





#### AUTHORS:

Heinrich T.J. Dahms<sup>1</sup> Richard Greenfield<sup>1</sup>



#### AFFILIATION:

<sup>1</sup>Department of Zoology, University of Johannesburg, Johannesburg, South Africa

#### CORRESPONDENCE TO:

Richard Greenfield

rgreenfield@uj.ac.za

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# A review of the environments, biota, and methods used in microplastics research in South Africa

Microplastics are small plastic materials often defined as those between 5 mm and 0.05 mm in size. Microplastics can have toxicological impacts on various biota, from gut blockages to the transport or leaching of toxicants used in their production or absorbed from the surrounding environment. Although microplastic research has increased significantly, microplastic research in Africa lags behind that of developed countries. South Africa is the African nation with the highest number of microplastic publications. We aimed to determine the current state of microplastic research in South Africa. A total of 46 publications on microplastics in South Africa have been produced. However, many of these publications use methods that might not be accurate in determining holistic descriptions of microplastics in the aquatic environment. Similarly, many ecologically relevant environments and species have not been investigated for microplastics in the country, including any atmospheric or terrestrial environment. We conclude that, although the research being produced in South Africa can be considered adequate, a singular standard method for sampling and assessing microplastics in South African environments is required. The production of such a standard method would be critical to use as a monitoring tool to determine and compare microplastic abundances across the country and globally.

- More than 40 publications on microplastics have been produced in South Africa.
- Microplastics have been discovered in multiple aquatic environments in South Africa, but have not been investigated in atmospheric or terrestrial environments.
- Polymer analysis was limited in published research.
- A standard method is required for comparing between studies.
- Terrestrial and atmospheric microplastic studies are required.

# Introduction

Plastics are polymers that consist of monomers chained together to produce products with unique characteristics.<sup>1</sup> These products could easily be moulded into any shape required for the product to be used. This ability has allowed for an explosion of plastic products, reaching over 322 million tonnes produced globally.2 These plastics are heavily resistant to degradation, which has allowed plastic to accumulate in the environment.3 These plastics are impacted in the environment by abiotic factors such as UV rays, temperature changes, and abrasion from wind, waves, and biota, which can degrade them and break them into smaller microscopic particles.<sup>2,3</sup> These microscopic plastics have been detected globally, with one of the first discoveries made by Gregory4 around the coastline of New Zealand. Gregory4 discovered tiny beads on the beaches and surrounding coastline in 1977, but only later in the 21st century had environmental scientists begun to investigate these small plastic particles. 5 These particles were then classified as microplastics (usually within the size range of 0.05-5 mm; however, this definition is still being debated currently).6

Microplastics have since been collected, described, and characterised globally in marine and freshwater environments, soil, biota, and the atmosphere. Microplastics have further been classified as either primary or secondary microplastics.<sup>2</sup> Primary microplastics are plastics already produced in the size range of microplastics, whereas secondary microplastics are plastics that break down from larger plastic products.<sup>3</sup> The classification system was produced to understand from where microplastics originate to determine their pathways into the environment.2

After discovering microplastics, their toxicity and potential impact on biota were investigated. Microplastics can have toxicological impacts on biota through three separate pathways. First, the physical nature of microplastics can lead to gut obstructions or reduce the ability of organisms to move or reproduce.<sup>2</sup> Second, microplastics can also leach chemicals that are used as additives to increase their lifespan and function; this would include additives such as flame retardants, bisphenols, and other endocrine-disrupting chemicals.<sup>2,3</sup> The third toxicological impact of microplastics is their ability to act as vectors for toxicants.3 Toxicants such as metals and persistent organic pollutants have been discovered on the surface of microplastics in concentrations significantly higher than those in the surrounding environments.3 Microplastics' physical and chemical impacts allow for the increased bioaccumulation of a toxicant and can prevent species from regulating these toxicants out of their circulatory systems.8 Other notable toxicological studies have discovered that microplastics can lead to coral bleaching9 and can reduce the ability of phytoplankton to photosynthesise10. It is, therefore, understandable that microplastic pollution impacts 12 of the 17 United Nations Sustainable Development Goals, such as Life Below Water, Life on Land, Clean Water and Sanitation, and Good Health and Well-Being.<sup>11</sup>

In this review, we aimed to determine the state of microplastic research in South Africa. The review focuses on the environments and biota investigated, the concentrations of microplastics found, the overall methods used within the country, the current trends, and shortfalls of the research in South Africa, and where more research is required.



### Method

#### Data collection

Data were collected using the primary academic search engines ScienceDirect, SpringerLink, Google Scholar and the research network ResearchGate. The primary keywords used for the search of papers were "South Africa" and "Microplastics". The papers were then screened by relevance (whether conducted in South Africa) and ordered chronologically from 2023 to 1990. The information recorded from the papers included the authors, title, year of publication, where in South Africa the study was conducted, whether it was conducted in freshwater, marine or terrestrial environments, whether biota were included in the study, which matrices were investigated (water, sediment, biota), the extraction methods used, the identification method, mean concentrations and whether quality control was performed in the study. Modelling and review papers were also included; no published studies that were conducted in South Africa were omitted. Documents that did not undergo peer review, such as dissertations, theses, preprints and reports, were not included. Microsoft Excel was used to produce graphs and tables. A map of the research conducted within South Africa was created on QGIS v3.26.2 to visualise and highlight areas where more research might be required.

# Microplastic research in Africa

Microplastic research within Africa has primarily focused more on the marine environment than freshwater environments - a global trend also found in developed nations.<sup>5,12</sup> Microplastic research in Africa has increased over the last decade; however, three recent reviews of microplastic research in Africa have found that African nations are still trailing behind developed countries. 12,13 This is concerning considering the large scale of plastic pollution on the African continent.12 The reviews found that microplastics have been discovered in multiple African environments in over 11 African countries, including the Nile River, where microplastics were found in two fish species. 12,14 Other environments include the Niger Delta, Lake Victoria, the southern Mediterranean Sea, and along the western coast of Africa.<sup>14</sup> These studies not only found microplastics in the water and sediment, but also in biota, such as snails, multiple freshwater and marine fish species, and birds.14 The review by Alimi et al.14 further highlights that microplastic research in Africa was primarily divided between freshwater (22) and marine (37) environments, which included studies that discovered microplastics in surface water (15), sediment (22), and in biota (22). Central Africa remains under-researched, with almost no microplastic research being conducted there, including any terrestrial animals on the continent.<sup>14</sup> The reviews on microplastics in Africa concluded that microplastics detected in Africa have been attributed to waste mismanagement, a rapid increase in the populations in African countries, and the import of plastic and e-waste from developed countries.12 Changes in policy regarding the import of plastic and more sustainable plastic use in Africa were suggested by Alimi et al.<sup>14</sup> to curb plastic pollution. Lastly, although research in Africa has increased, critical environments, such as pristine natural environments and wetlands within Africa, atmospheric deposition, and terrestrial environments, have not been researched, with awareness of microplastic pollution across Africa being limited in the general population.<sup>13</sup> The reviews of microplastics in Africa further elaborate that many studies did not include experimental controls, that they lack polymer analysis, and that there was limited information on the interactions between microplastics and metals in the African environment.12-14

# **Microplastics in South African environments**

Alimi et al.<sup>14</sup> found that South Africa was the leading African nation in microplastic research, followed by Nigeria and Tunisia. As of August 2023, 46 publications on microplastics in South Africa had been published (Table 1). Microplastic research in South Africa began in 1990 when Ryan<sup>15</sup> investigated temporal trends and abundance of plastic litter on Cape Town beaches and defined plastic between 2 mm and 20 mm as microplastic. As of August 2023, 20 publications are on aspects of the marine environment, and 17 publications are on the freshwater environment (Figure 1). It is important to acknowledge that

of all 46 publications, only 12 have investigated microplastics' presence or impacts on biota. No studies have investigated the presence or deposition of microplastics from the atmosphere. Similarly, no research has been done on microplastics in the terrestrial environment, including on any terrestrial organisms. This indicates a large gap in microplastic research within South Africa.

# Microplastics in the marine environment

In 1990, Ryan<sup>15</sup> discovered small plastic particles within the size range of 20 mm and smaller on beaches around Cape Town; this finding could be considered the first evidence of microplastics in South Africa. However, only in 2015 were microplastics – as defined today (5–0.05 mm) – researched in South Africa. Since the first study by Ryan<sup>15</sup>, researchers have discovered, quantified, and characterised microplastics collected on beaches along the coastline<sup>18</sup>, estuaries, within coastal water<sup>19,20</sup>, and within major harbours along the South African coastline (Figure 2).

From 1990 to August 2023, a total of 20 publications were produced on microplastics in marine environments, excluding reviews and coastal modelling studies. The primary matrices investigated in South African marine environments were sediment, water, and biota, although no studies had investigated all three matrices within a single study. Estuaries can be regarded as the most under-researched marine environment, with only four publications quantifying their microplastic pollution. These studies have determined the microplastic concentrations in water and sediment<sup>21</sup>, their presence in biota such as estuarine fish species<sup>22</sup>, and the deposition of microplastics among estuarine seagrass<sup>23</sup>. Estuaries are important environments which link microplastics that may be transported downstream from inland rivers into the ocean. The results of these initial studies could indicate that these environments, which are nurseries for multiple organisms, could be at risk of microplastic pollution. 16,22,23 No study has critically evaluated how many microplastics are expelled through river mouths into the surrounding oceans, although Weideman et al.<sup>24</sup> found little to no microplastics being expelled during two sampling excursions to the Orange River mouth.

The other studies were primarily focused on quantifying microplastics on beaches and coastlines to determine the type of pollutant and how the microplastics may be distributed. Ryan et al.<sup>20</sup> discovered consistent patterns of plastic debris on South African beaches, and de Villiers<sup>25</sup> discovered significant levels of microfibres on beaches. The authors stated that microplastics became deposited along the coastline from the oceans.<sup>20,25</sup> Similarly, Ryan et al.<sup>26</sup> investigated polyethylene pellets collected along the coastline and characterised the organic pollutants bound to the plastic, which can increase the microplastics' toxicity.

When the concentration and distribution of microplastics in water along the coastline were investigated, researchers found two opposing trends in the distribution. Naidoo and Glassom<sup>27</sup> discovered higher concentrations of microplastics along larger urban areas, and differences between seasonal sampling were discovered, which were attributed only to changes in wind direction and tides during sampling days. During the same year, Collins and Hermes<sup>28</sup> conducted a modelling study to determine how floating microplastics accumulate and are transported along the South African coastline. The model indicated that microplastics are released from the five major industrial zones along the South African coastline, depositing and accumulating on beaches.<sup>28</sup> The model also indicated that a third of the microplastics released into the ocean move to the South Atlantic and South Indian oceans, and can then be transported worldwide.<sup>28</sup> These results correlate to the findings made by Naidoo and Glassom<sup>27</sup>.

However, harbours around the coastline of South Africa were found to be areas where microplastics could increase in abundance and then be released. The first study to investigate South African harbours was conducted by Nel et al.<sup>29</sup> who investigated whether population demographics reflect microplastic loads. Nel et al.<sup>29</sup> discovered that harbours were a significant source of microplastic pollution. However, no significant spatial differences were discovered between populations, indicating that microplastics can rapidly be distributed by ocean currents and wind<sup>29</sup>, contrary to the study conducted by Collins and Hermes<sup>28,29</sup>.



 Table 1:
 Summary of publications on microplastics research in South Africa

Authors	Year	Where	Environment investigated	Biota included	Matrices investigated	Extraction method	Identification method
Ryan <sup>15</sup>	1990	Western Cape beaches	Marine		Sediment	Sieving	Visual
Ryan et al. <sup>16</sup>	2012	South African coastline	Marine		Sediment	Sieving	Visual
Nel and Froneman <sup>17</sup>	2015	South-eastern coastline	Marine		Sediment and water	Density separation, NaCl and filtering	Visual
Naidoo et al. <sup>16</sup>	2015	KwaZulu-Natal estuaries	Marine		Sediment and water	Density separation, NaCl and plankton net	Visual and FT-IR
Verster <sup>39</sup>	2017	Review	Review		N/A	N/A	N/A
Nel et al. <sup>29</sup>	2017	South African coastline	Marine		Sediment and water	Density separation, NaCl and filtering	Visual
Ryan et al. <sup>20</sup>	2018	South African beaches	Marine		Sediment and water	Density separation, NaCl	Visual
Reynolds and Ryan <sup>40</sup>	2018	Western Cape and North-West wetlands	Fresh water	Birds	Biota	Sieving	Visual
Nel et al.41	2018	Eastern Cape Bloukrans River	Fresh water	Macroinvertebrates	Sediment and biota	Density separation, NaCl and digestion	Visual
De Villiers <sup>25</sup>	2018	South African coastline	Marine		Sediment	Density separation, NaCl	Visual
Naidoo and Glassom <sup>27</sup>	2019	KwaZulu-Natal coastline	Marine		Water	Steel manta trawl	Visual
Dalu et al. <sup>50</sup>	2019	Limpopo Province reservoir	Fresh water		Sediment	Survey	Visual without microscope
Weideman et al. <sup>24</sup>	2019	Orange-Vaal River (multiple provinces)	Fresh water		Water	Neuston net and bulk water	Visual
Collins and Hermes <sup>28</sup>	2019	South African coastline model	Marine		N/A	N/A	N/A
Govender et al. <sup>21</sup>	2020	KwaZulu-Natal estuaries	Marine		Sediment and water	Density separation, NaCl; plankton net	Visual and FT-IR
Pereao et al. <sup>62</sup>	2020	Review	Review		N/A	N/A	N/A
Vilakati et al. <sup>59</sup>	2020	Western Cape seashore	Marine		Water	Plankton net	GC-MS, Visual, SEM-EDS, FTIR
Vetrimurugan et al. <sup>18</sup>	2020	KwaZulu-Natal coastline	Marine		Sediment	Density separation, ZnCl <sub>2</sub>	SEM-EDS
McGregor and Strydom <sup>33</sup>	2020	Eastern Cape coastline	Marine	Fish	Biota	Digestion	Visual
Naidoo et al. <sup>22</sup>	2020	KwaZulu-Natal coastline	Marine	Fish	Biota	Digestion	Visual and FT-IR
Sparks and Immelman <sup>34</sup>	2020	Agulhas	Marine	Fish	Biota	Digestion	Visual
Dahms et al. <sup>7</sup>	2020	Gauteng (Braamfontein Spruit)	Fresh water	Macroinvertebrates	Sediment, water, and biota	Density separation, NaCl and digestion	Visual
Weideman et al. <sup>24</sup>	2020	Orange-Vaal River (multiple provinces)	Fresh water		Water	Density separation NaCl, Bulk water, Neuston Net	Visual
Sparks <sup>35</sup>	2020	Western Cape coastline	Marine	Mussels	Biota	Digestion	Visual
Ryan <sup>20</sup>	2020	Adjacent oceans review	Review		N/A	N/A	N/A

...Table 1 continues on next page



Table 1 continued...

Authors	Year	Where	Environment investigated	Biota included	Matrices investigated	Extraction method	Identification method
Iroegbu et al. <sup>53</sup>	2020	Review	Review		N/A	N/A	N/A
Arabi et al. <sup>37</sup>	2020	Review	Review		N/A	N/A	N/A
Godfrey <sup>63</sup>	2020	Short note	Short note		N/A	N/A	N/A
Verster et al. <sup>52</sup>	2020	Review	Review		N/A	N/A	N/A
Mehlhorn et al. <sup>31</sup>	2021	KwaZulu-Natal harbours	Marine		Sediment	Handpicking	Visual and FT-IR
Vilakati et al. <sup>54</sup>	2021	Gauteng (WWTP)	Fresh water		Water	Net	GC-MS, Visual, SEM-EDS, FTIR
Preston-Whyte et al. <sup>32</sup>	2021	Durban	Marine		Sediment and water	Sieving and density separation, NaCl	Visual and FT-IR
Sparks et al. <sup>36</sup>	2021	Western Cape (retail mussels)	Marine	Mussels	Biota	Digestion	Visual and FT-IR
Dalu et al. <sup>51</sup>	2021	Limpopo WWTP (Mvudi river)	Fresh water		Sediment	Sieving	Visual
Bulannga and Schmidt <sup>56</sup>	2022	KwaZulu-Natal	Fresh water	Single cellular organism	Biota	N/A	SEM
Saad et al. <sup>44</sup>	2022	Vaal River (Gauteng, Free State)	Fresh water		Sediment	Density separation, NaI	Visual, SEM, Raman
Ramaremisa et al. <sup>45</sup>	2022	Vaal River (Gauteng, Free State)	Fresh water		Sediment and water	Density separation, Nal and plankton net	Visual, SEM, Raman
Saad et al. <sup>42</sup>	2022	Vaal River (Gauteng, Free State)	Fresh water	Fish	Biota	Digestion	Visual, Raman
Dahms et al. <sup>43</sup>	2022	Vaal River (Gauteng, Free State)	Fresh water	Fish	Sediment, Water, and biota	Density separation, NaCl and bulk water	Visual
Dalu et al. <sup>47</sup>	2023	Limpopo Province (Crocodile River)	Fresh water	Fish	Biota	Digestion	Visual, Nile Red
Boshoff et al. <sup>23</sup>	2023	Western Cape (estuaries)	Marine	Grass	Sediment and biota	Density separation, ZnCl <sub>2</sub>	Visual
Julius et al. <sup>19</sup>	2023	Western Cape coastline	Marine		Sediment and water	Density separation, NaCl and bulk water	Visual and FT-IR
Mutshekwa et al. <sup>49</sup>	2023	Limpopo Province (reservoirs)	Fresh water		Sediment	Density separation, NaCl	Visual and FT-IF
Nkosi et al. <sup>46</sup>	2023	Limpopo Province (Crocodile River)	Fresh water		Sediment and water	Density separation, ZnCl <sub>2</sub>	Visual, Nile Red
Apetogbor et al. <sup>48</sup>	2023	Western Cape (Plankenburg river)	Fresh water		Sediment and water	Density separation, NaCl	Visual and FT-IR
Owowenu et al. <sup>57</sup>	2023	Review	Review		N/A	N/A	N/A

In a multinational study, Matsuguma et al.<sup>30</sup> collected a singular core sample from Durban harbour, which had more microplastics than Tokyo Bay, which could indicate that microplastics may highly pollute South African harbours.<sup>30</sup> It is, however, critical to report that due to the various methods used across studies, it is impossible to compare microplastic concentrations between studies accurately.

Further studies by Mehlhorn et al.<sup>31</sup> and Preston-Whyte et al.<sup>32</sup> investigating the abundance and distribution of microplastics in harbour environments were conducted on the retention of microplastics in South African harbours. The results indicated that South African harbours are areas where microplastics can accumulate due to the input from rivers

and stormwater drains, be deposited on the sediment in the slow-moving water, and then be released into the ocean during increased flow.<sup>31,32</sup> These studies are essential to determine where more significant plastic pollution prevention is required to reduce microplastic pollution along the South African coastline. Whether biota that are found within and in the surrounding area of harbours are impacted by the microplastics is unknown, as biota have not been sampled and investigated in harbours.

Research on the impact or presence of microplastics on marine biota is limited in South Africa. Only five publications have determined the presence of microplastics in marine animals. Fish have been the predominant focus, with three of the studies determining the presence



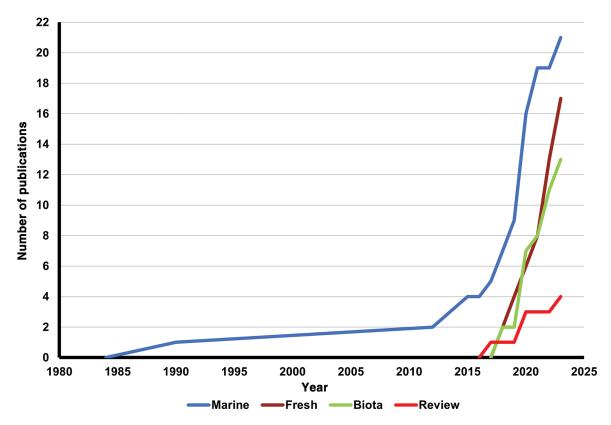


Figure 1: Line graphs of the total number of publications on microplastics in South Africa, from 1980 to July 2023.

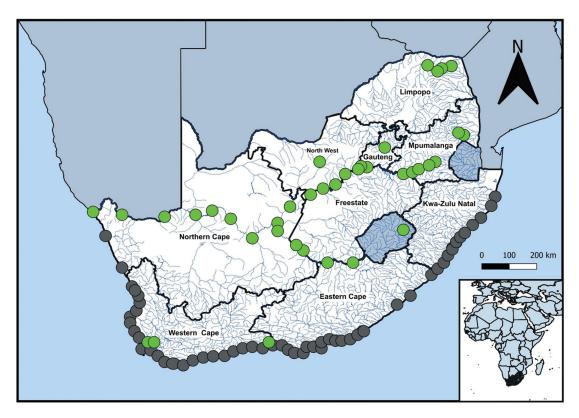


Figure 2: A map of areas where microplastics have been detected in South African marine (grey) and freshwater (green) environments.

of microplastics in marine and estuarine fish.  $^{33,34}$  The only other biota that have been researched was when Sparks $^{35}$  found microplastics in mussels along the coast of Cape Town, with means of  $2.33\pm0.2$  particles/g and  $0.27\pm0.5$  particles/organism. A secondary study by Sparks et al.  $^{36}$  investigated retail mussels and discovered a mean

of  $3.83\pm0.2$  (SE) particles per mussel. These findings indicated that people eating retail mussels were in danger of consuming microplastics. Although microplastics had been discovered in wild mussels and fish, this was the first significant identification that the South African population was ingesting microplastics.  $^{36}$ 



It is recommended that microplastic research in the marine environment must accurately determine the distribution of specific polymers along coastlines, beaches, and biota.37 It is also recommended that more species related to human consumption be investigated to determine whether local fishing villages and larger towns could ingest these organisms and their associated microplastics.<sup>2,3</sup> Questionnaire-based qualitative research could be used to determine the scale to which communities encounter or ingest microplastics by eating biota that are polluted with them. Aspects such as how the organisms are consumed, by whom, and how frequently could shed light on how populations might be impacted by microplastics. Critically, larger marine organisms such as sharks, birds and mammals must also be investigated to determine the presence of microplastics in marine ecosystems across the South African coastline. Finally, the impacts of microplastics on South African coral, zooplankton and phytoplankton must be investigated to understand their impact on these critical organisms.

#### Microplastics in the freshwater environment

In parallel with the global trend in microplastic research, freshwater environments in South Africa were investigated for microplastics only decades after the first publication on microplastics from marine environments.<sup>5</sup> The first discovery of microplastics in the ocean and research bias have been described as reasons for the dramatic difference in research between the two environments.<sup>38</sup> In a review, Verster et al.<sup>39</sup> highlighted the gap in microplastic research in freshwater environments, which was then followed by a significant increase in microplastic research in freshwater environments (Figure 2). The first research was published when Reynolds and Ryan<sup>40</sup> and Nel et al.<sup>41</sup> discovered microplastics in birds from contaminated wetlands<sup>40</sup> and in benthic macroinvertebrates in the Bloukrans River System, respectively41. Reynolds and Ryan40 detected mean microplastic concentrations of 1.53±0.64 particles per faecal sample from birds. Similarly, in the Bloukrans River, Nel et al.41 detected microplastics in the larvae of chironomid species, with means of 0.37±0.44 and 1.12±1.19 particles/mg ww for high and low flow. These results indicate that microplastics are within the environment and in animals from lower to higher orders of the trophic system. Nel et al.41 also detected microplastics within the sediment of the same river system. They found that microplastic abundance in invertebrates and sediment could be correlated over different seasons, indicating that the species could be used as an indicator for microplastic pollution within the sediment of a river system.41

This research was followed by the first evidence of microplastics in the Orange-Vaal River system.<sup>24</sup> Weideman et al.<sup>24</sup> investigated the most extensive and important river system in South Africa and discovered that the dams within it were not trapping floating microplastics. A mean concentration of 0.21±0.27 particles/L was detected using a 300-µm mesh plankton net, which prevented microplastics smaller than 300 um from being collected. However, they did not investigate any biota or sediment from the dams or try to determine how the microplastics were distributed through the system. Weideman et al.24 continued their research in the Orange-Vaal River system in 2020 and found limited long-distance transfer of microplastics in the system, with more microplastics in the upper sections of the river than downstream. They detected means of 2.3±7.2 microfibres/L in the wet season and 1.4+2.6 microfibres/L in the dry season. The authors did not investigate other matrices or aspects of the environment to understand why the transfer of microplastics was so limited. The importance of research in this system was so significant that four other studies of microplastics in the Vaal River were conducted. 42 Dahms et al. 43 investigated the water, sediment, and fish species Clarias gariepinus for microplastics, detecting them in all three matrices. Similarly, Saad et al.44 investigated another fish species, Cyprinus carpio and detected microplastics in the fish. Clarias gariepinus and Cyprinus carpio are important fish species economically and are a food source for subsistence fishers in the country, indicating that the microplastics ingested by these fishes might be ingested by humans. 43,44 Further studies by Ramaremi et al.45 and Saad et al.44 also detected microplastics in the sediment of the Vaal River, which were then compared to the water microplastics concentrations detected by Ramaremi et al.45 These publications pose the question of whether it is acceptable to investigate single components

of a river system, such as those done by Weideman et al.<sup>24</sup>, Ramaremi et al.<sup>45</sup> and Saad et al.<sup>42,44</sup>, or whether more holistic approaches must be considered to determine the entire distribution of microplastics in an ecosystem, such as studies conducted by Nel et al.<sup>41</sup>, Dahms et al.<sup>7,43</sup> and Boshoff et al.<sup>23</sup>

The only other major rivers in South Africa that have been investigated for microplastic pollution are the Crocodile River and Plankenberg River. The Crocodile River was investigated when Nkosi et al.46 determined the diversity of microplastics in water and sediment, and Dalu et al.47 discovered microplastics in freshwater fishes living near wastewater treatment plants (WWTPs). Dalu et al.47 determined that fish ingested greater amounts of microplastics in the wet season (10-119 particles per fish taxon) than in the dry season (11–34 particles per fish taxon), indicating more microplastics may be resuspended and ingested in the wet season. The first river in the Western Cape Province was investigated when microplastics were discovered in the Plankenberg River. 48 Seasonal variations of microplastics were found, with mean microplastic abundance in water higher in the spring (5.13±6.62 particles/L) than in the autumn (1.52±2.54 particles/L).48 The results of this study should form a baseline for monitoring and future research on microplastics in the system.48

Only one urban stream has been investigated. Dahms et al.<sup>7</sup> detected the first microplastics within the Braamfontein Spruit in Johannesburg, the largest city in South Africa. Dahms et al.<sup>7</sup> detected microplastics within all three matrices of the environment (water, sediment, biota) to provide a more holistic view of microplastics in the system. They<sup>7</sup> found an influence of environmental characteristics, such as increased water velocity leading to increased microplastics in water, and finer sediment grain sizes having higher microplastic abundances than larger grain sizes.<sup>7</sup> These results indicate that only determining microplastics within a single component of a river system with irregular sampling could give a false reading of the extent of microplastics in a system.<sup>7</sup> Dahms et al.<sup>7</sup> attributed sewage run-off as the leading cause of the increased microplastics in some areas in the system.

Aquatic environments that have rarely been investigated are pans, reservoirs, or isolated water bodies, with the only publications being those of Mutshekwa et al.<sup>49</sup> and Dalu et al.<sup>50</sup> who conducted a survey in which microplastics and other plastic debris were identified on the shoreline of a reservoir in the Limpopo Province. Microplastics in the reservoir were regarded as having a direct negative health impact on people dependent on the reservoir, highlighting how microplastics could be in isolated water bodies.

With microplastics in rivers being attributed to WWTPs, multiple studies have investigated their contribution to microplastics in South Africa. It was previously highlighted that Dalu et al.47 discovered that fish near a WWTP were ingesting microplastics. Other studies that have investigated microplastics include that of Dalu et al.51 who investigated the impacts of urbanisation and WWTPs on microplastic loads in the Mvudi River system in the Limpopo Province. The authors found no relationship between microplastics and the WWTP; however, they detected differences in microplastic types across seasons and determined the sources of microplastic pollution to be from atmospheric deposition, direct pollution or possibly broken drainage pipes, outside of WWTPs.51 This result is contrary to those of other studies worldwide, which have noted that WWTPs can expel billions of microplastics every day.<sup>2,52,53</sup> The other WWTPs investigated were in the Gauteng Province; Vilakati et al.54 attempted to characterise microplastics through pyrolysisgas chromatography-mass spectrometry (GC-MS). Vilakati et al.54 discovered four primary plastic polymers, with polyvinyl chloride (PVC) being the most abundant polymer found, representing 47.8% of the polymers found. The authors also characterised residues on the microplastics such as calcium, aluminium, and others related to additives used in the production of the plastic or from the surrounding environment. This characterisation is critical to understand how toxic microplastics are in the South African environment and how they can be related to toxicological testing. Currently, microplastic studies use concentrations and combinations of plastic polymers that cannot be



regarded as environmentally representative, not accurately reflecting what has been found in various environments, and therefore more research on the make-up of microplastic polymers is required.<sup>55</sup>

Of the microplastics studies in freshwater environments, seven included a biological component as either a bioindicator or to assess whether microplastics were entering aquatic food chains. Fish have primarily been the most popular organisms studied and were investigated in three of the studies, followed by benthic macroinvertebrates in two studies, with birds and ciliates being the only other organisms investigated.<sup>56</sup>

Although there has been a significant increase in microplastic research in freshwater systems, many publications have primarily determined and reported the microplastic abundance in only one or two ecosystem components. Only 13 studies have attempted to determine the microplastic abundance in two or more components of the environment, with only 2 studies investigating all components of the ecosystem to determine how the distribution is impacted. A review paper by Owowenu et al.<sup>57</sup> similarly highlighted how aspects such as flow, depth, sediment grain size, river width and discharge could impact the distribution of microplastics. Few papers have tried to determine how polymers were distributed across a river system; without accurate representation or understanding of how microplastics distribute, microplastic toxicological research cannot use environmentally relevant concentrations in toxicological studies.

From the review of all the literature presented here, it is recommended that microplastic studies in freshwater environments must incorporate all aspects of the system to determine accurately how microplastics are distributed. Freshwater environments remain under-researched in South Africa, with many critical environments, such as large wetlands and river systems, not having been investigated at all (Figure 2). Freshwater environments, such as large wetlands and river systems, not having been investigated at all (Figure 2). Freshwater environments, such as large wetlands and river systems, and sites of international importance that have not been investigated include Lake Saint Lucia, the Nylsvley wetland area, the Limpopo River system, the Kruger National Park, and the various caves and groundwater which are critical to the country. It is also recommended that the great watershed along the Drakensberg Escarpment is investigated, to determine whether atmospheric deposition pollutes the country's rivers at their origins.

# **Methods used in South Africa**

# Field sampling methods

Water and sediment have been the primary components of the environment that have been investigated in South Africa, Sampling methods have been similar to those used in other countries.<sup>2</sup> Sediment has been sampled the most consistently across studies with bulk sediment samples collected using spades, shovels, corers or other sediment sampling instruments, and then analysed. These methods are consistently used; however, for monitoring purposes, a standardised amount of sediment from various sampling points in the river, collected using a standard tool, would yield better results to compare between sites and studies. Microplastics are then extracted from the sediment through density separation methods. The primary solution used in density separation methods across the reviewed studies was NaCl at a density of 1.2 g.cm3 which was used in 12 of the reviewed publications (Table 1). The other studies used slightly denser solutions, such as Nal or ZnCl<sub>2</sub>, which can have densities of over 1.5 g.cm<sup>3</sup>, allowing for collection of denser microplastic polymers.<sup>58</sup> Although using a less dense medium such as NaCl is a much more environmentally friendly and cost-effective method, using it to collect microplastics from sediment may not be the most accurate method to determine microplastic profiles in sediment, as it would not allow for heavier polymers to be extracted.58 Due to various densities of plastics, the question of whether a singular standard solution density must be prescribed for microplastic analysis, has yet to be determined.58

Collection of water samples varied across studies; however, the most common method was sampling bulk water, filtered at the site, or using nets such as manta trawls, neuston, and plankton nets of various mesh sizes that might not collect all the sizes of microplastics present. Care must be taken when nets or containers made from plastic are used,

which could potentially contaminate the sample with microplastics. Contamination also needs to be considered when biota are sampled to ensure that nets used to catch and keep biota do not contaminate the samples. Finally, the most important aspect of sampling from environments is to contextualise the site from where the sample was collected. The season, flow, depth, sediment grain size, and discharge could all impact the distribution of the microplastics. If the site data cannot be contextualised, the results could provide a false indication of the total plastics sampled, regardless of the method used. 7,57 The contextualisation of the environment and a standardised method would similarly allow for a singular reporting unit of microplastics, compared to the various units seen in the studies reported in this review paper. Consideration must be given to contamination control in microplastic studies. In the review, eight of the studies failed to include information on the contamination control steps taken during the study. A singular contamination control guide could be produced for microplastics research during field and laboratory components of a study; however, input from all research groups would be required to find the most accurate system to use across studies.

### Microplastic identification

Identifying microplastic polymers in South Africa seems limited compared to that in developed countries. Only a limited number of publications had some form of polymer identification. Of those that had determined the polymer of plastic, the most available method seemed to be Fourier transform infrared (FT-IR) analysis conducted in 9 of the publications produced, with 21 papers not conducting any form of polymer analysis. This was followed by Raman spectroscopy, used in three publications, and pyrolysis-GC-MS<sup>59</sup>, conducted in two publications. Although recent publications by De Frond et al.<sup>60</sup> and Kotar et al.<sup>61</sup> have noted that visual identification can be an accurate method to determine larger sizes of microplastics (particles >0.02 mm), identifying how polymers distribute in the environment is crucial to determining the overall toxicological impact of microplastics in the environment. Similarly, polymer analysis has become a required aspect of microplastics research as a method to determine the accuracy of the visual identification of microplastics.<sup>60,61</sup>

### Recommendations for future research

Microplastics research should be conducted in more environments and biota in South Africa. Aquatic environments are crucial in a waterscarce country such as South Africa, and the impact of microplastics on freshwater environments has scarcely been investigated. There is also the need for a prescribed method for the sampling and analysis of microplastics in South African environments. For a standard monitoring tool, a standard method that is both cost- and time-effective but accessible and accurate in its analysis could be easily adopted by the various research institutes in the country. 62,63 Standardisation is crucial to compare microplastic concentrations across the country, as using different methods prevents researchers from comparing microplastic concentrations between studies. This review has found that half of the publications in the country lack polymer analysis, which could significantly impact the validity of the results. 60 This shortcoming could be due to a lack of resources, such as instruments or funding that can be used for polymer analysis. Making FT-IR analysis, the most conducted method of polymer analysis in South Africa, the prescribed and required tool for polymer analysis could enable a more accurate standard monitoring protocol in the country, although all instruments used in polymer identification have shortcomings. 60,61 A fast, accurate, and cost-effective method needs to be established so that reliable analysis can be adopted as a standard method at all research institutions across the country. A prescribed organism for microplastic levels in sediment and water must be identified; however, this has not yet been determined internationally. Most importantly, methods to sample microplastics accurately and consistently across studies must be determined. Investigating one or two components over a season could give a false microplastic abundance. Similarly, the lack of research on the wetlands in South Africa is concerning, as wetlands could be a hotspot for microplastic accumulation. The other environments that require research include terrestrial environments and atmospheric deposition in South Africa and globally. 12-14



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# **Competing interests**

We have no competing interests to declare.

### **Authors' contributions**

H.T.J.D.: Conceptualisation; data collection; methodology; data collection; data analysis; writing – the initial draft. R.G.: Conceptualisation; methodology; validation; writing – revision; student supervision; funding acquisition.

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