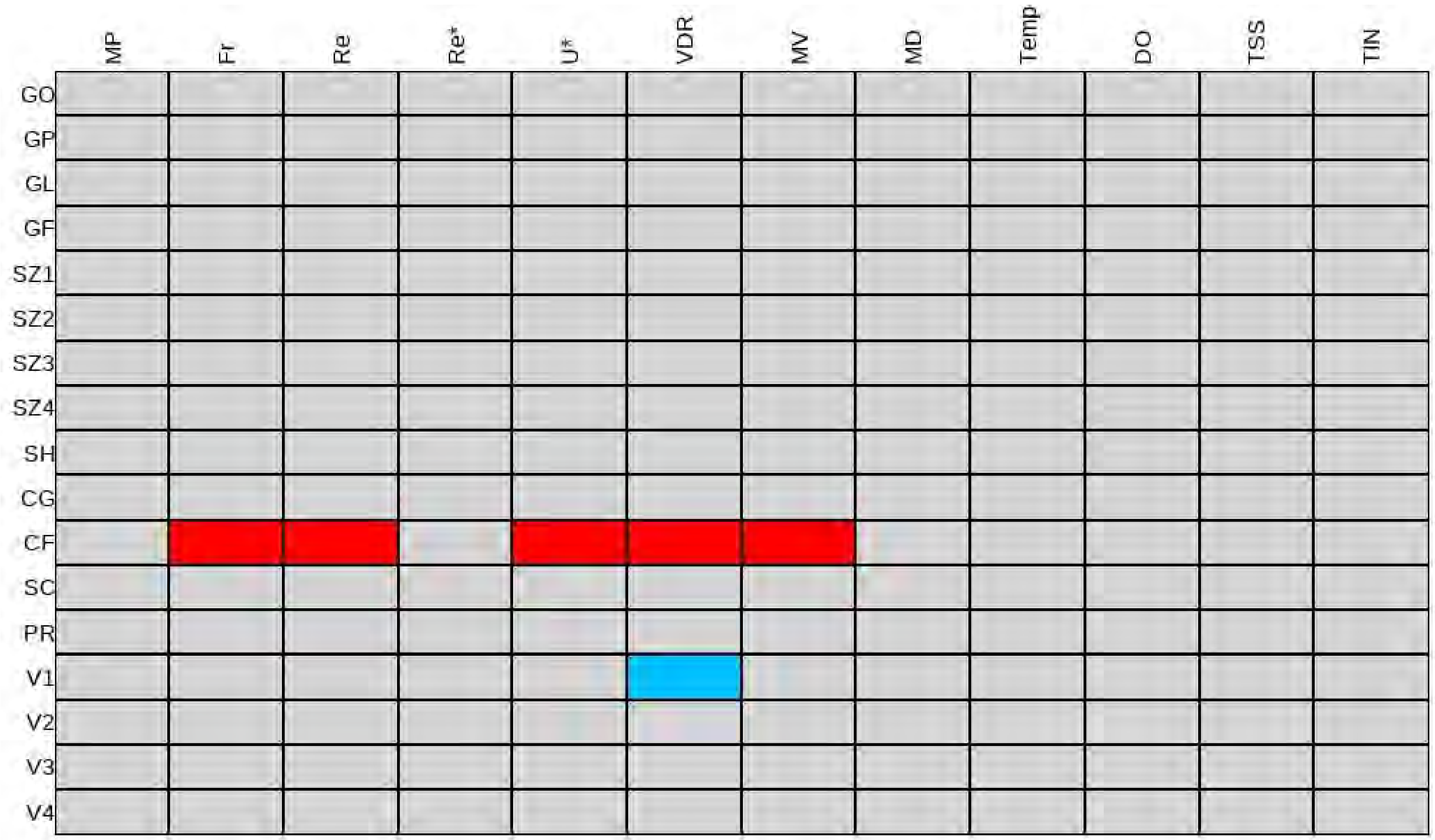


**Figure 5.5.** Results of the fourth corner analysis showing the correlation between macroinvertebrate traits and analysed environmental variables in the Swartkops and Buffalo rivers. Red indicates a significant positive correlation, and the blue indicates a significant negative correlation ($P \leq 0.05$). P-values were adjusted for multiple comparison using the false discovery rate (FDR) method.

## 5.5. Discussion

Given the global environmental concerns regarding microplastic pollution and the importance of rivers to humans and other aquatic organisms, the identification of traits useful for quantifying macroinvertebrate exposure to microplastics can be vital for biomonitoring studies. In the present study, the sink hydraulic zones (i.e., slow flow patches e.g., pools) were indicated with a higher likelihood to favour the exposure to and ingestion of microplastics by organisms compared to the flush hydraulic zone. This result is corroborated by the observation of Dahms *et al*. (2020) who observed that areas of increased depth and decreased flow allowed microplastics to settle onto the sediment where benthic macroinvertebrates and gathering collectors could ingest the microplastic fragments. Tibbetts *et al.* (2018) also reported that the greatest concentrations of microplastics were found in low velocity environments. Based on the RLQ results, predators were found to be predominantly associated with the sink hydraulic zones. However, there was a low likelihood of microplastic localisation in their guts, indicating that their association with sink hydraulic zones does not necessarily significantly correlate with the ingestion of microplastics by these taxa. While predators may mistake microplastics for food due to their colours and sizes resembling prey items (Lorenz *et al*., 2019), the uptake of these particles by organisms is influenced by their availability and concentration within the ecological niche. Additionally, many predators possess specialized structures for oxygen uptake apart from gills, while certain species like Hemipterans have piercing and sucking mouthparts (Picker *et al*., 2002). These adaptations are likely to decrease their likelihood of interacting with microplastics in the river environments.

Collector gatherers showed a higher likelihood of microplastic localisation in guts. This may be due to the fact that collector gatherers are likely to gather microplastics alongside fine particulate organic matter (FPOM) in sediments and other deposition areas with slower flow velocities. Shredders, similarly indicated with a high likelihood of ingestion, can pick up microplastics from aquatic plant surfaces or macrophytes they consume. Macrophytes have been suggested as potential vectors for microplastic transfer (Sfriso *et al*., 2021; Mateos-Cárdenas *et al*., 2022), while high concentrations of settled microplastics in riverine systems is a potential risk factor for deposit feeders and species typical of coarse sediment and subsurface environments, and hence habitats within which microplastics are likely to aggregate and be retained (Windsor *et al*., 2019). Scherer *et al*. (2017) observed that microplastic ingestion rates increase steadily with particle concentration until species reach their maximum feeding capacity. Taxa exposed to high microplastic concentrations may have adaptive features that confer resilience and mitigate potential damage. For instance, filtering collectors associated with the flush zone showed a lower likelihood of microplastic localisation in their guts, likely due to their adaptation to cope with high loads of suspended matter. Heavy presence of natural particles, as noted by Scherer *et al.* (2017), generally reduces microplastic uptake. In environments where microplastic concentrations correlate with high levels of organic and inorganic pollution, taxa sensitive to pollutants may be excluded, limiting their interaction with microplastics. Kataoka *et al*. (2019b) reported more advanced microplastic pollution in heavily polluted rivers with poor water quality compared to less polluted ones. Taxa tolerant of poor water quality are therefore more likely to interact with microplastics in such environments. However, in areas where microplastic concentrations do not correspond with poor water quality, taxa sensitive to microplastics are expected to show higher levels of microplastic localization in their guts or gills compared to taxa with traits indicating resilience to microplastic pollution.

Large body size was associated with the sink hydraulic zone and showed a non-significant positive correlation with microplastic concentrations, yet it exhibited a lower likelihood of microplastic localization in guts. This suggests that larger body size may limit interaction or exposure to microplastics, indicating a resilient trait against microplastic pollution. Larger organisms typically have a reduced surface area to volume ratio (Odume, 2020), which minimizes external contact with microplastics. Conversely, very small body size was associated with a higher likelihood of microplastic localization in guts. Small organisms generally have a larger surface area to volume ratio (Lu *et al*., 2022), potentially increasing their surface available for interacting with microplastics. Although very small body size was negatively correlated with microplastic concentrations (albeit non-significantly), it suggests that possessing a very small body size may be indicative of sensitivity to microplastics.

Furthermore, small-bodied taxa face morphological constraints in their guts, limiting their ability to ingest particles larger than those in the micron range. According to Ma *et al.* (2020), the size of microplastics is critical for their bioavailability. For example, Fernández (2001) found that under similar exposure conditions, *Artemia franciscana* ingested fewer microplastic particles compared to daphnids, due to its preference for smaller particles (< 50 µm). Kokalj *et al.* (2018) reported an exponential relationship between microplastic size and uptake rate in *Daphnia magna*, observing that fewer daphnids contained microplastics as particle size increased. Smaller microplastics have higher bioavailability because they are more readily ingested and translocated within organisms (Ma *et al*., 2020). Additionally, smaller microplastics may pose greater toxicity as they are retained longer, potentially causing adverse effects due to low egestion efficiency (Ma *et al.*, 2020). In the current study, ingested microplastics were predominantly found in the lower microplastic size range, consistent with their higher distribution in water samples as reported in Chapter three. This highlights significant ecological implications for taxa with very small body sizes, which show a higher likelihood of microplastic localisation in their guts.

The results concerning gill types indicate a higher likelihood of microplastic localisation in operculate gills compared to other gill types. The operculate gill type, which features a protective covering for the gills, may trap microplastics as water circulates through them. Both operculate, plate-like, and lamellate gill types showed negative correlations with microplastic concentrations, whereas the filamentous gill type exhibited a positive correlation. Although these correlations were generally not significant across all gill types, the pattern suggests that operculate gills may be sensitive to microplastic pollution, while filamentous gills could be more resilient. Filamentous gills are primarily external and lack an outer protective layer, potentially facilitating exposure to microplastics. However, they are less likely to retain microplastics within their gills compared to gill types with internal structures like operculate gills. These findings underscore the importance of gill morphology in influencing interactions with microplastics in aquatic organisms, highlighting potential differences in sensitivity and resilience among different gill types.

## 5.6 Conclusion

The findings of this study indicate that macroinvertebrates are more susceptible to microplastic exposure in sink hydraulic zones, characterised by slow-flowing to stagnant water patches. Taxa with large, medium, and small body sizes, as well as those with filtering collection, scraping, and predation feeding habits, showed a low likelihood of microplastic localisation in their guts. Similarly, taxa with filamentous, plate-like, and lamellate gill types exhibited a low likelihood of microplastic localisation in their gills. Conversely, taxa characterised by very small body size, shredding and collector-gathering feeding habits, demonstrated a higher likelihood of microplastic localisation in their guts. Those with operculate gill types also showed a higher likelihood of microplastic localisation in their gills. These results suggest that taxa with operculate gills, very small body sizes, shredding and collector-gathering feeding habits may be more sensitive to microplastic pollution. In contrast, taxa with filamentous gills, large body sizes, and filtering collection feeding habits are suggested to be resilient to microplastic pollution. Small body size enhances the surface area available for interaction with microplastics, potentially increasing susceptibility. Operculate gills, with their external covering, can trap microplastic particles within the gill structures.

Identifying sensitive and tolerant traits, along with ecological preferences related to microplastic pollution, is crucial for developing trait-based indices for biomonitoring microplastic pollution in riverine systems. This approach could provide valuable insights into the ecological impacts of microplastics and aid in effective environmental management strategies.

# CHAPTER 6: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 6.1 Introduction

Microplastic pollution of riverine systems is a threat to aquatic life and resources. A complete assessment of riverine microplastic pollution requires an understanding of transport dynamics and the fate of microplastics in rivers. Approaches that shed light on the transport dynamics and fate of microplastics in rivers are useful to determine species at risk of exposure to microplastics. The distribution of microplastics in rivers may vary spatially and temporally along different flow patches as a function of river hydro-geomorphology. The spatial and temporal variations may potentially create hotspots for microplastics and mediate the exposure of macroinvertebrates to microplastics. Macroinvertebrates are adapted to different gradients of flow patches or hydraulic biotopes through their traits which may expose them to different levels of exposure to microplastics. Understanding the patchy distribution and transport dynamics of microplastics and how macroinvertebrates are distributed and predisposed to microplastic pollution in riverine systems would provide a solid framework for predicting exposure to microplastics and determining the ecological effects.

Physicochemical variables such as nutrients and temperatures may also create an enabling environment for microbial colonisation of microplastics in rivers. Microbially colonised microplastics may serve as vectors of pathogenic microbes in rivers. It is thus important to establish the relationship between microplastics and water physicochemical properties. Land-use types in proximity to river systems draining urban and industrial areas may influence the type and concentrations of microplastics in rivers. Relating land-use types to riverine microplastics is useful for determining land-use types that are correlated with microplastic pollution. Understanding the relationship between land-use types and riverine microplastics is useful for designing mitigation measures aimed at preventing the further pollution of rivers.

Microplastic research has advanced in recent years. However, differences in both the sampling protocols and the scale of sampling employed in monitoring and evaluation studies globally have complicated the interpretation of results and comparisons of studies to understand the level of pollution in different rivers relative to others. Therefore, unless standardised, robust and efficient monitoring and evaluation approaches that are both diagnostic, predictive, and sufficiently sensitive to various impacts in urban landscapes are developed, the escalating microplastic pollution problems and the increasingly negative impacts on freshwater ecosystems function, biological diversity will be difficult to curtail. In this study, a multi-dimensional approach that integrated geomorphology and hydraulics was used in combination with the TBA to assess and evaluate microplastic pollution and potential macroinvertebrate exposure to microplastics in two river systems which drain urbanised catchments in the Eastern Cape of South Africa. Although, freshwater microplastic pollution monitoring has gained serious attention globally, this is the first study that combines different monitoring approaches for microplastics. It is therefore important to evaluate the benefits of undertaking this study and its contributions to the study of microplastics ecology. The purpose of this chapter, therefore, is to present a concise discussion of each of the approaches applied and evaluate their benefits to the global microplastic research space.

### 6.2 Microplastic abundance distribution, land use and physicochemical variables

Settled and suspended microplastics showed significant spatial variations in distribution across the study sites. Sites most heavily impacted were those affected by effluent discharge from WWTW, with the highest mean occurrences of suspended and settled microplastics found at sites S2 and S3 respectively. Both sites were in the lower reaches of the Swartkops River and were impacted by effluents from WWTW. Sites in the Swartkops were statistically different in the composition of physicochemical variables such as TSS, TIN, turbidity, EC, and orthophosphate phosphorus. Physicochemical variables showed differing relations with microplastic concentrations across the wet and dry seasons with the relation between physicochemical variables and microplastics more profound during the dry season. In the dry season, settled microplastics were associated with high levels of turbidity, TSS, TIN, orthophosphate phosphorus and increasing temperature, while suspended microplastics were closely associated with high EC, TIN, and orthophosphate phosphorus. The RDA model explained an unbiased variation of 88% between the physicochemical variables and microplastics, indicating a good ordination. Urban land use showed significant variations between suspended microplastics distribution and other land-use types such as rural, agricultural, industrial, and natural land. Fibres and fragments were the most abundant plastic shape while polyethylene and polypropylene were the most abundant plastic polymers. The smallest size class ($0.063 < 0.5$) of microplastics was the most abundant in surface water and in the sediments.

High concentrations of suspended microplastics in riverine systems are a potential risk factor for filter-feeding macroinvertebrates that usually collect fine particulate organic matter from the flowing water. The association of microplastics with high nutrients and temperature provides an enabling condition for the development of biofilms on microplastic surfaces, allowing bacteria to thrive. Biofilms can alter microplastic density and change their transport behaviour in the river water. Increased microplastic density can promote settling to the riverbed which removes such particles from the reach of the intensity of light required for photo-degradation of the plastic material. Colonisation by bacterial biofilm can promote the spread of bacterial colonies, including pathogenic colonies. In this way, microplastics serve as vectors of pathogenic microbial communities in rivers. The correlation of relationship between anthropogenic land cover and microplastics indicates that anthropogenic activities adjacent to rivers could be related to the type of microplastics that may be available a river. Land-based management approaches can be evaluated for their effectiveness based on the results of routine monitoring approaches that integrate land use and physicochemical variables in the study of microplastics. However, this site-level monitoring approach may not reveal microplastic hotspots, which are mostly located in patches of flow with limited flow velocities, riverbanks, backwaters, portions separated by fallen logs or weirs and other portions of the river that are not central to the river which may be missed in a longitudinal sampling approach. The implication is that microplastics may be underestimated and underreported for river systems with potentially high levels of microplastic concentrations. Quantitative assessment of microplastic mitigation measures that rely on evaluation reports may then use underestimated concentration results. It is also difficult to refer to the ecological effects of microplastic pollution using this evaluation method while results are difficult to compare for different rivers in the same and different regions, given the diversity in scale and methods used.

### 6.3 The hydraulic biotopes approach

Chapter 4 of this thesis provided the hydraulic biotope approach as a way of monitoring and evaluating microplastics in river systems. The approach is built on river geomorphology and hydrodynamics and, because it is targeted at different flow patches of the river, it is useful for unravelling potential microplastics hotspots. The different flow patches can also be characterised by hydraulic indices such as the Froude number, Reynolds number, and the velocity depth ratio which gives an indication of the hydrodynamic conditions at the time of sampling. Such hydraulic indices could be useful for inter-laboratory calibrations and study comparisons.

The results of the hydraulic biotopes approach shed light on the influence of riverine hydrodynamics on the spatiotemporal variations in microplastic distribution at the reach scale.

Given that riverine macroinvertebrates are strongly associated with a gradient of flow velocity, this approach, in combination with trait ecology discussed in Chapter 5, is useful for assessing the ecological effects of microplastics and for determining potential exposure of macroinvertebrates to microplastics.

The hydraulic biotope approach also shed light on microplastics hotspots in rivers that may be situated far away from known point sources of pollution. The incorporation of hydraulic indices in this study is useful for predictive and modelling studies of microplastic pollution. For instance, results obtained from the modelled data in this study revealed that the Froude number index showed a significantly decreasing trend in settled microplastic distribution with increasing *Fr* number values. The modelled data also predicted a significantly decreasing trend in suspended microplastics distribution at low *Fr* values, and an increasing trend from a higher *Fr* value of about 0.75. The results reinforced the assumption that settled microplastics are increasingly remobilised into suspension at higher *Fr* values which is a vital characteristic of fast-flowing hydraulic biotopes such as fast runs, riffles, and rapids. The determination of distinct patches of flow within river systems that can potentially have more concentrations of suspended and settled microplastics can shed light on macroinvertebrates species that are potentially at risk of exposure to microplastics.

### 6.4 The mechanistic trait-based approach

The macroinvertebrate traits-based approach (TBA) was undertaken at the family level taxonomic resolution. Flow patches with elevated concentrations of nutrients, TSS, high temperatures with increased water depth are major areas that local biota in the Buffalo and Swartkops rivers are at risk of exposure to microplastics. The TBA offered a basis for interpreting why certain macroinvertebrate families were absent from some hydraulic zones. The family-level TBA showed that macroinvertebrates that use lamellate and plate-like gills were more abundant in hydraulic zones with high levels of dissolved oxygen, decreasing markedly in hydraulic zones with low levels of dissolved oxygen. Taxa with operculated gills were more likely to clog in the sink hydraulic zones. The sink hydraulic zones were more likely to mediate ingestion. Notably, the relative abundances of taxa with operculated gills increased in sinks and were markedly reduced in the flush zone, while taxa bearing filamentous gills with a lesser likelihood of clogging increased in flushes where DO levels were also high. Microplastic ingestion increased, though moderately, in the sink zone which also had a higher concentration of nutrients and low levels of DO. Taxa with small body size and collector-gatherers indicating a higher likelihood of ingestion had higher abundances in the sinks, while filtering collectors were associated with the flush zones. Association of hydraulic biotopes with other pollutants was an increasing risk factor predisposing taxa to microplastics. Hence, there were increases in the relative abundance of taxa with large body size and operculate gills in sinks were DO is lower than in flushes. The TBA proved to be a useful approach that can be used in microplastic research to predict species at risk of exposure to microplastic pollution.

### 6.5 Conclusion

This study has contributed to the field of microplastic research in South Africa and globally by demonstrating the importance of an integrated approach for monitoring microplastic pollution at a scale that is ecologically relevant to uncover microplastic hotspots. It highlighted the limitations of the current monitoring and evaluation systems (Chapters 1 and 4), and then provided evidence of the comparative advantages of adapting the hydraulic biotope scale approach (Chapters 4 and 5) in freshwater monitoring of microplastic pollution. The trait-based approach used in this study shed light on the importance of integrating ecologically based methods for quantifying and predicting exposure to microplastics and linking field-based observations to theoretical concepts to guide management strategies. The results indicate that patches of rivers with low flow and high depth are potential sinks of microplastics, and macroinvertebrates associated with such patches are potentially more exposed to microplastics.

### 6.6 Research limitations

The following are important limitations of this research:

- Polymer confirmation of the lower size microplastics (i.e., particles less than 0.5 mm) was limited by the ATR-FTIR used for this study. In some instances, even fibres of higher dimensions, because of their shape, were difficult to transfer into the FTIR set-up. This technological limitation could mean that some microplastics were overlooked which could have an impact on the abundances reported. Microplastic monitoring studies could benefit from availability of appropriate and affordable technology for detecting and confirming the smaller size classes of microplastics, especially in the African continent.
- Despite recent efforts and the compilation of a macroinvertebrate trait database for South Africa, there are gaps in the available trait information. The trait-based approach used in this study could benefit from additional trait ecological preference information (e.g., life cycle and aquatic strategies) which were not used in this study due to scanty, insufficient or unavailability of trait information.

- Buckets were used to collect water samples for microplastic investigation. This methodology comes with a potential limitation. Depending on the flow velocity, the number of microplastics may be underestimated as some particles may not be collected due to the back pressure in the bucket when lifting out of the water.
- The sampling approach employed in this study to collect settled microplastics does not lend itself towards quantifying microplastics in majority of the common units that have been used for reporting settled stocks of microplastics in most literatures. This discrepancy could potentially limit the ability to directly compare findings with existing research on microplastics.

**6.7 Recommendations for further study and management**

Based on the investigation undertaken in this study, the following are recommended for further study and in managing microplastic pollution of rivers:

- The variables required for the calculation of the hydraulic index, roughness Reynolds number, used alongside the Froude and Reynolds number to characterise the different flow patches were time consuming to obtain. It is recommended that the Froude number, Reynolds number and the velocity depth ratio are sufficient to characterise the mean flow conditions and to discriminate between different flow patches.
- Given the magnitude of the microplastic pollution challenge, continuous monitoring and assessment studies are required for rivers draining urban catchments to assess the efficiency of land-based mitigation measures.
- One of the potential drawbacks of the TBA is the limited autecological information on Afro-tropical macroinvertebrates. It is recommended that applied research be directed towards the continuous enhancement of the already published database of macroinvertebrates in South Africa. It is also important to create an integrative traits database for the African continent as in Europe, Australia, and North America.
- The relationship between microplastics and the roughness Reynolds number showed an increasing trend with settled microplastics at high roughness values. However, limited data points made it difficult to draw a justifiable conclusion regarding the observed relationship. Extensive data points gathered over longer periods may provide a clearer picture of such a relationship.

## REFERENCES

Abbasi, S. (2021). Microplastics washout from the atmosphere during a monsoon rain event. *Journal of Hazardous Materials Advances*, *4*(September), 100035. https://doi.org/10.1016/j.hazadv.2021.100035

Abdala-Roberts, L., Galmán, A., Petry, W. K., Covelo, F., de la Fuente, M., Glauser, G., & Moreira, X. (2018). Interspecific variation in leaf functional and defensive traits in oak species and its underlying climatic drivers. *PLoS ONE*, *13*(8), 1–13. https://doi.org/10.1371/journal.pone.0202548

Akamagwuna, F. C., Mensah, P. K., Nnadozie, C. F., & Odume, O. N. (2019). Trait-based responses of Ephemeroptera, Plecoptera, and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *River Research and Applications*, *35*(7), 999–1012. https://doi.org/10.1002/rra.3458

Akindele, E. O., Ehlers, S. M., & Koop, J. H. E. (2019). First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. *Limnologica*, *78*. https://doi.org/10.1016/j.limno.2019.125708

Andrady, A. L., Barnes, P. W., Bornman, J. F., Gouin, T., Madronich, S., White, C. C., Zepp, R. G., & Jansen, M. A. K. (2022). Oxidation and fragmentation of plastics in a changing environment; from UV-radiation to biological degradation. *Science of the Total Environment*, *851*(May), 158022. https://doi.org/10.1016/j.scitotenv.2022.158022

Apetogbor, K., Pereao, O., Sparks, C., & Opeolu, B. (2022). Spatio-Temporal Distribution of Microplastics in Water and Sediment Samples of the Plankenburg River, Western Cape, South Africa. *SSRN Electronic Journal*, *323*(February), 121303. https://doi.org/10.2139/ssrn.4226452

APHA - (American Public Health Association). (2005). *Standard methods for the examination of water and waste water. Method 2540D, APHA, 21st Edition* (Vol. 2). https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/scientific/technical-documents/white-papers/apha-total-suspended-solids-procedure-white-paper.pdf

Aragaw, T. A. (2021). Microplastic pollution in African countries' water systems: a review on findings, applied methods, characteristics, impacts, and managements. *SN Applied*

*Sciences*, *3*(6). https://doi.org/10.1007/s42452-021-04619-z

Asefnejad, A., Khorasani, M. T., Behnamghader, A., Farsadzadeh, B., & Bonakdar, S. (2011). Manufacturing of biodegradable polyurethane scaffolds based on polycaprolactone using a phase separation method: physical properties and in vitro assay. *International Journal of Nanomedicine*, *6*(November 2014), 2375–2384. https://doi.org/10.2147/ijn.s15586

Atugoda, T., Vithanage, M., Wijesekara, H., Bolan, N., Sarmah, A. K., Bank, M. S., You, S., & Ok, Y. S. (2021). Interactions between microplastics, pharmaceuticals and personal care products: Implications for vector transport. *Environment International*, *149*, 106367. https://doi.org/10.1016/j.envint.2020.106367

Avio, C. G., Gorbi, S., & Regoli, F. (2015). Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Marine Environmental Research*, *111*, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014

Baiyegunhi, C., Liu, K., & Gwavava, O. (2017). Sedimentation rate and subsidence history of the southeastern Karoo Basin, South Africa, using 1D backstripping method. *Arabian Journal of Geosciences*, *10*(10). https://doi.org/10.1007/s12517-017-3009-x

Ballent, A., Purser, A., Mendes, P. D. J., Pando, S., & Thomsen, L. (2012). Physical transport properties of marine microplastic pollution. *Biogeosciences Discussions*, *9*(12), 18755–18798. https://doi.org/10.5194/bgd-9-18755-2012

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P., & Nessimian, J. L. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. *Hydrobiologia*, *575*(1), 83–94. https://doi.org/10.1007/s10750-006-0286-x

Bate, G., Smailes, P., & Adams, J. (2004). A water quality index for use with diatoms in the assessment of rivers. *Water SA*, *30*(4), 493–498. https://doi.org/10.4314/wsa.v30i4.5101

Bayo, J., Olmos, S., & López-Castellanos, J. (2020). Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. *Chemosphere*, *238*. https://doi.org/10.1016/j.chemosphere.2019.124593

Berlino, M., Mangano, M. C., De Vittor, C., & Sarà, G. (2021). Effects of microplastics on the functional traits of aquatic benthic organisms: A global-scale meta-analysis. In

*Environmental Pollution* (Vol. 285). Elsevier Ltd. https://doi.org/10.1016/j.envpol.2021.117174

Biswas, B., & Chandra Das, B. (2016). Hydraulic Parameters and Morphometric Variables Interactions in Bedrock Channel. *Quaestiones Geographicae*, *35*(3), 75–88. https://doi.org/10.1515/quageo-2016-0028

Blarer, P., & Burkhardt-Holm, P. (2016). Microplastics affect assimilation efficiency in the freshwater amphipod Gammarus fossarum. *Environmental Science and Pollution Research*, *23*(23), 23522–23532. https://doi.org/10.1007/s11356-016-7584-2

Blott, S. J., & Pye, K. (2001). Gradistat: A grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms*, *26*(11), 1237–1248. https://doi.org/10.1002/esp.261

Boos, J. P., Dichgans, F., Fleckenstein, J. H., Gilfedder, B. S., & Frei, S. (2024). Assessing the Behavior of Microplastics in Fluvial Systems: Infiltration and Retention Dynamics in Streambed Sediments. *Water Resources Research*, *60*(2), 1–16. https://doi.org/10.1029/2023WR035532

Boos, J. P., Gilfedder, B. S., & Frei, S. (2021). Tracking Microplastics Across the Streambed Interface: Using Laser-Induced-Fluorescence to Quantitatively Analyze Microplastic Transport in an Experimental Flume. *Water Resources Research*, *57*(12), 1–10. https://doi.org/10.1029/2021WR031064

Bravard, J. P., & Petit, F. (2009a). Geomorphology of Streams and Rivers. In *Encyclopedia of Inland Waters* (pp. 387–395). Elsevier Inc. https://doi.org/10.1016/B978-012370626-3.00043-0

Bravard, J. P., & Petit, F. (2009b). Geomorphology of Streams and Rivers. *Encyclopedia of Inland Waters*, *December*, 387–395. https://doi.org/10.1016/B978-012370626-3.00043-0

Buwono, N. R., Risjani, Y., & Soegianto, A. (2021). Distribution of microplastic in relation to water quality parameters in the Brantas River, East Java, Indonesia. *Environmental Technology and Innovation*, *24*, 101915. https://doi.org/10.1016/j.eti.2021.101915

Campanale, C., Savino, I., Pojar, I., Massarelli, C., & Uricchio, V. F. (2020). A practical overview of methodologies for sampling and analysis of microplastics in riverine environments. In *Sustainability (Switzerland)* (Vol. 12, Issue 17). MDPI AG.

https://doi.org/10.3390/SU12176755

Cao, J., Yang, Q., Jiang, J., Dalu, T., Kadushkin, A., Singh, J., Fakhrullin, R., Wang, F., Cai, X., & Li, R. (2022). Coronas of micro/nano plastics: a key determinant in their risk assessments. *Particle and Fibre Toxicology*, *19*(1), 1–25. https://doi.org/10.1186/s12989-022-00492-9

Carter, Alan; Hunter, Amy; Mack, Cherie-Lynn; Smith, Caitlin; Mgweba, T. (2016). *EOH Coastal and Environmental Services: Buffalo River Estuary situation assessment. Prepared for : Department of Environmental Affairs and the Nelson Mandela Metropolitan University. March 2016* (Issue March).

Cesarini, G., & Scalici, M. (2022). Riparian vegetation as a trap for plastic litter. *Environmental Pollution*, *292*(PB), 118410. https://doi.org/10.1016/j.envpol.2021.118410

Chen, H., Jia, Q., Zhao, X., Li, L., Nie, Y., Liu, H., & Ye, J. (2020). The occurrence of microplastics in water bodies in urban agglomerations: Impacts of drainage system overflow in wet weather, catchment land-uses, and environmental management practices. *Water Research*, *183*, 116073. https://doi.org/10.1016/j.watres.2020.116073

Chen, H., Qin, Y., Huang, H., & Xu, W. (2020). A regional difference analysis of microplastic pollution in global freshwater bodies based on a regression model. *Water (Switzerland)*, *12*(7). https://doi.org/10.3390/w12071889

Chen, X., Xiong, X., Jiang, X., Shi, H., & Wu, C. (2019). Sinking of floating plastic debris caused by biofilm development in a freshwater lake. *Chemosphere*, *222*, 856–864. https://doi.org/10.1016/j.chemosphere.2019.02.015

Chércoles Asensio, R., San Andrés Moya, M., De La Roja, J. M., & Gómez, M. (2009). Analytical characterization of polymers used in conservation and restoration by ATR-FTIR spectroscopy. *Analytical and Bioanalytical Chemistry*, *395*(7), 2081–2096. https://doi.org/10.1007/s00216-009-3201-2

Cheung, P. K., Hung, P. L., & Fok, L. (2019). River Microplastic Contamination and Dynamics upon a Rainfall Event in Hong Kong, China. *Environmental Processes*, *6*(1), 253–264. https://doi.org/10.1007/s40710-018-0345-0

Chevene, F., Dolédec, S., & Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwater Biology*, *31*(3), 295–309. https://doi.org/10.1111/j.1365-2427.1994.tb01742.x

Chigor, V. N., Sibanda, T., & Okoh, A. I. (2013). Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa. *Environmental Science and Pollution Research*, *20*(6), 4125–4136. https://doi.org/10.1007/s11356-012-1348-4

Christensen, N. D., Wisinger, C. E., Maynard, L. A., Chauhan, N., Schubert, J. T., Czuba, J. A., & Barone, J. R. (2020). Transport and characterization of microplastics in inland waterways. *Journal of Water Process Engineering*, *38*(September), 101640. https://doi.org/10.1016/j.jwpe.2020.101640

Collins, A. L., Anthony, S., Turner, T., & Hawley, J. (2007). Predicting the impact of projected change in agriculture by 2015 on annual mean fluvial suspended sediment concentrations across England and Wales. *IAHS-AISH Publication*, *314*, 28–37.

Cowger, W., Steinmetz, Z., Gray, A., Munno, K., Lynch, J., Hapich, H., Primpke, S., De Frond, H., Rochman, C., & Herodotou, O. (2021). Microplastic Spectral Classification Needs an Open Source Community: Open Specy to the Rescue! *Analytical Chemistry*, *93*(21), 7543–7548. https://doi.org/10.1021/acs.analchem.1c00123

Crew, A., Gregory-Eaves, I., & Ricciardi, A. (2020). Distribution, abundance, and diversity of microplastics in the upper St. Lawrence River. *Environmental Pollution*, *260*, 113994. https://doi.org/10.1016/j.envpol.2020.113994

Culp, J. M., Armanini, D. G., Dunbar, M. J., Orlofske, J. M., LeRoy Poff, N., Pollard, A. I., Yates, A. G., & Hose, G. C. (2011). Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integrated Environmental Assessment and Management*, *7*(2), 187–197. https://doi.org/10.1002/ieam.128

Cummins, K. W., & Klug, M. J. (1979). Feeding Ecology of Stream Invertebrates. *Annual Review of Ecology and Systematics*, *10*(1), 147–172. https://doi.org/10.1146/annurev.es.10.110179.001051

Cushing, C. E., Minshall, G. W., & Newbold, J. D. (1993). two Idaho streams. *Environmental Sciences*.

Cutroneo, L., Reboa, A., Besio, G., Borgogno, F., Canesi, L., Canuto, S., Dara, M., Enrile, F., Forioso, I., Greco, G., Lenoble, V., Malatesta, A., Mounier, S., Petrillo, M., Rovetta, R., Stocchino, A., Tesan, J., Vagge, G., & Capello, M. (2020). Correction to: Microplastics in seawater: sampling strategies, laboratory methodologies, and identification techniques applied to port environment (Environmental Science and Pollution Research, (2020), 27, 9, (8938-8952), 10.1007/s11356-020-07783-8). *Environmental Science and Pollution Research*, *27*(16), 20571. https://doi.org/10.1007/s11356-020-08704-5

Dahms, H. T. J., van Rensburg, G. J., & Greenfield, R. (2020a). The microplastic profile of an urban African stream. *Science of the Total Environment*, *731*. https://doi.org/10.1016/j.scitotenv.2020.138893

Dahms, H. T. J., van Rensburg, G. J., & Greenfield, R. (2020b). The microplastic profile of an urban African stream. *Science of the Total Environment*, *731*. https://doi.org/10.1016/j.scitotenv.2020.138893

Dawson, A. L., Bose, U., Ni, D., & Nelis, J. L. D. (2024). Unravelling protein corona formation on pristine and leached microplastics. *Microplastics and Nanoplastics*, *4*(1). https://doi.org/10.1186/s43591-024-00086-6

Dawson, S. K., Carmona, C. P., González-Suárez, M., Jönsson, M., Chichorro, F., Mallen-Cooper, M., Melero, Y., Moor, H., Simaika, J. P., & Duthie, A. B. (2021). The traits of "trait ecologists": An analysis of the use of trait and functional trait terminology. *Ecology and Evolution*, *11*(23), 16434–16445. https://doi.org/10.1002/ece3.8321

Day, JA., de Moor, IJ:, Stewart, BA:; Louw, A. (2001). Guides to the freshwater invertebrates of Southern Africa Volume 3: Crustacea II. Ostracoda, Copepoda and Branchiura. In *Water Research Commission* (Vol. 3).

de Carvalho, A. R., Garcia, F., Riem-Galliano, L., Tudesque, L., Albignac, M., ter Halle, A., & Cucherousset, J. (2021). Urbanization and hydrological conditions drive the spatial and temporal variability of microplastic pollution in the Garonne River. *Science of the Total Environment*, *769*. https://doi.org/10.1016/j.scitotenv.2020.144479

de los Santos, C. B., Krång, A. S., & Infantes, E. (2021). Microplastic retention by marine vegetated canopies: Simulations with seagrass meadows in a hydraulic flume. *Environmental Pollution*, *269*, 116050. https://doi.org/10.1016/j.envpol.2020.116050

de Moor, IJ., Day, JA., de Moor, F. (2003). Guides to the freshwater invertebrates of Southern

Africa. Volume 8: Insecta II. Hemiptera, Megaloptera, Neuroptera, Tricoptera and Lepidoptera. In *Water Research Commission, WRC Report No. TT214/03* (Issue Volume 8).

de Moor, IJ ., Day, JA., & de Moor, F. (2003). Guides to the freshwater invertebrates of Southern Africa. Volume 7: Insecta I. Ephemeroptera, Odonata and Plecoptera. In *Water Research Commission, WRC Report No. TT207/03* (Vol. 7).

de Smit, J. C., Anton, A., Martin, C., Rossbach, S., Bouma, T. J., & Duarte, C. M. (2021). Habitat-forming species trap microplastics into coastal sediment sinks. *Science of the Total Environment*, *772*, 145520. https://doi.org/10.1016/j.scitotenv.2021.145520

de Wet, C., & Odume, O. N. (2019). Developing a systemic-relational approach to environmental ethics in water resource management. *Environmental Science and Policy*, *93*(February 2018), 139–145. https://doi.org/10.1016/j.envsci.2018.12.030

DEA. (2018). *Department of Environmental Affairs: Draft Buffalo River estuarine management plan*.

Defontaine, S., Sous, D., Tesan, J., Monperrus, M., Lenoble, V., & Lanceleur, L. (2020). Microplastics in a salt-wedge estuary: Vertical structure and tidal dynamics. *Marine Pollution Bulletin*, *160*(September). https://doi.org/10.1016/j.marpolbul.2020.111688

Dehaut, A., Cassone, A. L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, *215*, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018

Dickens, C. W. S., & Graham, P. M. (2002). The south african scoring system (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science*, *27*(1), 1–10. https://doi.org/10.2989/16085914.2002.9626569

Dikareva, N., & Simon, K. S. (2019). Microplastic pollution in streams spanning an urbanisation gradient. *Environmental Pollution*, *250*, 292–299. https://doi.org/10.1016/j.envpol.2019.03.105

Ding, L., Mao, R. fan, Guo, X., Yang, X., Zhang, Q., & Yang, C. (2019). Microplastics in surface waters and sediments of the Wei River, in the northwest of China. *Science of the Total Environment*, *667*, 427–434. https://doi.org/10.1016/j.scitotenv.2019.02.332

Dolédec, Sylvain; Chessel, D; Ter Braak, C.J.F.; Champley, S. (1996). Matching species traits to environmental variables: a new three-table ordination method. *Environmental and Ecological Statistics*, *3*, 143–166. https://doi.org/10.1007/BF02427859

Dollar, E. S. J., James, C. S., Rogers, K. H., & Thoms, M. C. (2007). A framework for interdisciplinary understanding of rivers as ecosystems. *Geomorphology*, *89*(1-2 SPEC. ISS.), 147–162. https://doi.org/10.1016/j.geomorph.2006.07.022

Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, *22*(4), 1–20. https://doi.org/10.18637/jss.v022.i04

Duerdoth, C. P., Arnold, A., Murphy, J. F., Naden, P. S., Scarlett, P., Collins, A. L., Sear, D. A., & Jones, J. I. (2015). Assessment of a rapid method for quantitative reach-scale estimates of deposited fine sediment in rivers. *Geomorphology*, *230*, 37–50. https://doi.org/10.1016/j.geomorph.2014.11.003

DWAF. (2004). *DWAF - Department of Water Affairs and Forestry. Eastern Cape River Health Programme Draft Technical Report : Buffalo River Monitoring , 2002 - 2003*.

Eamrat, R., Taweesan, A., & Pussayanavin, T. (2022). Assessment of Microplastics Distribution and Related Water Quality in an Urban Canal, Thailand. *Pollution*, *8*(4), 1172–1184. https://doi.org/10.22059/POLL.2022.340679.1407

Environ-Fish Africa. (2009). *C . A . P . E . Estuaries Management Programme: Swartkops Integrated Environmental Management Plan*. *August 2009*, 74.

Environ-Fish Africa. (2011). *C.A.P.E. Estuary Management Programme: Integrated Management Plan: Swartkops Estuary and the Swartkops River valley and aloes nature reserves*. https://doi.org/10.1113/jphysiol.1954.sp005129

Eo, S., Hong, S. H., Song, Y. K., Han, G. M., & Shim, W. J. (2019). Spatiotemporal distribution and annual load of microplastics in the Nakdong River, South Korea. *Water Research*, *160*, 228–237. https://doi.org/10.1016/j.watres.2019.05.053

Fazey, F. M. C., & Ryan, P. G. (2016). Biofouling on buoyant marine plastics: An experimental study into the effect of size on surface longevity. *Environmental Pollution*, *210*, 354–360. https://doi.org/10.1016/j.envpol.2016.01.026

Fernández, R. G. (2001). Artemia bioencapsulation I. Effect of particle sizes on the filtering behavior of Artemia franciscana. *Journal of Crustacean Biology*, *21*(2), 435–442. https://doi.org/10.1163/20021975-99990144

Foekema, E. M., De Gruijter, C., Mergia, M. T., Van Franeker, J. A., Murk, A. J., & Koelmans, A. A. (2013). Plastic in north sea fish. *Environmental Science and Technology*, *47*(15), 8818–8824. https://doi.org/10.1021/es400931b

Francis, Y. (2008). Edgeworth, Francis Y. *The Concise Encyclopedia of Statistics*, 177–178. https://doi.org/10.1007/978-0-387-32833-1_124

Franzellitti, S., Canesi, L., Auguste, M., Wathsala, R. H. G. R., & Fabbri, E. (2019). Microplastic exposure and effects in aquatic organisms: A physiological perspective. *Environmental Toxicology and Pharmacology*, *68*(March), 37–51. https://doi.org/10.1016/j.etap.2019.03.009

Frehland, S., Kaegi, R., Hufenus, R., & Mitrano, D. M. (2020). Long-term assessment of nanoplastic particle and microplastic fiber flux through a pilot wastewater treatment plant using metal-doped plastics. *Water Research*, *182*, 115860. https://doi.org/10.1016/j.watres.2020.115860

Frei, S., Piehl, S., Gilfedder, B. S., Löder, M. G. J., Krutzke, J., Wilhelm, L., & Laforsch, C. (2019). Occurence of microplastics in the hyporheic zone of rivers. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-51741-5

Frias, J. P. G. L., & Nash, R. (2019). Microplastics: Finding a consensus on the definition. *Marine Pollution Bulletin*, *138*(November 2018), 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

Frissell, C. A., Liss, W. J., Warren, C. E., & Hurley, M. D. (1986). A hierarchical framework for stream habitat classification: Viewing streams in a watershed context. *Environmental Management*, *10*(2), 199–214. https://doi.org/10.1007/BF01867358

Gallitelli, L., Di Lollo, G., Adduce, C., Maggi, M. R., Trombetta, B., & Scalici, M. (2023). Aquatic plants entrap different size of plastics in indoor flume experiments. *Science of the Total Environment*, *863*(August 2022), 161051. https://doi.org/10.1016/j.scitotenv.2022.161051

Gerber, A., & Gabriel, M. (2002). Aquatic Invertebrates of South African Rivers. In *Institute of Water Quality Studies, Department of Water Affairs and Forestry. Illustrations,* *Version 2.*

González del Tánago, M., & García de Jalón, D. (2006). Hierarchical Classification of Rivers: A proposal for eco-geomorphic characterization of Spanish rivers within the European Water Frame Directive . *Limnetica*, *25*(3), 693–712.

Gordon, N. D., Mc Mahon, T. A., & Finlayson, B. L. (1992). (1992). Stream hydrology. An introduction for ecologists. In *Chichester: Wiley* (Second Edi). Chichester: Wiley. https://doi.org/10.1080/03632415.2016.1172482

Grbić, J., Helm, P., Athey, S., & Rochman, C. M. (2020). Microplastics entering northwestern Lake Ontario are diverse and linked to urban sources. *Water Research*, *174*. https://doi.org/10.1016/j.watres.2020.115623

Guidelli, E. J., Ramos, A. P., Zaniquelli, M. E. D., & Baffa, O. (2011). Green synthesis of colloidal silver nanoparticles using natural rubber latex extracted from Hevea brasiliensis. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *82*(1), 140–145. https://doi.org/10.1016/j.saa.2011.07.024

Guisan, A. Edwards, T, C, Hastie, T. (2002). Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, *157*(2–3), 89–100. https://doi.org/10.1111/j.1365-3040.1985.tb01209.x

Gurnell, A. M., Rinaldi, M., Belletti, B., Bizzi, S., Blamauer, B., Braca, G., Buijse, A. D., Bussettini, M., Camenen, B., Comiti, F., Demarchi, L., García de Jalón, D., González del Tánago, M., Grabowski, R. C., Gunn, I. D. M., Habersack, H., Hendriks, D., Henshaw, A. J., Klösch, M., … Ziliani, L. (2016). A multi-scale hierarchical framework for developing understanding of river behaviour to support river management. *Aquatic Sciences*, *78*(1), 1–16. https://doi.org/10.1007/s00027-015-0424-5

Han, N., Zhao, Q., Ao, H., Hu, H., & Wu, C. (2022). Horizontal transport of macro- and microplastics on soil surface by rainfall induced surface runoff as affected by vegetations. *Science of the Total Environment*, *831*(March), 154989. https://doi.org/10.1016/j.scitotenv.2022.154989

He, Beibei; Duodu, Godfred; Rintoul, Llew; Ayoko, Godwin; Goonetilleke, A. (2020). In fl uence of microplastics on nutrients and metal concentrations in river sediments *. *Environmental Pollution*, *263*, 114490. https://doi.org/10.1016/j.envpol.2020.114490

He, B., Smith, M., Egodawatta, P., Ayoko, G. A., Rintoul, L., & Goonetilleke, A. (2021). Dispersal and transport of microplastics in river sediments. *Environmental Pollution*, *279*, 116884. https://doi.org/10.1016/j.envpol.2021.116884

He, B., Wijesiri, B., Ayoko, G. A., Egodawatta, P., Rintoul, L., & Goonetilleke, A. (2020). Influential factors on microplastics occurrence in river sediments. *Science of the Total Environment*, *738*, 139901. https://doi.org/10.1016/j.scitotenv.2020.139901

He, S., Jia, M., Xiang, Y., Song, B., Xiong, W., Cao, J., Peng, H., Yang, Y., Wang, W., Yang, Z., & Zeng, G. (2022). Biofilm on microplastics in aqueous environment: Physicochemical properties and environmental implications. *Journal of Hazardous Materials*, *424*(PB), 127286. https://doi.org/10.1016/j.jhazmat.2021.127286

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environmental Science and Technology*, *46*(6), 3060–3075. https://doi.org/10.1021/es2031505

Hodkinson, I. D., Merritt, R. W., & Cummins, K. W. (1981). An Introduction to the Aquatic Insects of North America. *The Journal of Animal Ecology*, *50*(1), 330. https://doi.org/10.2307/4053

Hoellein, T. J., Shogren, A. J., Tank, J. L., Risteca, P., & Kelly, J. J. (2019). Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-40126-3

Horton, A. A., & Dixon, S. J. (2018). Microplastics: An introduction to environmental transport processes. *WIREs Water*, *5*(2). https://doi.org/10.1002/wat2.1268

Horton, A. A., Svendsen, C., Williams, R. J., Spurgeon, D. J., & Lahive, E. (2017). Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification. *Marine Pollution Bulletin*, *114*(1), 218–226. https://doi.org/10.1016/j.marpolbul.2016.09.004

Huang, Y., Li, W., Gao, J., Wang, F., Yang, W., Han, L., Lin, D., Min, B., Zhi, Y., Grieger, K., & Yao, J. (2021). Effect of microplastics on ecosystem functioning: Microbial nitrogen removal mediated by benthic invertebrates. *Science of the Total Environment*, *754*. https://doi.org/10.1016/j.scitotenv.2020.142133

Hübner, M. K., Michler-Kozma, D. N., & Gabel, F. (2020). Microplastic concentrations at the water surface are reduced by decreasing flow velocities caused by a reservoir. *Fundamental and Applied Limnology*, *194*(1), 49–56. https://doi.org/10.1127/fal/2020/1307

Hui, C., & McGeoch, M. A. (2006). Evolution of body size, range size, and food composition in a predator-prey metapopulation. *Ecological Complexity*, *3*(2), 148–159. https://doi.org/10.1016/j.ecocom.2005.12.003

Hurley, R., Woodward, J., & Rothwell, J. J. (2018). Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nature Geoscience*, *11*(4), 251–257. https://doi.org/10.1038/s41561-018-0080-1

Ibekwe, A. M., Ma, J., & Murinda, S. E. (2016). Bacterial community composition and structure in an Urban River impacted by different pollutant sources. *Science of the Total Environment*, *566–567*, 1176–1185. https://doi.org/10.1016/j.scitotenv.2016.05.168

Issac, M. N., & Kandasubramanian, B. (2021). Effect of microplastics in water and aquatic systems. *Environmental Science and Pollution Research*, *28*(March), 19562. https://doi.org/10.1007/s11356-021-13184-2/Published

J.I., Jones, J.F., Murphy, A.L., Collins, D.A. Sear, P.S., Naden, P.D., A. (2012). The impact of fine sediment on macroinvertebrates. *River Research and Applications*, *28*(8), 1055–1071. https://doi.org/10.1002/rra

Jafari, M., & Ansari-Pour, N. (2019). Why, when and how to adjust your P values? *Cell Journal*, *20*(4), 604–607. https://doi.org/10.22074/cellj.2019.5992

Jordaan, K., & Bezuidenhout, C. C. (2013). The impact of physico-chemical water quality parameters on bacterial diversity in the Vaal River, South Africa. *Water SA*, *39*(3), 385–396. https://doi.org/10.4314/wsa.v39i3.7

Jowett, I. G. (1993). A method for objectively identifying pool, run, and riffle habitats from physical measurements. *New Zealand Journal of Marine and Freshwater Research*, *27*(2), 241–248. https://doi.org/10.1080/00288330.1993.9516563

Jowett, I. G., & Richardson, J. (1990). Microhabitat preferences of benthic invertebrates in a new zealand river and the development of in-stream flow-habitat models for deleatidium spp. *New Zealand Journal of Marine and Freshwater Research*, *24*(1), 19–30.

https://doi.org/10.1080/00288330.1990.9516399

Jowett, I. G., Richardson, J., Biggs, B. J. F., Hickey, C. W., & Quinn, J. M. (1991). Microhabitat preferences of benthic invertebrates and the development of generalised deleatidiumspp habitat suitability curves, applied to four new zealand rivers. *New Zealand Journal of Marine and Freshwater Research*, *25*(2), 187–199. https://doi.org/10.1080/00288330.1991.9516470

Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez C., V., Beers, K. L., Balazs, G. H., Jones, T. T., Work, T. M., Brignac, K. C., Royer, S. J., Hyrenbach, K. D., Jensen, B. A., & Lynch, J. M. (2018). Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine Pollution Bulletin*, *127*(December), 704–716. https://doi.org/10.1016/j.marpolbul.2017.12.061

Justino, A. K. S., Ferreira, G. V. B., Fauvelle, V., Schmidt, N., Lenoble, V., Pelage, L., Martins, K., Travassos, P., & Lucena-Frédou, F. (2023). From prey to predators: Evidence of microplastic trophic transfer in tuna and large pelagic species in the southwestern Tropical Atlantic. *Environmental Pollution*, *327*(December 2022). https://doi.org/10.1016/j.envpol.2023.121532

Karaouzas, I., Theodoropoulos, C., Vourka, A., Gritzalis, K., & Skoulikidis, N. T. (2019). Stream invertebrate communities are primarily shaped by hydrological factors and ultimately fine-tuned by local habitat conditions. *Science of the Total Environment*, *665*, 290–299. https://doi.org/10.1016/j.scitotenv.2019.02.134

Kataoka, T., Nihei, Y., Kudou, K., & Hinata, H. (2019a). Assessment of the sources and inflow processes of microplastics in the river environments of Japan. *Environmental Pollution*, *244*, 958–965. https://doi.org/10.1016/j.envpol.2018.10.111

Kataoka, T., Nihei, Y., Kudou, K., & Hinata, H. (2019b). Assessment of the sources and inflow processes of microplastics in the river environments of Japan. *Environmental Pollution*, *244*, 958–965. https://doi.org/10.1016/j.envpol.2018.10.111

Khatmullina, L., & Isachenko, I. (2017). Settling velocity of microplastic particles of regular shapes. *Marine Pollution Bulletin*, *114*(2), 871–880. https://doi.org/10.1016/j.marpolbul.2016.11.024

Kiørboe, T., Visser, A., Andersen, K. H., & Browman, H. (2018). A trait-based approach to ocean ecology. *ICES Journal of Marine Science*, *75*(6), 1849–1863. https://doi.org/10.1093/icesjms/fsy090

Kiss, T., Fórián, S., Szatmári, G., & Sipos, G. (2021). Spatial distribution of microplastics in the fluvial sediments of a transboundary river – A case study of the Tisza River in Central Europe. *Science of the Total Environment*, *785*. https://doi.org/10.1016/j.scitotenv.2021.147306

Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and spatial distribution of microplastics in river shore sediments of the rhine-main area in Germany. *Environmental Science and Technology*, *49*(10), 6070–6076. https://doi.org/10.1021/acs.est.5b00492

Kleynhans, CJ, Thirion, C and Moolman, J. (2005). A Level I River Ecoregion classification System for South Africa, Lesotho and Swaziland. Report No. N/0000/00/REQ0104. Resource Quality Services, Department of Water Affairs and Forestry, Pretoria, South Africa. In *Remote Sensing of Land Use and Land Cover in Mountain Region*. https://doi.org/10.1007/978-981-13-7580-4_8

Kokalj, A. J., Kunej, U., & Skalar, T. (2018). Screening study of four environmentally relevant microplastic pollutants: Uptake and effects on Daphnia magna and Artemia franciscana. *Chemosphere*, *208*, 522–529. https://doi.org/10.1016/j.chemosphere.2018.05.172

Kondolf, G. M., Piégay, H., Schmitt, L., & Montgomery, D. R. (2016a). Geomorphic classification of rivers and streams. In *Tools in Fluvial Geomorphology* (Issue January). https://doi.org/10.1002/9781118648551.ch7

Kondolf, G. M., Piégay, H., Schmitt, L., & Montgomery, D. R. (2016b). Geomorphic classification of rivers and streams. In *Tools in Fluvial Geomorphology* (pp. 133–158). Wiley. https://doi.org/10.1002/9781118648551.ch7

Koutnik, V. S., Leonard, J., Alkidim, S., DePrima, F. J., Ravi, S., Hoek, E. M. V., & Mohanty, S. K. (2021). Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling. *Environmental Pollution*, *274*, 116552. https://doi.org/10.1016/j.envpol.2021.116552

Kowalski, N., Reichardt, A. M., & Waniek, J. J. (2016). Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Marine Pollution Bulletin*, *109*(1), 310–319. https://doi.org/10.1016/j.marpolbul.2016.05.064

Kühn, S., van Franeker, J. A., O'Donoghue, A. M., Swiers, A., Starkenburg, M., van Werven, B., Foekema, E., Hermsen, E., Egelkraut-Holtus, M., & Lindeboom, H. (2020). Details of plastic ingestion and fibre contamination in North Sea fishes. *Environmental Pollution*, *257*(November), 113569. https://doi.org/10.1016/j.envpol.2019.113569

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E. L., & van Franeker, J. A. (2017). The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, *115*(1–2), 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Kumar, R., Sharma, P., Verma, A., Jha, P. K., Singh, P., Gupta, P. K., Chandra, R., & Vara Prasad, P. V. (2021). Effect of physical characteristics and hydrodynamic conditions on transport and deposition of microplastics in riverine ecosystem. *Water (Switzerland)*, *13*(19). https://doi.org/10.3390/w13192710

Kunz, A., Schneider, F., Anthony, N., & Lin, H. T. (2023). Microplastics in rivers along an urban-rural gradient in an urban agglomeration: Correlation with land use, potential sources and pathways. *Environmental Pollution*, *321*(January), 121096. https://doi.org/10.1016/j.envpol.2023.121096

Lamouroux, N., Dolédec, S., & Gayraud, S. (2004). Biological traits of stream macroinvertebrate communities: Effects of microhabitat, reach, and basin filters. *Journal of the North American Benthological Society*, *23*(3), 449–466. https://doi.org/10.1899/0887-3593(2004)023<0449:BTOSMC>2.0.CO;2

Lavers, J. L., Stivaktakis, G., Hutton, I., & Bond, A. L. (2019). Detection of ultrafine plastics ingested by seabirds using tissue digestion. *Marine Pollution Bulletin*, *142*(January), 470–474. https://doi.org/10.1016/j.marpolbul.2019.04.001

Lebreton, L. C. M., Van Der Zwet, J., Damsteeg, J. W., Slat, B., Andrady, A., & Reisser, J. (2017). River plastic emissions to the world's oceans. *Nature Communications*, *8*, 1–10. https://doi.org/10.1038/ncomms15611

Lehotský, M. (2004). River morphology hierarchical classification (RMHC). *Acta*

*Universitatis Carolinae, Geographica*, *39*(1), 33–45.

Leterme, S. C., Tuuri, E. M., Drummond, W. J., Jones, R., & Gascooke, J. R. (2023). Microplastics in urban freshwater streams in Adelaide, Australia: A source of plastic pollution in the Gulf St Vincent. *Science of the Total Environment*, *856*(May 2022), 158672. https://doi.org/10.1016/j.scitotenv.2022.158672

Li, W., Zhang, Y., Wu, N., Zhao, Z., Xu, W., Ma, Y., & Niu, Z. (2019). Colonization Characteristics of Bacterial Communities on Plastic Debris Influenced by Environmental Factors and Polymer Types in the Haihe Estuary of Bohai Bay, China [Research-article]. *Environmental Science and Technology*, *53*(18), 10763–10773. https://doi.org/10.1021/acs.est.9b03659

Li, Y., Liu, S., Liu, M., Huang, W., Chen, K., Ding, Y., Wu, F., Ke, H., Lou, L., Lin, Y., Zhang, M., Liu, F., Wang, C., & Cai, M. (2021). Mid-Level Riverine Outflow Matters: A Case of Microplastic Transport in the Jiulong River, China. *Frontiers in Marine Science*, *8*(July), 1–11. https://doi.org/10.3389/fmars.2021.712727

Liro, M., Mikuś, P., & Wyżga, B. (2022). First insight into the macroplastic storage in a mountain river: The role of in-river vegetation cover, wood jams and channel morphology. *Science of the Total Environment*, *838*(March), 0–11. https://doi.org/10.1016/j.scitotenv.2022.156354

Liro, M., van Emmerik, T., Wyzga, B., Liro, J., & Mikuś, P. (2020). Macroplastic storage and remobilization in rivers. *Water (Switzerland)*, *12*(7). https://doi.org/10.3390/w12072055

Liu, Y., Zhang, J. Di, Tang, Y., He, Y., Li, Y. J., You, J. A., Breider, F., Tao, S., & Liu, W. X. (2021). Effects of anthropogenic discharge and hydraulic deposition on the distribution and accumulation of microplastics in surface sediments of a typical seagoing river: The Haihe River. *Journal of Hazardous Materials*, *404*(PB), 124180. https://doi.org/10.1016/j.jhazmat.2020.124180

López-Rojo, N., Pérez, J., Alonso, A., Correa-Araneda, F., & Boyero, L. (2020). Microplastics have lethal and sublethal effects on stream invertebrates and affect stream ecosystem functioning. *Environmental Pollution*, *259*. https://doi.org/10.1016/j.envpol.2019.113898

Lorenz, C., Roscher, L., Meyer, M. S., Hildebrandt, L., Prume, J., Löder, M. G. J., Primpke, S., & Gerdts, G. (2019). Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. *Environmental Pollution*, *252*, 1719–1729. https://doi.org/10.1016/j.envpol.2019.06.093

Lu, A., Li, J., Zheng, B., & Yin, X. (2022). Effect of Different Land Use Types on the Taxonomic and Functional Diversity of Macroinvertebrates in an Urban Area of Northern China. *Water (Switzerland)*, *14*(23). https://doi.org/10.3390/w14233793

Lu, X., Wang, X., Liu, X., & Singh, V. P. (2023). Dispersal and transport of microplastic particles under different flow conditions in riverine ecosystem. *Journal of Hazardous Materials*, *442*(September 2022), 130033. https://doi.org/10.1016/j.jhazmat.2022.130033

Lusher, A. L., Welden, N. A., Sobral, P., & Cole, M. (2017). Sampling, isolating and identifying microplastics ingested by fish and invertebrates. In *Analytical Methods* (Vol. 9, Issue 9, pp. 1346–1360). Royal Society of Chemistry. https://doi.org/10.1039/c6ay02415g

Lwanga, E. H., Beriot, N., Corradini, F., Silva, V., Yang, X., Baartman, J., Rezaei, M., van Schaik, L., Riksen, M., & Geissen, V. (2022). Review of microplastic sources, transport pathways and correlations with other soil stressors: a journey from agricultural sites into the environment. *Chemical and Biological Technologies in Agriculture*, *9*(1), 1–20. https://doi.org/10.1186/s40538-021-00278-9

Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., & Xing, B. (2020). Microplastics in aquatic environments: Toxicity to trigger ecological consequences. *Environmental Pollution*, *261*, 114089. https://doi.org/10.1016/j.envpol.2020.114089

ma, P., Wei Wang,  mu, Liu, H., Feng Chen,  yu, & Xia, J. (2019). Research on ecotoxicology of microplastics on freshwater aquatic organisms. *Environmental Pollutants and Bioavailability*, *31*(1), 131–137. https://doi.org/10.1080/26395940.2019.1580151

Maclear, L. G. A. (1995). *GH3874 Design and Constr of a Gwater Mon network - Swartkops River Alluvial Aquifer, Tech report*.

Mai, Y., Peng, S., Lai, Z., & Wang, X. (2021). Measurement, quantification, and potential risk of microplastics in the mainstream of the Pearl River (Xijiang River) and its estuary, Southern China. *Environmental Science and Pollution Research*, *28*(38), 53127–53140.

https://doi.org/10.1007/s11356-021-14395-3

Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. (2015). Microplastics profile along the Rhine River. *Scientific Reports*, *5*(December). https://doi.org/10.1038/srep17988

Masura, J., Baker, J., Foster, G., & Arthur, C. (2015). Laboratory Methods for the Analysis of Microplastics in the Marine Environment. *NOAA Marine Debris Program National*, *July*, 1–39. https://marinedebris.noaa.gov/sites/default/files/publications-files/noaa_microplastics_methods_manual.pdf

Mateos-Cárdenas, A., Moroney, A. von der G., van Pelt, F. N. A. M., O'Halloran, J., & Jansen, M. A. K. (2022). Trophic transfer of microplastics in a model freshwater microcosm; lack of a consumer avoidance response. *Food Webs*, *31*(July 2021). https://doi.org/10.1016/j.fooweb.2022.e00228

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an abundant and distinct microbial habitat in an urban river. *Environmental Science and Technology*, *48*(20), 11863–11871. https://doi.org/10.1021/es503610r

McCormick, A. R., Hoellein, T. J., London, M. G., Hittie, J., Scott, J. W., & Kelly, J. J. (2016). Microplastic in surface waters of urban rivers: Concentration, sources, and associated bacterial assemblages. *Ecosphere*, *7*(11). https://doi.org/10.1002/ecs2.1556

McGivney, E., Cederholm, L., Barth, A., Hakkarainen, M., Hamacher-Barth, E., Ogonowski, M., & Gorokhova, E. (2020). Rapid Physicochemical Changes in Microplastic Induced by Biofilm Formation. *Frontiers in Bioengineering and Biotechnology*, *8*(March), 1–14. https://doi.org/10.3389/fbioe.2020.00205

McNeish, R. E., Kim, L. H., Barrett, H. A., Mason, S. A., Kelly, J. J., & Hoellein, T. J. (2018). Microplastic in riverine fish is connected to species traits. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-29980-9

Meijer, L. J. J., van Emmerik, T., van der Ent, R., Schmidt, C., & Lebreton, L. (2021). More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Science Advances*, *7*(18), 1–14. https://doi.org/10.1126/sciadv.aaz5803

Menezes, S., Baird, D. J., & Soares, A. M. V. M. (2010). Beyond taxonomy: A review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. In *Journal of Applied Ecology* (Vol. 47, Issue 4, pp. 711–719).

https://doi.org/10.1111/j.1365-2664.2010.01819.x

Miao, L., Gao, Y., Adyel, T. M., Huo, Z., Liu, Z., Wu, J., & Hou, J. (2021). Effects of biofilm colonization on the sinking of microplastics in three freshwater environments. *Journal of Hazardous Materials*, *413*(December 2020), 125370. https://doi.org/10.1016/j.jhazmat.2021.125370

Miller, J., & Orbock Miller, S. M. (2020). A Geomorphic Framework for the Analysis of Microplastics in Riverine Sediments. *E3S Web of Conferences*, *202*, 1–10. https://doi.org/10.1051/e3sconf/202020201002

Minshall, G. W., Thomas, S. A., Newbold, J. D., Monaghan, M. T., & Cushing, C. E. (2000). Physical factors influencing fine organic particle transport and deposition in streams. *Journal of the North American Benthological Society*, *19*(1), 1–16. https://doi.org/10.2307/1468278

Mmachaka, T., Nel, M. A., Snow, B., & Adams, J. B. (2023). Reduction in pollution load to an urban estuary using a sustainable drainage system treatment train. *Marine Pollution Bulletin*, *194*(August). https://doi.org/10.1016/j.marpolbul.2023.115378

Mohamed Nor, N. H., & Obbard, J. P. (2014). Microplastics in Singapore's coastal mangrove ecosystems. *Marine Pollution Bulletin*, *79*(1–2), 278–283. https://doi.org/10.1016/j.marpolbul.2013.11.025

Moreschi, A. C., Callil, C. T., Christo, S. W., Ferreira Junior, A. L., Nardes, C., de Faria, É., & Girard, P. (2020). Filtration, assimilation and elimination of microplastics by freshwater bivalves. *Case Studies in Chemical and Environmental Engineering*, *2*(August). https://doi.org/10.1016/j.cscee.2020.100053

Moses, S. R., Löder, M. G. J., Herrmann, F., & Laforsch, C. (2023). Seasonal variations of microplastic pollution in the German River Weser. *Science of the Total Environment*, *902*(August). https://doi.org/10.1016/j.scitotenv.2023.166463

Moyal, J., Dave, P. H., Wu, M., Karimpour, S., Brar, S. K., Zhong, H., & Kwong, R. W. M. (2023). Impacts of Biofilm Formation on the Physicochemical Properties and Toxicity of Microplastics: A Concise Review. *Reviews of Environmental Contamination and Toxicology*, *261*(1), 1–17. https://doi.org/10.1007/s44169-023-00035-z

Natarajan, L., Jenifer, M. A., & Mukherjee, A. (2021). Eco-corona formation on the nanomaterials in the aquatic systems lessens their toxic impact: A comprehensive review. *Environmental Research*, *194*(December 2020), 110669. https://doi.org/10.1016/j.envres.2020.110669

Nel, L., Strydom, N. A., & Bouwman, H. (2015). Preliminary assessment of contaminants in the sediment and organisms of the Swartkops Estuary, South Africa. *Marine Pollution Bulletin*, *101*(2), 878–885. https://doi.org/10.1016/j.marpolbul.2015.11.015

Newbold, J. D., Thomas, S. A., Minshall, G. W., Cushing, C. E., & Georgian, T. (2005). Deposition, benthic residence, and resuspension of fine organic particles in a mountain stream. *Limnology and Oceanography*, *50*(5), 1571–1580. https://doi.org/10.4319/lo.2005.50.5.1571

Newbould, R. A., Powell, D. M., & Whelan, M. J. (2021). Macroplastic Debris Transfer in Rivers: A Travel Distance Approach. *Frontiers in Water*, *3*(September), 1–14. https://doi.org/10.3389/frwa.2021.724596

Newson, M. D., & Newson, C. L. (2000). Geomorphology, ecology and river channel habitat: Mesoscale approaches to basin-scle chailenges. *Progress in Physical Geography*, *24*(2), 195–217. https://doi.org/10.1177/030913330002400203

Nguyen, T. H., Tang, F. H. M., & Maggi, F. (2020). Sinking of microbial-associated microplastics in natural waters. *PLoS ONE*, *15*(2), 1–20. https://doi.org/10.1371/journal.pone.0228209

Niu, A., Song, L. Y., Xiong, Y. H., Lu, C. J., Junaid, M., & Pei, D. S. (2019). Impact of water quality on the microbial diversity in the surface water along the Three Gorge Reservoir (TGR), China. *Ecotoxicology and Environmental Safety*, *181*(March), 412–418. https://doi.org/10.1016/j.ecoenv.2019.06.023

Nizzetto, L., Bussi, G., Futter, M. N., Butterfield, D., & Whitehead, P. G. (2016). A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environmental Science: Processes and Impacts*, *18*(8), 1050–1059. https://doi.org/10.1039/c6em00206d

Nuelle, M., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution*, *184*(2014), 161–169. https://doi.org/10.1016/j.envpol.2013.07.027

Nuelle, M. T., Dekiff, J. H., Remy, D., & Fries, E. (2014). A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution*, *184*, 161–169. https://doi.org/10.1016/j.envpol.2013.07.027

O'Keeffe, J. H., van Ginkel, C. E., Hughes, D. A., Hill, T. R., & Ashton, P. J. (1996). *A situation analysis of water quality in the catchment of the Buffalo River, Eastern Cape, with special emphasis on the impacts of low cost, high-density urban development on water quality* (Vol. 1, Issue 40).

Odume, O. N. (2014). *An evaluation of macroinvertebrate-based biomonitoring and ecotoxicological assessments of deteriorating environmental water quality in the Swartkops River, South Africa*. Rhodes University.

Odume, O. N. (2020). Searching for urban pollution signature and sensitive macroinvertebrate traits and ecological preferences in a river in the Eastern Cape of South Africa. *Ecological Indicators*, *108*(September 2019), 105759. https://doi.org/10.1016/j.ecolind.2019.105759

Odume, O. N., Akamagwuna, F. C., Ntloko, P., Dallas, H. F., Nnadozie, C. F., & Barber-James, H. M. (2023). A trait database for southern African freshwater invertebrates. *African Journal of Aquatic Science*, *48*(1), 64–70. https://doi.org/10.2989/16085914.2022.2142505

Odume, O. N., & Muller, W. J. (2011). Diversity and structure of Chironomidae communities in relation to water quality differences in the Swartkops River. *Physics and Chemistry of the Earth*, *36*(14–15), 929–938. https://doi.org/10.1016/j.pce.2011.07.063

Odume, O. N., Muller, W. J., Arimoro, F. O., & Palmer, C. G. (2012). The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River, South Africa: A multimetric approach. *African Journal of Aquatic Science*, *37*(2), 191–200. https://doi.org/10.2989/16085914.2012.670613

Odume, O. N., Muller, W. J., Palmer, C. G., & Arimoro, F. O. (2012). Mentum deformities in Chironomidae communities as indicators of anthropogenic impacts in Swartkops River. *Physics and Chemistry of the Earth*, *50–52*, 140–148. https://doi.org/10.1016/j.pce.2012.08.005

Odume, O. N., Onyima, B. N., Nnadozie, C. F., Omovoh, G. O., Mmachaka, T., Omovoh, B.

O., Uku, J. E., Akamagwuna, F. C., & Arimoro, F. O. (2022). Governance and Institutional Drivers of Ecological Degradation in Urban River Ecosystems: Insights from Case Studies in African Cities. *Sustainability (Switzerland)*, *14*(21). https://doi.org/10.3390/su142114147

Ohoro, C. R., Adeniji, A. O., Semerjian, L., Okoh, O. O., & Okoh, A. I. (2021). Occurrence and distribution of pharmaceuticals in surface water and sediment of Buffalo and Sundays River estuaries, South Africa and their ecological risk assessment. *Emerging Contaminants*, *7*, 187–195. https://doi.org/10.1016/j.emcon.2021.09.002

Oliveira, J., Belchior, A., da Silva, V. D., Rotter, A., Petrovski, Ž., Almeida, P. L., Lourenço, N. D., & Gaudêncio, S. P. (2020). Marine Environmental Plastic Pollution: Mitigation by Microorganism Degradation and Recycling Valorization. *Frontiers in Marine Science*, *7*(December). https://doi.org/10.3389/fmars.2020.567126

Omarjee, A., Taljaard, S., Ramjukadh, C. L., & van Niekerk, L. (2021). pH variability in catchment flows to estuaries – A South African perspective. *Estuarine, Coastal and Shelf Science*, *262*(September), 107605. https://doi.org/10.1016/j.ecss.2021.107605

Osorio, E. D., Tanchuling, M. A. N., & Diola, M. B. L. D. (2021). Microplastics Occurrence in Surface Waters and Sediments in Five River Mouths of Manila Bay. *Frontiers in Environmental Science*, *9*(September), 1–14. https://doi.org/10.3389/fenvs.2021.719274

Owolabi, S. T., Madi, K., Kalumba, A. M., & Baiyegunhi, C. (2021). A geomagnetic analysis for lineament detection and lithologic characterization impacting groundwater prospecting; a case study of Buffalo catchment, Eastern Cape, South Africa. *Groundwater for Sustainable Development*, *12*, 100531. https://doi.org/10.1016/j.gsd.2020.100531

Owowenu, E. K., Nnadozie, C. F., Akamagwuna, F., Siwe, X. N., & Uku, J. E. (2023). A critical review of environmental factors influencing the transport dynamics of microplastics in riverine systems : implications for ecological studies. *Aquatic Ecology*, *57*, *557–57*(0123456789). https://doi.org/10.1007/s10452-023-10029-7

Pastuchová, Z., Lehotský, M., & Grešková, A. (2008). Influence of morphohydraulic habitat structure on invertebrate communities (Ephemeroptera, Plecoptera and Trichoptera). *Biologia*, *63*(5), 720–729. https://doi.org/10.2478/s11756-008-0116-x

Pedersen, E. J., Miller, D. L., Simpson, G. L., & Ross, N. (2019). Hierarchical generalized additive models in ecology: An introduction with mgcv. *PeerJ*, *2019*(5). https://doi.org/10.7717/peerj.6876

Picker, M., Griffiths, C., Weaving, A. (2002). *Field guide to Insects of South Africa*. Struik publishers, South Africa.

Pilière, A. F. H., Verberk, W. C. E. P., Gräwe, M., Breure, A. M., Dyer, S. D., Posthuma, L., de Zwart, D., Huijbregts, M. A. J., & Schipper, A. M. (2016). On the importance of trait interrelationships for understanding environmental responses of stream macroinvertebrates. *Freshwater Biology*, *61*(2), 181–194. https://doi.org/10.1111/fwb.12690

Poff, N. L., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P., & Kondratieff, B. C. (2006). Poffetal_2006_FunctionalTraitNiche_JNABS insects. *Journal of the North American Benthological Society*, *25*(4), 730–755. http://rydberg.biology.colostate.edu/poff/Public/poffpubs/Poffetal_2006_FunctionalTraitNiche_JNABS.pdf%0Ahttp://www.jnabs.org/doi/abs/10.1899/0887-3593(2006)025%5B0730:FTNONA%5D2.0.CO%3B2

Poff, N. L. R. (1997). Landscape filters and species traits: Towards mechanistic understanding and prediction in stream ecology. *Journal of the North American Benthological Society*, *16*(2), 391–409. https://doi.org/10.2307/1468026

Pollard, A. I., & Yuan, L. L. (2010). Assessing the consistency of response metrics of the invertebrate benthos: A comparison of trait- and identity-based measures. *Freshwater Biology*, *55*(7), 1420–1429. https://doi.org/10.1111/j.1365-2427.2009.02235.x

Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2019). Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC - Trends in Analytical Chemistry*, *110*, 150–159. https://doi.org/10.1016/j.trac.2018.10.029

Quibell, G., & Belcher, A. (1996). *Zwartkops River Water Resources Management Plan: A situation assessment and development of a catchment water resources amnagement plan*. *June*.

Rakib, M. R. J., Al Nahian, S., Madadi, R., Haider, S. M. B., De-La-Torre, G. E., Walker, T. R., Jonathan, M. P., Cowger, W., Khandaker, M. U., & Idris, A. M. (2023).

Spatiotemporal trends and characteristics of microplastic contamination in a large river-dominated estuary. *Environmental Science: Processes and Impacts*, *25*(5), 929–940. https://doi.org/10.1039/d3em00014a

Ramírez, A., & Gutiérrez-Fonseca, P. E. (2014). Functional feeding groups of aquatic insect families in Latin America: A critical analysis and review of existing literature. *Revista de Biologia Tropical*, *62*(May), 155–167. https://doi.org/10.15517/rbt.v62i0.15785

Rex M, C., Debroy, A., Nirmala, M. J., & Mukherjee, A. (2023). Ecotoxicological significance of bio-corona formation on micro/nanoplastics in aquatic organisms. *RSC Advances*, *13*(33), 22905–22917. https://doi.org/10.1039/d3ra04054b

Rezaei, M., Riksen, M. J. P. M., Sirjani, E., Sameni, A., & Geissen, V. (2019). Wind erosion as a driver for transport of light density microplastics. *Science of the Total Environment*, *669*, 273–281. https://doi.org/10.1016/j.scitotenv.2019.02.382

RHP. (2004). *State-of-rivers report :The River Health Programme. Buffalo River system*.

RHP. (2005). River Health Programme: State of rivers report. The Buffalo River System. In *Water Wheel*.

Robson, B. J., Barmuta, L. A., & Fairweather, P. G. (2005). Methodological and conceptual issues in the search for a relationship between animal body-size distributions and benthic habitat architecture. *Marine and Freshwater Research*, *56*(1), 1–11. https://doi.org/10.1071/MF04210

Rosgen, D. L. (1994). C A T E N A A classification of natural rivers. *Catena*, *22*, 169–199. https://wildlandhydrology.com/resources/docs/Stream Classification/Rosgen_1994_A_Classification_of_Natural_Rivers.pdf

Rowntree, K. M. and, & Wadeson, R. A. (Roy A. (1999). *A hierarchical geomorphological model for the classification of selected South African rivers : final report to the Water Research Commission*. [WRC].

Scherer, C., Brennholt, N., Reifferscheid, G., & Wagner, M. (2017). Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/s41598-017-17191-7

Schlichting, H. (1979). *Boundary -layer Theory* (Seventh ed). McGrraw-Hill.

Schmera, D., Heino, J., & Podani, J. (2022). Characterising functional strategies and trait space of freshwater macroinvertebrates. *Scientific Reports*, *12*(1), 1–9. https://doi.org/10.1038/s41598-022-16472-0

Schmera, D., Heino, J., Podani, J., Erős, T., & Dolédec, S. (2017). Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. *Hydrobiologia*, *787*(1), 27–44. https://doi.org/10.1007/s10750-016-2974-5

Schorer, M., & Eisele, M. (1997). Accumulation of inorganic and organic pollutants by biofilms in the aquatic environment. In *Water, Air, & Soil Pollution* (Vol. 99, Issues 1–4, pp. 651–659). https://doi.org/10.1007/bf02406904

Schreyers, L., van Emmerik, T., Nguyen, T. L., Castrop, E., Phung, N. A., Kieu-Le, T. C., Strady, E., Biermann, L., & van der Ploeg, M. (2021). Plastic Plants: The Role of Water Hyacinths in Plastic Transport in Tropical Rivers. *Frontiers in Environmental Science*, *9*(May), 1–9. https://doi.org/10.3389/fenvs.2021.686334

Sequeira, I. F., Prata, J. C., da Costa, J. P., Duarte, A. C., & Rocha-Santos, T. (2020). Worldwide contamination of fish with microplastics: A brief global overview. *Marine Pollution Bulletin*, *160*(September), 111681. https://doi.org/10.1016/j.marpolbul.2020.111681

Serna, J. P., Fernandez, D., Velez, F., Ruiz, J., Breckling, B., & Aguirre, N. (2022). Altitudinal distribution of the functional feeding groups of aquatic macroinvertebrates using an ecological network in Andean streams. *Revista de Biologia Tropical*, *70*(1), 82–95. https://doi.org/10.15517/rev.biol.trop..v70i1.46904

Sfriso, A. A., Tomio, Y., Juhmani, A. S., Sfriso, A., Munari, C., & Mistri, M. (2021). Macrophytes: A temporary sink for microplastics in transitional water systems. *Water (Switzerland)*, *13*(21), 1–12. https://doi.org/10.3390/w13213032

Shekoohiyan, S., & Akbarzadeh, A. (2022). The abundance of microplastic pollution along the Jajroud river of Tehran: Estimating the water quality index and the ecological risk. *Ecological Indicators*, *145*(August), 109629. https://doi.org/10.1016/j.ecolind.2022.109629

Southwood, T. R. E. (1977). Habitat, the templet for ecological strategies? – Presidential Address to British Ecological Society, 5 January 1977. *Journal of Animal Ecology*, *46*(January), 337–365.

Southwood, T. R. E. (1988). Tactics, strategies and templets. *Oikos*, *52*, 3–18.

Stabnikova, O., Stabnikov, V., Marinin, A., Klavins, M., Klavins, L., & Vaseashta, A. (2021). Microbial life on the surface of microplastics in natural waters. *Applied Sciences (Switzerland)*, *11*(24), 1–19. https://doi.org/10.3390/app112411692

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., & Gomes, R. L. (2020). Freshwater microplastic concentrations vary through both space and time. *Environmental Pollution*, *263*, 114481. https://doi.org/10.1016/j.envpol.2020.114481

Statzner, B., & Bêche, L. A. (2010). Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshwater Biology*, *55*(SUPPL. 1), 80–119. https://doi.org/10.1111/j.1365-2427.2009.02369.x

Statzner, B., Gore, J. A., & Resh, V. H. (1988). Hydraulic Stream Ecology: Observed Patterns and Potential Applications. *Journal of the North American Benthological Society*, *7*(4), 307–360. https://doi.org/10.2307/1467296

Steer, M., Cole, M., Thompson, R. C., & Lindeque, P. K. (2017). Microplastic ingestion in fish larvae in the western English Channel. *Environmental Pollution*, *226*, 250–259. https://doi.org/10.1016/j.envpol.2017.03.062

Stock, F., Kochleus, C., Bänsch-Baltruschat, B., Brennholt, N., & Reifferscheid, G. (2019). Sampling techniques and preparation methods for microplastic analyses in the aquatic environment – A review. *TrAC - Trends in Analytical Chemistry*, *113*, 84–92. https://doi.org/10.1016/j.trac.2019.01.014

Su, L., Sharp, S. M., Pettigrove, V. J., Craig, N. J., Nan, B., Du, F., & Shi, H. (2020). Superimposed microplastic pollution in a coastal metropolis. *Water Research*, *168*, 115140. https://doi.org/10.1016/j.watres.2019.115140

Talbot, R., & Chang, H. (2021). Microplastics in freshwater: A global review of factors affecting spatial and temporal variations. *Environmental Pollution*, *292*(2021). https://doi.org/10.1016/j.envpol.2021.118393

Talbot, R., & Chang, H. (2022). Microplastics in freshwater: A global review of factors affecting spatial and temporal variations. *Environmental Pollution*, *292*(PB), 118393. https://doi.org/10.1016/j.envpol.2021.118393

Tang, K. H. D. (2024). Terrestrial and Aquatic Plastisphere: Formation, Characteristics, and Influencing Factors. *Sustainability (Switzerland)* , *16*(5). https://doi.org/10.3390/su16052163

Tang, Y., Liu, Y., Chen, Y., Zhang, W., Zhao, J., He, S., Yang, C., Zhang, T., Tang, C., Zhang, C., & Yang, Z. (2021). A review: Research progress on microplastic pollutants in aquatic environments. *Science of the Total Environment*, *766*, 142572. https://doi.org/10.1016/j.scitotenv.2020.142572

Thomas, S. A., Newbold, J. D., Monaghan, M. T., Minshall, G. W., Georgian, T., & Cushing, C. E. (2001). The influence of particle size on seston deposition in streams. *Limnology and Oceanography*, *46*(6), 1415–1424. https://doi.org/10.4319/lo.2001.46.6.1415

Thomson, J. R., Taylor, M. P., Fryirs, K. A., & Brierley, G. J. (2001). A geomorphological framework for river characterization and habitat assessment. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *11*(5), 373–389. https://doi.org/10.1002/aqc.467

Tibbetts, J., Krause, S., Lynch, I., & Smith, G. H. S. (2018). Abundance, distribution, and drivers of microplastic contamination in urban river environments. *Water (Switzerland)*, *10*(11). https://doi.org/10.3390/w10111597

Tien, C. J., Wang, Z. X., & Chen, C. S. (2020). Microplastics in water, sediment and fish from the Fengshan River system: Relationship to aquatic factors and accumulation of polycyclic aromatic hydrocarbons by fish. *Environmental Pollution*, *265*, 114962. https://doi.org/10.1016/j.envpol.2020.114962

Tirkey, A., & Upadhyay, L. S. B. (2021). Microplastics: An overview on separation, identification and characterization of microplastics. *Marine Pollution Bulletin*, *170*(June), 112604. https://doi.org/10.1016/j.marpolbul.2021.112604

Tongo, I., & Erhunmwunse, N. O. (2022). Effects of ingestion of polyethylene microplastics on survival rate, opercular respiration rate and swimming performance of African catfish (Clarias gariepinus). *Journal of Hazardous Materials*, *423*(PB), 127237. https://doi.org/10.1016/j.jhazmat.2021.127237

Townsend, C. R., & Hildrew, A. G. (1994). Species traits in relation to a habitat templet for river systems. *Freshwater Biology*, *31*(3), 265–275. https://doi.org/10.1111/j.1365-2427.1994.tb01740.x

USEPA - United States Enivronmental Protection Agency. (1983). *EPA methods for chemcial analysis of water and wastes. Residue*.

van Emmerik, T., Mellink, Y., Hauk, R., Waldschläger, K., & Schreyers, L. (2022). Rivers as Plastic Reservoirs. *Frontiers in Water*, *3*(January), 1–8. https://doi.org/10.3389/frwa.2021.786936

Verberk, W. C. E. P., Van Noordwijk, C. G. E., & Hildrew, A. G. (2013). Delivering on a promise: Integrating species traits to transform descriptive community ecology into a predictive science. *Freshwater Science*, *32*(2), 531–547. https://doi.org/10.1899/12-092.1

Vieira, B. N. K. M., Poff, N. L., Carlisle, D. M., Ii, S. R. M., Koski, M. L., Kondratieff, B. C., & Ii, S. R. M. (2006). A Database of Lotic Invertebrate Traits for North America. *Director*, 19. http://pubs.water.usgs.gov/ds187

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*(5), 882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x

Vlok, W. (2002). Guides to the freshwater invertebrates of Southern Africa. Volume 4: Crustacea III. Bathynellacea, Amphipoda, Isopoda, Spelaeogriphacea, Tanaidacea and Decapoda. In *African Journal of Aquatic Science* (Vol. 27, Issue 1). https://doi.org/10.2989/16085914.2002.9626580

Wadeson, R. A. (1994). A geomorphological approach to the identification and classification of instream flow environments. *Southern African Journal of Aquatic Sciences*, *20*(1–2), 38–61. https://doi.org/10.1080/10183469.1994.9631349

Wadeson, R. A. (1996). The development of the hydraulic biotope concept within a catchment based hierarchical geomorphological model. In *PHD Thesis*. Rhodes University, South Africa.

Wadeson, R. A., & Rowntree, K. M. (1998). Application of the hydraulic biotope concept to the classification of instream habitats. *Aquatic Ecosystem Health and Management*, *1*(2), 143–157. https://doi.org/10.1080/14634989808656911

Wagner, M., & Lambert, S. (2018). Freshwater Microplastics. In *Springer Nature* (Vol. 58). http://www.springer.com/series/698

Wallace, J. B., & Webster, J. R. (1996). The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, *41*(1), 115–139. https://doi.org/10.1146/annurev.en.41.010196.000555

Wang, T., Wang, J., Lei, Q., Zhao, Y., Wang, L., Wang, X., & Zhang, W. (2021). Microplastic pollution in sophisticated urban river systems: Combined influence of land-use types and physicochemical characteristics. *Environmental Pollution*, *287*(June), 117604. https://doi.org/10.1016/j.envpol.2021.117604

Wang, Z. M., Wagner, J., Ghosal, S., Bedi, G., & Wall, S. (2017). SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. *Science of the Total Environment*, *603–604*, 616–626. https://doi.org/10.1016/j.scitotenv.2017.06.047

Wegscheider, B., Monk, W. A., Lento, J., Haralampides, K., Ndong, M., Linnansaari, T., & Allen Curry, R. (2023). Developing environmental flow targets for benthic macroinvertebrates in large rivers using hydraulic habitat associations and taxa thresholds. *Ecological Indicators*, *146*, 109821. https://doi.org/10.1016/j.ecolind.2022.109821

Weideman, E. A., Perold, V., & Ryan, P. G. (2019). Little evidence that dams in the Orange–Vaal River system trap floating microplastics or microfibres. *Marine Pollution Bulletin*, *149*(October), 110664. https://doi.org/10.1016/j.marpolbul.2019.110664

Welden, N. A. C., & Cowie, P. R. (2016a). Environment and gut morphology influence microplastic retention in langoustine, Nephrops norvegicus. *Environmental Pollution*, *214*, 859–865. https://doi.org/10.1016/j.envpol.2016.03.067

Welden, N. A. C., & Cowie, P. R. (2016b). Long-term microplastic retention causes reduced body condition in the langoustine, Nephrops norvegicus. *Environmental Pollution*, *218*, 895–900. https://doi.org/10.1016/j.envpol.2016.08.020

Windsor, F. M., Tilley, R. M., Tyler, C. R., & Ormerod, S. J. (2019). Microplastic ingestion by riverine macroinvertebrates. *Science of the Total Environment*, *646*, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society. Series B: Statistical Methodology*, *73*(1), 3–36. https://doi.org/10.1111/j.1467-

9868.2010.00749.x

Xia, F., Yao, Q., Zhang, J., & Wang, D. (2021). Effects of seasonal variation and resuspension on microplastics in river sediments. *Environmental Pollution*, *286*(May), 117403. https://doi.org/10.1016/j.envpol.2021.117403

Yahaya, A., Okoh, O. O., Agunbiade, F. O., & Okoh, A. I. (2019). Occurrence of phenolic derivatives in Buffalo River of Eastern Cape South Africa: Exposure risk evaluation. *Ecotoxicology and Environmental Safety*, *171*(August 2018), 887–893. https://doi.org/10.1016/j.ecoenv.2019.01.037

Yan, M., Wang, L., Dai, Y., Sun, H., & Liu, C. (2021a). Behavior of Microplastics in Inland Waters: Aggregation, Settlement, and Transport. *Bulletin of Environmental Contamination and Toxicology*, *107*(4), 700–709. https://doi.org/10.1007/s00128-020-03087-2

Yan, M., Wang, L., Dai, Y., Sun, H., & Liu, C. (2021b). Behavior of Microplastics in Inland Waters: Aggregation, Settlement, and Transport. *Bulletin of Environmental Contamination and Toxicology*, *107*(4), 700–709. https://doi.org/10.1007/s00128-020-03087-2

Yano, K. A., Geronimo, F. K., Reyes, N. J., & Kim, L. H. (2021). Characterization and comparison of microplastic occurrence in point and non-point pollution sources. *Science of the Total Environment*, *797*, 148939. https://doi.org/10.1016/j.scitotenv.2021.148939

Yao, S., Li, X., Wang, T., Jiang, X., Song, Y., & Arp, H. P. H. (2023). Soil Metabolome Impacts the Formation of the Eco-corona and Adsorption Processes on Microplastic Surfaces. *Environmental Science and Technology*, *57*(21), 8139–8148. https://doi.org/10.1021/acs.est.3c01877

Yonkos, L. T., Friedel, E. A., Perez-Reyes, A. C., Ghosal, S., & Arthur, C. D. (2014). Microplastics in four estuarine rivers in the chesapeake bay, U.S.A. *Environmental Science and Technology*, *48*(24), 14195–14202. https://doi.org/10.1021/es5036317

Zhang, C., Zhou, H., Cui, Y., Wang, C., Li, Y., & Zhang, D. (2019). Microplastics in offshore sediment in the Yellow Sea and East China Sea, China. *Environmental Pollution*, *244*, 827–833. https://doi.org/10.1016/j.envpol.2018.10.102

Zhang, H. (2017). Transport of microplastics in coastal seas. *Estuarine, Coastal and Shelf*

*Science*, *199*, 74–86. https://doi.org/10.1016/j.ecss.2017.09.032

**APPENDICES**

**Appendix A.** Kruskal-Wallis and Dunn's analysis of physicochemical parameters

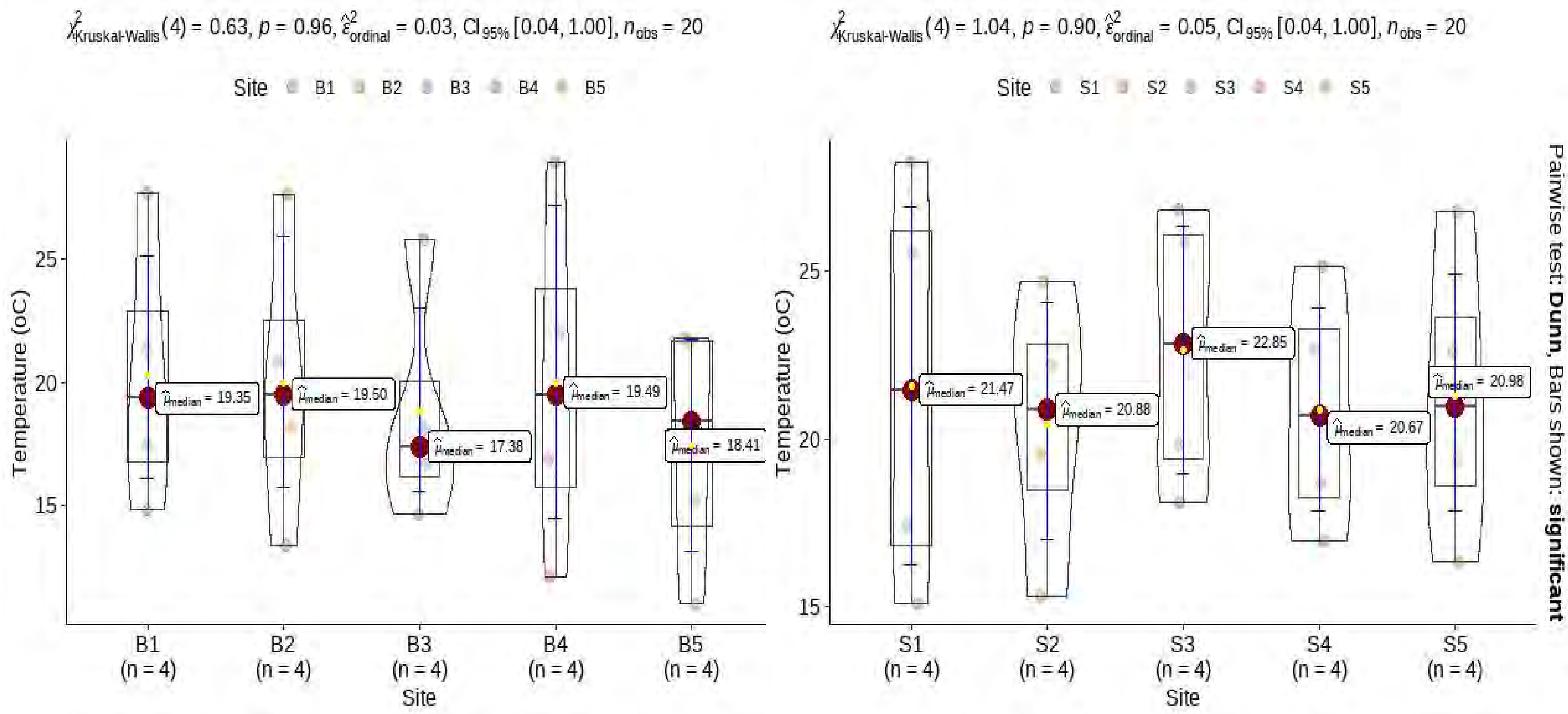


**Figure A1.** Comparison of Temperature (°C) across the sites in the Buffalo and the Swartkops Rivers

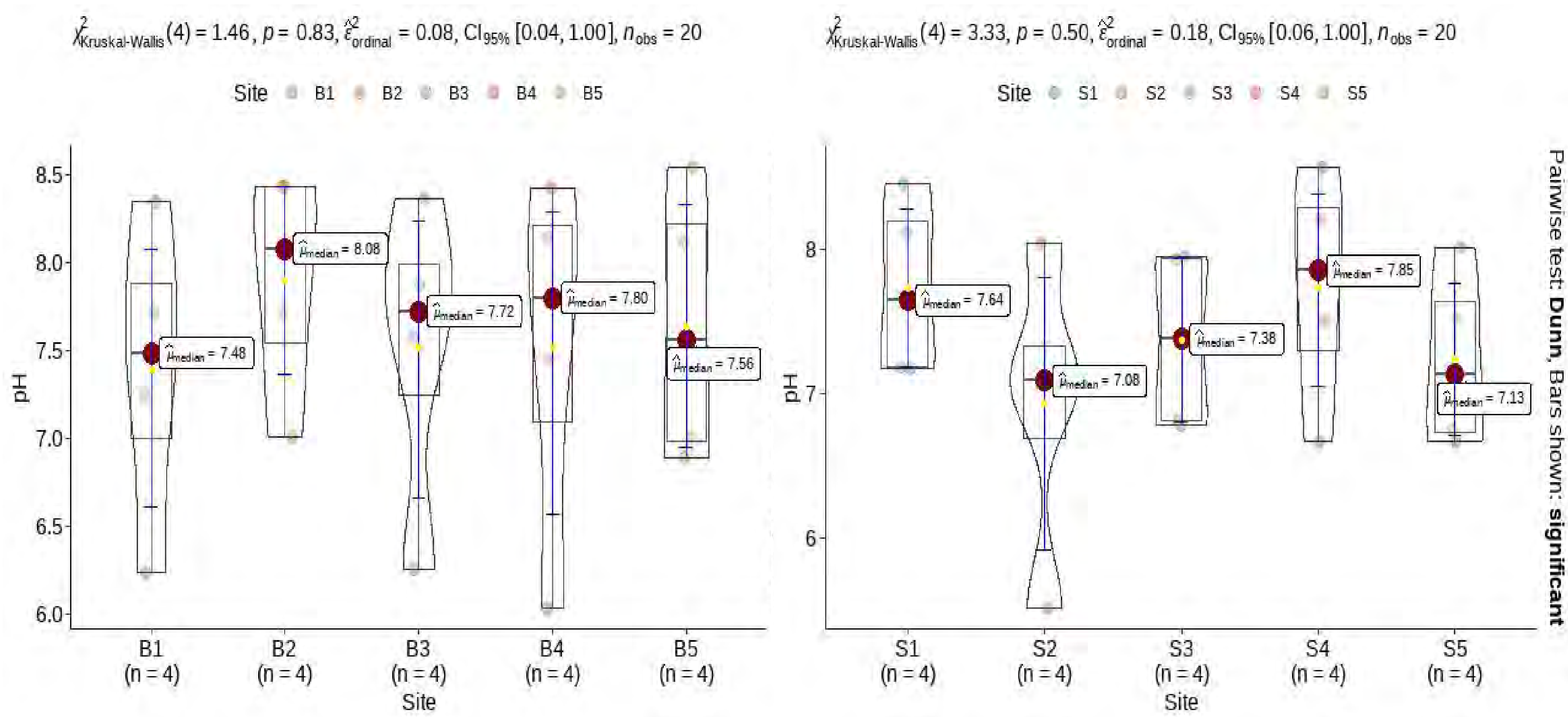


**Figure A2.** Comparison of pH across the sites in the Buffalo and the Swartkops Rivers

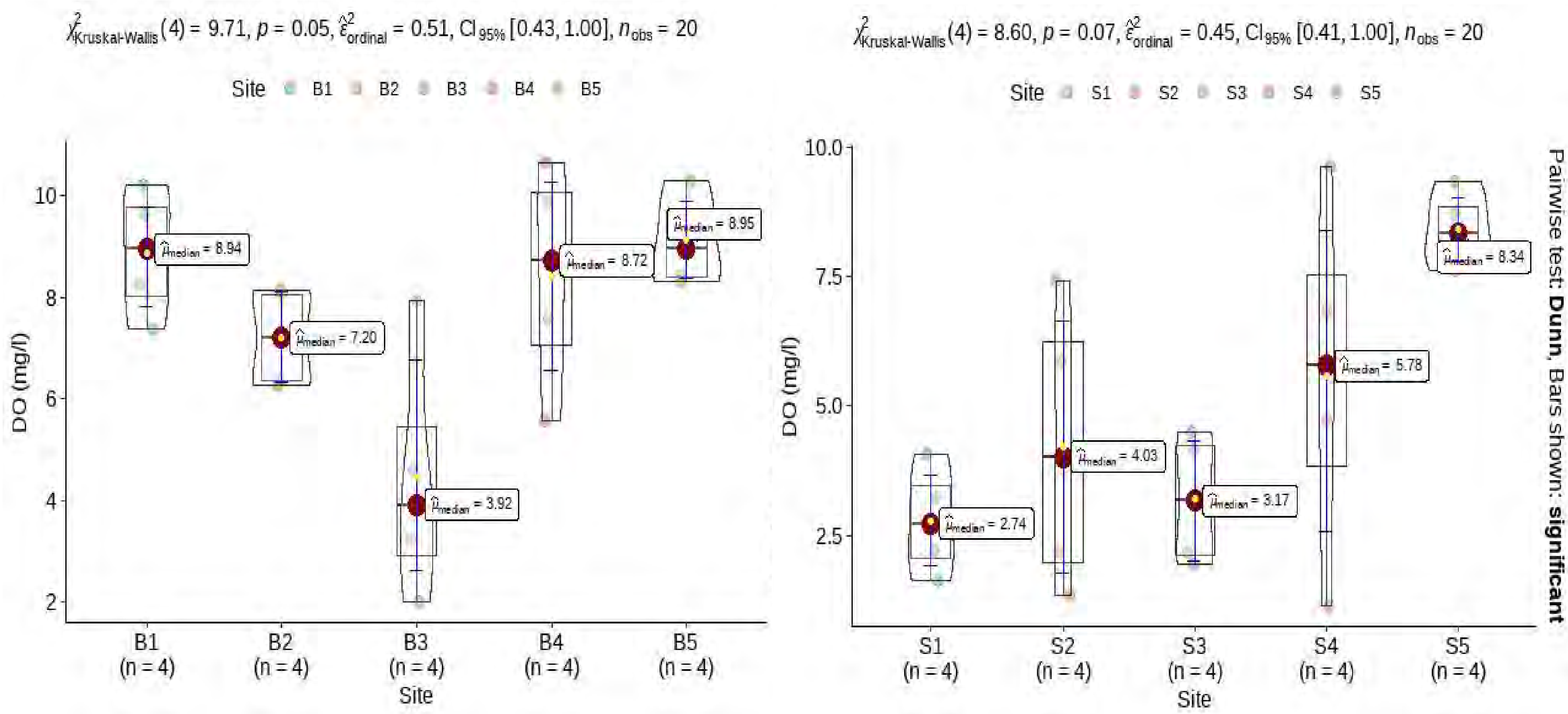


**Figure A3.** Comparison of DO (mg/l) across the sites in the Buffalo and the Swartkops Rivers

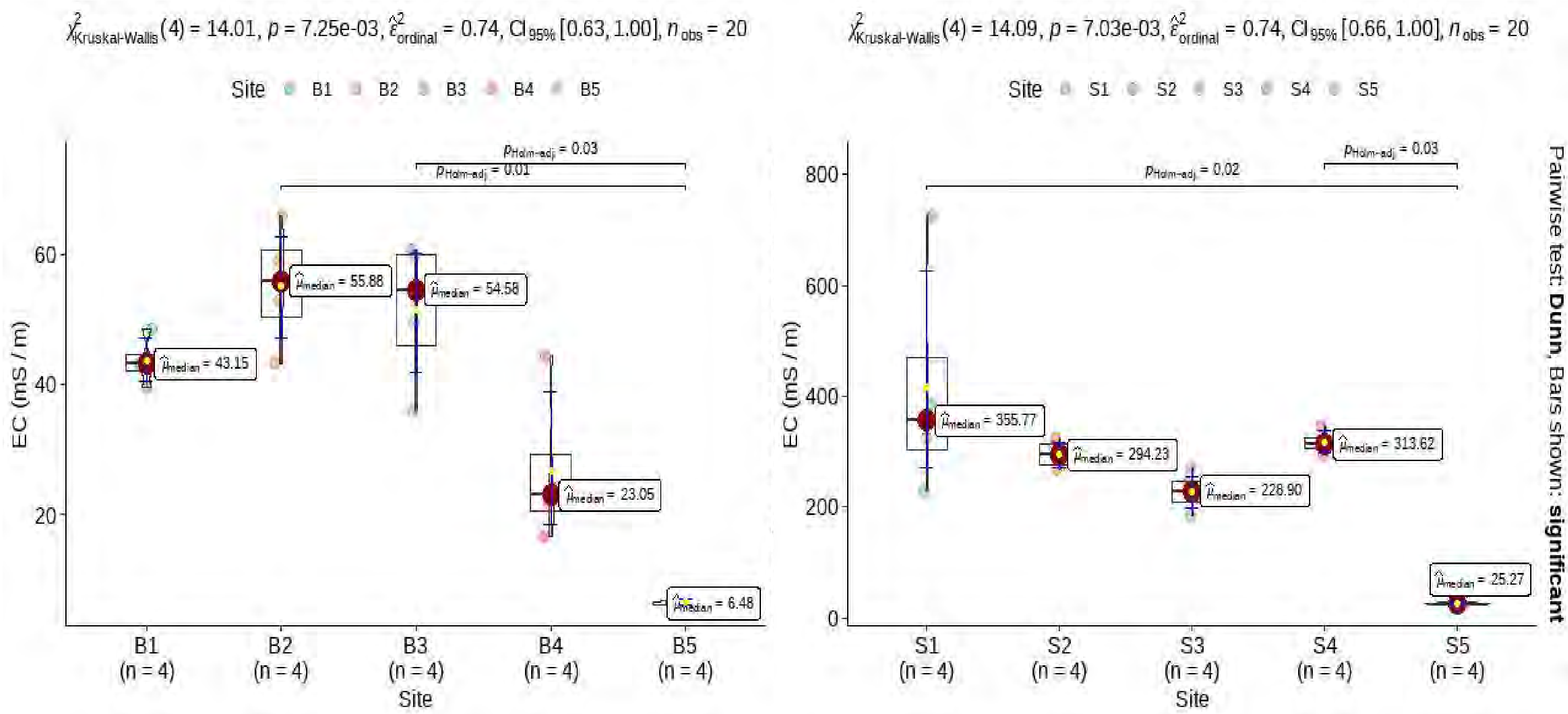


**Figure A4.** Comparison of EC (mS/m) across the sites in the Buffalo and the Swartkops Rivers

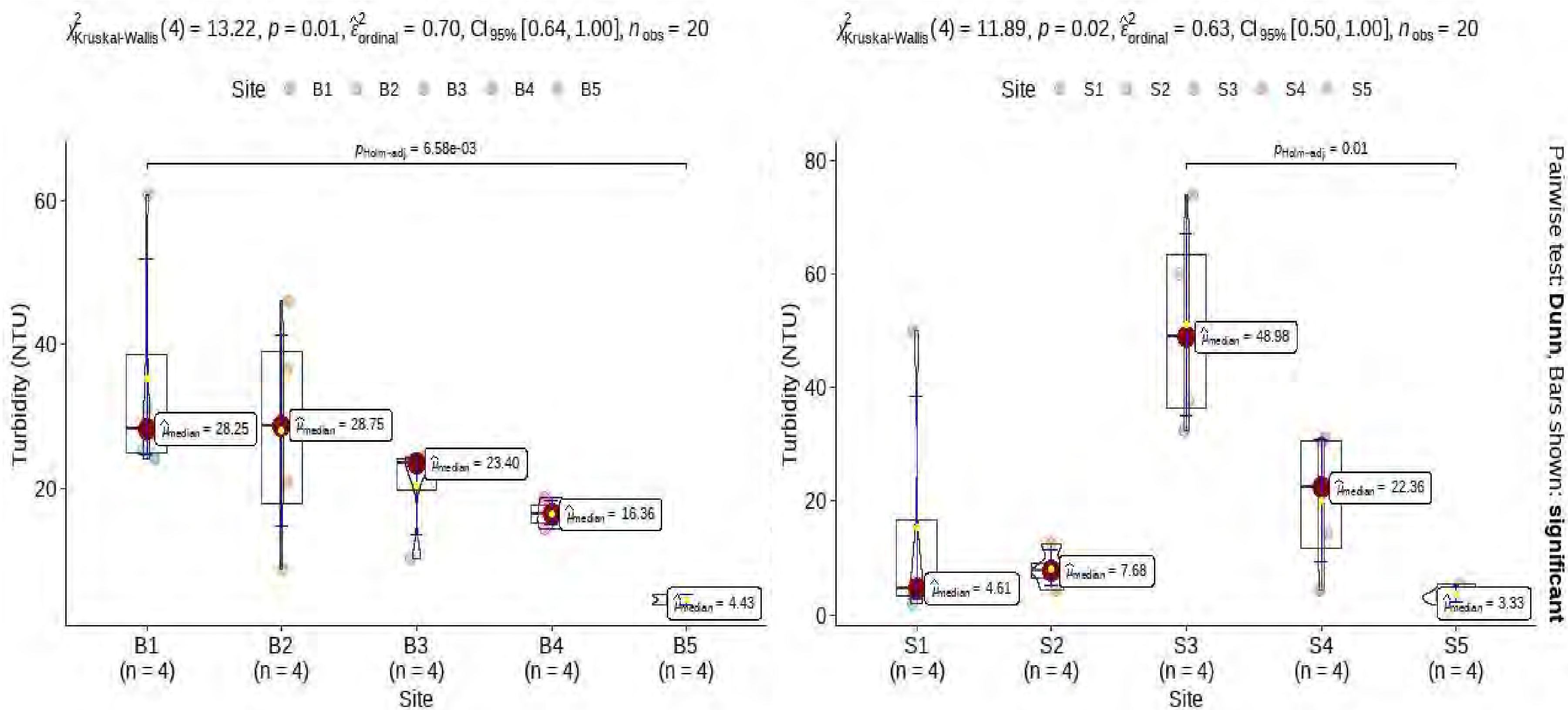


**Figure A5.** Comparison of Turbidity (NTU) across the sites in the Buffalo and the Swartkops Rivers

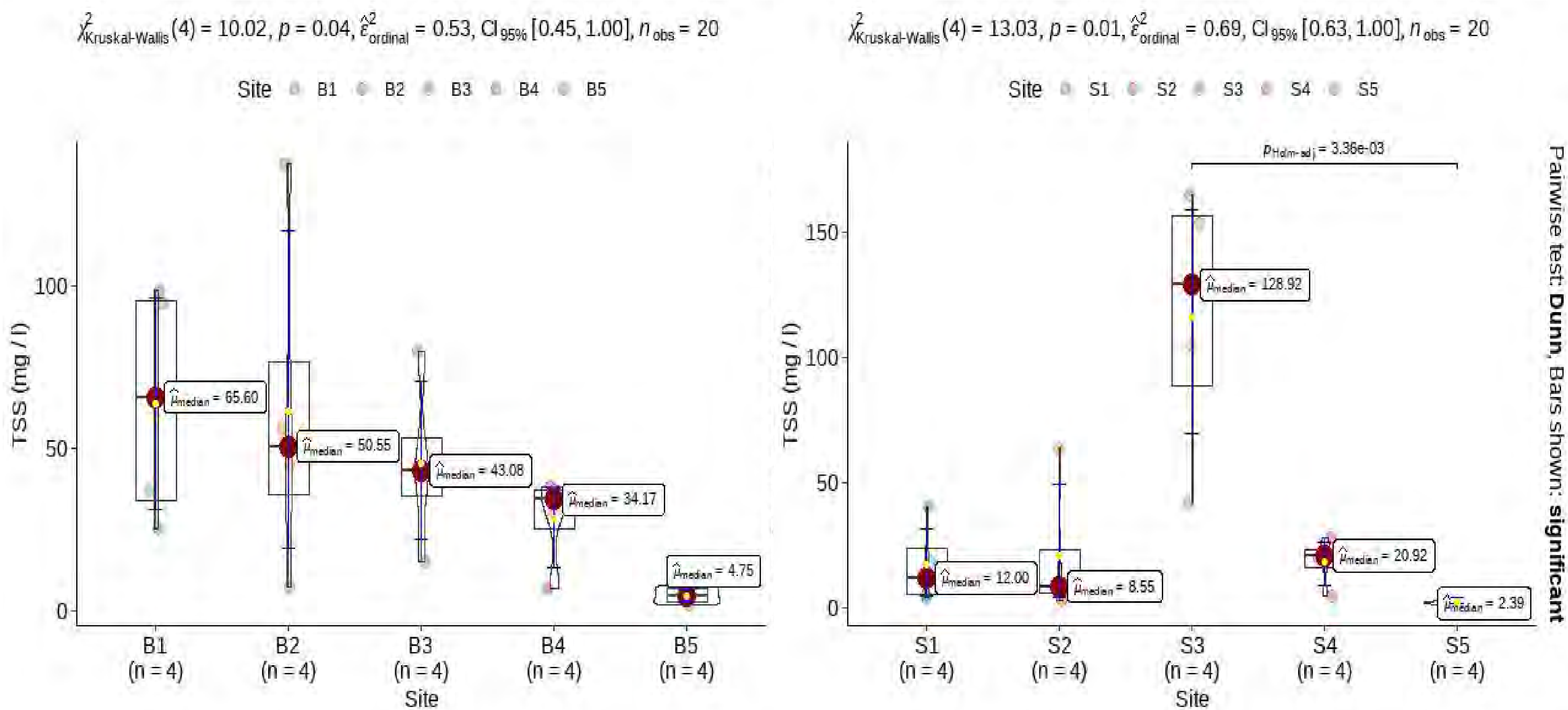


**Figure A6.** Comparison of TSS (mg/l) across the sites in the Buffalo and the Swartkops Rivers

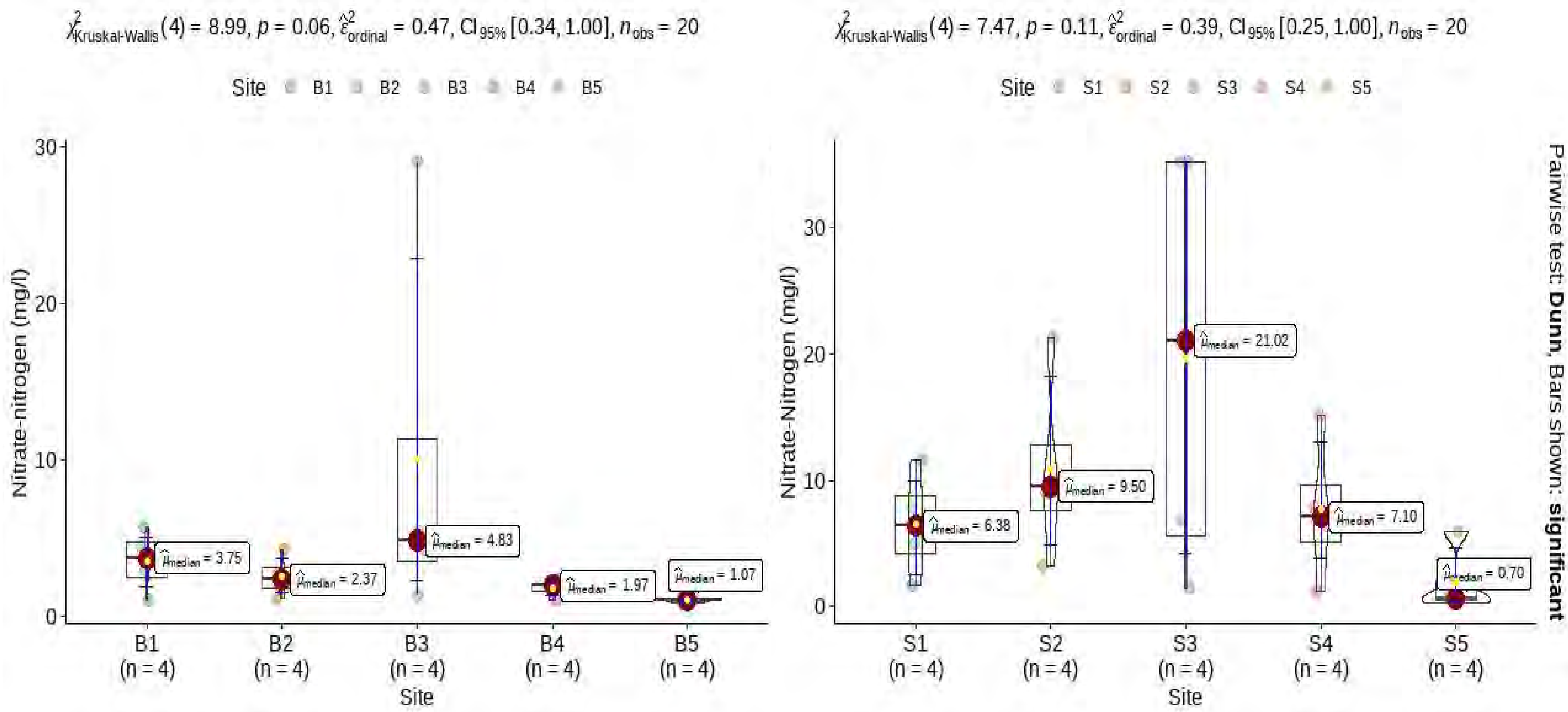


**Figure A7.** Comparison of $NO_3^-N$ (mg/l) across the sites in the Buffalo and the Swartkops Rivers

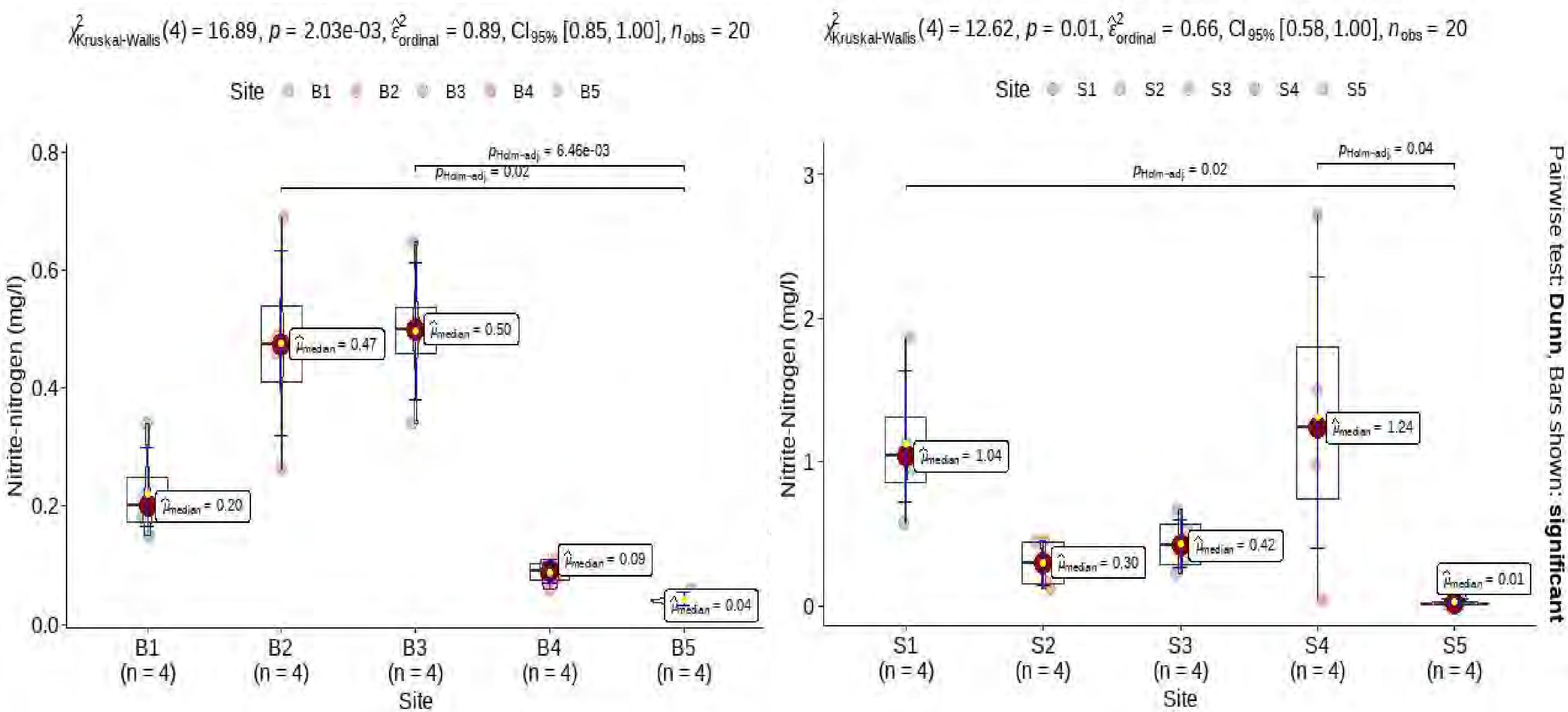


**Figure A8.** Comparison of $NO_2^-N$ (mg/l) across the sites in the Buffalo and the Swartkops Rivers

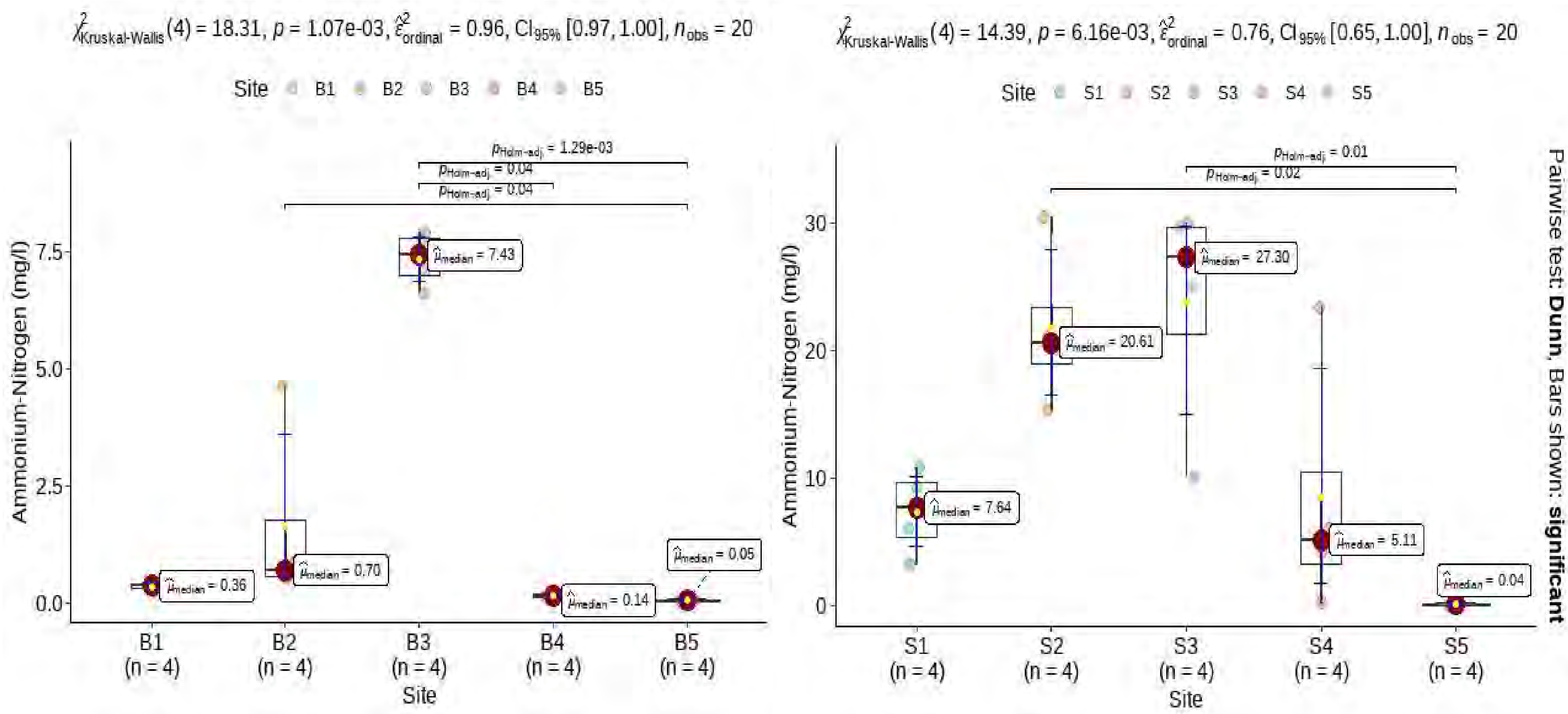


**Figure A9.** Comparison of $NH_4^-N$ (mg/l) across the sites in the Buffalo and the Swartkops Rivers

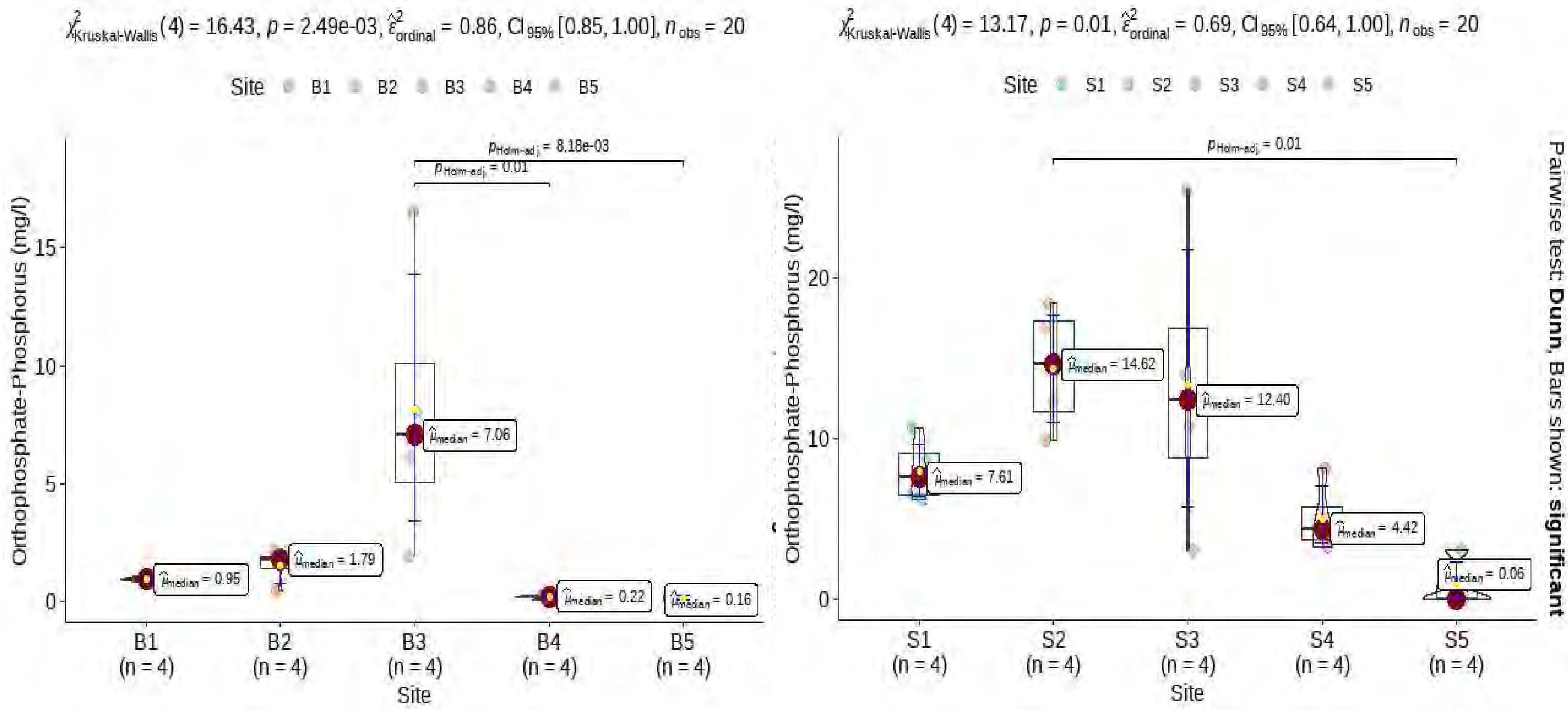


**Figure A10.** Comparison of $PO_4^-$-P (mg/l) across the sites in the Buffalo and the Swartkops Rivers

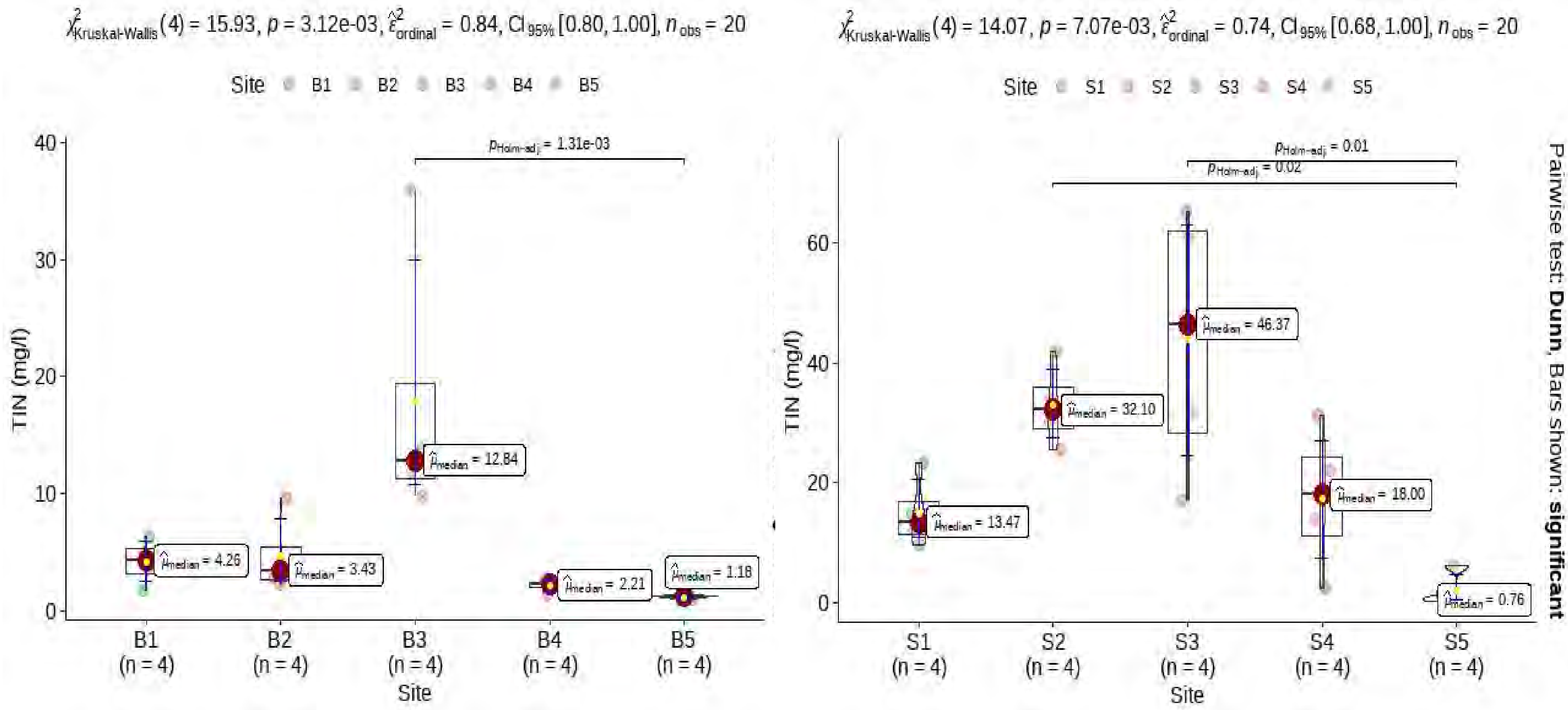


**Figure A11.** Comparison of TIN (mg/l) across the sites in the Buffalo and the Swartkops Rivers

**Appendix B.** Polymer spectra of selected microplastics

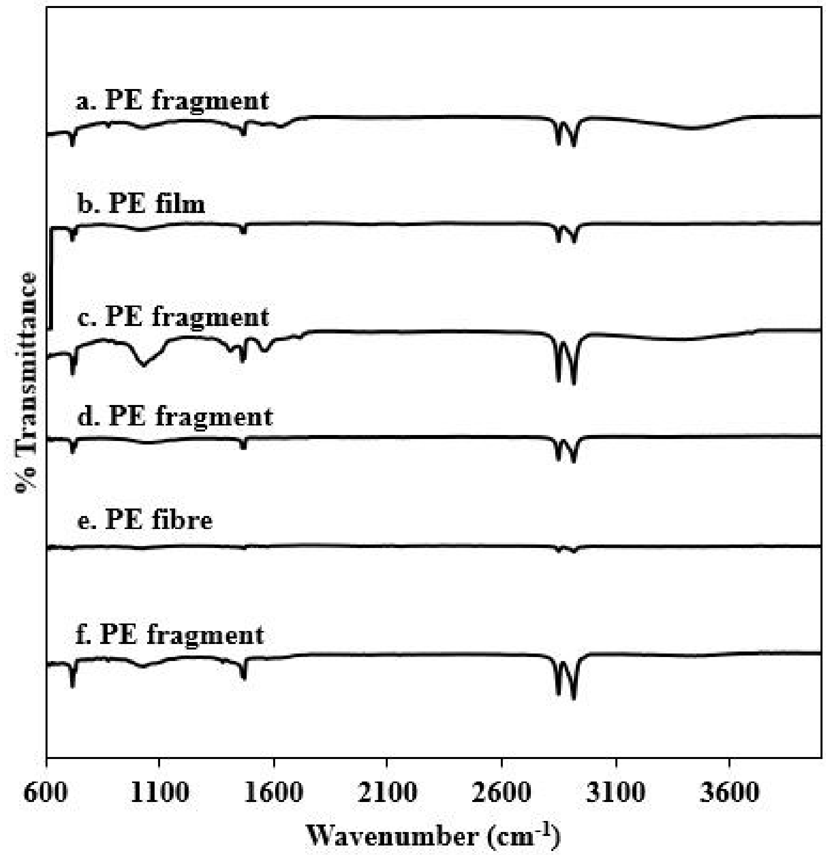


**Figure B1.** Polymer spectra of selected polyethylene particles (spectra "a – f" are indicated for microplastic particles in Appendix C, Plate C1 – C6)

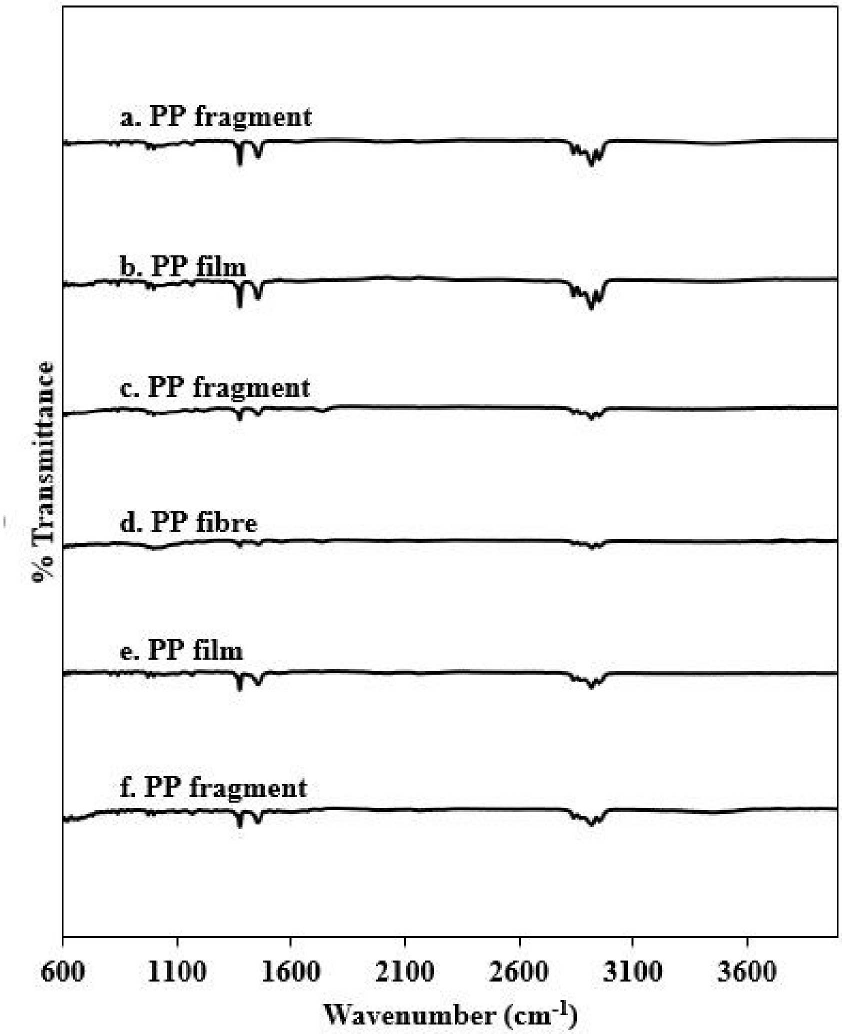


**Figure B2.** Polymer spectra of selected polypropylene particles (spectra "a – f" are indicated for microplastics particles in Appendix C, Plate C7 – C12)

**Appendix C.** Examples of microplastic particles from water samples that were subjected to FTIR polymer confirmation test.

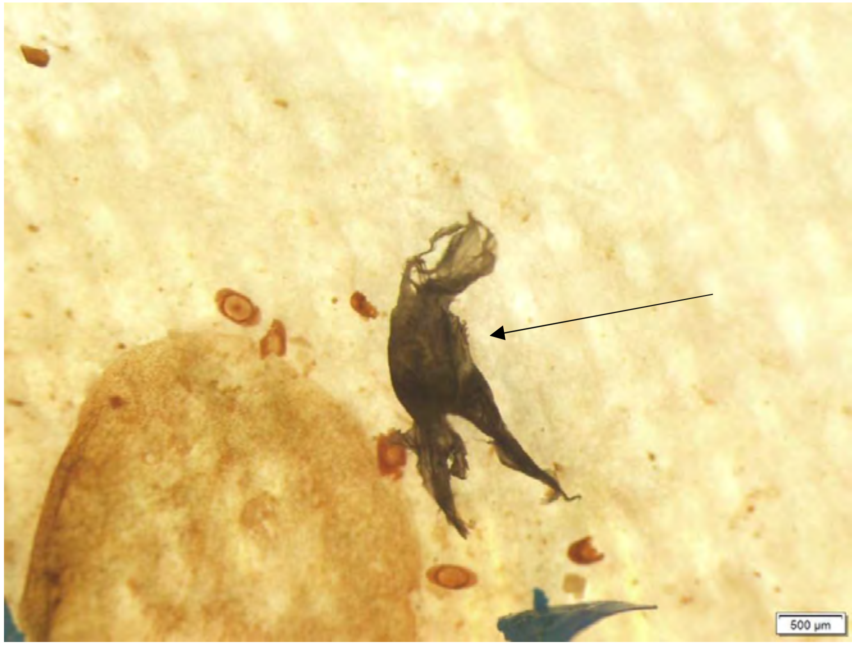


**Plate C1.** Polyethylene fragment (FTIR spectrum indicated as alphabet 'a' in Appendix B, Figure B1)

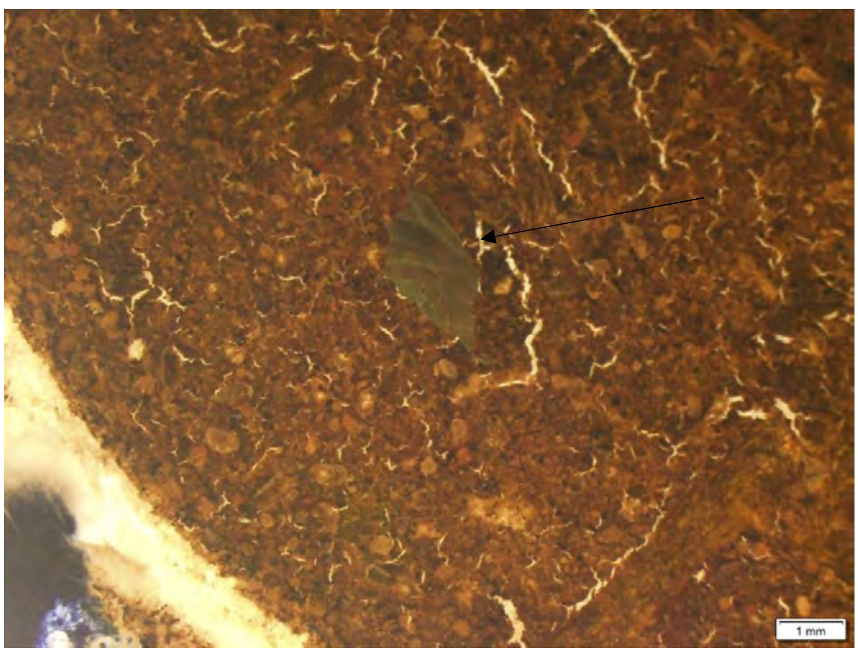


**Plate C2.** Polyethylene film (FTIR spectrum indicated as alphabet 'b' in Appendix B, Figure B1)

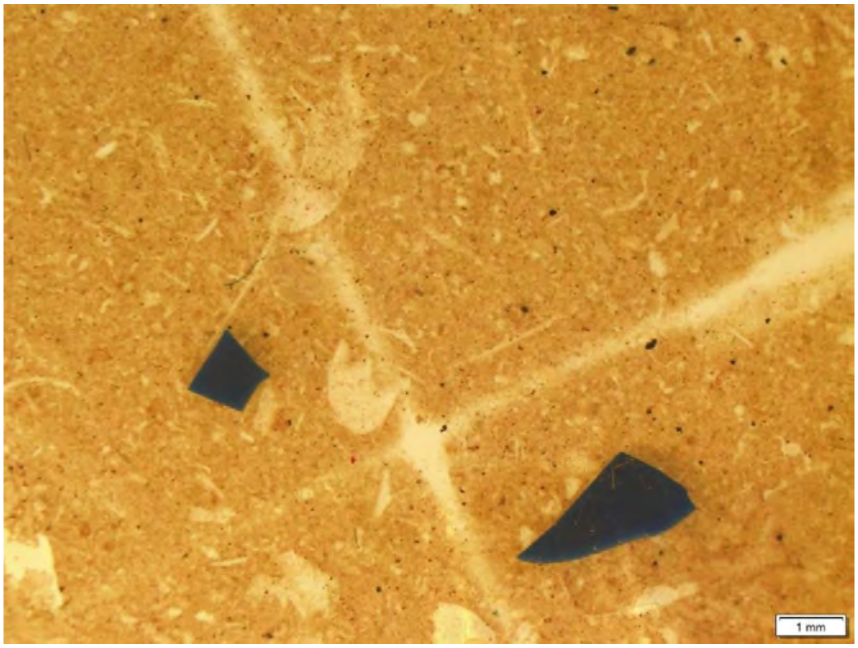

**Plate C3.** Polyethylene fragment (FTIR spectrum indicated as alphabet 'c' in Appendix B, Figure B1)

**Plate C4.** Polyethylene fragment (FTIR spectrum indicated as alphabet 'd' in Appendix B, Figure B1)

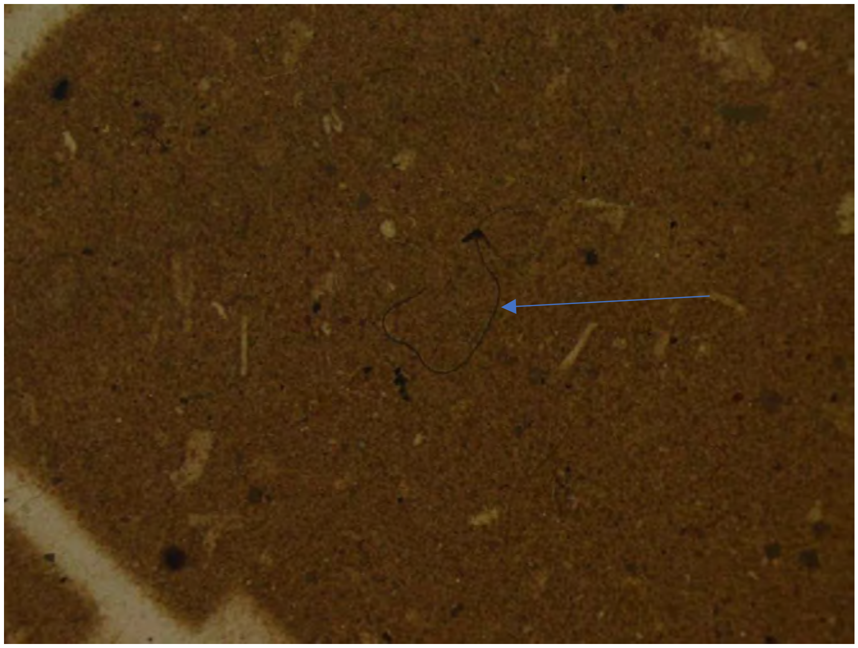

**Plate C5.** Polyethylene fibre (FTIR spectrum indicated as alphabet 'e' in Appendix B, Figure B1)

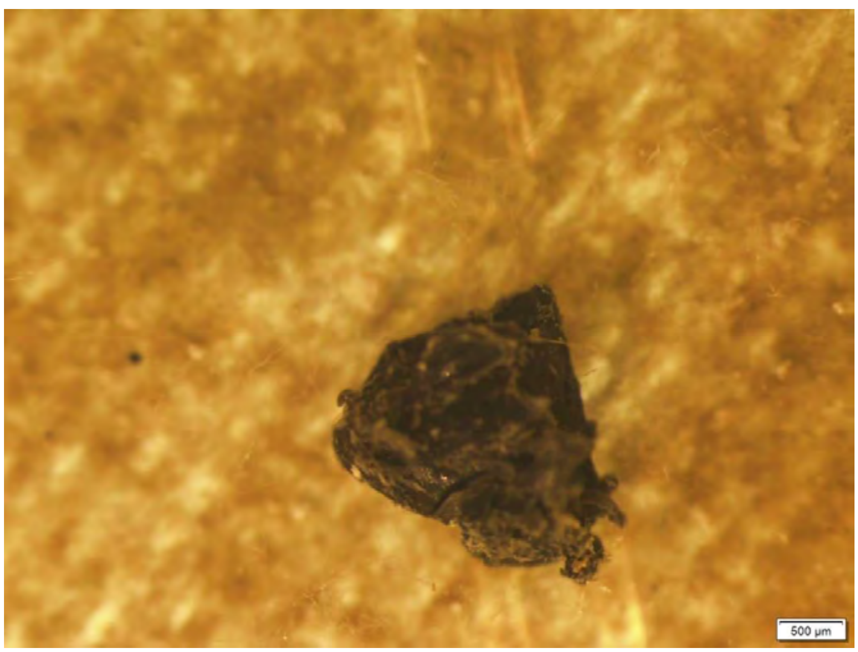


**Plate C6.** Polyethylene fragment (FTIR spectrum indicated as alphabet 'f' in Appendix B, Figure B1)

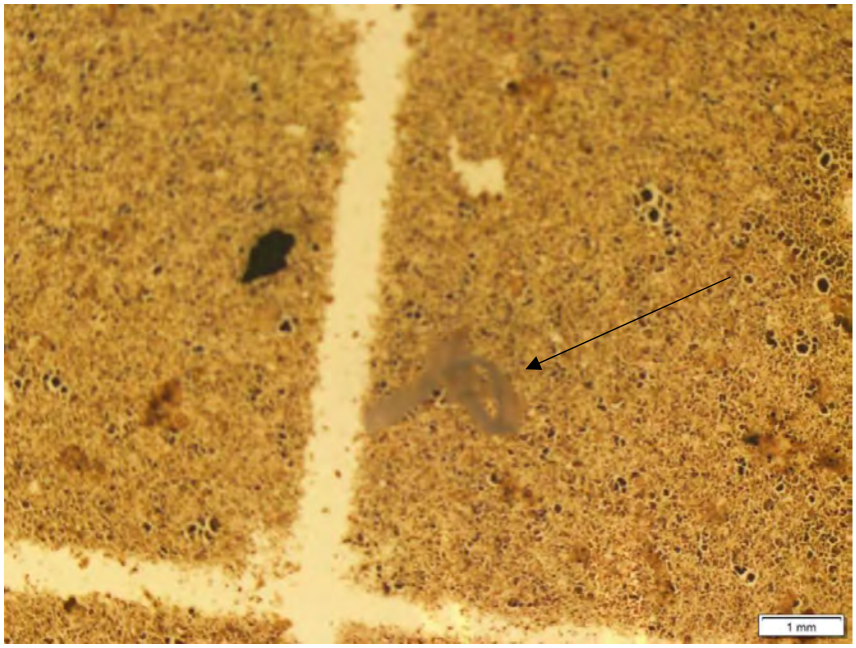

**Plate C7.** Polypropylene fragment (FTIR spectrum indicated as alphabet 'a' in Appendix B, Figure B2)

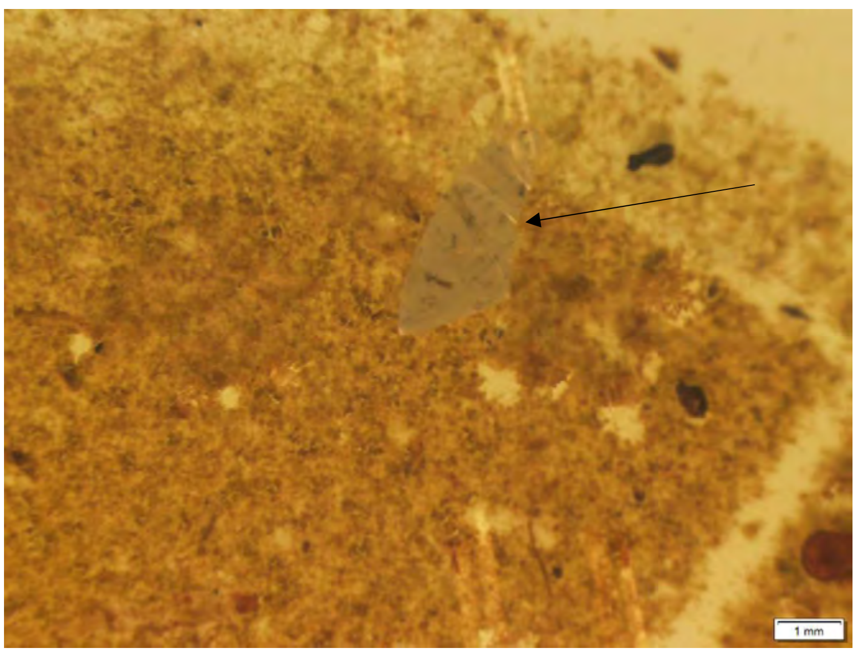

**Plate C8.** Polypropylene film (FTIR spectrum indicated as alphabet 'b' in Appendix B, Figure B2)

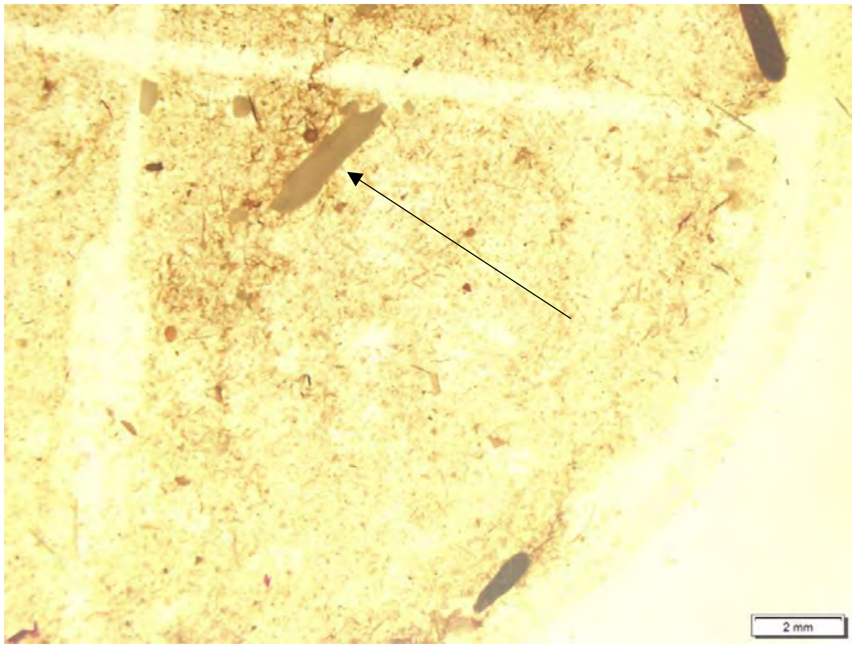

**Plate C9.** Polypropylene fragment (FTIR spectrum indicated as alphabet ‘c’ in Appendix B, Figure B2)

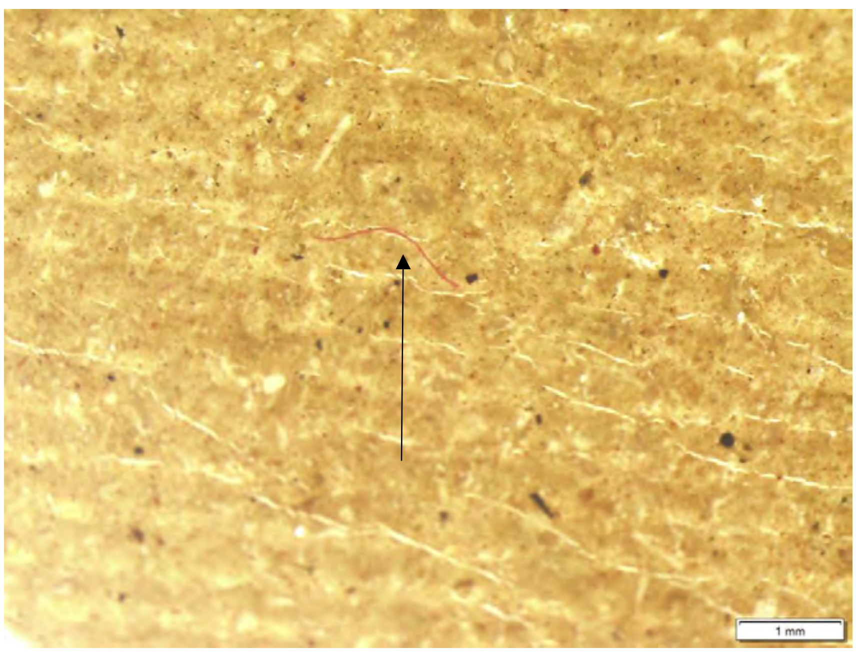

**Plate C10.** Polypropylene fibre (FTIR spectrum indicated as alphabet ‘d’ in Appendix B, Figure B2)

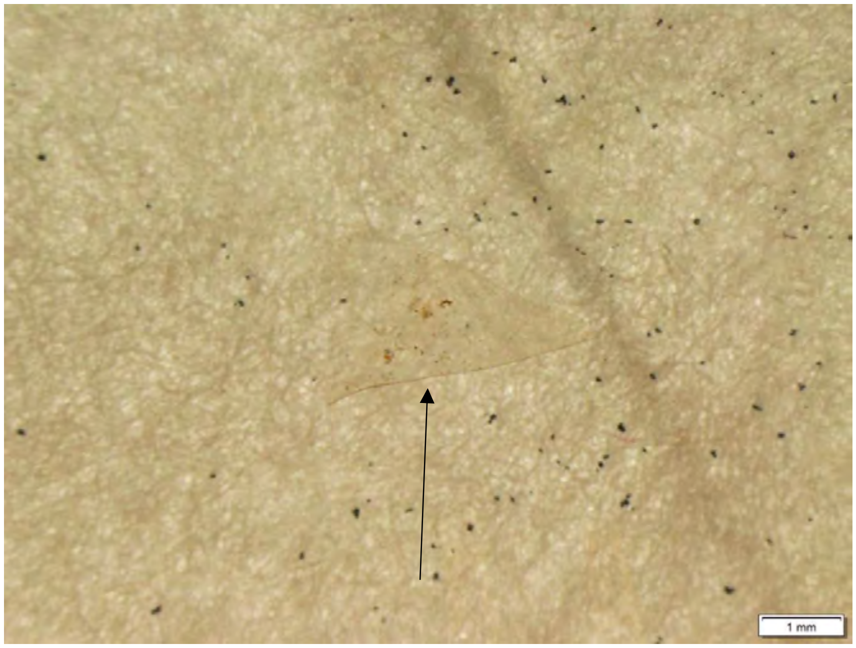


**Plate C11.** Polypropylene film (FTIR spectrum indicated as alphabet ‘e’ in Appendix B2, Figure B2)


**Plate C12.** Polypropylene fragment (FTIR spectrum indicated as alphabet ‘f’ in Appendix B, Figure B2)

**Appendix D**. Mann Whitney U comparisons of the hydraulic indices between the hydraulic zones

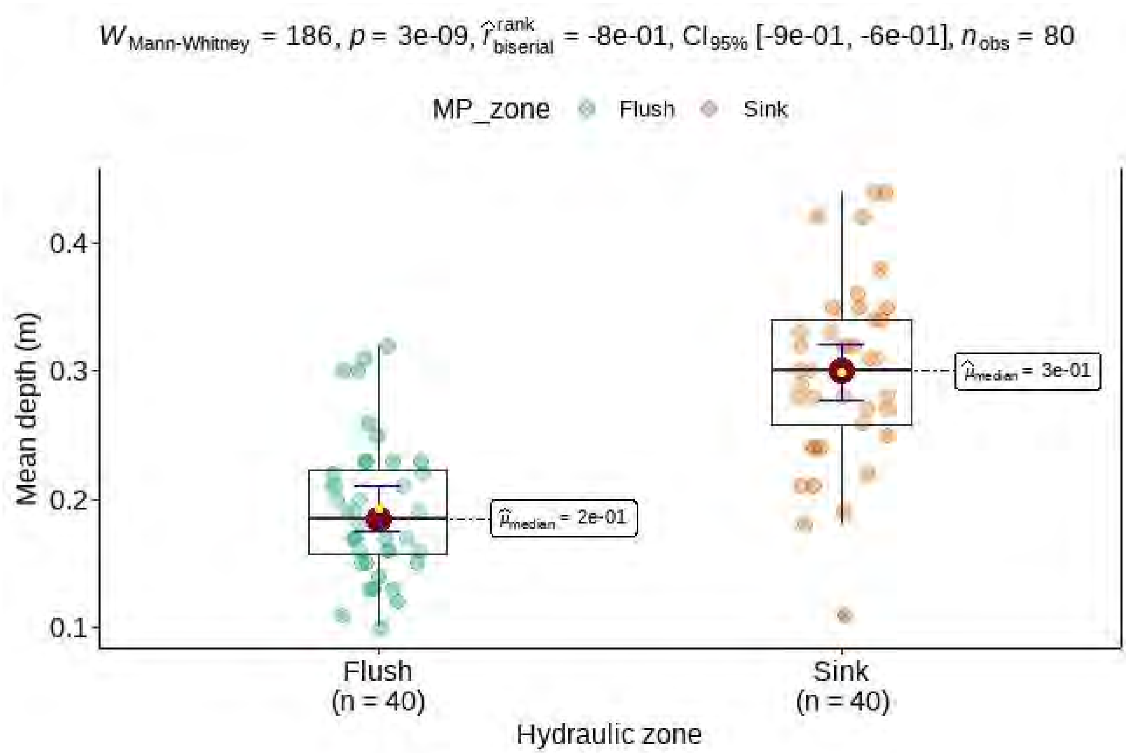


**Figure D1.** Comparison of depth between the hydraulic zones

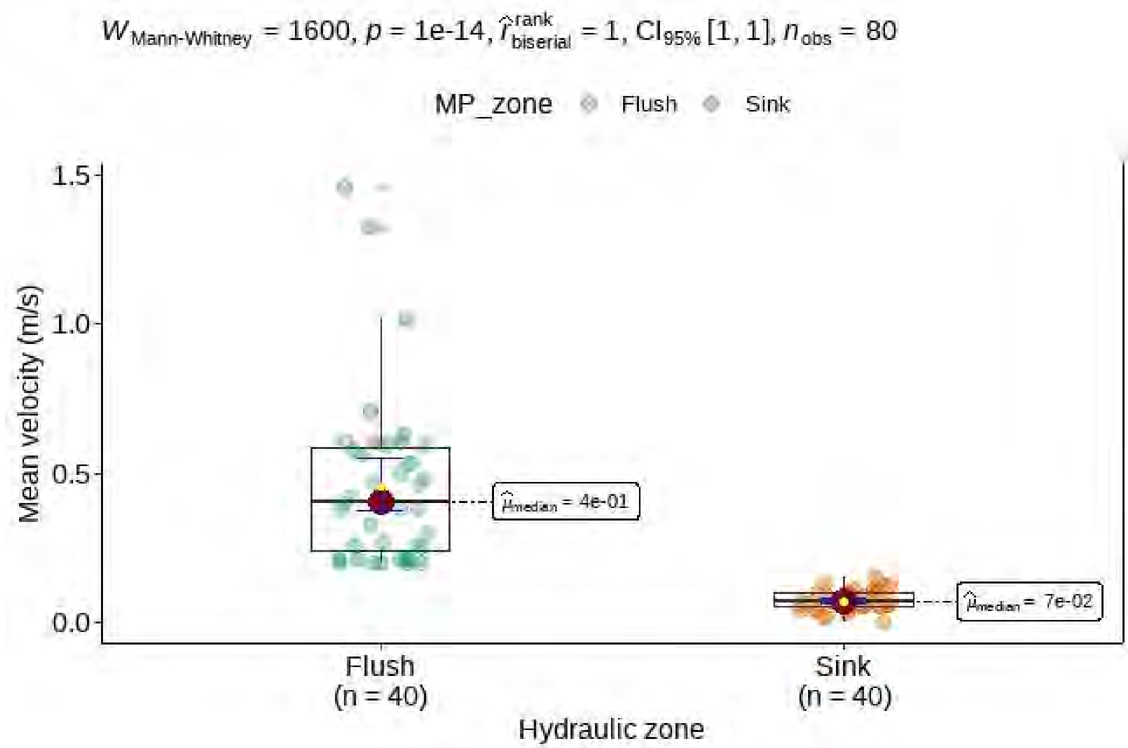


**Figure D2.** Comparison of velocity between the hydraulic zones

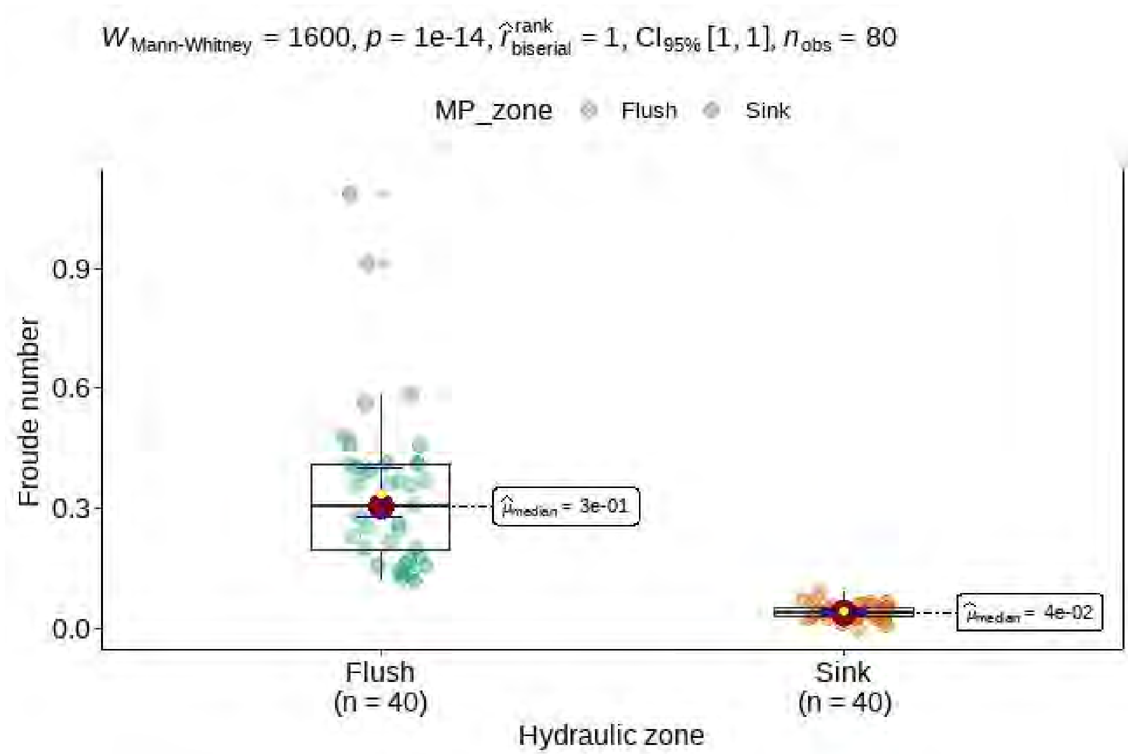


**Figure D3.** Comparison of the Froude number (*Fr*) between the hydraulic zones

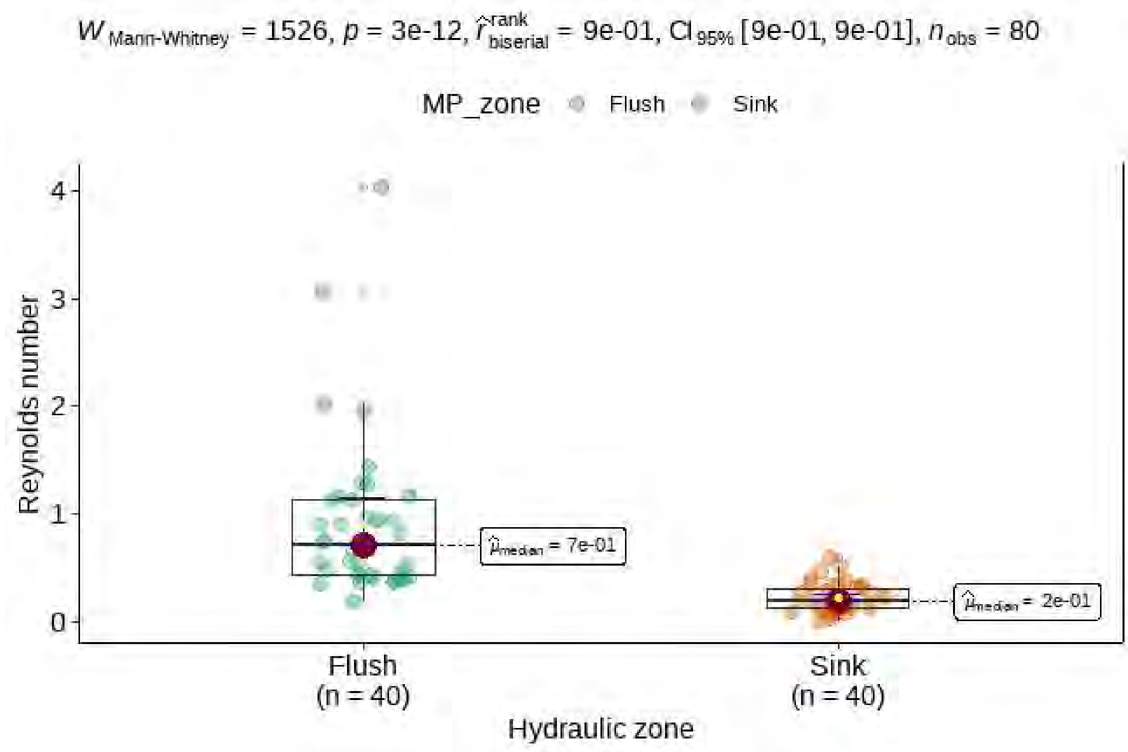


**Figure D4.** Comparison of the Reynolds number (*Re*) between the hydraulic zones

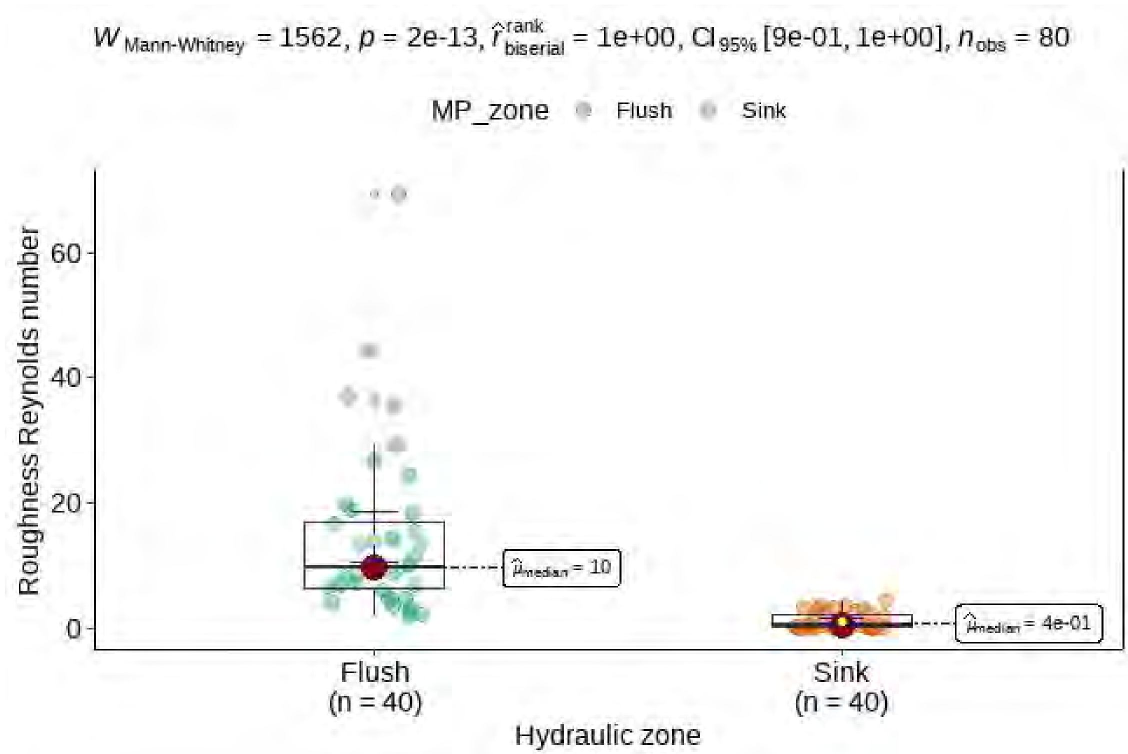


**Figure D5.** Comparison of the ‘roughness’ Reynolds number (*Re**) between the hydraulic zones

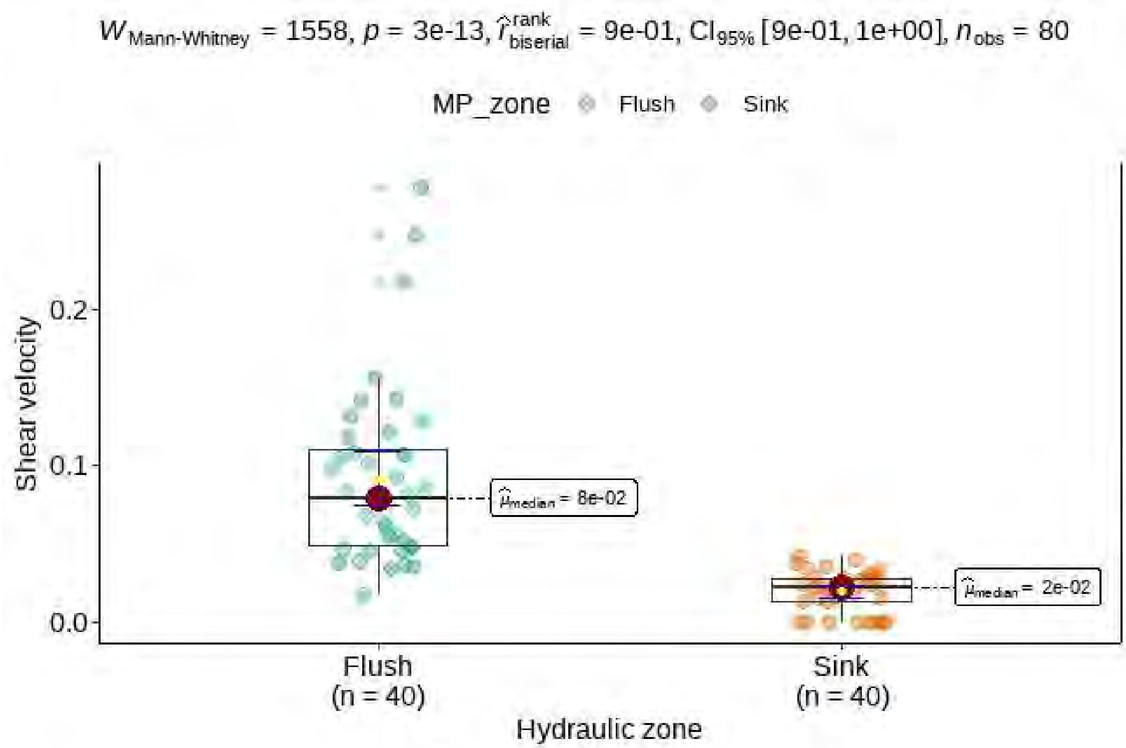


**Figure D6.** Comparison of the Shear velocity (*U**) between the hydraulic zones

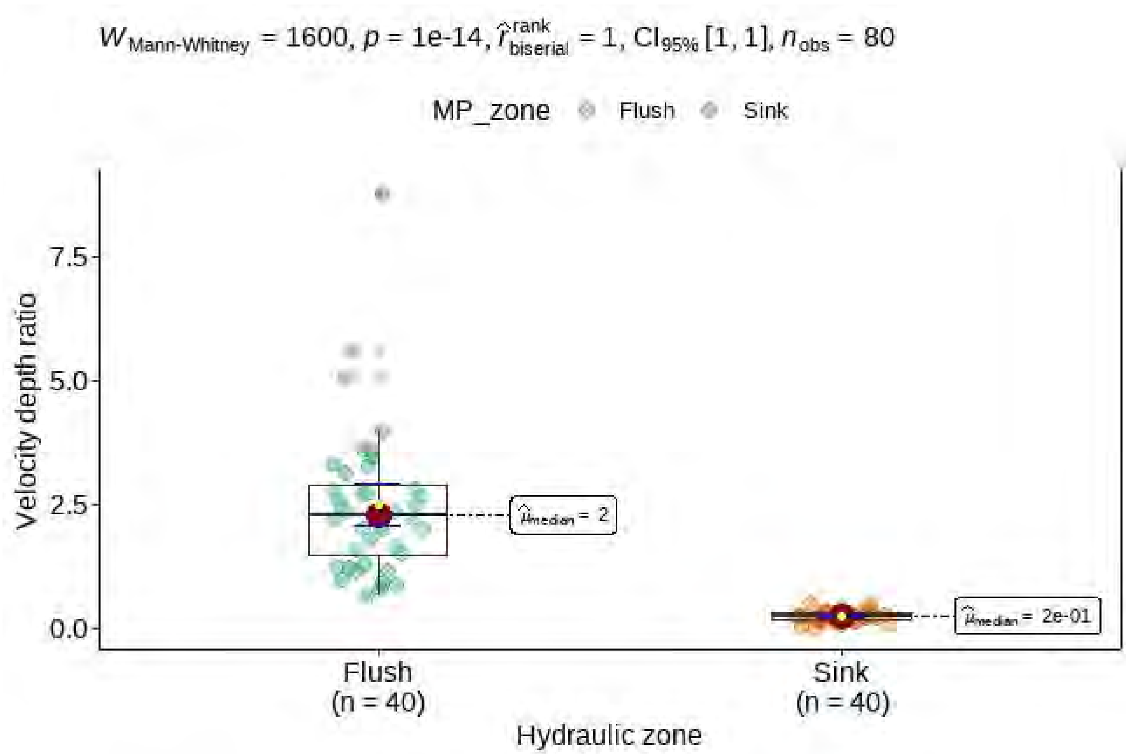


**Figure D7.** Comparison of the velocity depth ratio (*VDR*) between the hydraulic zones

**Appendix E.** Range of data derived for the hydraulic zones.

**Table E1.** Range of data derived for the hydraulic zones (spring, wet season)

| **Sites** | **B1** | **B2** | **B3** | **B4** | **B5** | **S1** | **S2** | **S3** | **S4** | **S5** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush |
| **Geomorphic unit type** | Rapid | Riffle | Riffle | Run | Run | Rapid | Run | Run | Run | Run |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 30.00 | 36.00 | 26.00 | 22.00 | 22.00 | 37.00 | 27.00 | 27.00 | 31.0 | 19.00 |
| **Width(m)** | 6.90 | 13.00 | 5.60 | 9.10 | 4.00 | 17.00 | 23.10 | 4.30 | 10.4 | 4.40 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT8 | FT7 | FT5 | FT5 | FT5 | FT7 | FT5 | FT5 | FT5 | FT5 |
| **Velocity ($ms^{-1}$)** | 1.32 | 0.21 | 0.21 | 0.20 | 0.21 | 1.46 | 0.26 | 0.20 | 0.33 | 0.27 |
| **Depth (m)** | 0.15 | 0.32 | 0.21 | 0.21 | 0.16 | 0.26 | 0.17 | 0.17 | 0.16 | 0.15 |
| **Roughness height (m)** | 0.20 | 0.18 | 0.25 | 0.13 | 0.20 | 0.25 | 0.18 | 0.22 | 0.17 | 0.12 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S11 | S8, S9 | S10 | S8 | S9 | S10, S8 | S8 | S8 | S7 | S8 |
| **Sorting** | Moderate | Moderate | Poor | Poor | Moderate | Poor | Good | Moderate | Poor | Moderate |
| **Packing (1 -5)** | 1 | 1 | 1 | 4 | 1 | 5 | 2 | 3 | 4 | 2 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 10 | 15 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Single logs** | 0 | 0 | 0 | 3 | 4 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 1.09 | 0.12 | 0.15 | 0.13 | 0.17 | 0.91 | 0.20 | 0.16 | 0.26 | 0.22 |
| ***Re*** | 202661 | 66534 | 44144 | 54705 | 35257 | 403401 | 47425 | 39306 | 55462 | 43831 |
| ***Re**** | 44268 | 8820 | 9250 | 6110 | 7200 | 69500 | 8640 | 7400 | 10065 | 6600 |
| ***U**** | 0.217 | 0.049 | 0.037 | 0.047 | 0.036 | 0.278 | 0.048 | 0.034 | 0.061 | 0.055 |
| ***VDR*** | 8.80 | 0.66 | 1.00 | 0.80 | 1.31 | 5.62 | 1.53 | 1.18 | 2.06 | 1.80 |

**Table E2.** Range of data derived for the hydraulic zones (summer, wet season)

| **Sites** | **B1** | **B2** | **B3** | **B4** | **B5** | **S1** | **S2** | **S3** | **S4** | **S5** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush |
| **Geomorphic unit type** | Riffle | Riffle | Riffle | Run | Run | Riffle | Run | Run | Run | Run |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 32.00 | 37.00 | 28.00 | 25.00 | 39.00 | 38.00 | 35.00 | 29.00 | 34.00 | 27.00 |
| **Width(m)** | 7.50 | 10.80 | 12.30 | 7.00 | 5.00 | 17.20 | 24.00 | 5.90 | 10.80 | 3.50 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT7 | FT7 | FT7 | FT7 | FT7 | FT5 | FT5 | FT5 | FT5 | FT7 |
| **Velocity ($ms^{-1}$)** | 0.46 | 0.59 | 0.53 | 0.47 | 0.61 | 0.21 | 0.20 | 0.42 | 0.26 | 0.56 |
| **Depth (m)** | 0.17 | 0.21 | 0.21 | 0.13 | 0.12 | 0.17 | 0.17 | 0.19 | 0.13 | 0.14 |
| **Roughness height (m)** | 0.11 | 0.23 | 0.17 | 0.08 | 0.17 | 0.18 | 0.10 | 0.16 | 0.04 | 0.10 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S7 | S8, S9 | S10 | S7 | S8 | S9 | S8 | S8 | S6 | S7 |
| **Sorting** | Moderate | Moderate | Poor | Poor | Moderate | Poor | Good | Moderate | Poor | Moderate |
| **Packing (1 -5)** | 1 | 1 | 1 | 4 | 1 | 4 | 2 | 3 | 4 | 2 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 0 | 10 | 20 | 5 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 60 | 45 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Single logs** | 0 | 4 | 1 | 3 | 5 | 0 | 0 | 0 | 1 | 2 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 25 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.36 | 0.41 | 0.37 | 0.42 | 0.56 | 0.16 | 0.16 | 0.31 | 0.23 | 0.48 |
| ***Re*** | 94764 | 143568 | 128670 | 74512 | 76012 | 40248 | 37736 | 94215 | 38020 | 91163 |
| ***Re**** | 11413 | 24610 | 18360 | 8400 | 16660 | 6840 | 4600 | 13440 | 2880 | 4600 |
| ***U**** | 0.101 | 0.107 | 0.108 | 0.105 | 0.098 | 0.038 | 0.046 | 0.084 | 0.072 | 0.046 |
| ***VDR*** | 2.71 | 2.81 | 2.52 | 3.62 | 5.08 | 1.24 | 1.18 | 2.21 | 2.00 | 4.00 |

**Table E3.** Range of data derived for the hydraulic zones (autumn, dry season)

| **Sites** | **B1** | **B2** | **B3** | **B4** | **B5** | **S1** | **S2** | **S3** | **S4** | **S5** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush |
| **Geomorphic unit type** | Riffle | Riffle | Riffle | Run | Run | Riffle | Run | Run | Run | Run |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 31.00 | 33.00 | 29.00 | 25.00 | 39.00 | 39.00 | 32.00 | 31.00 | 34.00 | 22.00 |
| **Width(m)** | 7.70 | 9.70 | 13.40 | 7.00 | 5.39 | 16.50 | 23.00 | 3.00 | 10.00 | 2.90 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT8 | FT6 | FT6 | FT7 | FT7 | FT7 | FT5 | FT5 | FT5 | FT5 |
| **Velocity ($ms^{-1}$)** | 0.63 | 1.02 | 0.71 | 0.50 | 0.48 | 0.38 | 0.20 | 0.25 | 0.21 | 0.58 |
| **Depth (m)** | 0.19 | 0.31 | 0.30 | 0.20 | 0.30 | 0.23 | 0.10 | 0.20 | 0.23 | 0.16 |
| **Roughness height (m)** | 0.09 | 0.15 | 0.25 | 0.08 | 0.34 | 0.18 | 0.09 | 0.12 | 0.05 | 0.12 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S7 | S9 | S10 | S7 | S8 | S9 | S8 | S7 | S6 | S7 |
| **Sorting** | Moderate | Moderate | Poor | Poor | Moderate | Poor | Good | Moderate | Poor | Moderate |
| **Packing (1 -5)** | 1 | 1 | 1 | 4 | 1 | 4 | 2 | 3 | 4 | 2 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 10 | 15 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 3 | 5 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.46 | 0.59 | 0.41 | 0.36 | 0.28 | 0.25 | 0.20 | 0.18 | 0.14 | 0.46 |
| ***Re*** | 111869 | 306990 | 195413 | 93458 | 127434 | 82453 | 19418 | 49020 | 46442 | 90097 |
| ***Re**** | 13416 | 37050 | 35750 | 10240 | 29240 | 14040 | 3510 | 6840 | 3200 | 14520 |
| ***U**** | 0.156 | 0.247 | 0.143 | 0.128 | 0.086 | 0.078 | 0.039 | 0.057 | 0.064 | 0.121 |
| ***VDR*** | 3.32 | 3.29 | 2.37 | 2.50 | 1.60 | 1.65 | 2.00 | 1.25 | 0.91 | 3.63 |

**Table E4.** Range of data derived for the hydraulic zones (winter, dry season)

| **Sites** | **B1** | **B2** | **B3** | **B4** | **B5** | **S1** | **S2** | **S3** | **S4** | **S5** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush | Flush |
| **Geomorphic unit type** | Riffle | Riffle | Riffle | Run | Run | Riffle | Run | Run | Run | Run |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 30.00 | 38.00 | 27.00 | 25.00 | 38.00 | 36.00 | 34.00 | 27.00 | 32.00 | 20.00 |
| **Width(m)** | 6.70 | 9.90 | 15.50 | 6.90 | 7.20 | 16.00 | 23.00 | 2.9 | 7.80 | 2.90 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT8 | FT8 | FT7 | FT5 | FT5 | FT5 | FT5 | FT7 | FT5 | FT5 |
| **Velocity ($ms^{-1}$)** | 0.59 | 0.60 | 0.60 | 0.41 | 0.40 | 0.38 | 0.20 | 0.53 | 0.30 | 0.38 |
| **Depth (m)** | 0.22 | 0.23 | 0.22 | 0.13 | 0.18 | 0.16 | 0.23 | 0.19 | 0.15 | 0.11 |
| **Roughness height (m)** | 0.11 | 0.15 | 0.25 | 0.08 | 0.34 | 0.18 | 0.09 | 0.12 | 0.05 | 0.12 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S7 | S9 | S10 | S7 | S8 | S9 | S8 | S8 | S6 | S7 |
| **Sorting** | Moderate | Moderate | Poor | Poor | Moderate | Poor | Good | Moderate | Poor | Moderate |
| **Packing (1 -5)** | 1 | 1 | 1 | 4 | 1 | 4 | 2 | 3 | 4 | 2 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 15 | 15 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 4 | 5 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.40 | 0.40 | 0.41 | 0.36 | 0.30 | 0.30 | 0.13 | 0.39 | 0.25 | 0.37 |
| ***Re*** | 113859 | 115966 | 115789 | 43333 | 56250 | 53333 | 40000 | 97767 | 41284 | 37658 |
| ***Re**** | 15620 | 19800 | 26750 | 7360 | 19040 | 2040 | 4680 | 14160 | 4050 | 2160 |
| ***U**** | 0.142 | 0.132 | 0.107 | 0.092 | 0.056 | 0.068 | 0.052 | 0.118 | 0.081 | 0.018 |
| ***VDR*** | 2.68 | 2.61 | 2.73 | 3.15 | 2.22 | 2.38 | 0.87 | 2.79 | 2.00 | 3.45 |

**Table E5.** Range of data derived for the hydraulic zones (spring, wet season)

| Sites | B1 | B2 | B3 | B4 | B5 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink |
| **Geomorphic unit type** | Pool | Pool | Pool | Pool | Pool | Backwater | Pool | Pool | Pool | Pool |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 16.00 | 15.00 | 14.00 | 17.00 | 13.00 | 15.00 | 28.00 | 22.00 | 21.00 | 20.00 |
| **Width(m)** | 13.00 | 7.00 | 5.60 | 10.80 | 10.50 | 8.00 | 11.00 | 8.00 | 6.00 | 5.00 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT2 | FT3 | FT3 | FT2 | FT3 | FT1 | FT3 | FT2 | FT2 | FT3 |
| **Velocity ($ms^{-1}$)** | 0.09 | 0.13 | 0.10 | 0.05 | 0.11 | 0.00 | 0.06 | 0.04 | 0.02 | 0.12 |
| **Depth (m)** | 0.27 | 0.44 | 0.21 | 0.19 | 0.30 | 0.24 | 0.32 | 0.29 | 0.44 | 0.33 |
| **Roughness height (m)** | 0.12 | 0.09 | 0.15 | 0.01 | 0.12 | 0.06 | 0.04 | 0.04 | 0.00 | 0.00 |
| ***Substrate*** | | | | | | | | | | |
| Substrate class | S7 | S7 | S9 | S3 | S7 | S5 | S7 | S1 | S3 | S1 |
| **Sorting** | Good | Poor | Poor | Poor | Moderate | Poor | Good | Moderate | Moderate | Good |
| **Packing (1 -5)** | 2 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 5 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 5 | 15 | 20 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.06 | 0.06 | 0.07 | 0.04 | 0.06 | 0.00 | 0.03 | 0.024 | 0.01 | 0.07 |
| ***Re*** | 25103 | 59521 | 18918 | 9510 | 33777 | 0.00 | 19733 | 13078 | 9442 | 41121 |
| ***Re**** | 2668 | 2668 | 3150 | 210 | 3276 | 0.00 | 840 | 520 | 0.00 | 0.00 |
| ***U**** | 0.023 | 0.023 | 0.020 | 0.021 | 0.028 | 0.00 | 0.021 | 0.013 | 0.00 | 0.00 |
| ***VDR*** | 0.33 | 0.33 | 0.48 | 0.26 | 0.37 | 0.00 | 0.19 | 0.14 | 0.36 | 0.36 |

**Table E6.** Range of data derived for the hydraulic zones (summer, wet season)

| **Sites** | **B1** | **B2** | **B3** | **B4** | **B5** | **S1** | **S2** | **S3** | **S4** | **S5** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink |
| **Geomorphic unit type** | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 23.00 | 19.00 | 21.00 | 21.00 | 19.00 | 20.00 | 31.00 | 25.00 | 27.00 | 25.00 |
| **Width(m)** | 12.80 | 7.80 | 4.80 | 6.90 | 9.80 | 7.90 | 12.00 | 8.20 | 6.20 | 5.10 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT2 | FT3 | FT3 | FT2 | FT3 | FT3 | FT5 | FT2 | FT2 | FT3 |
| **Velocity ($ms^{-1}$)** | 0.09 | 0.15 | 0.10 | 0.06 | 0.10 | 0.06 | 0.07 | 0.07 | 0.02 | 0.12 |
| **Depth (m)** | 0.30 | 0.28 | 0.35 | 0.26 | 0.30 | 0.24 | 0.31 | 0.35 | 0.35 | 0.33 |
| **Roughness height (m)** | 0.014 | 0.08 | 0.11 | 0.01 | 0.15 | 0.02 | 0.04 | 0.04 | 0.00 | 0.00 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S2 | S7 | S9 | S3 | S7 | S5 | S7 | S1 | S3 | S1 |
| **Sorting** | Good | Poor | Poor | Poor | Moderate | Poor | Good | Moderate | Moderate | Good |
| **Packing (1 -5)** | 2 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 5 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 5 | 15 | 5 | 5 | 0 | 10 | 20 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 25 | 20 | 25 | 15 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.05 | 0.09 | 0.05 | 0.04 | 0.06 | 0.04 | 0.04 | 0.04 | 0.01 | 0.07 |
| ***Re*** | 31839 | 51157 | 39370 | 19071 | 31283 | 18438 | 24192 | 28258 | 7856 | 46262 |
| ***Re**** | 532 | 3360 | 3080 | 243 | 3552 | 440 | 960 | 960 | 0.00 | 0.00 |
| ***U**** | 0.038 | 0.042 | 0.028 | 0.027 | 0.024 | 0.022 | 0.024 | 0.024 | 0.00 | 0.00 |
| ***VDR*** | 0.30 | 0.54 | 0.29 | 0.23 | 0.33 | 0.25 | 0.23 | 0.20 | 0.06 | 0.36 |

**Table E7.** Range of data derived for the hydraulic zones (autumn, dry season)

| Sites | B1 | B2 | B3 | B4 | B5 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink |
| **Geomorphic unit type** | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 21.00 | 22.00 | 19.00 | 21.00 | 15.00 | 17.00 | 32.00 | 24.00 | 23.00 | 21.00 |
| **Width(m)** | 7.60 | 6.40 | 4.70 | 7.50 | 5.28 | 8.00 | 12.00 | 7.90 | 4.60 | 4.10 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT2 | FT3 | FT2 | FT2 | FT2 | FT2 | FT2 | FT2 | FT2 | FT3 |
| **Velocity ($ms^{-1}$)** | 0.08 | 0.11 | 0.07 | 0.06 | 0.07 | 0.06 | 0.05 | 0.04 | 0.04 | 0.08 |
| **Depth (m)** | 0.36 | 0.34 | 0.28 | 0.42 | 0.28 | 0.28 | 0.31 | 0.22 | 0.21 | 0.42 |
| **Roughness height (m)** | 0.01 | 0.08 | 0.10 | 0.01 | 0.01 | 0.02 | 0.02 | 0.04 | 0.00 | 0.00 |
| ***Substrate*** | | | | | | | | | | |
| **Substrate class** | S2 | S7 | S9 | S3 | S2 | S5 | S7 | S1 | S3 | S1 |
| **Sorting** | Good | Poor | Poor | Poor | Moderate | Poor | Good | Moderate | Moderate | Good |
| **Packing (1 -5)** | 2 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 5 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 5 | 15 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.04 | 0.06 | 0.04 | 0.03 | 0.04 | 0.04 | 0.03 | 0.03 | 0.03 | 0.04 |
| ***Re*** | 26915 | 34953 | 17818 | 22909 | 17345 | 15555 | 15500 | 8808 | 8155 | 33267 |
| ***Re**** | 432 | 2640 | 1900 | 324 | 310 | 460 | 380 | 520 | 0.00 | 0.00 |
| ***U**** | 0.036 | 0.033 | 0.019 | 0.027 | 0.031 | 0.023 | 0.019 | 0.013 | 0.00 | 0.00 |
| ***VDR*** | 0.22 | 0.32 | 0.25 | 0.14 | 0.25 | 0.21 | 0.16 | 0.18 | 0.19 | 0.19 |

**Table E8.** Range of data derived for the hydraulic zones (winter, dry season)

| Sites | B1 | B2 | B3 | B4 | B5 | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Hydraulic zone** | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink | Sink |
| **Geomorphic unit type** | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool | Pool |
| ***Dimensions*** | | | | | | | | | | |
| **Length (m)** | 18.00 | 19.00 | 21.00 | 19.00 | 18.00 | 19.00 | 35.00 | 22.00 | 21.00 | 20.00 |
| **Width(m)** | 12.00 | 6.90 | 7.50 | 8.20 | 4.55 | 7.00 | 12.00 | 8.00 | 5.00 | 4.20 |
| ***Hydraulic variables*** | | | | | | | | | | |
| **Flow type** | FT1 | FT3 | FT2 | FT2 | FT3 | FT3 | FT3 | FT2 | FT2 | FT3 |
| **Velocity ($ms^{-1}$)** | 0.08 | 0.10 | 0.07 | 0.05 | 0.07 | 0.06 | 0.04 | 0.05 | 0.03 | 0.10 |
| **Depth (m)** | 0.32 | 0.32 | 0.24 | 0.34 | 0.18 | 0.25 | 0.32 | 0.27 | 0.11 | 0.38 |
| **Roughness height (m)** | 0.01 | 0.08 | 0.15 | 0.01 | 0.01 | 0.02 | 0.02 | 0.04 | 0.00 | 0.00 |
| ***Substrate*** | | | | | | | | | | |
| Substrate class | S2 | S7 | S9 | S3 | S2 | S5 | S7 | S1 | S3 | S1 |
| **Sorting** | Good | Poor | Poor | Poor | Moderate | Poor | Good | Moderate | Moderate | Good |
| **Packing (1 -5)** | 2 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 5 |
| ***Aquatic vegetation*** | | | | | | | | | | |
| **Emergent (%)** | 5 | 5 | 15 | 5 | 15 | 5 | 5 | 0 | 10 | 0 |
| **Floating (%)** | 0 | 0 | 0 | 0 | 0 | 20 | 15 | 20 | 10 | 0 |
| ***Organic matter*** | | | | | | | | | | |
| **Multiple logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Single logs** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Twigs and leaves** | 5 | 5 | 10 | 10 | 20 | 20 | 15 | 10 | 20 | 5 |
| **Detritus** | 5 | 5 | 5 | 10 | 20 | 20 | 15 | 5 | 20 | 5 |
| ***Derived indices*** | | | | | | | | | | |
| ***Fr*** | 0.05 | 0.06 | 0.05 | 0.03 | 0.05 | 0.04 | 0.02 | 0.03 | 0.03 | 0.05 |
| ***Re*** | 22456 | 26890 | 14483 | 13821 | 10000 | 13274 | 11531 | 12500 | 3084 | 34862 |
| ***Re**** | 420 | 2320 | 2400 | 207 | 280 | 4460 | 320 | 680 | 0.00 | 2160 |
| ***U**** | 0.035 | 0.029 | 0.016 | 0.023 | 0.028 | 0.023 | 0.016 | 0.017 | 0.00 | 0.018 |
| ***VDR*** | 0.25 | 0.31 | 0.29 | 0.15 | 0.39 | 0.24 | 0.13 | 0.19 | 0.27 | 3.45 |

**Appendix F.** Fuzzy coding of macroinvertebrate traits and ecological preferences in the Swartkops and Buffalo rivers

**Table F1.** Fuzzy coding of macroinvertebrate traits and ecological preferences in the Swartkops and Buffalo rivers. Code: Gill type GO (operculate gill), GP (ovate / plate-like gill), GL (lamellate gill), GF (filamentous / finger-like gill); Body size (mm) SZ1 (≤5), SZ2 (> 5 to 10), SZ3 (> 10 to 20), SZ4 (> 20); feeding habit SH (shredder), CG (collector-gatherer), CF (collector-filterer), SC (scrapers, grazers and brushers), PR (predators); Hydraulic preference (velocity m/s) V1 (<0.1), V2 (0.1 < 0.3), V3 (0.3 – 0.6), V4 (> 0.6).

| Traits | Gill type | | | | Body size | | | | Feeding habit | | | | | Hydraulic preference | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Taxon ↓ / Code ⟶** | **GO** | **GP** | **GL** | **GF** | **SZ1** | **SZ2** | **SZ3** | **SZ4** | **SH** | **CG** | **CF** | **SC** | **PR** | **V1** | **V2** | **V3** | **V4** |
| **CRUSTACEA** | | | | | | | | | | | | | | | | | |
| Potamonautidae | 1 | 0 | 5 | 1 | 0 | 1 | 1 | 3 | 1 | 1 | 0 | 3 | 0 | 5 | 5 | 5 | 5 |
| **EPHEMEROPTERA** | | | | | | | | | | | | | | | | | |
| Leptophlebiidae | 1 | 0 | 5 | 3 | 0 | 5 | 3 | 0 | 1 | 3 | 1 | 3 | 0 | 1 | 3 | 5 | 3 |
| Baetidae | 0 | 3 | 1 | 1 | 3 | 5 | 0 | 0 | 1 | 5 | 1 | 3 | 0 | 3 | 3 | 5 | 5 |
| Caenidae | 5 | 0 | 0 | 3 | 3 | 5 | 0 | 0 | 0 | 5 | 0 | 3 | 0 | 5 | 3 | 3 | 3 |
| Heptageniidae | 0 | 5 | 3 | 1 | 0 | 1 | 5 | 0 | 0 | 5 | 0 | 3 | 0 | 1 | 5 | 5 | 3 |
| Tricorythidae | 0 | 0 | 5 | 0 | 0 | 1 | 5 | 0 | 0 | 5 | 0 | 3 | 0 | 1 | 1 | 3 | 5 |
| **ODONATA** | | | | | | | | | | | | | | | | | |
| Coenagrionidae | 0 | 1 | 5 | 0 | 0 | 1 | 5 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 3 | 1 | 1 |
| Aeshnidae | 0 | 1 | 5 | 0 | 0 | 0 | 5 | 5 | 0 | 0 | 1 | 1 | 5 | 3 | 3 | 5 | 3 |
| Gomphidae | 0 | 1 | 5 | 0 | 0 | 1 | 5 | 3 | 0 | 0 | 1 | 1 | 5 | 5 | 5 | 3 | 3 |
| Libellulidae | 0 | 1 | 5 | 0 | 0 | 3 | 5 | 1 | 0 | 0 | 1 | 1 | 5 | 3 | 3 | 5 | 3 |
| **HEMIPTERA** | | | | | | | | | | | | | | | | | |
| Naucoridae | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 0 | 0 | 0 | 5 | 3 | 5 | 3 | 1 |
| Belostomatidae | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 5 | 0 | 0 | 0 | 0 | 5 | 5 | 1 | 0 | 0 |
| **TRICHOPTERA** | | | | | | | | | | | | | | | | | |
| Hydropsychidae | 0 | 0 | 1 | 5 | 0 | 1 | 5 | 0 | 1 | 0 | 5 | 0 | 1 | 1 | 3 | 5 | 5 |
| **DIPTERA** | | | | | | | | | | | | | | | | | |
| Chironomidae | 0 | 0 | 0 | 5 | 3 | 5 | 1 | 1 | 1 | 5 | 0 | 3 | 3 | 5 | 5 | 5 | 5 |
| Simuliidae | 0 | 0 | 0 | 5 | 3 | 5 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 1 | 1 | 3 | 5 |
| **PLECOPTERA** | | | | | | | | | | | | | | | | | |
| Perlidae | 0 | 0 | 1 | 5 | 0 | 0 | 5 | 3 | 1 | 0 | 0 | 0 | 5 | 1 | 3 | 5 | 3 |
| **COLEOPTERA** | | | | | | | | | | | | | | | | | |
| Hydrophilidae | 0 | 3 | 0 | 3 | 1 | 5 | 3 | 1 | 3 | 5 | 0 | 0 | 5 | 3 | 3 | 3 | 3 |

**Appendix G.** Morphology of ingested microplastics

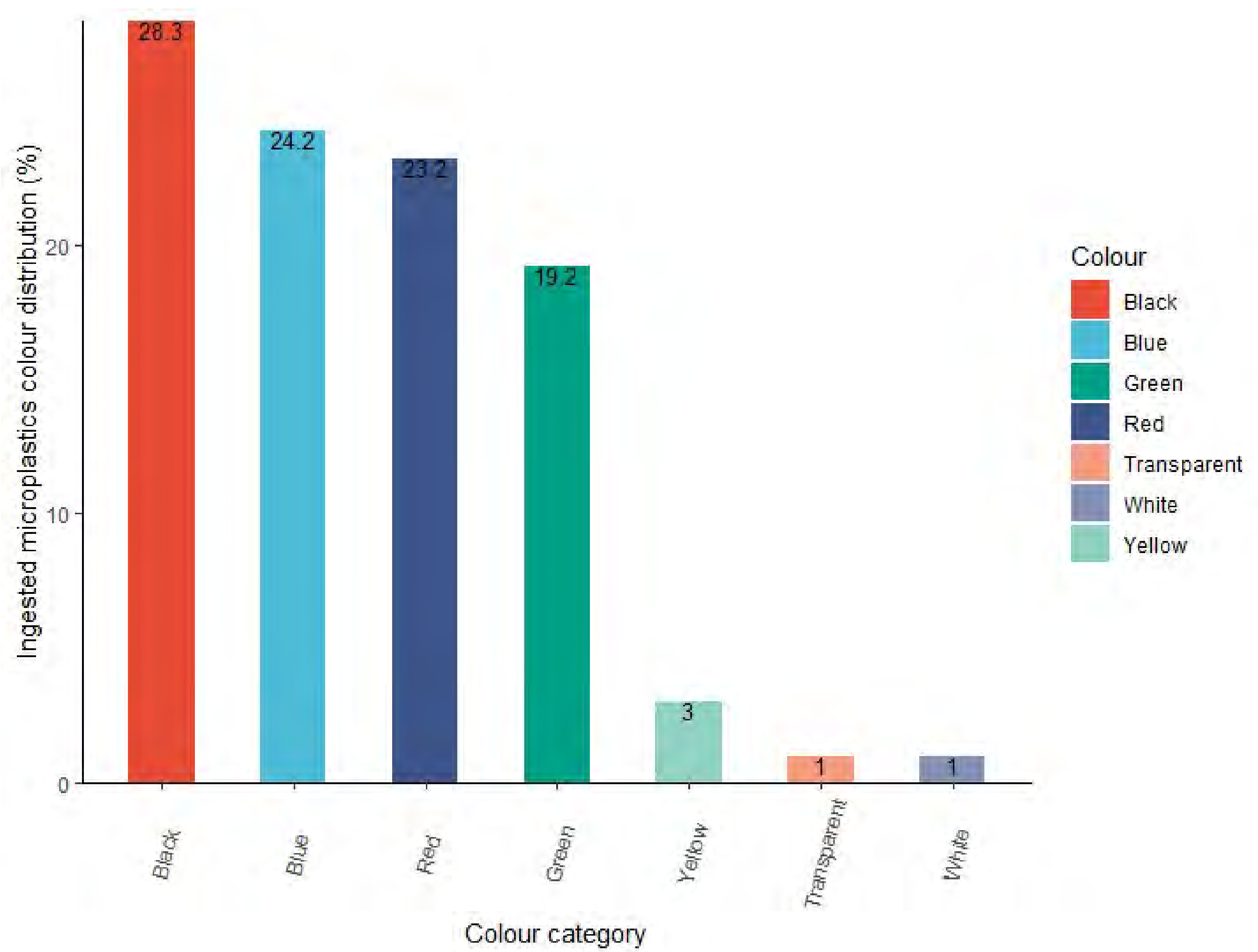


**Figure G1.** Colour distribution of ingested microplastics

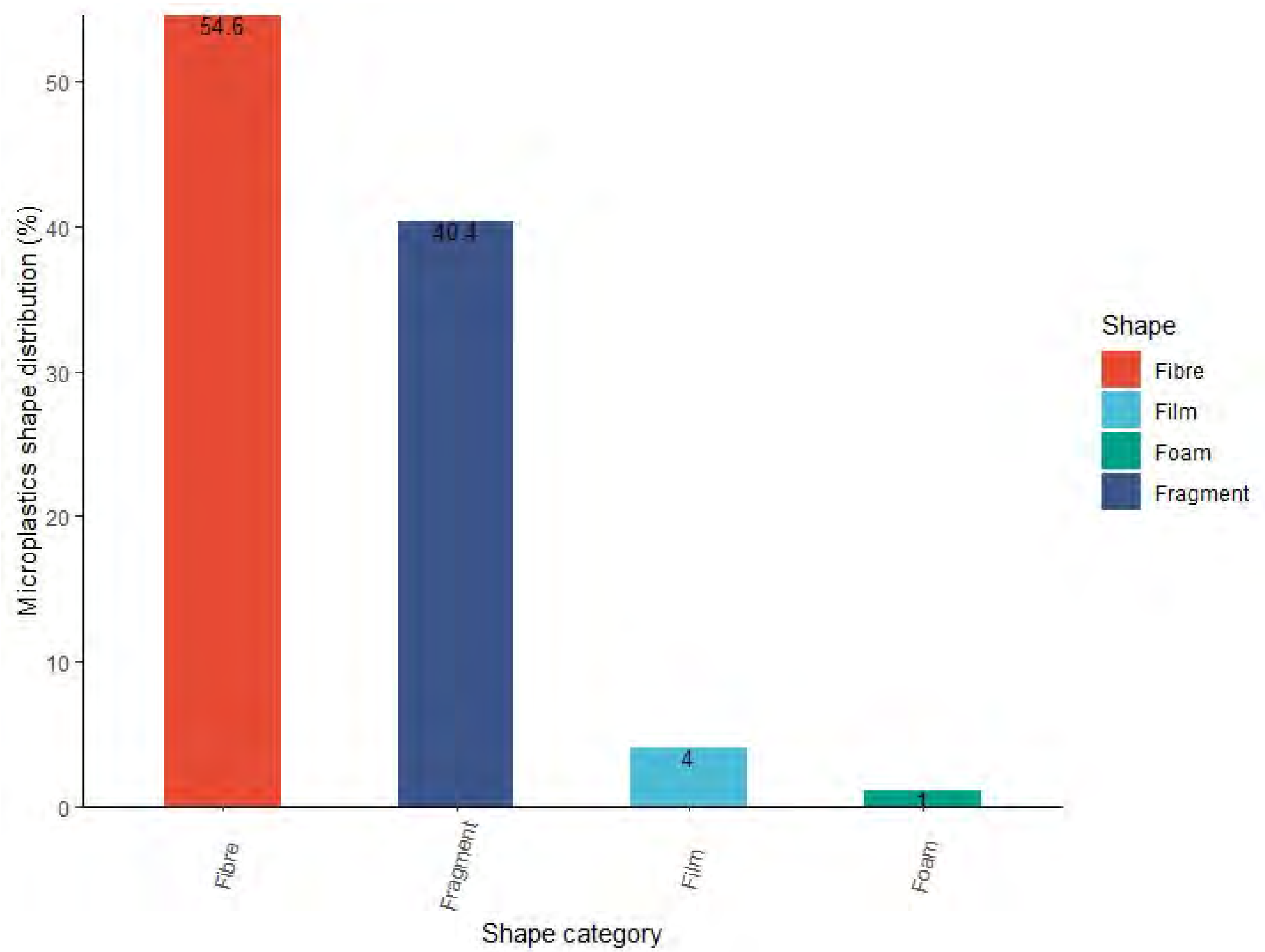


**Figure G2.** Shape distribution of ingested microplastics

**Appendix H.** Plates of ingested microplastics particles

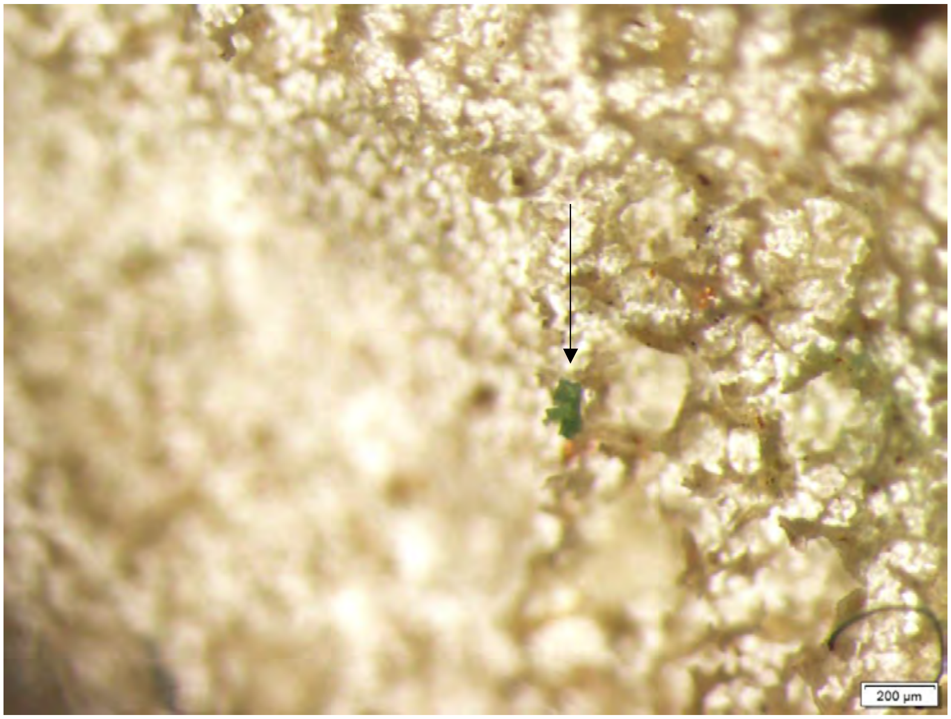


**Plate H 1.** Green fragment

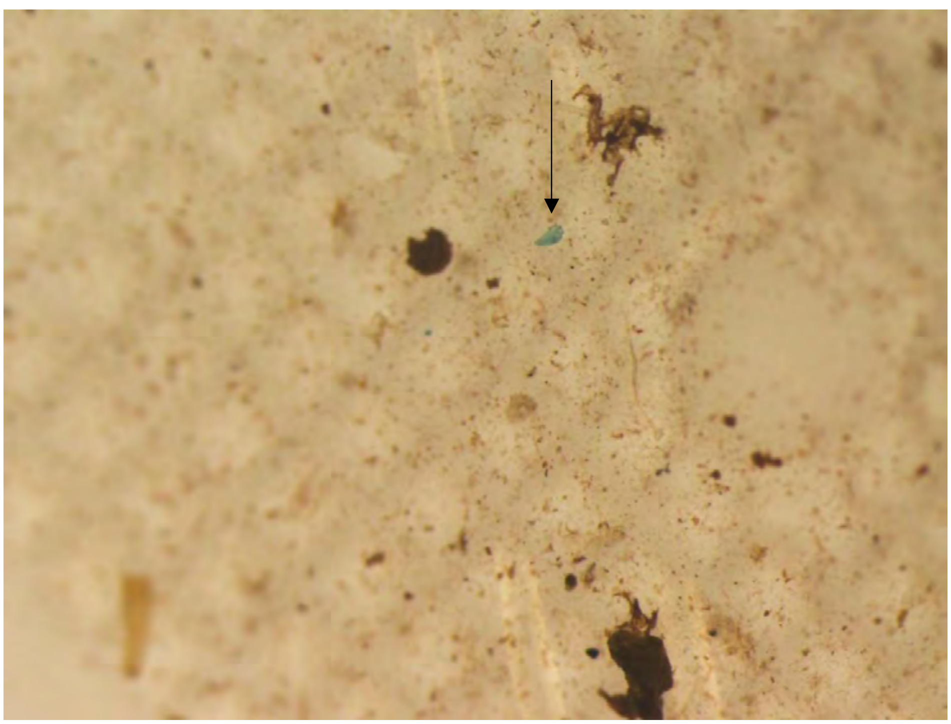

**Plate H 2.** Blue fragment

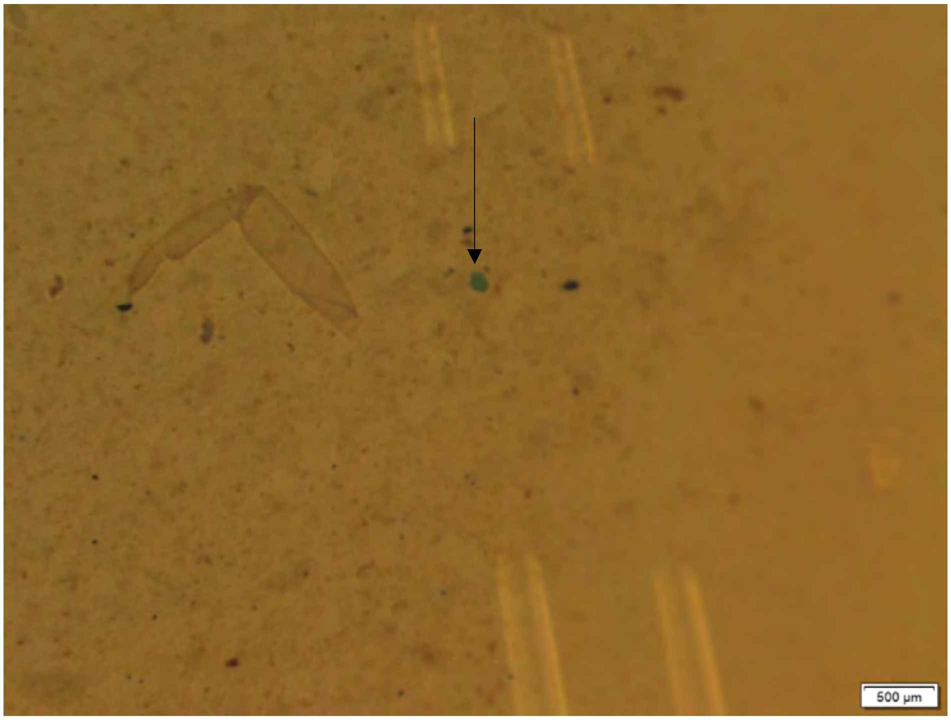


**Plate H 3.** Green fragment

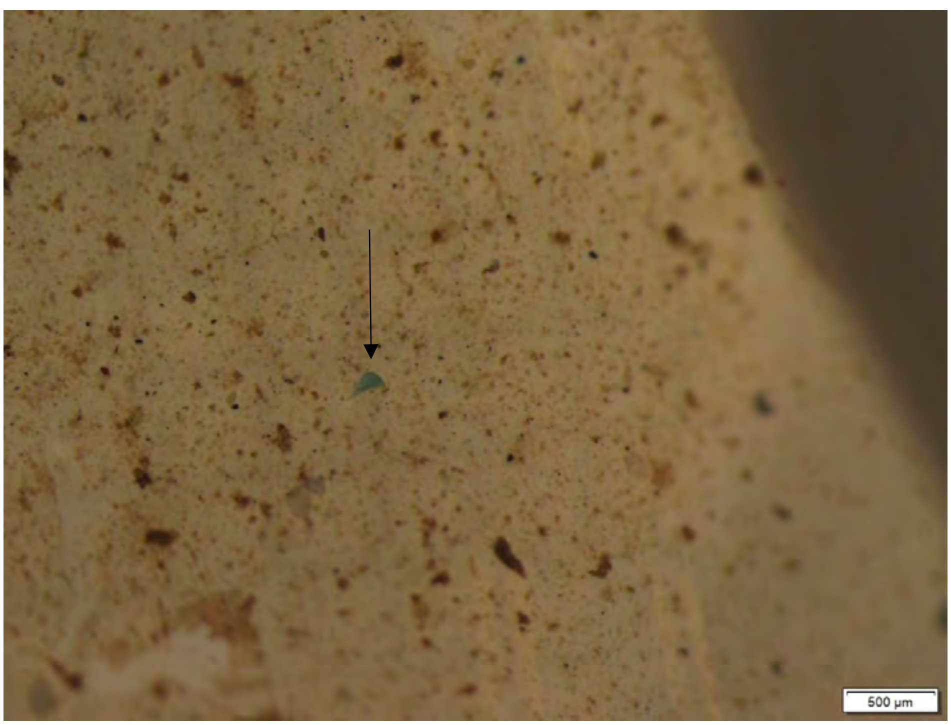


**Plate H 4.** Blue fragment

**Plate H 5.** Blue fragment

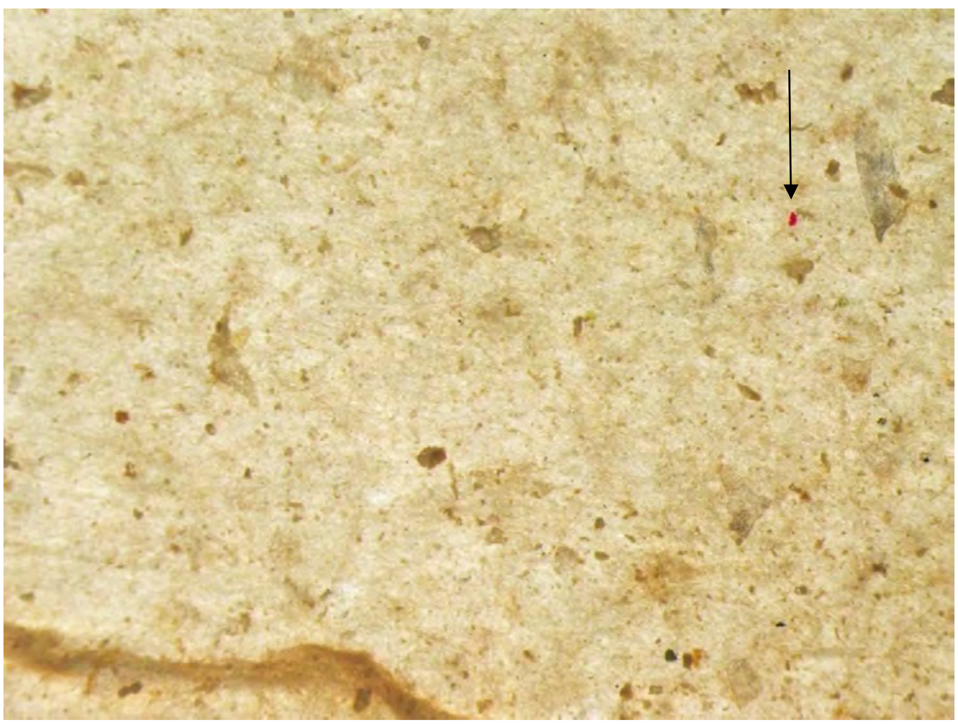

**Plate H 6.** Red fragment

**Plate H 7.** Blue fibre

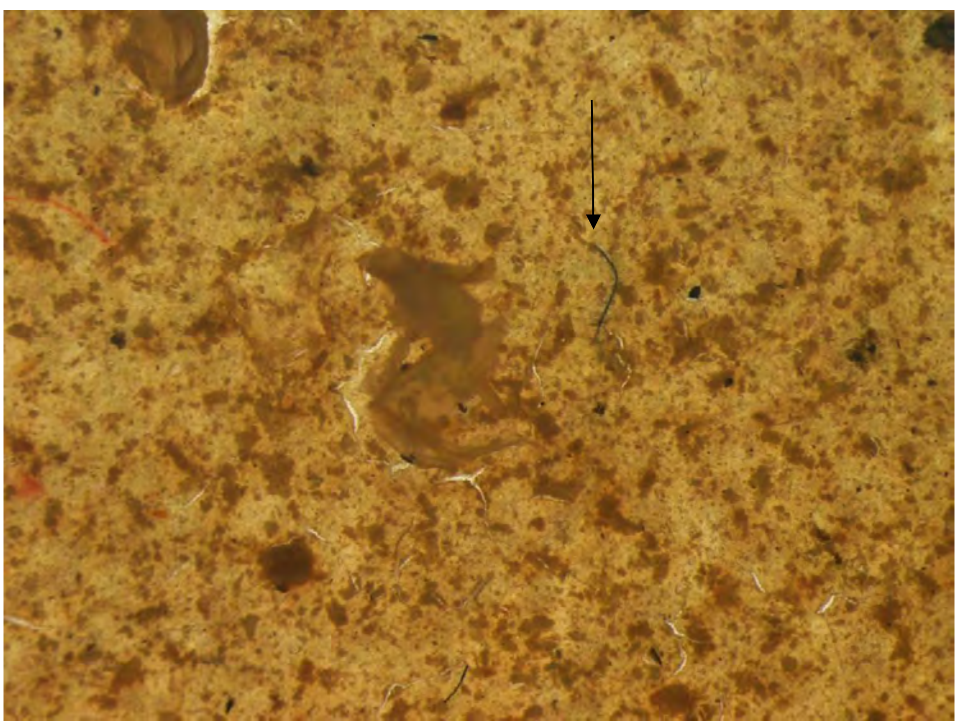

**Plate H 8.** Black fibre

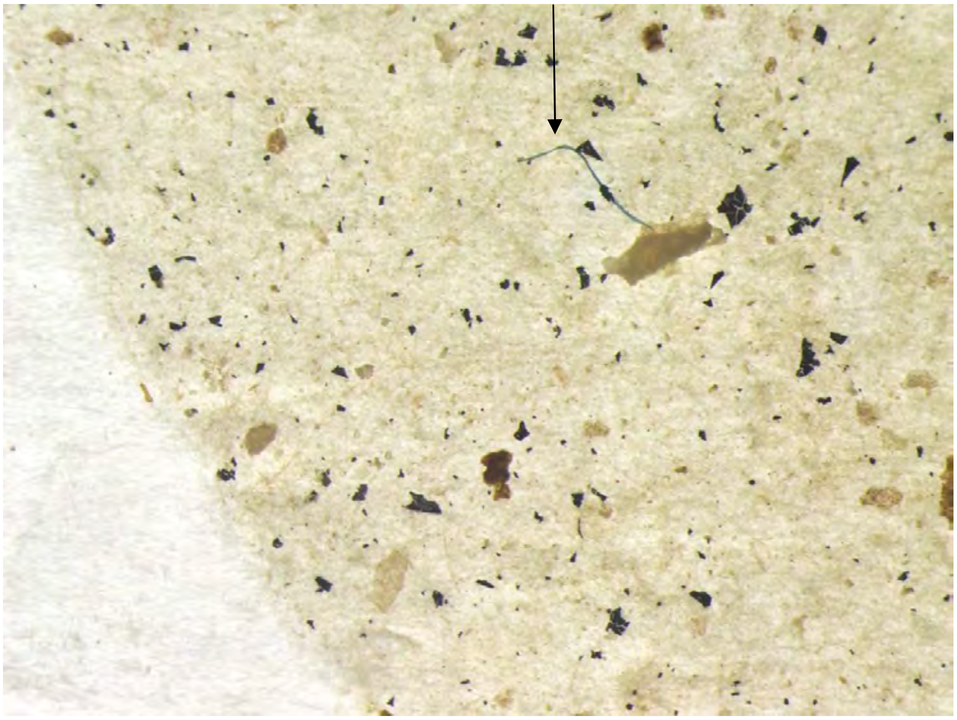

**Plate H 9.** Blue fibre

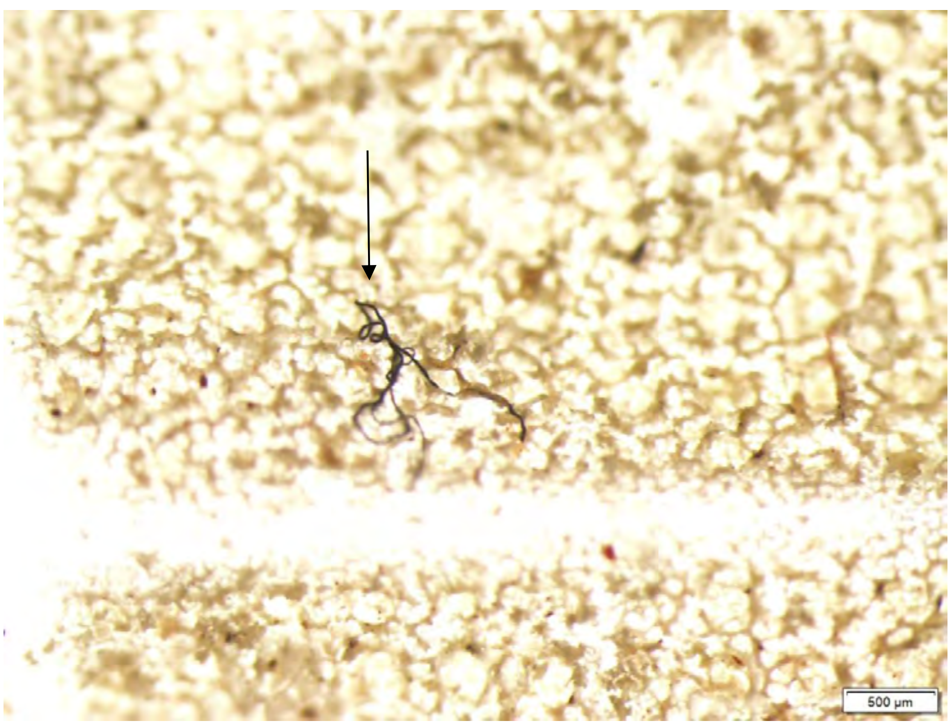


**Plate H 10.** Black fibre

**Appendix I.** RLQ analysis plots

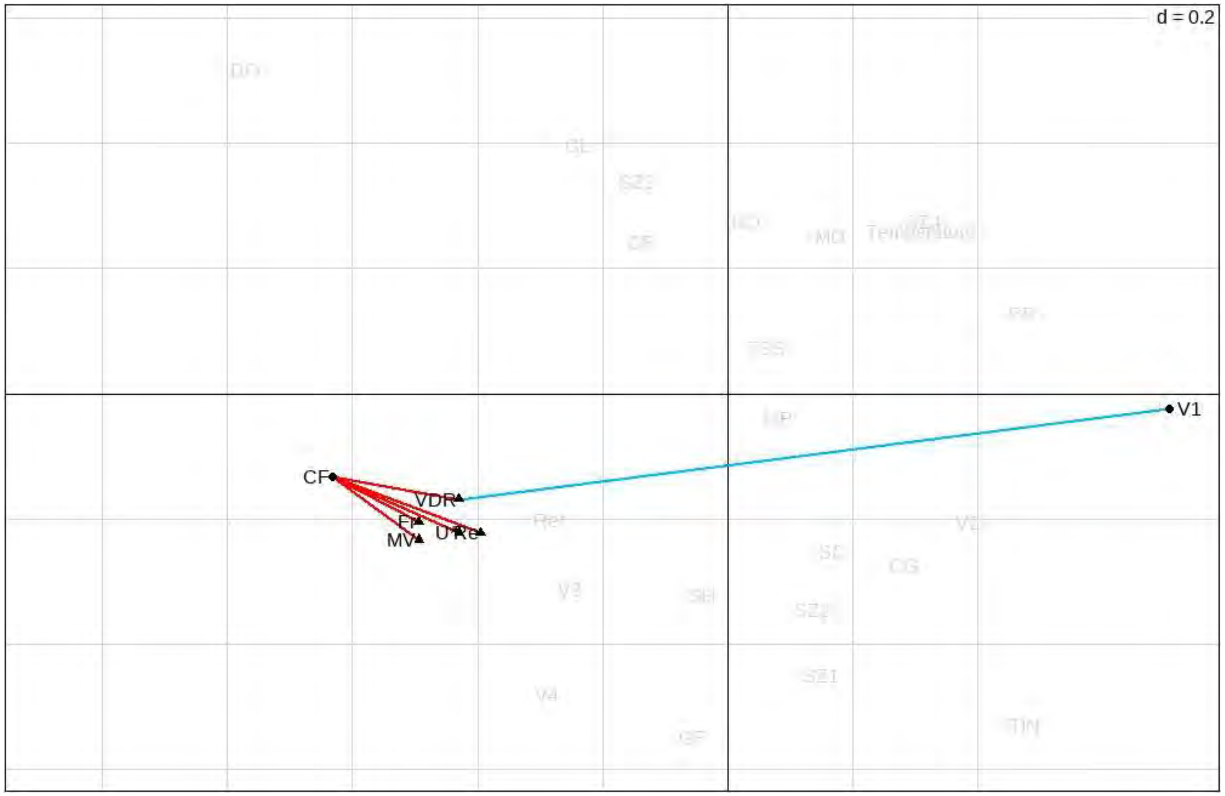


**Figure I 1.** RLQ analysis biplot showing significant associations revealed by the fourth-corner approach. *P*-values adjusted for multiple comparisons using the FDR method and a significant level α = 0.05. Blue lines indicate significant negative associations, red lines indicate significant positive associations. Only traits and environmental variables that have at least one significant association are represented.

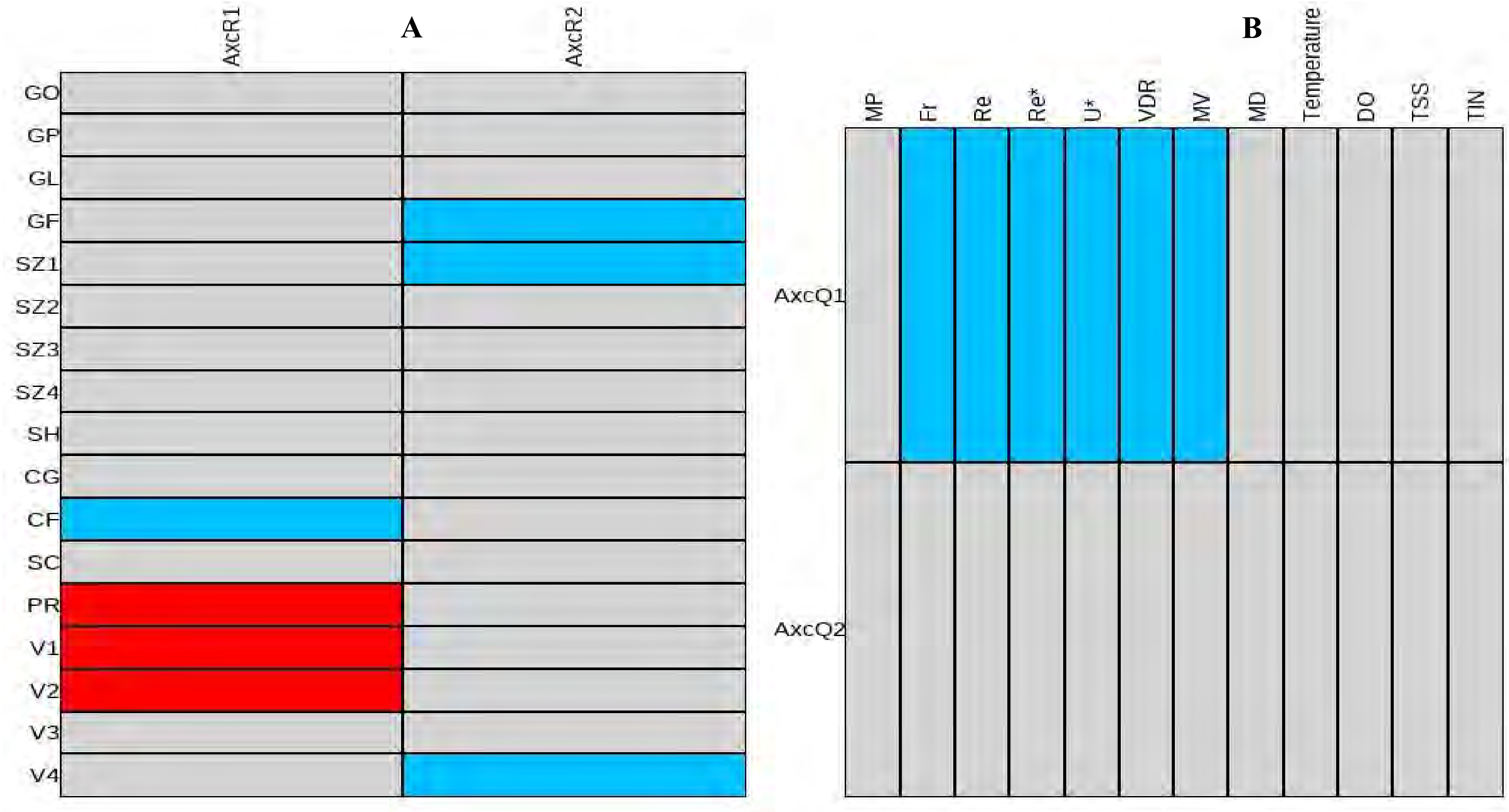


**Figure 1 2.** RLQ analysis results showing significant links between RLQ axes and traits (A) and between R LQ axes and environmental variables (B), revealed by the fourth-corner approach. *P*-values adjusted for multiple comparisons using the fdr method and a significant level α = 0.05. Colours indicate significance.

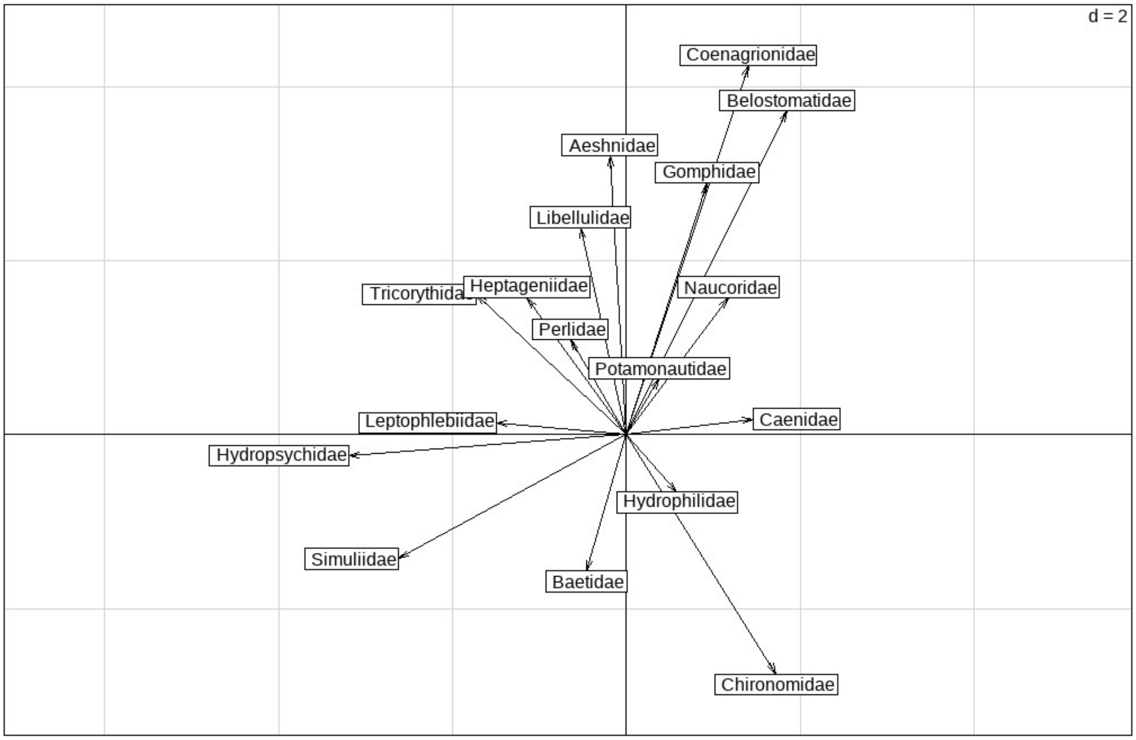


**Figure 1 3.** RLQ analysis showing the distribution of macroinvertebrate in relation to the hydraulic zones