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Water or sediment? Assessing seasonal microplastic accumulation from wastewater treatment works

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ABSTRACT

Microplastics have become a major environmental concern around the world due to their potential impact on ecosystem functioning and biota. Microplastics enter freshwater systems through a variety of sources, with wastewater treatment work discharges being the most important source. The study aimed to determine the seasonal (i.e., hot–wet, cool–dry) variation in water and sediment microplastic abundances up- and down-stream of wastewater treatment works across two subtropical river systems (i.e., Crocodile and Luvuvhu) in South Africa. Overall, we found that microplastic type and distribution often did not show clear seasonal and site differences in water, hence microplastics were widespread across the studied systems and microplastic concentrations did not relate clearly to wastewater treatment works. This was further indicated by microplastic risk assessments which showed high pollution loads upstream. However, there were significant differences in sediment microplastic loads across seasons, indicating a source-sink effect towards the hot-wet season. The non-metric multidimensional scaling ordination based on microplastic densities for water and sediment discriminated slightly among systems, with major overlaps across the different locations and seasons. As a result, the current research indicates that seasonal context influences differences in microplastic concentrations, with the hot–wet season being associated with the high pollution loads, particularly within the sediments where this was more pronounced indicating the sink-source effect which is linked to sediments and not water.

Key words: aquatic ecosystem, environmental change, microplastics, plastic pollution, rivers, wastewater treatment work

HIGHLIGHTS

- Fragments were the dominant microplastic group.
- Microplastic type and distribution often did not show clear seasonal and site variations.
- Microplastic risk assessments indicated high pollution loads upstream.
- No clear impacts of wastewater treatment work.
- Results useful in informing stakeholders on how to manage plastics and establish programmes.

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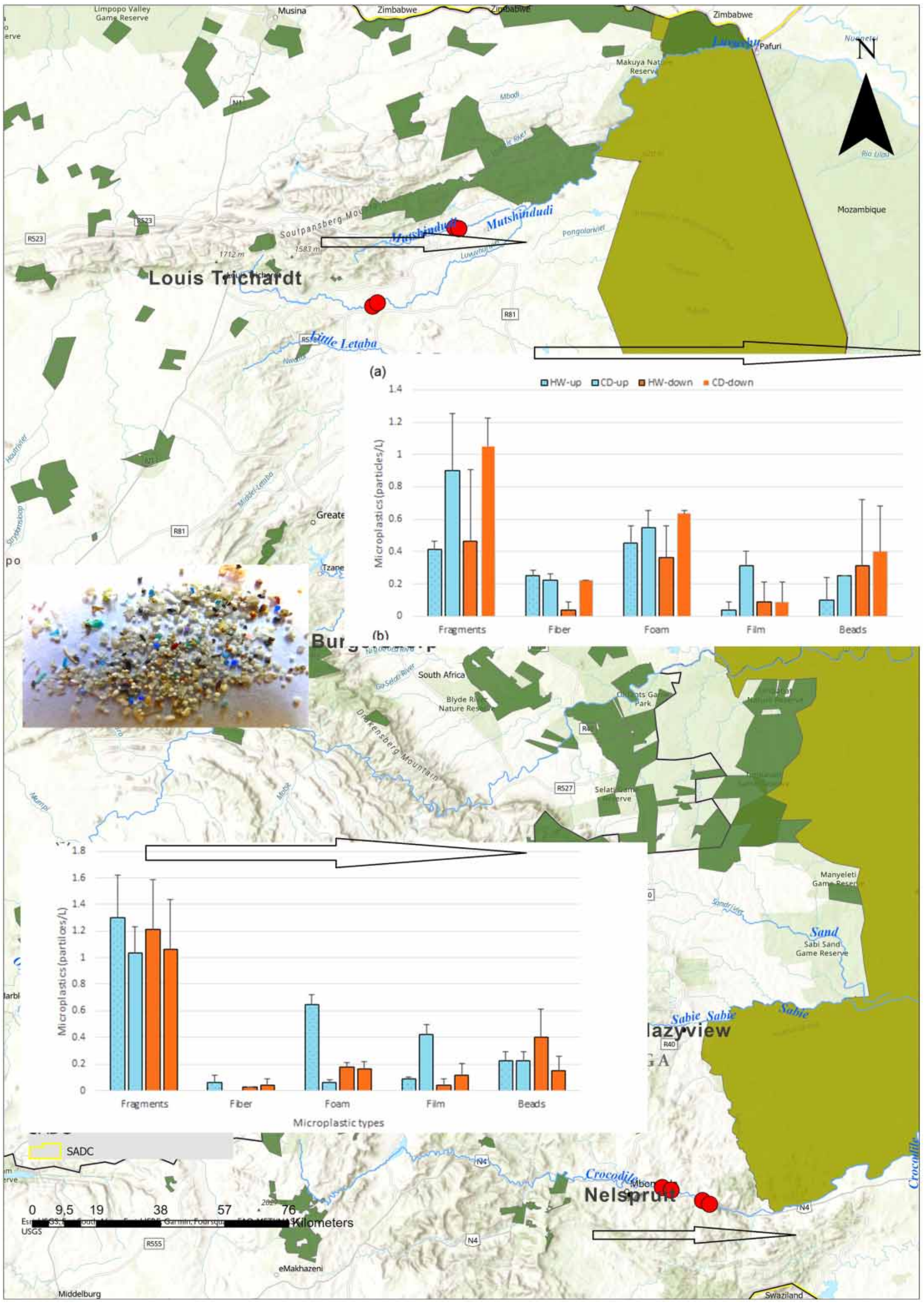
GRAPHICAL ABSTRACT



1. INTRODUCTION

Plastics are organic synthetic polymers derived from the monomerization of gas and crude oil (Cole *et al.* 2011). Since the early 1940s, there has been a rapid increase in the number of plastics produced, resulting in their widespread use (Dalu & Tavengwa 2022). Despite its numerous societal benefits, plastic has become the focus of growing ecological concern due to the proportion of globally generated waste (Barnes *et al.* 2009). Due to poor waste management, human behaviour, and unintentional loss, plastic may end up in aquatic environments with serious consequences (Derraik 2002; Cuthbert *et al.* 2019). Plastic can accumulate throughout the world's aquatic and terrestrial ecosystems, and further disintegrates into smaller fragments called 'microplastics' (Cole *et al.* 2011; Hidalgo-Ruz *et al.* 2012; Horton *et al.* 2017).

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Although the impacts of microplastics on many aquatic ecosystems are still not fully understood, laboratory experiments on these pollutants have been linked to negative consequences for some biota (Avio *et al.* 2017; Cuthbert *et al.* 2019). In addition to their widespread presence, microplastics are an environmental concern due to their potential to harm a variety of aquatic organisms either physically or chemically, from zooplankton to mammals (Miranda & de Carvalho-Souza 2016; Yardy *et al.* 2022). Microplastics can block animal digestive tracts and provide a viable pathway for the transfer of trace organic and inorganic contaminants to the exposed organisms (Cole *et al.* 2011). They may also endanger human health if consumed, particularly through contaminated aquatic food such as fish and shrimp (Miranda & de Carvalho-Souza 2016).

The detection and measurement of microplastics in aquatic habitats have increased recently (Murphy *et al.* 2016). It is challenging to pinpoint the precise origin of these contaminants due to many potential sources. However, studies (e.g., Nel & Froneman 2015; Wang *et al.* 2017; Dalu *et al.* 2019a, 2019b, 2021) have shown that their existence in aquatic ecosystems is frequently linked to places with intensive industrial activity, including harbours and metropolitan areas. Although the presence of microplastics in coastal ecosystems has been relatively thoroughly established, information on estuarine and freshwater habitats' origins remains relatively sparse (Anderson *et al.* 2016). This is despite streams, rivers, and estuaries being thought to be a major source of inland microplastic pollution to marine habitats (Eerkes-Medrano *et al.* 2015).

Rivers can be major sources of plastics in coastal environments, with approximately 1.15–2.41 million tonnes of plastic predicted to be released into the oceans each year, and dams are estimated to capture approximately 65% of plastics from entering rivers (Lebreton *et al.* 2017). Urban runoff, sewage effluent, and sludge applied to agricultural regions are only a few examples of the many different sources of microplastic contamination that may affect freshwater ecosystems (Eriksen *et al.* 2013). According to McCormick *et al.* (2014), the concentration of microplastic contamination increased downstream of wastewater treatment works. However, Klein *et al.* (2015) observed no connection between the prevalence of microplastics and the location of sewage treatment works, industrial activities, and/or population density. Because of the current growing population and rapid industrialisation and urbanisation, which are likely to increase the amounts of microplastics in wastewater, the significance of wastewater treatment work effluent as a microplastic source may increase in the future (Browne *et al.* 2011).

Wastewater treatment works can serve as both a barrier and an entry point for microplastics into the aquatic environment (McCormick *et al.* 2014; Dalu & Tavengwa 2022). Conventional wastewater treatment with primary and secondary treatment processes can remove up to 99% of microplastics from wastewater, with most microplastics removed during the pre-treatment phase (Carr *et al.* 2016). Despite their high reduction capability, conventional wastewater treatment works may be a significant source of microplastics due to the large volumes of effluent discharged. Therefore, increasing the quality of final effluents has been a constant requirement for wastewater treatment over the last few decades. However, technologies used to improve the final effluent quality are not specifically designed to remove microplastics (Mason *et al.* 2016).

This research will aim to contribute to a better understanding of the effects of wastewater treatment works on subtropical river systems. Thus, we aimed to assess differences in microplastic abundances up- and down-stream of wastewater treatment works in two subtropical river systems in the provinces of Mpumalanga and Limpopo, South Africa (Crocodile and Luvuvhu Rivers). Furthermore, sediment pollution load indices based on microplastic data were calculated for all sites and systems to calculate the level of impact of these pollutants on the natural environment. We hypothesised that the (i) Luvuvhu River catchment system will be less impacted by microplastics compared to the Crocodile River as it does not have many adjacent communities linked to wastewater treatment works and is rural, whereas the Crocodile River is highly disturbed by human activities and will have increased microplastic loads within its system and (ii) the microplastic pollution load will be greater downstream of the wastewater treatment works due to increased microplastic loads from the discharge points. The study will provide data which the local municipality can use to develop adequate management strategies to reduce plastic pollution.

2. MATERIALS AND METHODS

2.1. Study sites

The Crocodile River (east) is a river system found within the Mpumalanga province of South Africa and has a catchment area of 10,446 km^2, with an average channel width of 45 m. The river originates in the Steenkamp

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Mountains north of Dullstroom, Mpumalanga province, and then flows through the Kwena Dam, Montrose waterfall, Schoemanskloof, Mbombela (or Nelspruit), and Kruger National Park, before emptying into the Komati River system. Most of the river's water is used for irrigation purposes (Harrison *et al.* 2018). The average catchment annual rainfall and temperature are 800 mm and 12–14 °C, respectively. The Crocodile River is dominated by bedrock (i.e., dolerite intrusions and basaltic lava) and sandy pools. The system is dominated by trees such as *Acacia*, *Combretum*, *Sclerocarya*, and *Terminalia*. Anthropogenic activities have increased in recent decades due to socioeconomic drivers such as agriculture, population growth, climate variability, floodgate construction, and untreated wastewater disposal (Stone-Jovicich *et al.* 2011).

The Luvuvhu River is a river system found within South Africa's northern Limpopo province, with its origins being the Soutpansberg Mountains (Mazibuko *et al.* 2021). The Luvuvhu River flows for over 200 km across a variety of landscapes before joining the Limpopo River at the edge of the Kruger National Park and the border with Zimbabwe. The catchment area of the systems is about 5,941 km^2. The average annual precipitation, total evaporation, and temperatures are 608 mm, 1,678 mm, and 21–25 °C, respectively. The land use activities are commercial forestry estates (4%), subsistence agriculture and grazing (50%), cultivated lands (including irrigated lands representing 3%) (13%), protected game reserve areas (30%) and urban areas (3%). Most of the population is found around the Thohoyandou area (Jewitt *et al.* 2004).

Four sites were selected upstream and downstream of two sewage treatment works in each system (i.e., Crocodile River – Kingston Vale and KaNyamazane; Luvuvhu River – Thohoyandouand Matatshe Prison) (Figure 1). Sampling was conducted in March–April 2022 (hot–wet season) and August (cool–dry season) from all four sites per system.

2.2. Data collection

2.2.1. Water

Water for microplastic abundance determination was collected from four sites per system ($n = 3$ subsamples per site) over two seasons (i.e., wet (March/April 2022) and dry (August 2022)) according to Bouwman *et al.* (2005) and Hidalgo-Ruz *et al.* (2012). The sample collection was based on the bulk sampling method where 90 L of river water collected using a metal bucket (15 L), and then was filtered through a 63-μm sieve mesh per site and season. Following each collection, the sieve was rinsed with distilled water to ensure that all microplastics passed uncontaminated into the receiving glass jar before further analysis in the laboratory (Bouwman *et al.* 2005; Masura *et al.* 2015). In the laboratory, the samples were filtered onto 2-μm mesh size cellulose membrane filter paper (diameter 47 mm) and immediately placed in labelled petridishes. The filter membranes were then dried at room temperature for 3–4 days before being examined under a Nikon DS-L3 camera head microscope at magnification range of 10 × –80 × , with the field of view calculated at 20× magnification (Masura *et al.* 2015). Visual counting was used to categorise particles based on their colours, shapes, and sizes (Bouwman *et al.* 2005; Wang *et al.* 2017). To aid in the identification of the samples, we used Nile Red dye (CAS 7385-67-3, HYD0718-500 mg, Hycultec, Beutelsbach) to stain all materials on the filter membranes. The microplastics were classified into the following commonly used morphological shapes: beads, fibre, fragment, film, and foam (Norén & Linell 2007). Colours were also classified as follows: black, blue, red, colourless, and other, which are representative of commonly occurring colours in most microplastic–aquatic studies (Barrows *et al.* 2018). Particles were considered microplastics if they had unnatural colouration (e.g., bright colouration, multicoloured) and/or unnatural shape (e.g., sharp edges, perfectly spherical; Hidalgo-Ruz *et al.* 2012).

2.2.2. Sediment

Sediment samples (2.0 kg, $n = 2$ per site) were collected from the upper 5-cm layer at the four different locations per system and season using a quadrat placed randomly along the river littoral zones. The sediment samples were stored in a labelled ziplock bag and transported to the laboratory for further identification. In the laboratory, the samples were dried in an oven at 50 °C for 48–72 h, or until they reached a consistent weight (Hidalgo-Ruz *et al.* 2012). A 500 g of subsample was sieved through a 2-mm mesh steel sieve to remove large plant materials and pebbles/stones. The sieved residual sediment was weighed to be used to determine the number of microplastic particles per kilogram of dry weight (*dwt*). Large microplastics (2–5 mm) that were found in the material left on the sieve, were counted as part of the overall microplastic count (Galgani *et al.* 2013). In a clean 5-L glass beaker, a 63-μm mesh-filtered hypersaturated saline zinc chloride ($ZnCl_2$) separation solution (700 g L^{-1}, density 1.6 g cm^{-3}) was added to each sieved 500 g of subsamples before being mixed

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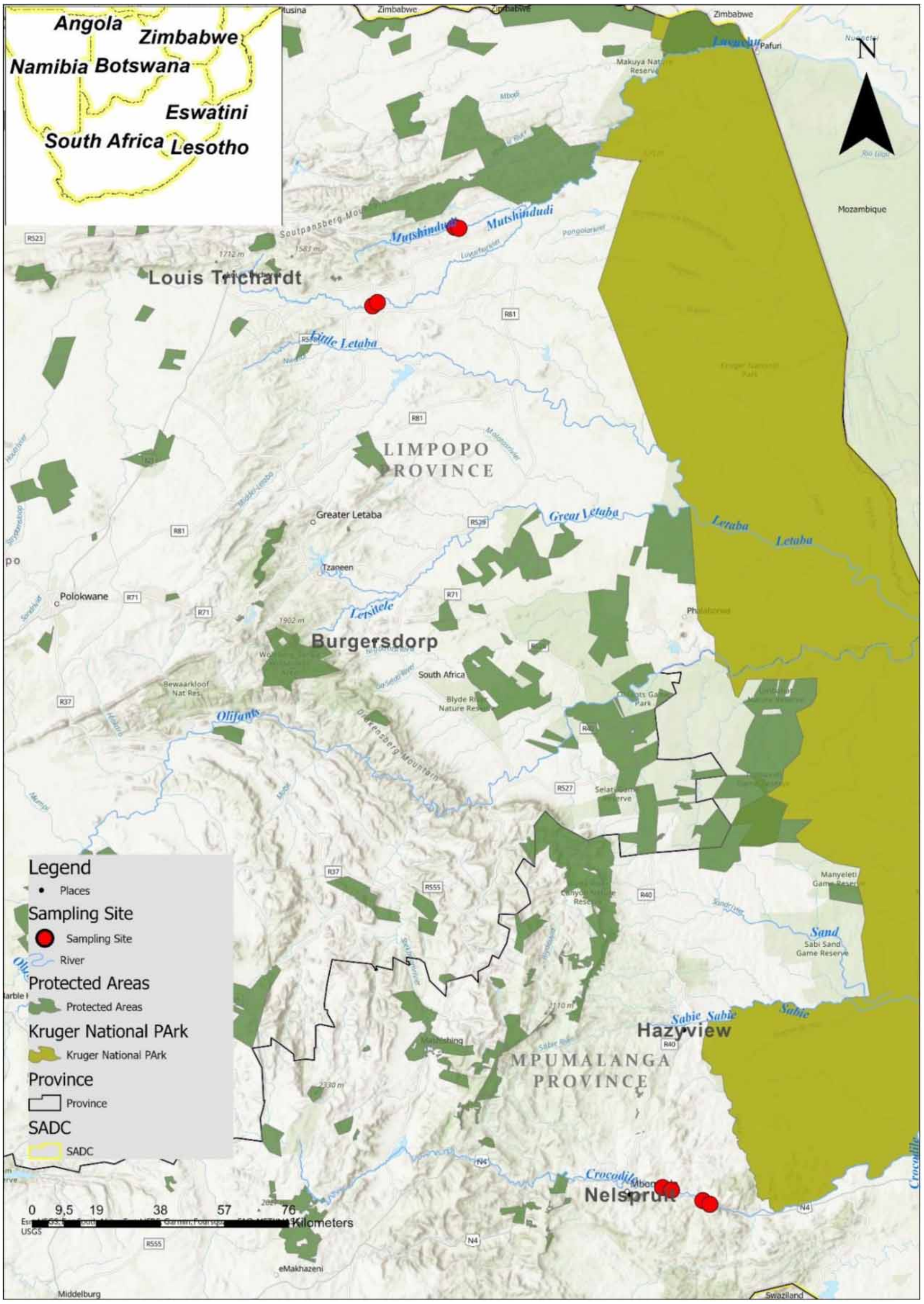

Figure 1 | The map highlighting the Crocodile (south) and Luvuvhu (north) River catchment study sites in red across the two provinces of Limpopo (north) and Mpumalanga (south), South Africa.

vigorously to disaggregate and suspend plastic particles. This was then allowed to stand for 24 h to allow the denser particles to sediment before the samples were filtered through a 63-μm mesh; the process was repeated at least four times per sample (Lusher *et al.* 2015). Similar to the water samples, sediment samples were filtered

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onto 2-μm mesh size cellulose membrane filter paper (diameter 47 mm) and immediately placed in labelled petridishes. The filter membranes were then dried at room temperature for 3–4 days before being examined under a Nikon DS-L3 camera head microscope at a magnification range of 10 × –80 × , with the field of view calculated at 20× magnification (Masura *et al.* 2015). All microplastics were classified into morphological shapes (i.e., fragments, beads, fibre, foam, film) and colour, similar to the water samples, after staining the samples with Nile Red dye.

2.3. Sediment microplastic risk assessment

Microplastic indices based on the modification of the Tomlinson *et al.* (1980) (pollution load index) for sediment metals were applied to provide comparative assessments of the potential effects of microplastics within the natural aquatic environment (see Kabir *et al.* 2021). The microplastic contamination factor assesses microplastic concentrations compared to background concentrations, with the following risk categories: low (<1), moderate (1–3), high (3–6), very high (>6).

$$\text{Contamination factor} = \frac{A_{\text{sample}}}{B_{\text{background}}}$$

where A is the concentration of microplastics at a particular site, and B is the concentration of background microplastic concentration from the control pristine site with little to no anthropogenic inputs (i.e., 20 particles kg^{-1}).

Microplastic pollution load index (MpPLI) allows for the standardisation, with a high degree of accuracy, of microplastic concentrations found in the river sediment for each system. Any site is considered to be polluted or in poor condition when MpPLI > 1 and no pollution when < 1. The MpPLI for each river system was calculated according to modified Tomlinson *et al.* (1980) equations based on Kabir *et al.* (2021):

$$MpPLI_{\text{river}} = (CF_1 \times \ CF_2 \ \times \cdots \times CF_n)^{1/n}$$

where i represents the site, n the number of sites in a river system, CF_n is the contamination factor at a particular site n. Therefore, the $MpPLI_{river}$ is the riverine microplastic pollution load index.

2.4. Data analysis

The data were assessed for homogeneity of variance and normality using Levene's and Shapiro–Wilk's W tests, respectively, and were found to conform to parametric assumptions. A three-way analysis of variance (ANOVA) was used to analyse the differences in total microplastic 'species' (i.e., *different particle types* – fragments, beads, fibre, foam, film) and diversity indices among sites (i.e., *upstream* – CR1, CR3, LR1, LR3; *downstream* – CR2, CR4, LR2, LR4), systems (i.e., Crocodile River, Luvuvhu River) and seasons (i.e., cool–dry, hot–wet), with significant variables being further assessed for pairwise differences using Tukey's post-hoc analysis. All statistical analyses were carried out in SPSS version 25.0 (SPSS Inc. 2007).

Diversity analysis (i.e., Shannon–Wiener diversity and evenness indices) were calculated based on the modified Battisti *et al.* (2017) and Dalu *et al.* (2019a) equations for microplastics, where species were substituted by microplastic morphological shapes. Furthermore, spatiotemporal variation in microplastic particles was analysed using non-metric multi-dimensional scaling (*n*-MDS) based on Bray–Curtis similarity measures for the water and sediment microplastic abundances across locations, systems, and seasons.

3. RESULTS

3.1. Water microplastic abundances

Microplastic densities within the Crocodile River generally had high densities downstream of the wastewater treatment works across the two seasons (Figure 2(a)). During the hot–wet season, mean total microplastic densities of 3.3 and 4.3 particles L^{-1} for the upstream and downstream sites, respectively, were recorded, with fragments being dominant. During the cool–dry season, similar patterns to the hot–wet season were observed, with high mean total microplastic densities being recorded downstream (mean 5.4 particles L^{-1}), with fragments being dominant (Figure 2(a)).

Within the Luvuvhu River system, high mean total microplastic was observed downstream during both the hot–wet (total mean 4.9 particles L^{-1}) and cool–dry (total mean 4.5 particles L^{-1}) seasons, respectively. During the

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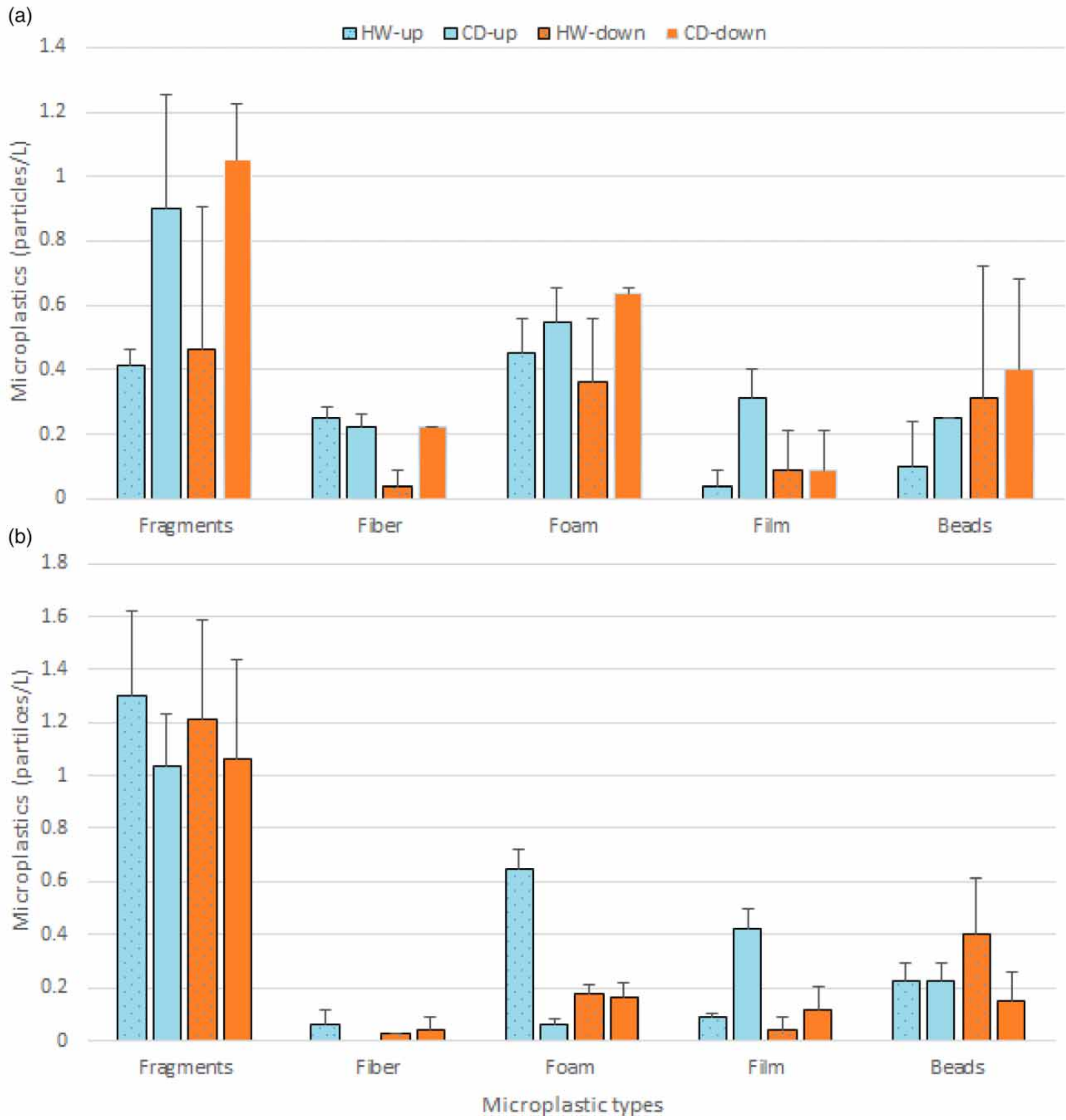

Figure 2 | Water microplastic types (particles L^{-1}, mean $\pm$ standard deviation) were collected in the (a) Crocodile and (b) Luvuvhu River systems. CD, cool–dry season; HW, hot–wet season.

hot–wet season, the upstream sites were dominated by fragments (mean 1.30 particles L^{-1}) and foam (mean 0.65 particles L^{-1}), with the downstream sites also being dominated by fragments (mean 1.21 particles L^{-1}) (Figure 2(b)). Similar to the hot–wet season, the cool–dry was dominated by fragments both upstream and downstream (Figure 2(b)).

We observed no significant differences ($p > 0.05$) across systems, seasons, and locations/sites for total microplastic abundances (Table 1). However, we observed significant fragment ($F = 8.06$, $p = 0.009$), fibre ($F = 8.50$, $p = 0.008$), foam ($F = 8.27$, $p = 0.008$), and film ($F = 5.07$, $p = 0.034$) differences across systems, with beads being significantly different across seasons ($F = 6.41$, $p = 0.018$) and sites ($F = 4.65$, $p = 0.041$) (Table 1).

Microplastic are classified into colours (black, blue, white, red, green, yellow, transparent, and other colours). Within the Crocodile River, transparent colours (48.7%), blue (36.3%), and white (27.0%) were observed to be high among other colours across the downstream of the wastewater treatment in both seasons, whereas the Luvuvhu River system was also dominated with transparent (35.7%), black (35.3%), and blue (30.9%) colours in both rivers and seasons.

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Table 1 | ANOVA results for water, sediment and combined (water, sediment) microplastic densities across different systems (i.e., Crocodile and Luvuvhu Rivers), seasons (i.e., hot–wet, cool–dry) and sites (i.e., upstream, downstream) of a sewage treatment works

	Systems		Seasons		Sites	
Variable	***F***	***p***	***F***	***p***	***F***	***p***
Water						
Fragments	**8.06**	**0.009**	1.76	0.197	2.11	0.159
Fibre	**8.50**	**0.008**	2.56	0.122	0.04	0.835
Foam	**8.27**	**0.008**	0.47	0.498	0.29	0.594
Film	**5.07**	**0.034**	0.13	0.722	0.42	0.523
Beads	0.28	0.603	**6.41**	**0.018**	**4.65**	**0.041**
Total	1.10	0.305	3.43	0.076	0.31	0.584
Sediment						
Fragments	3.31	0.081	**6.22**	**0.020**	2.01	0.169
Fibre	**14.74**	**0.001**	0.29	0.597	0.25	0.621
Foam	2.85	0.104	**14.41**	**0.001**	1.11	0.302
Film	0.18	0.894	**6.34**	**0.019**	0.02	0.878
Beads	0.14	0.708	**12.34**	**0.002**	0.07	0.797
Total	0.45	0.509	**23.40**	**< 0.001**	1.49	0.235
Combined						
Fragments	**10.44**	**0.002**	1.04	0.313	0.02	0.901
Fibre	0.02	0.894	0.98	0.327	0.01	0.904
Foam	0.43	0.514	**5.63**	**0.022**	1.32	0.257
Film	1.65	0.205	**4.85**	**0.032**	0.27	0.604
Beads	0.39	0.535	1.52	0.224	1.29	0.262
Total	1.51	0.225	3.41	0.071	1.51	0.226

Note: Significant values are indicated in bold.

3.2. Sediment microplastic abundances

Microplastic densities within the Crocodile River were generally high at the upstream sites of the wastewater treatment works during the hot–wet season (total mean 314.5 particles kg^{-1} *dwt*) compared to downstream sites (total mean 290.5 particles kg^{-1} *dwt*) (Figure 3(a)). During the cool–dry season, a reverse trend was observed, with downstream sites having a high microplastic densities (total mean 103 particles kg^{-1} *dwt*) compared to upstream sites (total mean 100.8 particles kg^{-1} *dwt*). Fragments (mean 87.5 particles kg^{-1} *dwt*) and fibres (mean 67.5 particles kg^{-1} *dwt*) remained dominant at the upstream site during the hot–wet season. Whereas fibres (mean 83.8 particles kg^{-1} *dwt*) and film (mean 75.0 particles kg^{-1} *dwt*) were high at the downstream site. Within the cool–dry season, fibres (mean 53.8 particles kg^{-1} *dwt*) and fragments (mean 21.3 particles kg^{-1} *dwt*) were dominant along the upstream site. During the cool–dry season, fibres (mean 46.3 particles kg^{-1} *dwt*) and beads (mean 21.3 particles kg^{-1} *dwt*) remained dominant at the downstream sites of the wastewater treatment works (Figure 3(a)).

Within the Luvuvhu River system, high mean total of microplastic densities (total mean 327.0 particles kg^{-1} *dwt*) observed upstream during the hot–wet season (Figure 3(b)). Whereas during the hot–wet season, fragments (mean 91.0 particles kg^{-1} *dwt*) and beads (mean 83 particles kg^{-1} *dwt*) remained dominant in the upstream site of the wastewater treatment works. Meanwhile, the downstream was dominated by beads (mean 71 particles kg^{-1} *dwt*) and fragments (mean 49 particles kg^{-1} *dwt*). During the cool–dry season, films (mean 48 particles kg^{-1} *dwt*), fibres (mean 46 particles kg^{-1}), and fragments were dominant in the upstream site of the wastewater treatment works (Figure 3(b)). Fragments (mean 32 particles kg^{-1} *dwt*) and fibres (mean 29 particles kg^{-1} *dwt*) were dominant in the cool–dry season at the downstream site.

We observed that fibres ($F = 14.74$, $p = 0.003$) were significantly different across systems (Table 1). Across seasons all microplastic types and total abundances were found to be significantly different ($p < 0.05$) with the

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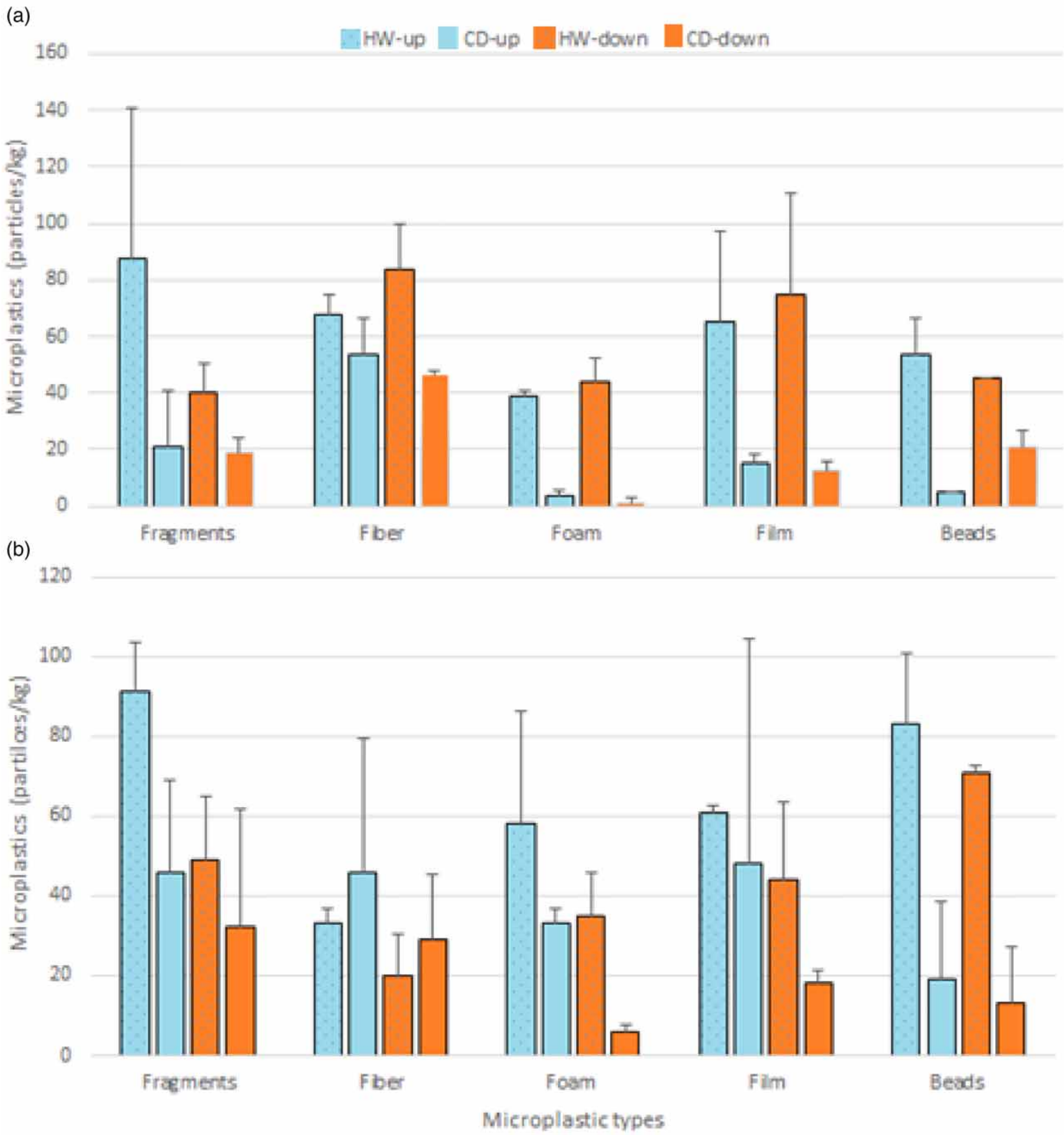

Figure 3 | Sediment microplastic abundances (particles kg^{-1} *dwt*; mean ± standard deviation) measured in the (a) Crocodile and (b) Luvuvhu river systems. CD, cool–dry season; HW, hot–wet season.

exception of fibres ($F = 0.29$, $p = 0.597$) which were not significantly different across seasons. Across the different study sites, up and downstream of sewage treatment works, no significant differences ($p > 0.05$) were observed (Table 1).

3.3. Microplastic diversity indices

3.3.1. Water

'Microplastic type' richness values for the Crocodile River range from a mean of 2.8 to 4 across both seasons, with low mean richness values being observed upstream (Figure 4(a)). For the Luvuvhu River system, the mean richness values were similar across sites (mean 3.5) during the hot–wet season, whereas in the cool–dry season slightly higher mean richness values were observed downstream (mean 3.8) (Figure 4(a)). Richness showed weakly significant seasonal variation ($F = 4.26$, $p = 0.050$) (Table 2).

The Shannon–Wiener richness values were generally low (mean range 0.70–1.15) for both systems. Within the Crocodile River system, low Shannon–Weiner indices were observed downstream during both seasons, whereas

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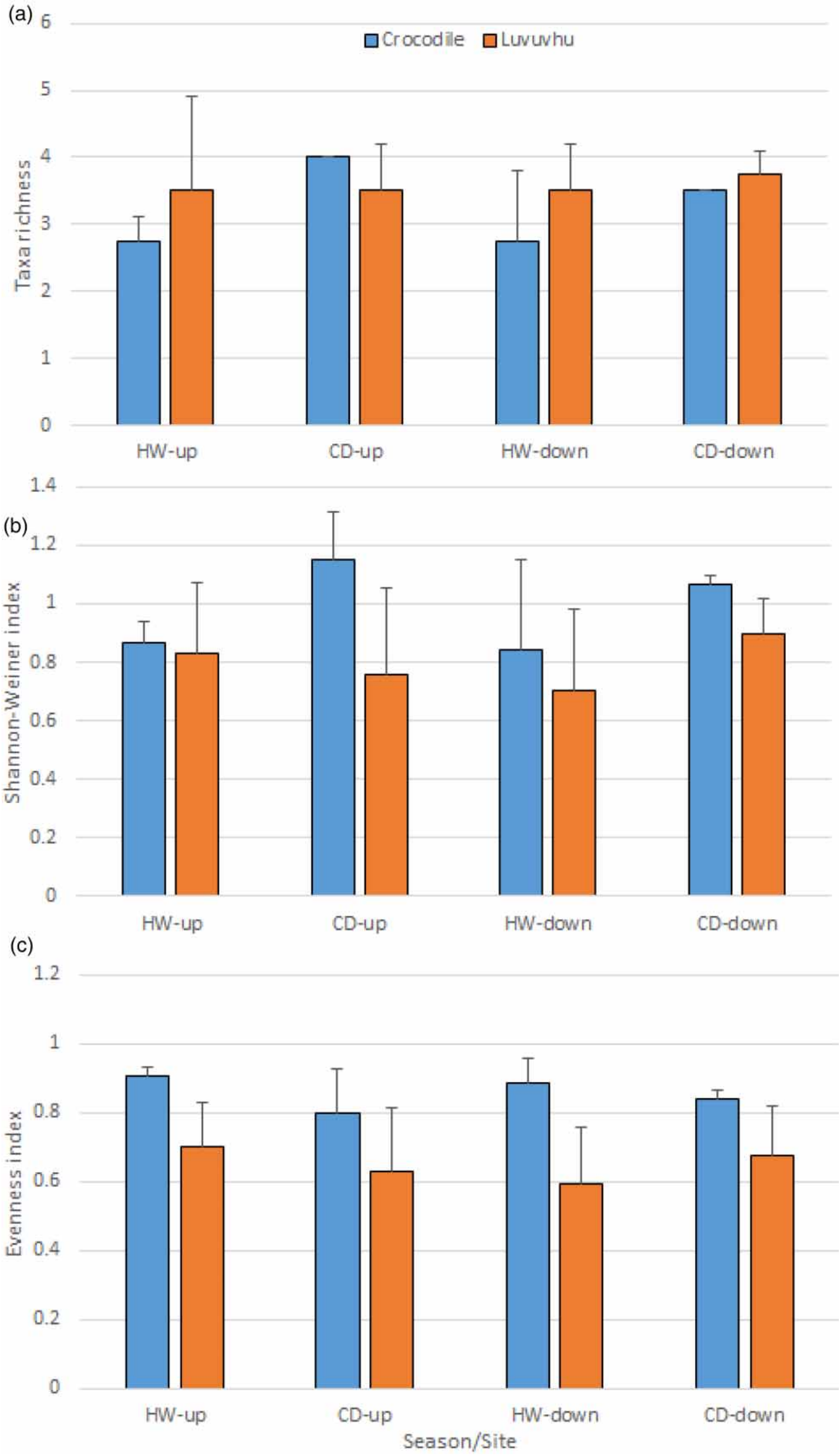

Figure 4 | Diversity indices [(a) taxa richness, (b) Shannon–Wiener, (c) evenness] observed in water microplastics in the Crocodile and Luvuvhu river systems. CD, cool–dry season; HW, hot–wet season.

in the Luvuvhu River system during the hot–wet season, low Shannon–Wiener indices were observed in the downstream site (mean 0.70). However, during the cool–dry season, high values were observed downstream (mean 0.90) (Figure 4(b)). For hot–wet season evenness in both systems, the values were high upstream and low downstream, whereas, during the cool–dry season, an opposite trend was observed (Figure 4(c)). Evenness showed significant system variation ($F = 21.90$, $p < 0.001$) (Table 2).

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Table 2 | ANOVA results for water, sediment and combined (water, sediment) microplastic diversity index measures across different systems (i.e., Crocodile and Luvuvhu Rivers), seasons (i.e., hot–wet and cool–dry) and sites (i.e., upstream and downstream) of a sewage treatment work

	System		Season		Sites	
Variable	***F***	***p***	***F***	***p***	***F***	***p***
Water						
Microplastic richness	1.81	0.189	**4.26**	**0.050**	0.25	0.624
Shannon–Wiener	2.25	0.145	2.72	0.112	0.34	0.564
Evenness	**21.9**	< **0.001**	0.74	0.399	0.19	0.668
Sediment						
Microplastic richness	0.30	0.586	**9.38**	**0.005**	0.30	0.586
Shannon–Wiener	0.31	0.585	**10.62**	**0.003**	0.02	0.884
Evenness	0.24	0.630	0.06	0.804	0.37	0.550
Combined						
Microplastic richness	0.36	0.551	0.02	0.897	0.004	0.950
Shannon–Wiener	1.68	0.200	0.22	0.638	0.000	0.990
Evenness	**8.28**	**0.006**	0.52	0.474	0.005	0.945

Note: Significant values are indicated in bold.

3.3.2. Sediments

'Microplastic type' richness values for the Crocodile River ranged from a mean 4.3 to 5.0 across seasons, with similar lower mean richness values being observed from upstream to downstream sites (Figure 5(a)). For the Luvuvhu River system, the mean richness values were similar across sites (mean 4.8) during the hot–wet season. Whereas, in the cool–dry seasons low mean richness values were observed at the upstream (mean 4.0) and downstream (mean 0.5) sites (Figure 5(a)). Richness showed significant seasonal variation ($F = 9.38$, $p = 0.005$) (Table 2).

The Shannon–Weiner diversity index values were generally high (mean range 1.1–1.5) for both seasons within the Crocodile River system. Whereas in the Luvuvhu River system during the cool–dry season, low Shannon–Weiner index values were observed at the upstream site (mean 1.1). However, during the hot–wet season, high values were observed at the upstream site (mean 1.4). Shannon–Weiner diversity index values showed a significant seasonal variation ($F = 10.62$, $p = 0.003$) (Figure 5(b)). For the evenness index for both systems, the values were low at the upstream and downstream sites, with the hot–wet season, an opposite trend was observed (Figure 5(c)). Evenness showed no significant ($p > 0.05$) seasonal, system, or site variation.

The *n*-MDS ordination based on microplastic densities for water and sediment discriminated slightly among systems, with major overlaps across the different locations and seasons (Figure 6). The overlap observed among systems, especially within the sediments could be attributed to differences in catchment anthropogenic activities (Figure 6).

3.4. Sediment microplastic risk assessment

The microplastic contamination factor for the Crocodile River hot–wet upstream sites (contamination factor (CF) mean 15.6 ± 3.5, risk category – very high) was generally high compared to the cool–dry upstream sites (CF 4.9 ± 0.1, risk category – high). Similar patterns were observed for the Crocodile River system, with very high-risk category for the hot–wet downstream site (CF mean 14.4 ± 6.7), whereas the cool–dry downstream site had a high-risk category (CF mean 5.0 ± 0.7). For the Luvuvhu River system, both season upstream sites had very high microplastic contamination factor risk (hot–wet mean 16.3 ± 6.0; cool–dry mean 9.6 ± 6.1). Similarly, the downstream sites for both seasons (hot–wet mean 10.9 ± 4.2; cool–dry mean 6.4 ± 0.2) also recorded very high-risk microplastic contamination categories.

The MpPLI both systems indicated microplastic pollution (>1; Crocodile PLI 8.4; Luvuvhu PLI 9.4). The pollution load for the Crocodile River was high during the hot–wet season (PLI range 13.3–15.3). The hot–wet season upstream site had a pollution load of 15.3, with the downstream site having a pollution load of 13.3.

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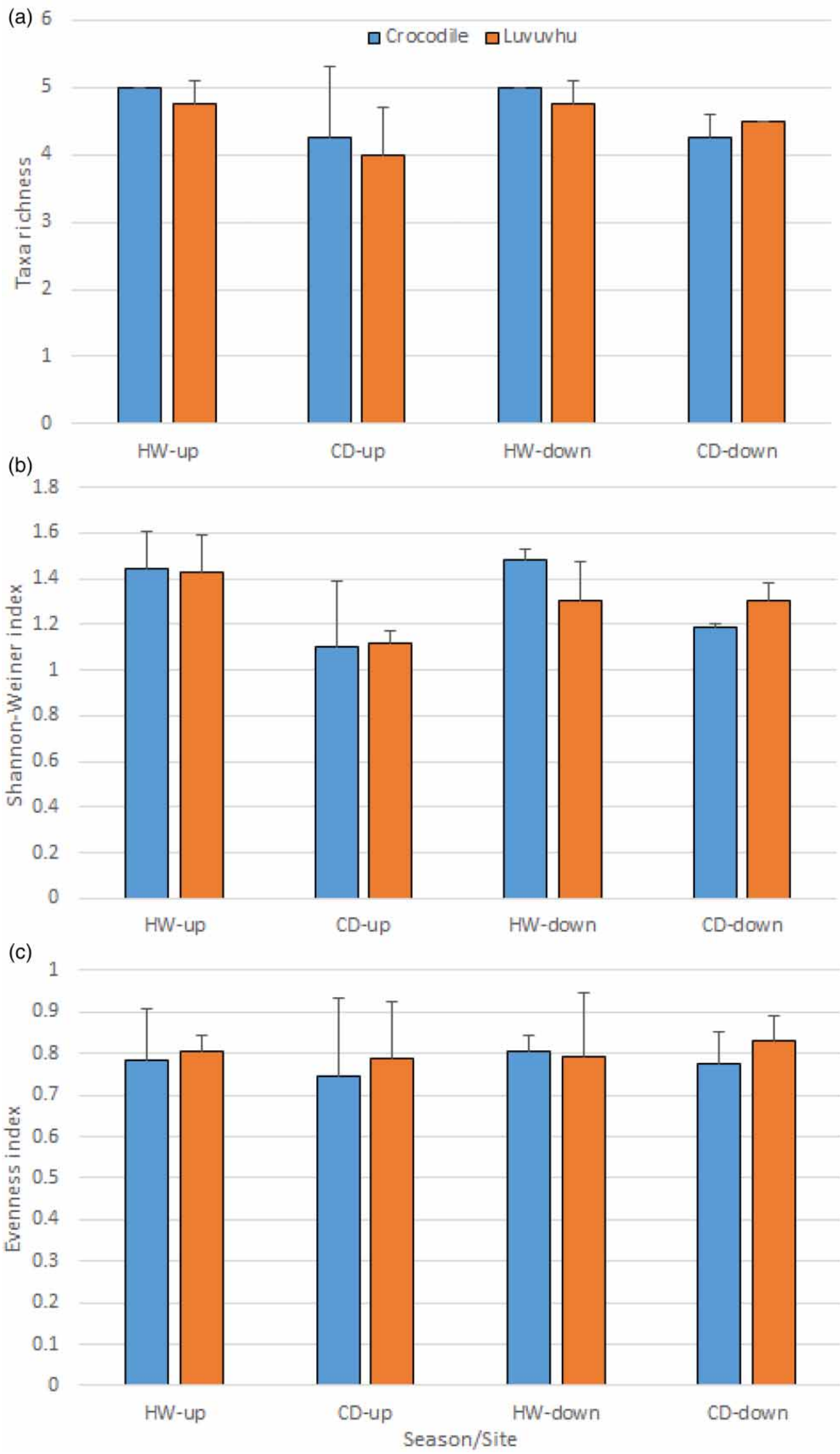

Figure 5 | Diversity indices [(a) taxa richness, (b) Shannon–Weiner, (c) evenness] observed in sediment microplastics in the Crocodile and Luvuvhu river systems. CD, cool–dry season; HW, hot–wet season; up, upstream; down, downstream.

During the cool–dry season, slightly high pollution load was observed in the downstream site (PLI 5.0) compared to the downstream site (PLI 4.9). Seasonal patterns were clearly observed in the Luvuvhu River, with high pollution load during the hot–wet season (PLI range 10.2–14.1) compared to the cool–dry (PLI range 5.9–8.2). Furthermore, upstream sites (mean, hot–wet PLI 14.1, cool–dry PLI 8.2) had high pollution load for the both seasons compared to the downstream sites (mean, hot–wet PLI 10.2, cool–dry PLI 5.9).

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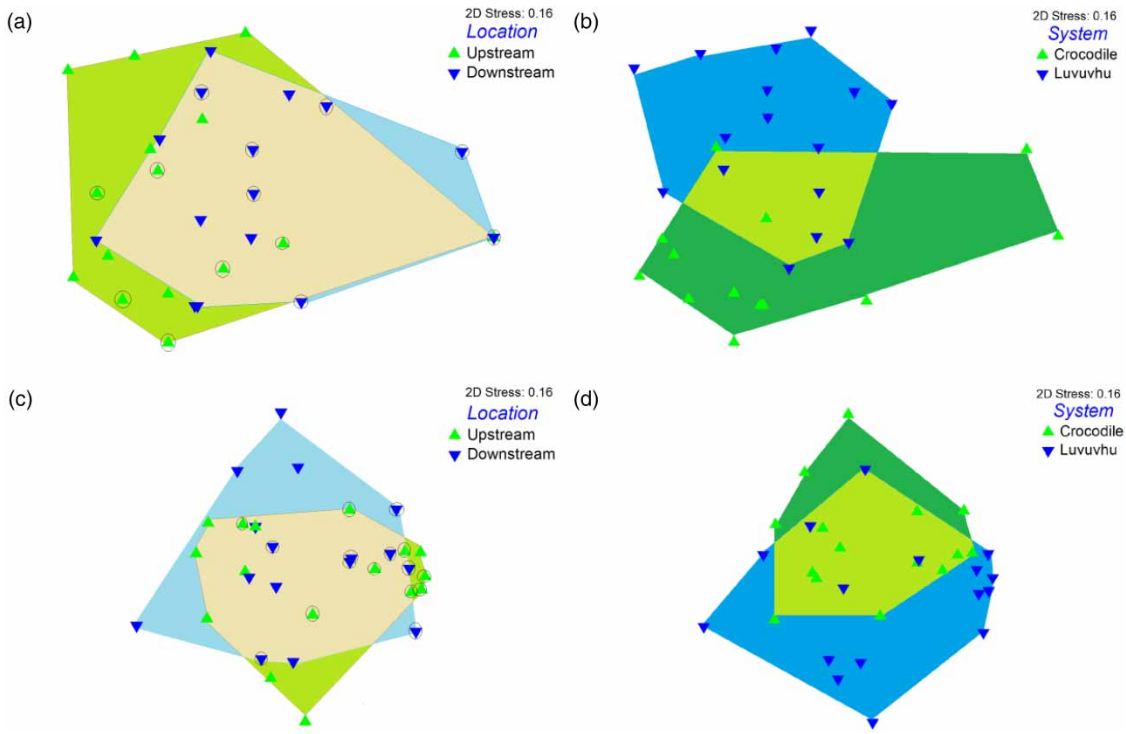

Figure 6 | *n*-MDS ordination highlighting variation of microplastic densities across locations and systems. Seasons are indicated by circled triangles within location panels (a) and (b).

4. DISCUSSION

The study aimed to determine the seasonal variation in water and sediment microplastic abundances upstream and downstream of wastewater treatment works in two river systems (i.e., Crocodile and Luvuvhu) across two seasons (hot–wet and cool–dry). Overall, we found that microplastic type and distribution often did not show clear site variation, hence microplastics were widespread across the studied systems and microplastic concentrations did not relate clearly to wastewater treatment works as further indicated by microplastic risk assessments. The Crocodile River had a high microplastic concentration in water and sediment samples during the cool–dry and hot–wet seasons due to its location and proximity to the highly urbanised and populated provincial capital of Mpumalanga Province, Nelspruit. In the Luvuvhu River system, however, high levels of sediment were detected during the hot–wet season. As a result, the current research indicates that seasonal context influences differences in microplastic concentrations, with the hot–wet season being associated with the high pollution loads, particularly within the sediments where this was more pronounced indicating that the sink-source effect which is linked to sediments and not water, similar to Nel *et al.* (2017).

Fragments were observed to be dominant in the current study and might have been primarily derived from household wastewater breakdown of macroplastics (Liu *et al.* 2018). While the majority of microplastics are removed during wastewater treatment, a significant amount is released into the nearby aquatic environment (Carr *et al.* 2016). Upgraded and state-of-the-art wastewater treatment works are a feasible solution for the removal or reducing microplastic pollution, however, this is a challenge within most of the developing world, including the current study area, due to the high capital costs of such facilities hence high microplastic loads will continue to be observed downstream of wastewater treatment works discharge points (Liu *et al.* 2018). As a result, while some wastewater treatment works can remove microbeads with an efficiency of more than 90%, other microplastic types cannot be effectively removed, resulting in microfibres causing significant environmental pollution downstream of wastewater treatment works (Carr *et al.* 2016; Murphy *et al.* 2016). However, overall, the results showed a lack of effect of wastewater treatment works on either water or sediment, with fragments of microplastics dominating the river systems both upstream and downstream of the wastewater treatment works.

The lack of differences among sites suggests the presence of other significant sources of microplastics along the river systems (Dris *et al.* 2015). Informal and formal human settlements, water abstraction, riparian brick

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making, washing, and bathing, subsistence and commercial agriculture, sewage discharge/spillage, and solid waste disposal/dumping are among the major contributors of microplastics upstream that were identified in the current study and these additional contributors could have dampened the 'signal' or effect from wastewater treatment works. Furthermore, Browne *et al.* (2011) and McCormick *et al.* (2014) discovered high concentrations of microplastics in sediment collected near effluent discharge points and these findings were in contrast with the current study, with sediment microplastic densities being similar both upstream and downstream of the sewage treatment works, indicating non-point sources aside from wastewater treatment works.

Furthermore, our hypothesis suggested that the Luvuvhu River catchment system will be less impacted by microplastics compared to the Crocodile River as it does not have many adjacent communities linked to wastewater treatment works and is rural, whereas the Crocodile River is highly disturbed by human activities and is urban. However, assessing the microplastic PLI, we observed slightly high microplastic pollution in Luvuvhu River thereby we reject our initial hypothesis. The dominance of fragments within the Luvuvhu River system could have contributed to increased pollution loading as we observed more water abstraction, riparian brick making, washing and bathing, subsistence and commercial agriculture, and illegal solid waste disposal/dumping which all contribute to increased microplastic pollution loads within the rivers. Our findings suggest that differences in microplastic loads were influenced by the seasonal context in the case of sediment, with the hot–wet season being associated with high pollution loads along the Crocodile and Luvuvhu river systems based on the sediment microplastic risk assessment. In contrast, a study by Liu *et al.* (2018) and Dalu *et al.* (2021) observed that high microplastic loads were associated with the cool–dry (i.e., winter) season which was associated with reduced water flows which resulted in increased sediment deposition. These studies observed a strong relationship between seasonality, suspension, and deposition, with dry periods being marked by high rates of deposition where river sediments serve as a sink and wet periods marked by microplastic suspension where sediments serve as a source (Nel *et al.* 2017, 2018).

External factors such as particle characteristics, river flow, water depth, substrate factors, and even bottom topography can all influence particle distribution (Eerkes-Medrano *et al.* 2015; Klein *et al.* 2015), and hence should be investigated. Klein *et al.* (2015) highlighted that rivers are dynamic ecosystems that can quickly deplete local sinks due to changes in hydrodynamics. The Crocodile and Luvuvhu River systems may be considered a major sink for microplastic pollution during the hot–wet season, with the mean ranging from 3.3 to 4.3 particles L^{-1} for upstream and downstream sites in the Crocodile River. Microplastic levels in the Luvuvhu River system range between (mean 4.9 particles L^{-1}) and cool–dry season (mean 4.5 particles L^{-1}). However, because marine environments have been identified as the final sink for microplastic particles, rivers' roles as sinks are only temporary. This was evident in the current study, as summer microplastic densities were lower than winter densities in water samples, most likely due to increased water flow, which resulted in downstream transport of any microplastics that accumulated in the sediment during the winter.

Besseling *et al.* (2017) have indicated most plastic particles discharged from wastewater treatment works were retained within the system and this poses a serious threat to organisms that live in the sediment and highlights freshwater systems as potential microplastic sinks. Both river systems appeared to be moderately polluted during the cool–dry season, with heavy microplastic contamination being observed during the hot–wet season. As a result, both river systems could be considered an important exporter of microplastics during the summer (i.e., hot–wet) season. It is also suggested that both river systems are a good indicator of microplastic dynamics for many regional rivers that run through cities and rural areas as these indicated similar patterns of microplastic pollution loads.

Microplastics can absorb persistently, bioaccumulative, and toxic substances such as persistent organic pollutants and metals (Rios *et al.* 2010). Pollutants absorbed through ingestion may be transferred to aquatic organisms. The interactions of these compounds within the bodies of these aquatic organisms may change the distribution, biotransformation, and/or toxicity of environmental contaminants. This may result in an increase in the concentration of contaminants and the possibility of these being incorporated into superior trophic chains, endangering the health of animals including humans (Teuten *et al.* 2009; Hidalgo-Ruz *et al.* 2012). However, many of the effects and processes involving the presence and accumulation of microplastics in the marine environment remain unknown, as do the long-term consequences. As a result, understanding all of these issues will be important for future research.

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5. CONCLUSIONS

The accumulation of microplastic in aquatic environments is arguably the most obvious among multiples human pressures. The result of this study shows that all water and sediment samples from the Crocodile and Luvuvhu river systems contained microplastics, indicating that severity of microplastic pollution in these rivers. Upstream of the wastewater treatment work in both rivers had relatively high microplastic abundance compared to downstream and this was supported by the high sediment microplastic risk assessments, indicating that there are other sources contributing to microplastic pollution besides the wastewater treatment facility such as water abstraction, riparian brick making, washing and bathing, subsistence and commercial agriculture and illegal solid waste disposal/dumping. Microplastics were found in high concentrations in the Crocodile River sediments and water samples than in the Luvuvhu River system, which suggest that urbanisation and high human population can greatly affect the distribution of microplastic in aquatic system. Fragments were the dominant microplastic type in both systems. Results of this study will be useful in informing water managers and other stakeholders about how to manage plastic litter and establish programmes aimed at reducing plastic pollution. More research is needed to fully understand the sources, fate, effects, and temporal changes associated with microplastics.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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