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ABUNDANCE AND DISTRIBUTION OF MICROPLASTICS IN BENTHIC SEDIMENTS AND CLADOCERA TAXA IN A SUBTROPICAL AUSTRAL RESERVOIR

By

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ABSTRACT

The pollution of the environment by microplastics has become a global issue to ecosystems. The presence of microplastics in aquatic ecosystems poses threats to biota such as ingestion, slow reproduction, and death. The present study assessed the abundance and distribution of microplastics in benthic sediments and Cladocera taxa in a subtropical austral reservoir. A Pearson's correlation analysis was used to assess the relationship between microplastic abundance and physicochemical variables such as pH, salinity, temperature, resistivity, oxygen potential reduction, conductivity, total dissolved solids, and Chlorophyll–*a*. Cladocera taxa were also collected and placed in a labelled test tube, where they were digested using 50ML Hydrogen peroxide. The findings of this study generally showed high densities of microplastics during the cool-dry season for both sediments and Cladocera taxa. Microplastic shapes were dominated by fibres and the colour scheme was dominated by transparent. For sediments, the abundance of microplastics negatively correlated with only three physicochemical variables: chlorophyll–*a* concentration, temperature, and resistivity, during the hot-wet season. Whereas it positively correlated with pH and salinity. For Cladocera, the abundance of microplastics positively correlated with only two physicochemical variables: conductivity and salinity, during the cool-dry season. High microplastic abundances were recorded during the season of high rainfall, indicating that the Nandoni reservoir has multiple sources of microplastic pollution. The Luvuvhu River is a major source of microplastic pollution, as it feeds large amounts of water into the reservoir during rainy season. Rain also contributes to microplastics in the reservoir by washing contaminants from surrounding terrestrial environments into the reservoir. As more studies focuses on quantifying microplastic pollution in oceans and rivers, the present study highlights the significance of including reservoirs as mechanisms for microplastic retention. The study also highlights a base for identifying and addressing the issue of microplastic pollution and its effect on reservoirs and the biota found within the reservoir.

It will also help all stakeholders (e.g., community members, conservationists, and institutions) to come together to discuss and provide possibly solutions for microplastic pollution locally and internationally. More studies, especially laboratory experimental studies should be conducted to measure the uptake of microplastics by Cladocera taxa. Additionally, scientists should publicise their findings to the general public in order to increase awareness about microplastic contamination and to spur action to reduce or eliminate plastic pollution of freshwater environment.

Keywords: Microplastic, Nandoni reservoir, sediments, Cladocera taxa, freshwater pollution.

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DECLARATION

I, Nombuso N Themba student number 201705958, hereby declare that the study on "Abundance and distribution of microplastics in benthic sediments and cladocera taxa in a subtropical austral reservoir" has not been submitted by anyone else or to any other institution and is my own research work and that all sources that I used have been acknowledged by the means of references at the end of this dissertation.

Signature:  Date: 30 November 2023

CHAPTER ONE: GENERAL BACKGROUND

Introduction

Plastic pollution is a major global issue and its impacts on ecosystems lack comprehensive understanding (Dalu et al., 2020). Plastics are organic synthetic polymers derived from the monomerization of gas and crude oil (Cole et al., 2011) and possess qualities that are ideally suited for a broad number of applications such as packaging, building and construction, home and sports equipment, cars, electronics, and agriculture (Adhwaryu and Alam, 2021). The durability, flexibility, lightweight, low cost, and waterproof properties of plastic allow it to be used in a variety of applications, resulting in its accumulation in the environment (Pellini et al., 2018, Razeghi et al., 2021, Dalu et al., 2023).

Since the early 1940s, there has been a rapid increase in the demand for plastic products propelling higher plastic production volumes (Dalu & Tavengwa 2022). Plastic have been found worldwide, including our oceans, sediments, surface waters, animals, and air (Lebreton et al., 2017; Chang et al., 2020). Each year, the world produces approximately 300 million tons of plastic (PlasticsEurope, 2019). It has been estimated that approximately 8 300 million metric tons (Mt) of virgin plastics have been manufactured to date (Geyer et al., 2017). A recent study estimates the global plastic waste production to be approximately 6.3 billion Mt, with 9% recycled, 12% incinerated (burned) and 79% discarded directly in open dumps and the natural environment (Geyer et al., 2017). A study by PlasticsEurope. (2019) estimated that just 20% of plastic waste gets recycled or burned globally.

Before 1980, fewer plastics were burned or recycled, as a result, all plastics were thrown away (Ritchie and Roser, 2018). Between the years 1980 and 1990, recycling and incineration rates increased by an average of approximately 0.7 % per year (Geyer et al., 2017). It is estimated that humans produced approximately 9.5 billion tonnes of plastic by 2019, which is more than one tonne of plastic for each individual alive today (Ritchie and Roser, 2018). The development of waste plastic is significantly influenced by the primary use of plastic and the duration of the products exposure to the environment (Geyer et al., 2017). Most plastics end up in landfills or are dumped into aquatic habitats. More than 5 trillion plastic fragments weighing more than 250 000 tons have been estimated to cause havoc on aquatic environments, such as oceans and inland waters. According to Sharma et al. (2021) between 74% and 94% of all ingested plastics make their way into various parts of the environment, especially aquatic environments.

The polymers that makeup plastics can have different chemical compositions, leading to variations in the potential dangers (Sharma et al., 2021). Carbon–based polymers, polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), polyethylene (PE), and polyethylene terephthalate (PET) are the types of plastics that are mostly discovered in the environment (Sharma et al. 2021). It has been reported that polystyrene microplastics induce toxic effects in organisms such as zebrafish (Veneman et al., 2017). Similarly, Lei et al. (2018) reported intestinal alterations with rising glutathione s-transferase (GST) activity and oxidative damage in zebrafish after exposure to polyethylene, polypropylene, polyvinyl chloride, and polyamide microplastics.

Microplastic pollution in aquatic environments has been a ubiquitous concern in recent years, owing to their rapid production combined with poor waste management practices (Nkosi et al., 2023). Microplastics are typically described as plastic trash that is less than 5 millimetres in diameter (Liu et al., 2020; Song et al., 2020; El Hadri et al., 2020). Microplastics can be found everywhere on Earth, from the soil, water to the atmosphere (Sun et al., 2021). According to

Wright and Kelly (2017), because of the slow rates at which they degrade, microplastics can persist in nature for a very long time and cause significant contamination to ecosystems. Microplastics have the potential to act as a medium for the spreading of chemical contaminants and infections (Caruso, 2019; Wu et al., 2019). Microplastics can enter aquatic habitats through various pathways when atmospheric conditions are favourable for their deposition in the environment.

Microplastics are created when large pieces of plastics go through various degradation processes. For the past years, the presence of microplastics in the hydrosphere has been regarded as an environmental issue because it threatens ecosystems globally (Haque and Fan, 2023). Direct manufacturing in the plastics industry and weathering, oxidation, and degradation of bulky waste plastics in the environment are major contributors to the production of microplastics (He et al., 2022). Microplastics are products of human activities and mostly derive from two sources (primary and secondary sources).

Microplastics can be classified into two categories i.e., primary and secondary microplastics which are the two categories that can be differentiated based on where the microplastics originated (Yang et al., 2022). Both types of microplastics are discharged directly into the aquatic environment through human activities such as littering. Primary microplastics refer to the direct use of plastic microbeads in specific cosmetics and other products, which are discharged directly into the aquatic environment through human activities. Primary microplastics are also known as primary microbeads. Whereas secondary microplastics are plastic fragments formed by larger plastic waste undergoing degradation processes that cause fragmentation and volume reduction (Yang et al., 2022). Processes of biodegradation, physical degradation, photodegradation, and chemical degradation all contribute to the formation of secondary microplastics during the deterioration of plastic (Corcoran et al. 2019).

After being introduced into the environment, these microplastic particles have the potential to be transported into aquatic ecosystems, to freshwater systems, either through direct inputs from the primary sources of contamination, such as wind or rainy events that wash plastics from land to surface waters (Lambert et al., 2017; Duis and Coors, 2016). Microplastics have the potential to spread throughout the ecosystem via the effluents and runoffs of wastewater treatment plants (Chen, 2019). They accumulate in the water column and/or in the sediments that act as temporary sinks (Blair et al., 2017). Other factors such as flow velocity, water depth, storms, floods, and/or anthropogenic activities may further influence the physical and temporal detention (Rocha–Santos and Duarte, 2015).

According to Klein et al. (2015), freshwater systems serve as a conduit for the movement of microplastics from terrestrial ecosystems to aquatic habitats. It is estimated that rivers transport between 70 % and 80 % of the plastic that ends up in these systems (GESAMP, 2010). In addition, microplastics have been found in reservoirs, lakes, and rivers within the water column and/or sediments on all continents (Faure et al., 2015; Mani et al., 2016; Su et al., 2016; Biginagwa et al., 2016; Anderson et al., 2017; Watkins et al., 2019; Huang et al., 2021).

When introduced to aquatic environments, microplastics can also be ingested by aquatic organisms such as fish (Waldschlager et al., 2020), birds, zooplankton and turtles. Field (Sun et al., 2017, Steer et al., 2017) and laboratory studies (Setälä et al., 2014) have shown that zooplankton ingest microplastics. Zooplankton is a primary consumer; it grazes on phytoplankton and transfer energy to higher trophic levels along the food chain and are therefore considered essential sources of prey for numerous marine organisms. This also means that microplastics ingested by zooplankton can be transferred to other organisms that feed on zooplankton. The presence of microplastics in lower trophic levels raises the possibility that microplastics and/or their contaminants (i.e., adsorbed or constituent chemicals) may be transferred into and up the food chain.

Microplastic with sorbed co–contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) can also be deposited in sediment surfaces. The aggregation of microplastic particles with organic matter in sediments can increase particle size and density, resulting in increased microplastic sedimentation rates (Long et al., 2022; Nel et al., 2019). The greatest concern is the potentially wide range of effects microplastics pose to ecosystems and public health due to microplastic ingestion by aquatic organisms and trophic transference through entire food webs (Teuten et al., 2009; Browne et al., 2008; Farrell and Nelson 2013; Nel et al., 2018; Cuthbert et al., 2019). The threat of microplastic contamination can be expressed as chemical toxicity and physical damage due to the vast volume of microplastics thrown into water bodies Moreover, microplastics can absorb persistent organic contaminants, which can be transferred to living tissues (Panno et al., 2019) through direct or indirect ingestion and become carriers of various metals (Zhou et al., 2019). Moreover, their bioaccumulation and invasion of the food chain pose a severe risk to the living world, including humans (Tien et al., 2020; X. X. Zhang et al., 2021).

The abundance of microplastics found is dependent on how close they were to the source of contamination. However, the values were comparable to those discovered in other aquatic habitats, such as marine systems, which included anywhere from tens to thousands of microplastics (Horton et al., 2017). Most freshwater systems are in regions with a high population density and, as a result, extensive anthropogenic activities, both of which contribute to an increase in the number of microplastics found in these regions. Freshwater systems have a significant economic value associated with them because humans extensively use freshwater systems in their day–to–day activities (Eerkes–Medrano et al., 2015).

Problem statement

The South African government categorised the plastic industry as an important sector for economic growth in enhancing export, innovation and recycling (Monaco et al., 2019). However, the country is eleventh globally as a main contributor of marine plastic debris, despite waste disposal protocols and legislation to promote recycling being in place (Verster et al., 2017). Excessive plastic production has placed a strain on aquatic ecosystems as unwanted microplastics enter aquatic environments through wastewater discharge, degradation of larger plastic items and user discards (Barnes et al., 2009). Thus, the widespread abundance of microplastics in the environment is directly due to human activities (Ma et al., 2019).

Water is a strained resource in South Africa, much like it is in many other developing countries. In addition, The demand for water is increasing due to human population growth and development. As a direct consequence of this, many reservoirs (e.g., Nandoni reservoir) have been constructed to provide enough water for everyone. According to Guo et al. (2021), reservoirs, which can be the deepest points in a landscape, they best represent the local area.. In recent years, researchers have noticed that reservoirs may contain trace to large amounts of microplastics as they act as sinks (Watkins et al., 2019; Huang et al., 2020). Despite performing various important functions for ecosystems, reservoirs have received a relatively low level of attention regarding the potential impacts of microplastic contamination (Guo et al., 2021).

Reservoirs can serve as regional models for ecosystem types, potentially highlighting the sources, nature, and extent of microplastic pollutants (Mbedzi et al., 2020). Studies focusing on microplastics occurrence and distribution in freshwater sediments (Mbedzi et al., 2020) and zooplankton are not enough within Southern Hemisphere.

Aims

To investigate the presence, distribution and effects of microplastics on pelagic invertebrates and benthic sediments of a subtropical reservoir.

Objectives

1. Assess the spatio–temporal abundances and distribution of microplastics found in benthic sediments and Cladocera taxa of the Nandoni reservoir across the hot–wet and cool–dry seasons and investigate other factors contributing to microplastic pollution in the Nandoni reservoir.
2. Investigate the relationship between sediment and Cladocera microplastic abundances with physicochemical variables within Nandoni reservoir.

Hypothesis

1. High abundances of microplastics will be found in near the dam wall within the benthic sediments and Cladocera taxa because that is where free–floating microplastic particles will mostly be likely to accumulate

CHAPTER TWO: LITERATURE REVIEW

Plastic pollution in aquatic environments is a growing problem worldwide because it is one of the hardest pollutants to eliminate (Kolandhasamy et al., 2018; Bianchi et al., 2020). Aquatic ecosystems can be polluted by several activities, such as pollution caused by industrialisation, farming, mining, and the growth of residential areas, which has led to the production of much plastic for business and personal use (Tang et al., 2021). Plastic pollution was also caused by bad waste management, careless people, and the accidental loss of plastic and a synthetic polymer with several uses.

Since the 1950s plastic materials have basically permeated everyday life (Scherer et al., 2017). Besides massive economic and social benefits of synthetic polymers, unsustainable resource management has resulted in plastic materials entering the environment (Scherer et al., 2017). Their main advantages (e.g., durability, lightweight) promote the accumulation and mobility in ecosystems (Scherer et al., 2017). When exposed to abiotic and biotic weathering processes, polymers fragment into increasingly smaller particles (Lambert and Wagner, 2016; Weinstein et al., 2016) referred to as microplastics (Thompson, 2004; Liu et al., 2020). Microplastic pollution continues to proliferate in freshwater, marine and terrestrial environments, but with their biotic implications remaining poorly understood (Cuthbert et al., 2019).

Over the last decade, numerous studies have reported the ubiquitous presence of microplastics in surface waters and sediments (Dalu et al., 2023, Nkosi et al., 2023, Mutshekwa et al., 2023. Primarily focused on marine systems, an increasing number of recent studies demonstrate that freshwater compartments are contaminated to a comparable degree (Imhof et al., 2016). Studies have shown that microplastics are building up in freshwater environments like lakes (Freeet al., 2014), rivers (Castaned et al., 2014), and streams (Mani et al., 2015). According to Horton et al. (2017), many rivers are now seen as important hotspots for microplastics because they are close to sources of plastic pollution, like cities and industries. Microplastics have been found in the water column and sediments of freshwater systems, as well as in fish that live in rivers (Castaned et al., 2014; Su et al., 2016; Collard et al., 2018; McNeish et al., 2018).

Microplastics are described as pieces of plastic that are smaller than 5 mm (Hidalgo–Ruz et al., 2012). Connors et al. (2017) say that there is no smaller size cut–off for this definition right now. Since the 1970s, when the first reports of micro–sized particles were made public (Carpenter and Smith, 1972; Carpenter et al., 1972; Colton et al., 1974), plastic pollution in water has been a cause for concern. Microplastics have been found in many places, like the air, land, freshwater, and the ocean (Tsuji et al., 2006). Microplastics get into aquatic environments worldwide, hence they are a global environmental problem. They were found in all levels of aquatic ecosystems, which shows that they pose serious risks to biota. Laboratory studies with invertebrates documented their adverse effects across various taxa, including reduced feeding, weight and fertility as well as induced mortality and inflammation (Lee et al., 2013; von Moos et al., 2012; Cole et al., 2015; Besseling et al., 2013.

Owing to their small size, microplastics are potentially available for a broad range of aquatic species (Scherer et al., 2017). Indeed, microplastic ingestion has been shown for several field collected marine and freshwater species (Frias et al., 2014; Mbedzi et al., 2020; Dalu et al., 2023). Additionally, ecologists have frequently used polymer beads to investigate the feeding behavior of pelagic freshwater zooplankton. In their attempt to categorize filtering capacities of different species and their ability to feed on bacteria and algae, they collected essential data for a better understanding of community assemblages in various habitats (Børsheim and

Andersen, 1987; Bern, 1990). So far, some laboratory and field studies have demonstrated microplastic contamination in plankton, aquatic invertebrates, and vertebrates (mammals and fish), and terrestrial organisms (earthworm) (Cole et al., 2011; Ivar do Sul and Costa, 2014; Lwanga et al., 2016). Bivalves and seabirds are amongst the many organisms that ingest microplastics (Egbeocha et al., 2018).

Smaller organisms such as Zooplankton have been shown to consume microplastics (Yu et al., 2020). These organisms play vital ecological roles in marine and freshwater ecosystems. Zooplankton encompass both freshwater and marine holoplankton and meroplankton, which exhibit very different life histories (Yu et al., 2020). High microplastic to zooplankton ratios have been documented in aquatic ecosystems such as the north Pacific gyre and Mediterranean Sea (Moore et al., 2001, Collignon et al., 2012), and both holoplankton and meroplankton have been reported to ingest microplastics in the field (Sun et al., 2017, Steer et al., 2017) and laboratory studies (Setälä et al., 2014). As a primary consumer, zooplankton graze on phytoplankton and transfer energy to higher trophic levels along the food chain and are therefore considered essential sources of prey for numerous marine organisms. They also play a vital role in nutrient cycling by feeding in surface water and packaging the organic matter into dense faeces which facilitate the transport of carbon and nutrients to deep waters (Turner, 2015). The presence of microplastics in lower trophic levels raises the possibility that microplastics sand/or their contaminants (i.e., adsorbed, or constituent chemicals) may be transferred into and up the food chain, potentially to species consumed by humans (Ivar do Sul and Costa, 2014; Lusher, 2015). Microplastic exposure has the ability to alter intricate predator-prey relationships, with the most significant effects occurring at lower food web levels (Foley et al., 2018). Cladocerans, for example, provide top-down control of phytoplankton in freshwater ecosystems. Mechanisms that contribute to lower grazing pressure may have a significant impact on biological control, resulting in higher primary producer biomass (Sommer and Sommer, 2006). Although we are unaware of any direct studies into this, it has been proposed that the high susceptibility of zooplankton to the effects of microplastics, as well as the potential for microplastic exposure to reduce feeding by primary consumers, could lead to an increase in microalgae as top-down regulation is reduced (Prata et al., 2019). The direct and indirect ecological consequences of microplastics on zooplankton will vary not only with the kind and concentration of microplastics, but also among zooplankton taxa, depending on shape and feeding style (Frydkjær et al., 2017; Scherer et al., 2017; Botterell et al., 2019). Crustaceous zooplankton are morphologically and taxonomically different, but they face the same challenge: collecting food from a dilute suspension (Scherer et al., 2017). Some zooplankton feed selectively and in ambush, whereas others filter feed (Kiørboe, 2011). Microplastics can complicate these different feeding techniques even more. For example, cladocerans are typically highly efficient filter feeders with higher ingestion rates than copepods, but they cannot easily reject microplastics or unwanted food items, whereas copepods are more selective for preferred food and thus potentially less likely to ingest microplastics (Sommer and Sommer, 2006). any negative impact microplastics have on zooplankton has the potential to subsequently affect different trophic levels and key ecological processes within the aquatic environment (Yu et al., 2020).

Thompson et al. (2004) found microplastics on beaches, in shore and deep–water sediments, and in marine and freshwater environments' surface and subsurface waters. Also, there is evidence that microplastics made in factories are getting into water directly through the runoff from consumer goods (Maynard, 2006).

Shoreline accumulation is another way that trash builds up and this happens often because of discharges from nearby settlements. Materials used in packaging (i.e., polypropylene, polyethylene), rope (i.e., polyamide), and clothing (i.e., acrylic, polyester) that break down into microscopic pieces have also been found on beaches (Browne et al., 2011). Every year, at least 8 million tons of plastic end up in the aquatic environment, like the reservoirs and seas/oceans (Moore, 2007). The most common type of aquatic waste is trash which can be found in both shallow and deep water. Estimates say that between 60 % and 80 % of all waste in aquatic environments, such as aquatic debris, is made of plastic (Napper and Thompson, 2020). In some places, it can make up as much as 90–95 % of all the trash in aquatic habitats.

Plastic is often used for one–time packaging items that are quickly thrown away and could end up in water if they are not handled properly (Napper and Thompson, 2020). Plastics can also last longer than other water materials because they are strong and do not break down easily by natural processes. Plastics could stay in aquatic settings for years, decades, or even longer (Napper and Thompson, 2020). But it has been hard to figure out how long plastics last. Many times, plastics do not completely break down. Instead, they break up into smaller pieces, which are called "microplastics". These plastic pieces get sucked into whirlpools in the aquatic environments, where they build up into huge floating islands of plastic like the Great Pacific Garbage Patch, which is the biggest in the world at 1.6 million km² and 80 000 tonnes (Cozar et al., 2014). Plastic trash not properly discharged can now be found floating in the world's waterways.

Water is one of the most vital and abundant components of the ecosystem. All living organisms require water for survival and growth (Patil et al., 2012). As of now, only Earth is the planet with around 70% water. The environment is heavily polluted with dangerous chemicals due to human population growth, industrialization, agricultural fertilizer use, and other human activities (Patil et al., 2012). Water must be tested before usage for drinking, residential, agricultural, or industrial purposes. Water must be tested for many physic-chemical variables. The selection of parameters for testing water is completely based on what purpose will we utilize that water for, and how much quality and cleanliness do we require? Water contains various forms of pollutants, including floating, dissolving, suspended, microbiological, and bacterial. Physical tests should assess temperature, color, odor, pH, turbidity, and TDS. Chemical tests should assess BOD, COD, dissolved oxygen, alkalinity, hardness, and other parameters(Patil et al., 2012) . To improve water quality and purity, test for trace metals, heavy metals, and pesticide residue. Drinking water must pass all tests and contain the required mineral levels. Only developed countries strictly check all of these requirements. The low quantity of heavy metal and organic pesticide contaminants in water necessitates advanced analytical devices and qualified personnel (Patil et al., 2012). Studying physicochemical parameters is crucial for accurately assessing water quality and comparing results to standard values. Aftab Begum et al. (2005) investigated several physicochemical analysis of untreated fertilizer wastewater. The study found excessive levels of EC, TDS, TSS, BOD, COD, and ammonia in the effluent, exceeding the acceptable limits set by CPCB (1995). Fungal examination confirmed the presence of 15 species on Malt Extract Agar (MEA) medium, indicating contamination. Dey Kallol et al. (2005) examined physiochemical characteristics in samples from the rivers Koel, Shankha, and Brahmani. It has been found that dilution during the rainy season reduces significantly higher metal content levels. The enrichment of metals through bio-magnification and bioaccumulation in edible components in water has been shown to significantly impact the water quality of the river Brahamani, causing public concern.

CHAPTER THREE: MATERIALS AND METHODS

Study area.

Nandoni reservoir (22°59′20″S 30°36′27″E) (Nandoni meaning "the iron smelting ovens" in the Venda language), previously known as the Mutoti Reservoir, is located in Vhembe District Municipality, Limpopo Province of South Africa along the Luvuvhu River near the villages of ha–Mutoti, ha–Budeli, and ha–Mphego, and just a few kilometres from Thohoyandou (Makhera et al., 2011). The overall length of the dam wall is an earth–fill and concrete structure of 2 215 m, height of the dam wall being 47 m, with a spillway of 200 m in length to accommodate the regional maximum flood of 4 650 m^3 s^{-1}. The reservoir approximate catchment area, total length and total capacity of 1 380 km^2, 2 215 m and 16.4 million m^3, respectively (Makherana et al., 2022). The construction of the Nandoni reservoir was started in 1998 and was completed in 2005. The mean air temperature around the reservoir in summer is 23 °C and winter is 17 °C (DWAF, 2012). The mean annual precipitation for the entire catchment varies between 610 and 800 mm, with a mean annual runoff of 519 million m^3 (Heath and Claassen, 1999). The topography of the reservoir area is comprised of low–lying, undulating terrain which is underlain by a gneiss sequence of the Soutpansberg group. The soil in most parts has been eroded due to continuous agricultural activities.

The reservoir is a tourist destination as it provides recreational activities such as fishing (Makherana et al., 2022). The reservoir is surrounded by lodges, a park and villages. Nandoni is a freshwater reservoir that was constructed to provide raw water for domestic use for rural areas in the northern part of the Limpopo province, from Malamulele and Lambani in the east to Sinthumule/Kutama in the west and Giyani (DWAF, 2004). There is also an illegal dumping site adjacent to the reservoir (Mbedzi et al., 2020). With all the human activities happening in and around the reservoir, it is subjected to pollution, especially plastic pollution which can have negative impacts on the organisms and/or the ecosystem, and potentially the humans.

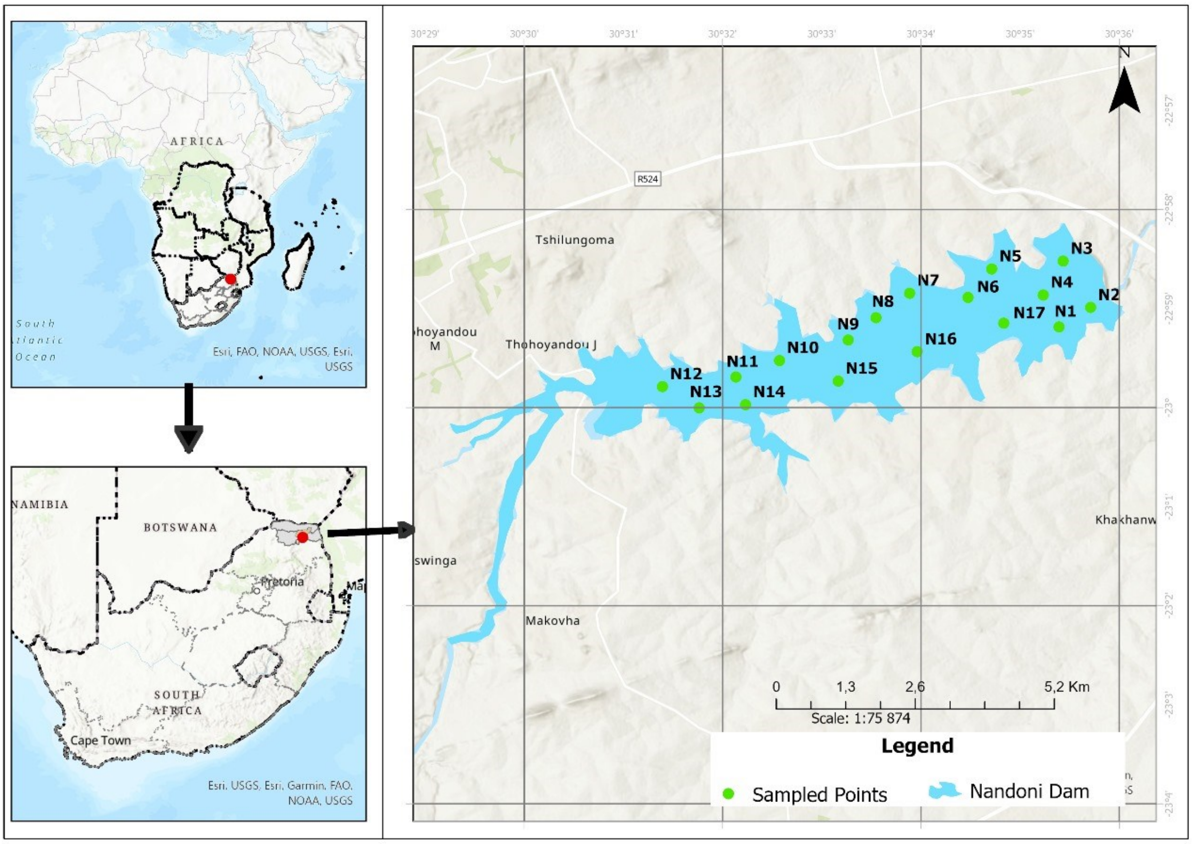

Figure 1. Map highlighting the study sites in the Nandoni reservoir, Limpopo province of South Africa.

Seventeen sites were randomly selected across the entire reservoir, with sampling occurring during the hot–wet (i.e., high water level) and cool–dry (i.e., low water level) seasons from the selected sites within the Nandoni reservoir in Limpopo Province of South Africa.

Data collection

Physicochemical variables

At each site (n = 2) and season, the water temperature (°C), pH, conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), salinity (ppm), resistivity (Ω) and oxygen reduction potential (mV) were measured with a portable multiparameter handheld meter on both sides on the boat. Furthermore, a 250 mL of water was collected for chlorophyll–a (chl–a) concentration determination later on in the laboratory. In the chl–a concentration was determined based on the spectrophotometry: monochromatic method with acidification according to Aminot and Rey (2001). In brief, the 250 mL water samples were vacuum filtered through a 0.7 µm glass fibre filter (GFF) (47 mm diameter). The GFFs were then added into individual labelled 15 mL glass test tubes containing 10 mL of 90 % acetone for chl–a extraction in the fridge over a 24 h period. Afterwards, using a Jenway spectrophotometer of 2 nm maximum bandwidth and stoppered cuvettes with a path length of up to 5 cm, the sample extracts were transferred from the glass tubes to the cuvette by careful pipetting after centrifuging at 3000 rpm for 5 mins. The absorbance of the sample extract was measured at 750 nm ($E750_o$) and 665 nm ($E665_o$) against a 90 % acetone blank. There afterwards, a 0.2 mL 1 % v/v hydrochloric acid was added in the cuvette and mixed, before waiting for 2–5 mins. The absorbance was again measured at 750 nm ($E750_a$) and 665 nm ($E665_a$) against a 90 % acetone blank. The chl–a concentrations were measured according to Lorenzen (1967) equation:

$$Chlorophyll\ (mg\ m^{-3}) = 11.4K\ ((E665_o - E750_o) - (E665_a - E750_a))V_e/LV_f$$

Where, L – cuvette light–path in cm, V_e – extraction volume in mL, V_f – filtered volume in L, R – maximum absorbance ratio of E_{665o} / E_{665a} in the absence of phaeopigments = 1.7 ad K – R/(R – 1) = 2.43

Sediment

Sediments were collected in 17 different locations using a stainless–steel Erkman sediment grab (capacity of 3.5 L, dimensions 152 mm × 152 mm × 152 mm, with a 30 m cable) (Royal Eijkelkamp, Giesbeek). A single sediment sample (2 kg) per site and season was collected and stored in labelled Ziplock bag for transportation to the laboratory. Not all sites had sediment collected i.e., 10 and 15 sites for hot–wet and cool–dry season, respectively. In the laboratory, sediment samples were dried in an oven at 70 °C for 72 h, or until they reached a consistent weight (Hidalgo–Ruz et al., 2012). A 0.2 kg subsample was sieved through a 2 mm mesh steel sieve to remove large pebbles and debris. The material retained in the sieve was analysed to include the large microplastics (500 µm–5 mm) in the total microplastic count. After sieving, 750 mL of clean glass containers were used to digest the organic matter from the sediment subsample with 70 mL of 30 % stabilised hydrogen peroxide. This process was then followed by density separation with 500 mL of $ZnCl_2$ solution. The sample was stirred vigorously, allowing the trapped particles between the sediment particles to be released and suspended before the mixture was left to settle for 24 h. The mixture was then carefully decanted and filtered through a 2 µm cellulose membrane filter paper (Nel et al., 2018; Mbedzi, 2020) and placed in a petri dish for later identification and quantification of microplastics.

Zooplankton

Zooplankton samples were collected from 17 sites over two seasons (i.e., hot–wet, cool–dry; n = 17 per season) using vertical tows so as to get representative zooplankton taxa i.e., Cladocera. A zooplankton net (diameter 40 cm, mesh size 63 µm) was used to collect the samples and preserved in 70 % ethanol for later processing in the laboratory. From the collected zooplankton, Cladocera were separated, and the identification of taxa was done using a dichotomic identification keys by Elenbaas (1994) and Fernando (2002) using an Olympus dissecting microscope at ×200 magnification. At least 50 Cladocera taxa were placed in separate labelled glass test tubes per site and the glass test tubes were covered with an aluminium foil to avoid external contaminants from getting in. Hydrogen peroxide (i.e., 50 mL) was added to digest the Cladocera for microplastic extraction. The sample extract was then vacuum filtered onto a 2 µm cellulose membrane filter, which was then placed into a clean petri dish waiting for further analysis.

Microplastic quantification

This study considered microplastics between 2 µm and 5 mm. After extraction, the samples were identified and quantified using an Olympus dissecting microscope at ×200 magnification. All suspected possible microplastic were examined and enumerated based on their colour (i.e., black/blue, white, yellow, red, green, transparent, other) and shape (fragments, fibre, film, foam, beads). The particles that possessed an unnatural colouration (e.g., multi–coloured, bright colourations) and an unnatural shape (e.g., perfectly spherical, and sharp edges) were counted as microplastics (Mbedzi et al., 2020). The study also used Nile Red dye (CAS 7385–67–3, HYD0718–500 mg, Hycultec, Beutelsbach) to stain the initially suspected microplastics. Once stained a blue light fluorescein was used to fluoresce and identify the initially suspected microplastics. (Nalbone et al., 2021). Nile Red dye was used to confirm microplastics as it exploits the hydrophobic properties of plastic by staining it and illuminating it under a blue fluorescein light. The identified microplastics were quantified as the number of particles per individual in Cladocera samples and particles kg^{-1} dry weight (dwt) in the sediment samples.

Data analysis

Geographical positions of study locations were used to produce microplastic distribution maps based on the estimated abundances in sediments and Cladocera using geographical information systems (ESRI, 2011). A one–way ANOVA was used to assess the differences in physicochemical variables and microplastic types across sediments and Cladocera according to sites (i.e., N1 – N17) and seasons (i.e., hot–wet, cool–dry) using STATISTICA version 12. Before multivariate analysis, all physicochemical variables and microplastic abundance data were log (x + 1) and square transformed to reduce heteroscedasticity. Furthermore, the data was tested for homogeneity of variances and normality and was found to meet the assumptions of a parametric test. The total number of microplastic particles 'species' in each Cladocera and sediment sample (a measure of γ–diversity; Magurran 2004) was calculated for each site and season. A measure of microplastic particles 'species' turnover inside each site and season (i.e., the Whittaker β–diversity corresponding to the internal heterogeneity in a 'community' or in a site) was calculated as $\beta W = \gamma/\text{mean } \alpha$ (Koleff et al., 2003). The Shannon–Wiener diversity index (H′) and evenness for microplastics were also calculated according to Battisti et al. (2018).

We further examined the differences in microplastic particles "communities" and identified the primary types that contributed to the differences using analyses of similarities (ANOSIM) and similarity percentages–particles "species" contributions (SIMPER) in PRIMER 5.0. Correlations were used to analyse the relationships of microplastic types with physicochemical variables and sediment microplastics abundances in STATISTICA version 12.

CHAPTER FOUR: RESULTS

Physicochemical variables

The results of the Nandoni reservoir physicochemical variables parameters during the hot–wet and cool–dry seasons are highlighted in Table 1. During the study, water pH was generally slightly acidic during the hot–wet season (mean range 5.92 – 6.14). However, during the cool–dry season water pH was neutral (mean range 7.02 – 7.03). The water temperature also showed seasonal trends, with high temperatures recorded during the hot–wet season (mean range 22.00 – 22.70 °C), and low temperatures during the cool–dry season (mean range 17.9 – 20.6 °C). Significant seasonal differences in pH ($F = 39.505$, $p = 0.001$), total dissolved solids ($F = 11.583$, $p = 0.001$), oxygen reduction potential ($F = 22.623$, $p = 0.001$), temperature ($F = 7.440$, $p = 0.001$), resistivity ($F = 129.744$, $p = 0.001$) and chlorophyll–*a* concentration ($F = 7.784$, $p = 0.009$) was observed (Table 2). The mean electrical conductivity range in the reservoir was 117.70 – 233.90 µS cm^{-1} for the hot–wet season and 188.7 – 246.5 µS cm^{-1} for the cool–dry season (Table 1). There was no significant seasonal differences ($p > 0.05$) observed in conductivity and salinity (Table 2).

Table 1. Mean (±standard deviation) of physicochemical variables measured from Nandoni reservoir across the two seasons.

Variable	**Hot–wet**		**Cool–dry**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
pH	5.92 – 6.14	6.02 ± 0.05	7.02 – 7.03	7.03 ± 0.003
Conductivity (µS cm^{-1})	117.70 – 233.90	216.62 ± 19.23	188.70 – 246.50	222.43 ± 13.32
Total dissolved solids (mg L^{-1})	18.02 – 215.50	104.29 ± 30.64	74.12 – 95.30	86.2 ± 4.54
Salinity (ppm)	95.91 – 113.90	107.03 ± 4.64	90.30 – 116.60	108.19 ± 5.73
Oxygen reduction potential (mV)	52.80 – 603.30	76.01 ± 93.43	–22.30 to 0.50	–0.27 ± 3.89
Temperature (°C)	22.20 – 22.70	22.39 ± 0.18	17.90 – 20.60	18.84 ± 0.7
Resistivity (Ω)	3.79 – 5.65	5.06 ± 0.34	5.20 – 6.33	5.91 ± 0.27
Chlorophyll–*a* (mg m^{-3})	1.11 – 34.40	9.09 ± 8.72	1.11 – 600.58	118.36 ± 161.28

Table 2. One–way ANOVA results based on seasons for physicochemical variables. Bold values indicate significant differences at $p < 0.05$

Variable	DF	F	*p*
pH	**1**	**39.505**	**<0.001**
Conductivity	1	2.103	0.152
Total dissolved solids	**1**	**11.583**	**0.001**
Salinity	1	0.850	0.360
Oxygen reduction potential	**1**	**22.623**	**<0.001**
Temperature	**1**	**7.440**	**<0.001**
Resistivity	**1**	**129.744**	**<0.001**
Chlorophyll–*a*	**1**	**7.784**	**0.009**

Sediment microplastic abundances

Generally, sediment microplastics had an overall average density of 224.1 ± 151.5 and 189 ± 135.5 particles kg^{-1} dry weight (dwt), for hot–wet and cool–dry seasons, respectively (Figures 2 and 3a,b). Fibres, beads and fragments were dominant during the hot-wet season compared to the cool-dry season, while film and foam were more dominant during the cool-dry season compared to the hot-wet season (Figure 2a – f). During the hot-wet season, Fibres dominated as the more prevalent microplastic type (mean 63 particles kg^{-1} dwt), followed by bead (mean 61.2 particles kg^{-1} dwt), film (mean 54 particles kg^{-1} dwt) and fragment (mean 43.2 particles kg^{-1} dwt). Foam was the least dominant type (mean 2.7 particles kg^{-1} dwt) (Figure 2a – f). Film (mean 61.8 particles kg^{-1} dwt) was the most dominant microplastic type during the cool-dry season, followed by fibres (mean 47.4 particles kg^{-1} dwt), bead (mean 33.6 particles kg^{-1} dwt) and fragment (mean 29.4 particles kg^{-1} dwt). Foam continued to be the least dominant type (mean 16.8 particles kg^{-1} dwt) (Figure 2a – f). Transparent microplastics dominated the colour scheme for both seasons.

Based on ANOVA analysis, there were no significant seasonal differences ($p > 0.05$) observed between sediments *vs* microplastic type (Table 3). The hot–wet season had high microplastic abundances (mean range 6 – 51 particles kg^{-1} dwt) compared to the cool–dry season (mean range 1 – 46 particles kg^{-1} dwt). During the hot–wet season, site 5 which is located upstream of the reservoir had the highest microplastic abundances (mean 459 particles kg^{-1} dwt) (Figure 3a), whereas sites 10, 13 and 14 had low microplastic densities, with a maximum microplastic density of 54 particles kg^{-1} dwt recorded at site 10. During the cool–dry season, sites 1 and 3 had high microplastic densities (mean 378 particles kg^{-1} dwt) and (mean 414 particles kg^{-1} dwt), respectively (Figure 3b). Sites 2, 7, 12 and 13 had very low microplastic densities (i.e., sites 2 = 18, 7 = 54, 12 = 18 and 13 = 9 particles kg^{-1} dwt) (Figure 3b).

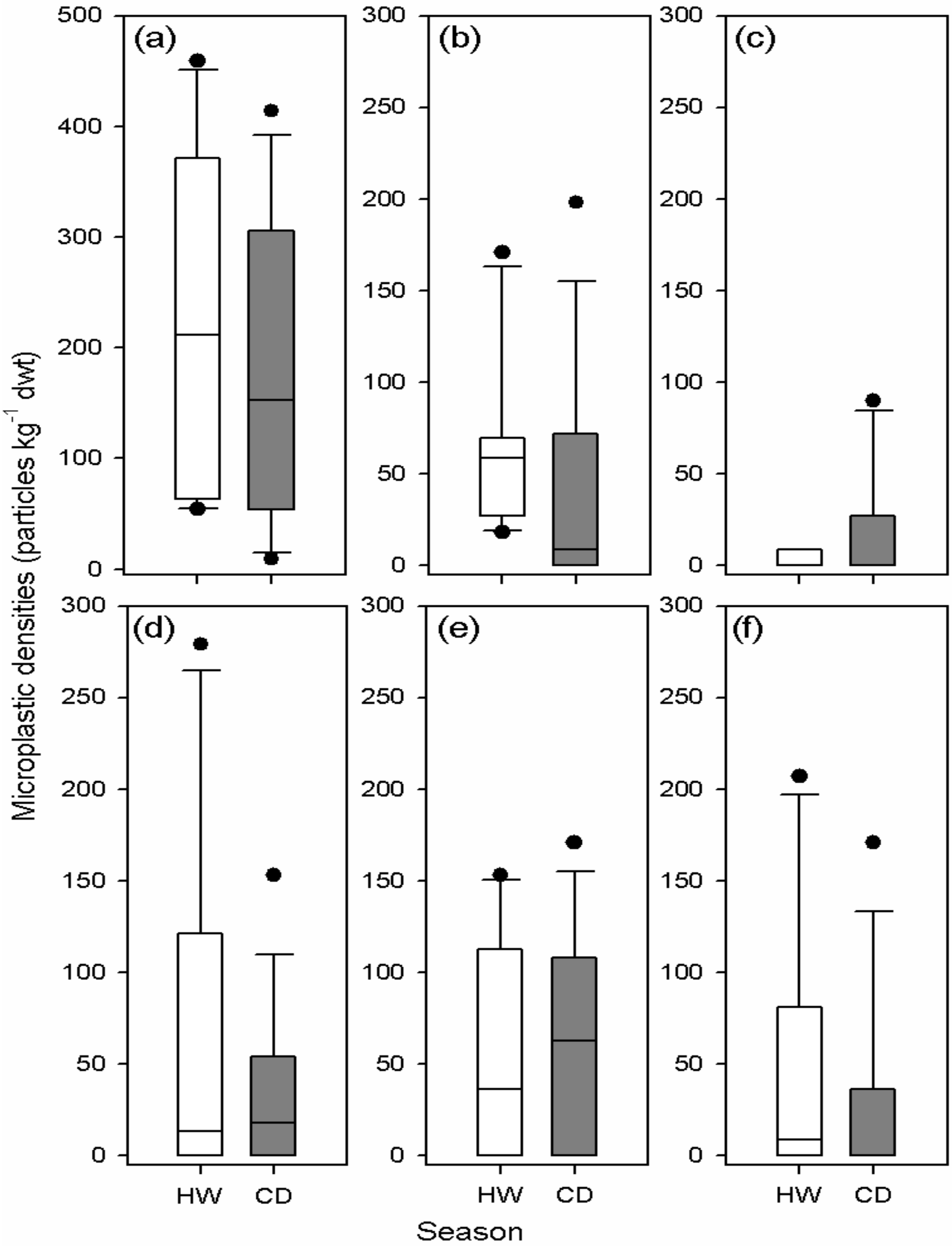

Figure 2. Box and whisker plots highlighting the variation in sediment microplastics densities from Nandoni reservoir: (a) total densities, (b) fibres, (c) foam, (d) beads, (e) film, and (f) fragments. Abbreviations: CD – cool-dry season, HW – hot-wet season

During the hot-wet season, high distribution of microplastics was recorded upstream/ mouth of the reservoir (mean range 144 – 459 particles kg^{-1} dwt) (Figure 3a). Site 5 had the highest microplastic densities upstream (mean 459 particles kg^{-1} dwt). Downstream of the reservoir had very low microplastic distribution (mean range 54 – 63 particles kg^{-1} dwt). Site 10 had the lowest microplastic densities downstream (Figure 3a). Similar trends were observed during the cool-dry season. The upstream sites had high distribution of microplastics (Figure 3b). Sites 1 and 3 had the highest microplastic densities upstream. Whereas the downstream sites had low microplastic distribution (mean range 9 – 153 particles kg^{-1} dwt) except for site 14 which had slightly higher microplastic distribution (mean 315 particles kg^{-1} dwt) (Figure 3b).

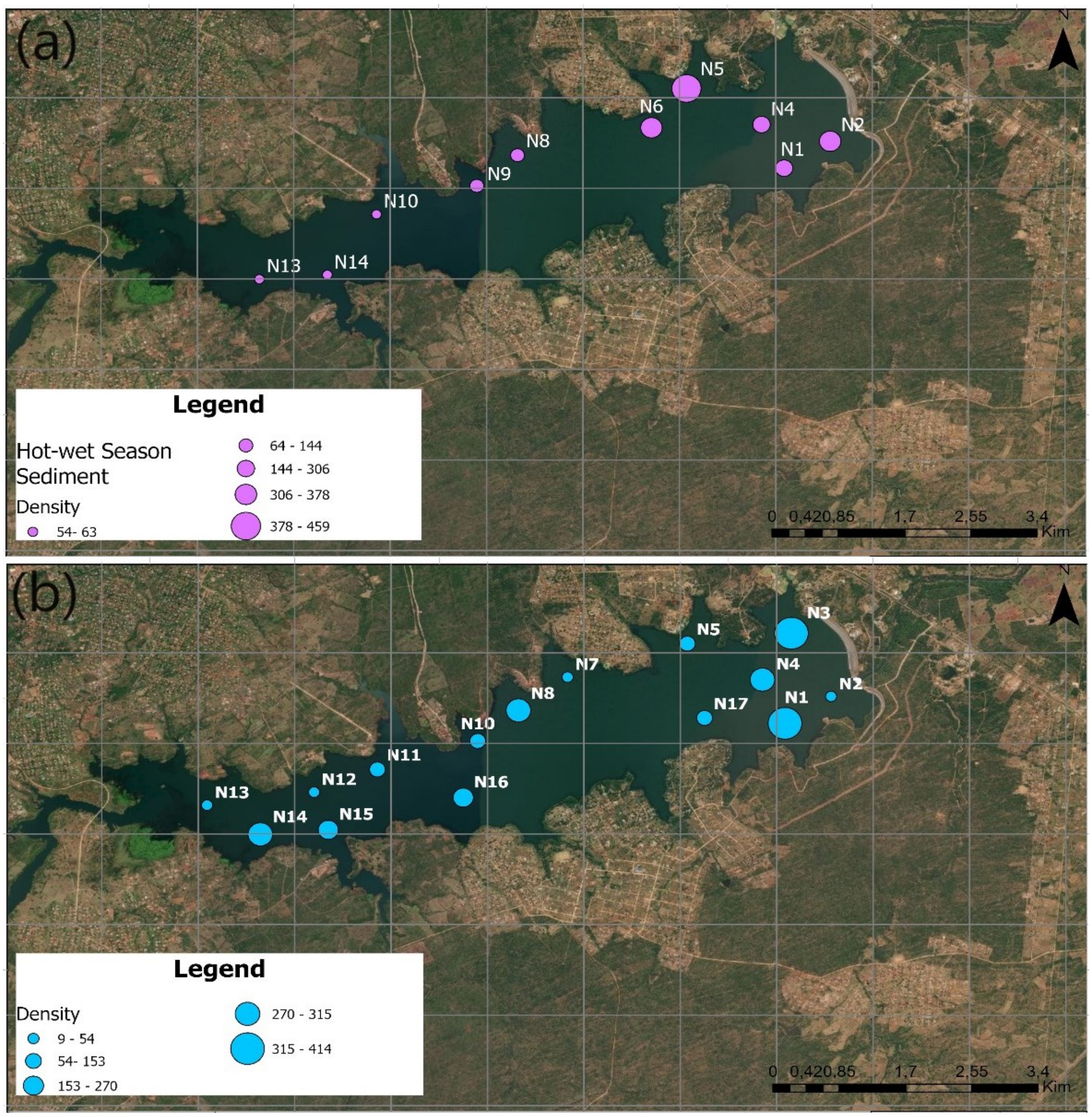

Figure 3. Distribution map of sediment microplastics in the Nandoni reservoir of South Africa from (a) hot–wet and (b) cool–dry season

For microplastic type richness, sediments had high richness during the hot-wet (mean 4) whilst the cool-dry season had low microplastic type richness (mean 3.7) (Figure 5a). High Shannon–Weiner diversity index values were observed during the hot-wet season (mean 1.1). Shannon–Weiner diversity index values did not show significant seasonal variation (F = 0.305, df = 1, p = 0.586) (Table 3). The evenness index was generally high during the hot-wet season (mean

0.8). The Whittaker β–diversity values were high during the cool-dry season (mean 0.6) (Figure 5a). There were no significant seasonal differences ($p > 0.05$) observed for diversity indices for sediment microplastics (Table 3).

Table 3. One–way ANOVA results based on seasons for microplastic colours and morphology in sediments and Cladocera. Bold values indicate significant differences at $p < 0.05$

Microplastic type	DF	Sediment		DF	Cladocera	
		F	*p*		F	*p*
Fibre	1	0.493	0.490	1	0.497	0.486
Foam	1	1.926	0.178	1	0.630	0.434
Bead	1	1.039	0.319	**1**	**4.190**	**0.050**
Film	1	0.103	0.751	1	0.006	0.939
Fragment	1	0.337	0.567	1	0.194	0.663
Total	1	0.367	0.551	1	0.131	0.720
Taxa richness	1	0.229	0.637	1	0.001	0.998
Shannon–Wiener	1	0.305	0.586	1	1.553	0.222
Simpson	1	0.900	0.353	1	2.623	0.116
Evenness	1	0.575	0.456	**1**	**8.378**	**0.007**

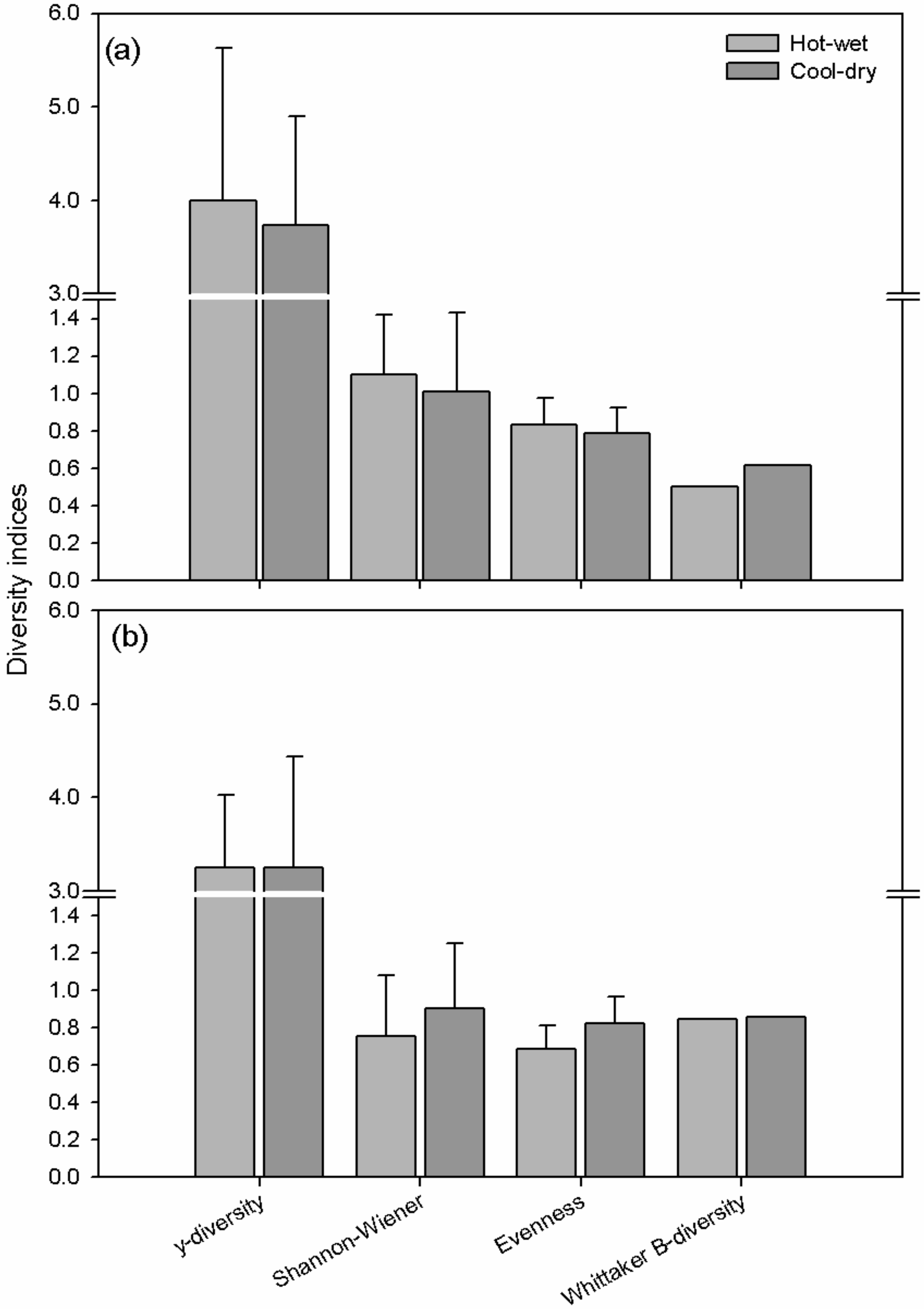

Figure 4. Microplastics diversity indices for (a) sediment and (b) Cladocera in Nandoni reservoir

The ANOSIM results had a Global R value of –0.027 and was not significant (p = 0.596). The SIMPER analysis showed average similarity of 51.2 % and 37.2 % during the hot–wet and cool–dry, respectively. During the hot–wet season, fibres (63.7 %), film (14.8 %) and beads (12.3 %) were the major drivers of the similarities, whereas, film (36.5 %), fibres (29.0 %), and beads (21.5 %) were the key drivers of similarities during the cool–dry season. A pairwise comparison among the two seasons identified 57.7 % dissimilarities being driven mostly by fibres (24.4 %), film (23.6 %), beads (22.7 %), fragments (18.3 %) and foam (11.0 %).

Cladocera microplastic abundances

Three dominant Cladocera taxa were identified in the Nandoni reservoir. Microplastics had an overall average density of 0.3 ± 0.2 particles individual organism^{-1} for the hot–wet and cool–dry seasons. Fibres, foam and film were more dominant during the hot-wet season compared to the cool-dry season. Whereas beads and fragments were more dominant during the cool-dry season compared to the hot-wet season (figure 5b – f). Fibre was the most abundant microplastic type during the hot-wet season (mean 10.8 particles individual organism^{-1}), followed by film (mean 1.5 particles individual organism^{-1}), foam (mean 0.9 particles individual organism^{-1}), fragment (mean 0.5 particles individual organism^{-1}) and bead (mean 0.1 particles individual organism^{-1}) (Figure 5b – f). Fibres continued to be the dominant microplastic type (mean 8.5 particles individual organism^{-1}) for the cool-dry season, followed by film and bead (mean 1.4 particles individual organism^{-1}) each, fragment (mean 0.7 particles individual organism^{-1}) and foam (mean 0.5 particles individual organism^{-1}) (figure 5b – f). Transparent microplastics dominated the colour scheme for both seasons.

Based on the ANOVA analysis, we observed significant seasonal differences between Cladocera and bead (F = 4.190, p = 0.050) (Table 3).

During the hot–wet season, site 9 had high microplastic densities (mean 0.80 particles individual organism^{-1}), whereas site 15 had low microplastic densities (mean 0.06 particles individual organism^{-1}) (Figure 5a). During the cool–dry season, sites 1 (0.80 particles individual organism^{-1}) and 2 (0.88 particles individual organism^{-1}) had high microplastic densities (Figure 5a). Sites 15 (0.06 particles individual organism^{-1}), 16 (0.06 particles individual organism^{-1}) and 17 (0.04 particles individual organism^{-1}) had low microplastic densities (Figure 5a).

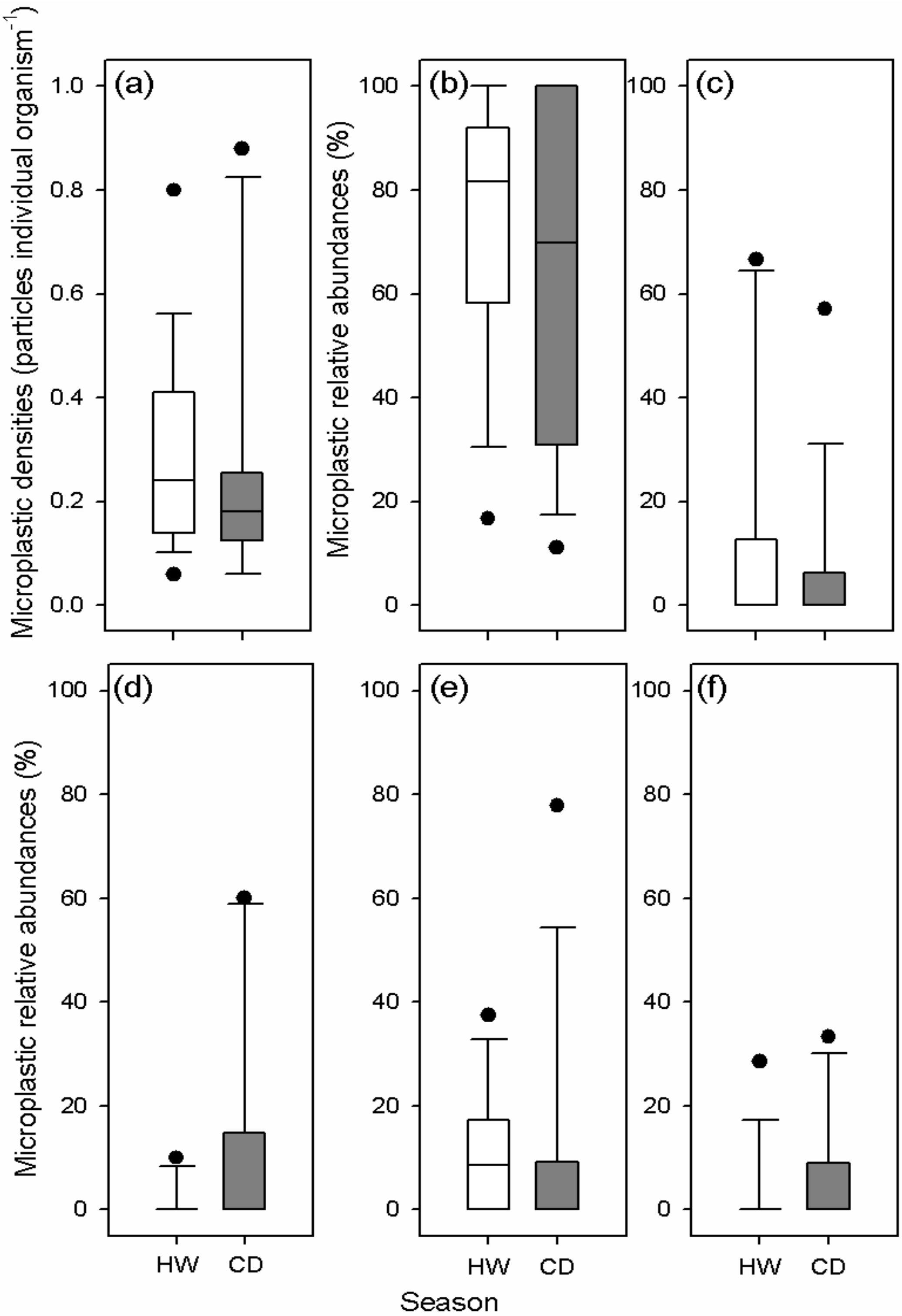

Figure 5. Box and whisker plots highlighting the variation in (a) total Cladocera microplastics densities and (b – f) percentage microplastic relative abundances in Cladocera from Nandoni reservoir: (b) fibres, (c) foam, (d) beads, (e) film, and (f) fragments. Abbreviations: CD – cool-dry season, HW – hot-wet season

During the hot-wet season, high distribution of microplastic was mostly recorded upstream and mid-stream of the reservoir. Site 9, which is located mid-stream had the highest microplastic densities (mean 0.80). However, site 15 which is also found mid-stream had the lowest microplastic densities (mean 0.06) (Figure 6a). During the cool-dry season, an opposite pattern was observed. Most of the upstream and downstream sites had high distribution of microplastics. The sites with the least microplastic densities were found mid-stream (sites 7, 9, 15 and 16) and upstream (sites 5 and 17).

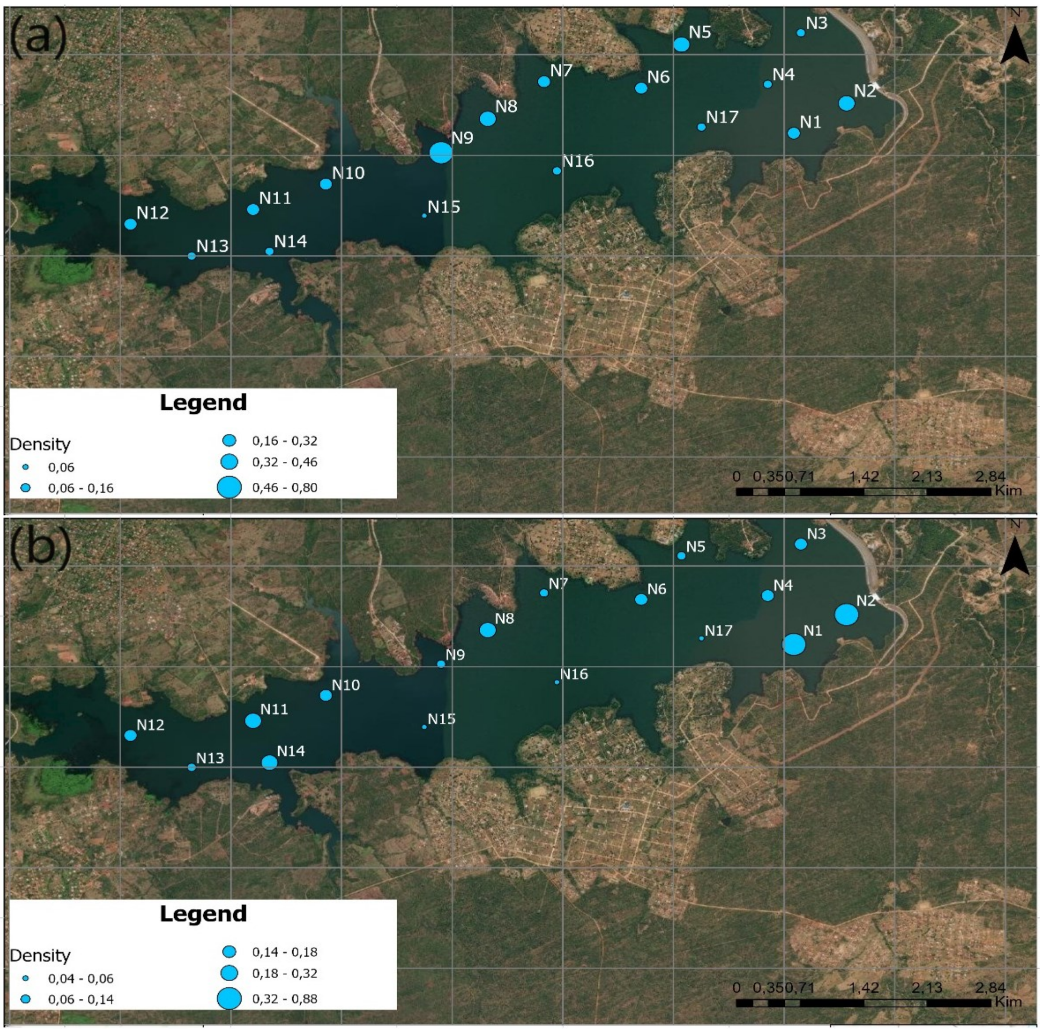

Figure 6. Distribution map of Cladocera microplastics in the Nandoni reservoir of South Africa from (a) hot–wet and (b) cool–dry season

For microplastic type richness, the mean richness values were similar across seasons (mean 3.25) (Figure 5b). High Shannon–Weiner diversity index values were observed during the cool-dry season (mean 0.9) (Figure 5b). Shannon–Weiner diversity index values did not show significant seasonal variation (F = 1.553, df = 1, p = 0.222) (Table 3). The evenness index was generally high during the cool-dry season. The evenness index showed significant seasonal differences (F = 8.378, p = 0.007) across seasons (Table 3). The Whittaker β–diversity values were similar across seasons (mean 0.8) (Figure 5b). We observed significant seasonal differences for beads (F = 4.190, df = 1, p = 0.050) and evenness (F = 8.378, df = 1, p = 0.007) (Table 3).

The ANOSIM results had a Global R value of 0.015 and was not significant (p = 0.301). The SIMPER analysis showed average similarity of 58.6 % and 51.1 % during the hot–wet and cool–dry, respectively. During the hot–wet season, fibres (83.1 %) and film (11.0 %) were the major drivers of the similarities, whereas fibres (85.5 %) and beads (4.7 %) were the key drivers of similarities during the cool–dry season. A pairwise comparison among the two seasons identified 45.7 % dissimilarities being driven mostly by fibres (33.5 %), film (23.2 %), foam (16.4 %), beads (14.1 %) and fragments (12.9 %).

Relationship among physicochemical variables, sediment and Cladocera microplastic abundances

During the hot–wet season, we observed significant negative relationship among sediments with chlorophyll–*a* concentration (r = –0.64, p = 0.048), temperature (r = –0.88, p = 0.001) and resistivity (r = –0.70, p = 0.024), whereas a positive significant relationship was observed for pH (r = 0.77, p = 0.010) and salinity (r = 0.76, p = 0.011) (Table 4). However, no significant relationships ($p > 0.05$) were observed between sediments and conductivity, total dissolved solids and oxygen reduction potential (Table 4). During the cool–dry season, no significant relationships ($p > 0.05$) were observed between sediments and physicochemical variables.

For Cladocera, we observed no significant differences with physicochemical variables during the hot–wet season (Table 4). Most of the physicochemical variables were found not to be significantly different ($p > 0.05$) during the cool–dry season, with positive significant relationships being observed between Cladocera and –conductivity ($r = 0.65$, $p = 0.007$) and salinity ($r = 0.53$, $p = 0.035$) (Table 4). For the combined (i.e., all seasons) data, we observed no significant differences ($p > 0.05$) between physicochemical variables and sediments, we also did not observe significant differences between physicochemical variables and Cladocera (Table 4).

Table 4. Pearson correlation results highlighting the relationship among physicochemical variables, sediment and Cladocera microplastic abundances in Nandoni reservoir. Values in parenthesis (i.e., brackets) *p*–values and bold values indicate significant differences at $p < 0.05$

Variable	Hot–wet		Cool–dry		All seasons	
	Sediment	Cladocera	Sediment	Cladocera	Sediment	Cladocera
Cladocera	0.10 (0.774)		0.02 (0.957)		0.23 (0.198)	
Chlorophyll–*a*	**–0.64 (0.048)**	–0.15 (0.556)	–0.27 (0.329)	–0.31 (0.243)	0.13 (0.445)	–0.30 (0.083)
pH	**0.77 (0.010)**	0.25 (0.334)	–0.46 (0.085)	0.13 (0.638)	0.22 (0.210)	–0.12 (0.488)
Conductivity	0.55 (0.099)	0.29 (0.261)	0.51 (0.055)	**0.65 (0.007)**	**0.41 (0.016)**	**0.35 (0.043)**
Total dissolved solids	0.49 (0.149)	–0.20 (0.451)	0.33 (0.232)	0.23 (0.386)	–0.19 (0.278)	–0.01 (0.972)
Salinity	**0.76 (0.011)**	0.31 (0.221)	0.30 (0.275)	**0.53 (0.035)**	**0.36 (0.035)**	**0.36 (0.037)**
Oxygen reduction potential	–0.44 (0.203)	–0.001 (0.998)	0.11 (0.698)	0.12 (0.671)	–0.20 (0.272)	0.12 (0.506)
Temperature	**–0.88 (0.001)**	0.17 (0.526)	–0.16 (0.561)	–0.37 (0.159)	–0.20 (0.254)	0.10 (0.577)
Resistivity	**–0.70 (0.024)**	–0.14 (0.594)	–0.17 (0.551)	–0.33 (0.212)	0.06 (0.730)	–0.21 (0.234)

CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Discussion

The study assessed the spatio–temporal variation in microplastic abundances and distribution in benthic sediments and Cladocera taxa of the Nandoni reservoir across the hot–wet (i.e., high water level) and cool–dry (i.e., low water level) seasons. The Nandoni reservoir was contaminated with microplastics, as these particles were detected in the Cladocera taxa and sediments. These results shows that microplastics are ubiquitous in the Nandoni reservoir, which might pose harm to zooplankton and other aquatic biota. As hypothesized, we observed high abundances of microplastics near the dam wall within the benthic sediments and within the Cladocera taxa as that is where free–floating microplastic particles are most likely accumulating. There were no clear significant seasonal differences in microplastic types observed within sediment samples.

Microplastic types

During the hot-wet season, fibres were the most dominant microplastic type, which is consistent with previous studies (e.g., Di and Wang, 2018; Liu et la., 2021; Nkosi et al., 2023). The abundance of these fibres within the natural environment could be caused by airborne deposition, fishing, agricultural activities occurring in and/or near the reservoir. According to Dalu et al. (2023), fishing tools or gear like fishing nets are formed from agricultural plastic mulch breaking down and finding their way to the nearby water bodies. One potential big source of fibres in Nandoni is laundry during weekdays (Figure 7). Synthetic clothing is a major source of fibres in aquatic systems and when synthetic clothing are washed, tiny fibres could potential be released into the natural waters (De Falco et al., 2019; Gaylarde et al., 2021).

Furthermore, cigarette butts which were found littered along the shorelines of the reservoir could be another potential source of fibres in the system as cigarette butts contain cellulose acetate, a type of plastic that breaks down into fibres within the natural environment. Also tyre wear could result in fibres, when they rub against the road causing small rubber pieces to break off releasing microplastics into the environment (Kole et al., 2017; Arias et al., 2022). In contrast, studies by Ballent et al. (2016), Horton et al. (2017) and Dalu et al. (2023) found fragments to be the most dominant microplastic type. Rodrigues et al. (2018) also highlighted that fragments were the most dominant particles in Antuã River.

During the cool-dry season, film was found to be the most dominant microplastic type among sediments. According to Nizzetto et al. (2016) microplastics especially films with large particle sizes are more likely to accumulate under slow water flow, which leads to an increase in their density and deposition. Film plastics are a major source of microplastics in aquatic systems which are a form of secondary microplastics. These microplastics are formed when macroplastic pieces break down into small pieces through a variety of processes, such as weathering, abrasion, and biological activity (Di and Wang, 2018; Nava et al., 2023). Thus, there dominance could have been due to a number of ways such as people littering particularly plastic bags and bottles on land and/or in waterways, which was a common occurrence within the study area. This litter can be washed into rivers and reservoirs by rain and/or wind from the terrestrial environment and be further broken down by river processes such as physical abrasion (Min et al., 2023). Litter dumping was a common occurrence along the shores of Nandoni reservoir, which could have contributed to film dominance.

Figure 7. Some of the potential sources of microplastics (i.e., fibres) within Nandoni reservoir. Women washing laundry on a weekend is common occurrence in the rivers that drain into Nandoni reservoir and also along the reservoir shorelines.

Microplastic abundance

Microplastics were more dominant during the hot-wet season compared to the cool-dry season, which is consistent with other studies (e.g., Guo et al., 2021, Dalu et al., 2023). The Nandoni reservoir receives more water from Luvuvhu River, which is highly polluted (Dalu et al., 2023).

This suggests that wet deposition (Brahney et al., 2020) or microplastic resuspension due to rainfall disturbance (Zhang et al., 2020) probably leads to increased microplastic pollution. Rain is a significant factor influencing microplastic deposition in aquatic bodies (Dris et al., 2016). Wind and rain can cause direct inputs of microplastics from adjacent terrestrial areas and shoreline to aquatic environments (Fischer et al., 2016). According to Guo et al. (2021), sediments are more likely to be a sink for terrestrial-derived microplastics during the hot-rainy season. However, other factors such as anthropogenic activities can also increase the diversity of microplastics since there are more diverse sources if inputs of plastic waste (Zhang et al., 2017). In contrast, a study by Dalu et al. (2021) and Nkosi et al. (2023) found that high microplastic abundances were associated with the cool-dry (i.e., winter) season due to reduced water flow which facilitated microplastic deposition within the sediments or concentration within the water column. Song et al. (2020) highlighted that flood discharge can transfer numerous microplastics to areas above the reservoir leading to an increase in microplastic abundances within the reservoirs. Guo et al. (2021) observed that the abundance of microplastic within reservoirs was high in the dry season compared to the hot-wet season, due to continuous input from human activities and the reduced water storage, which leads to high microplastic concentrations. During the hot-wet season, sediments had high abundances of microplastics downstream sites (i.e., sites closer to the dam wall) compared to the upstream (i.e., or at the mouth of the reservoir). Similar trends were observed during the cool-dry season and these results were contrast with findings from Watkins et al. (2019) who found that majority of the reservoirs, had high microplastic concentrations upstream than downstream. Liu et al. (2022) also found that microplastics accumulated heavily near the dam wall of the Guanyingyan Reservoir, thereby supporting our study findings.

Transparent was the dominant colour in both sediments and Cladocera taxa for both seasons, which was consistent with findings by Dalu et al. (2023) and Nkosi et al. (2023). According to Nkosi et al. (2023), microplastic colour helps to identify the origin of microplastics. The results suggest that fishing nets, illegal waste disposal and synthetic fibres might be the main cause of fibres in the reservoir.

During the hot-wet season, Cladocera taxa had high abundances of microplastic downstream (i.e., closer to the dam wall) and middle of the reservoir. The results suggest that Cladocera taxa ingest the large amount of microplastics that are currently accumulating or being washed into the reservoir. A relatively high abundance of microplastics in the middle parts of reservoir may result from significant thermal stratification during summer and formation of a metalimnion in the middle layer. The metalimnion of a water body is the layer where the temperature and thus the density gradient changes fast (Gau et al., 2021). Therefore, particles often accumulate in the metalimnion, which can lead to ingestion of microplastic by Cladocera taxa. However, almost similar trends were observed during the cool-dry season. The cool-dry season had high microplastic abundances in the upper and lower reaches of the reservoir. The upstream sites could be receiving microplastics from the Luvuvhu River while the lower reach sites had high microplastic abundances which could be caused by trapping of microplastics by the dam wall. Microplastics from upstream transported by river water cannot pass the dam wall and therefore accumulate behind the dam. According to Zhang et al. (2015), microplastics in the surface water can only be removed by sedimentation to the bottom of the reservoir (Van Cauwenberghe et al., 2013), or completely degraded, or accidentally ingested by aquatic organisms such as zooplankton. The accumulation of microplastics can be determined by the loadings occurring within the upper reaches of the reservoir (i.e., mouth).

In the present study, we used taxa richness, Shannon–Wiener diversity, evenness, and Whittaker β–diversity, to try and interpret the seasonal variation of the diversity of microplastics sources. For sediments, Shannon–Weiner diversity index values did not show significant seasonal variation, and this suggested that the sources of microplastics in the Nandoni reservoir did not vary across seasons. We observed slightly high Shannon–Weiner diversity index values during the hot-wet season for sediments but an opposite trend for Cladocera taxa, which according to Fang et al. (2021), suggest that the hot-wet season might have more microplastic sources. Sources such as fishing nets, illegal dumping, agriculture, and wastewater runoff were recorded in this study area. However, for Cladocera, we observed high Shannon–Weiner diversity index values were during the cool-dry season, which suggest it had more sources of microplastics. The slow water velocity during this season causes microplastic particles to float on the water surface, making it easy for Cladocera to ingest it. Jiwarungueangkul et al. (2021), showed that microplastic abundances in estuarine in the rainy season are lower than those in the dry season. This may be caused by the runoff flux effects on microplastic input (Kang et al., 2015; Cheung et al., 2016; Fan et al., 2019; Li et al., 2020; Sukhsangchan et al., 2020). Although high runoff flux in the rainy season can bring suspended microplastics into the lakes and estuaries (Kang et al., 2015; Fok and Cheung, 2015; Liu et al., 2019), the stronger hydrodynamic forces may make the deposition of microplastics difficult (Vermeiren et al., 2016; Krelling et al., 2017). This leads to a lesser amount of microplastics deposition in the rainy season. This highlights the seasonal variations in microplastic abundance in reservoirs. Wind can also cause direct deposition of microplastics into water bodies, thus, the strong winds during the cool-dry season particularly towards the end of the season could have also contributed as a source of microplastic contamination in the reservoir.

Relationship among physicochemical variables, sediment and Cladocera microplastic abundances. Water physicochemical variables were measured to microplastics' concentrations to try to interpret their relationships with microplastics' abundances. During the hot–wet season, we observed significant negative relationships among sediments with chlorophyll–*a* concentration, temperature, and resistivity. Chlorophyll–*a* is used as measure of the amount of algal biomass growing in water bodies, thus this negative relationship may imply that microplastics abundances in sediments are affected by increased algal biomass. The negative relationship between temperature and microplastic abundances may imply that microplastic in sediments are abundant in low temperatures. This also means that the abundance of microplastics in the sediments of the Nandoni Reservoir are not depended on the temperature. In contrast, a study showed that higher temperatures can enhance the interaction between microplastics, sediments and sediment microorganisms and accelerate the microplastic degradation and transformation processes, whereas lower temperatures can slow down these processes (Guo et al., 2024). Resistivity is a measure of fluid ability to conduct electricity, which can help explain and determine if there are dissolved ions (salts) or associated contaminants in the Nandoni Reservoir. Furthermore, the negative relationship suggests that microplastics accumulation are affected by the number of contamination sources. This can be linked to the Luvuvhu River as the major source of microplastic contamination in the Nandoni reservoir. We observed a positive significant relationship for pH and salinity. According to Buwono et al. (2021), drastic changes in pH are a water pollution indicator, hence the positive relationship may imply that microplastics are abundant in areas are highly alkaline. Nkosi et al. (2023), also highlighted a positive correlation between microplastics abundances and pH levels. Salinity is the concentration of dissolved salt in a water body; thus, the positive relationship suggests that microplastics become more abundant in water bodies containing high salt concentration. For Cladocera taxa, we observed positive significant relationships with conductivity and salinity during the cool–dry season. Conductivity is the ability to move heat or electricity from one place to another, thus, the positive relationship may imply that microplastics become more abundant in areas with high conductivity.

Conclusions

The result of the current study shows the complexity and provides evidence of microplastic pollution in the sediments and Cladocera taxa. As more studies are conducted to quantify microplastic pollution in oceans and Rivers, this study underlines the significance of including reservoirs as mechanisms for the retention of microplastics. The study also highlights a foundation for identifying and addressing the issue of microplastic pollution. It will also help all stakeholders (e.g., community members, conservationists, and institutions) to come together to discuss and provide possibly solutions for microplastic pollution locally.In both sediments and Cladocera taxa, microplastic pollution, microplastic concentration were high during the hot-wet season. This connection indicates that the Nandoni reservoir receives most of its microplastics from the Luvuvhu River, which has been found to contain high traces of microplastics.Additionally, although both sediments and Cladocera taxa were dominated by fibres, the distribution of microplastic types varied across sites and location. The density and ingestion of microplastics by Cladocera taxa was significantly high. Cladocera taxa is a primary consumer and plays an important role in nutrient cycling and the transfer of energy to higher trophic levels through food chain. This is an indication that Cladocera taxa may transfer microplastics to other aquatic organisms and possibly humans. Microplastics consumed by lower trophic level creatures facilitate bioaccumulation and transmission up the food chain and to new geographic location. The direct and indirect ecological consequences of microplastics on zooplankton vary not only with the kind and concentration of microplastics, but also among zooplankton taxa, depending on shape and feeding style. Crustaceous zooplankton are morphologically and taxonomically different, but they face the same challenge: collecting food from a dilute suspension. Some zooplankton feed selectively and in ambush, whereas others filter feed. Microplastics can complicate these different feeding techniques even more. For example, cladocerans are typically highly efficient filter feeders with higher ingestion rates than copepods, but they cannot easily reject microplastics or unwanted food items. These findings raise a great concern regarding the long-term exposure of animals and human populations to microplastics. and highlight the urgent need of more developmental and reproductive studies regarding the microplastics paradigm.

Recommendations

To completely understand the environmental factors that can potentially influence differences in the consequences of microplastic exposure on freshwater biota, both temporary and spatially, regular monitoring of microplastic pollution and its effects on freshwater organisms is essential. Also, South Africa can have a national footprint of microplastics in freshwater reservoirs, this could be significant for policy recommendations and implementation. It is equally important for scientists to publicise their findings to the general public in order to increase awareness about microplastic contamination and to spur action to reduce or eliminate plastic pollution of freshwater environment.

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