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Distribution of microplastic colour and count in the Crocodile River, South Africa

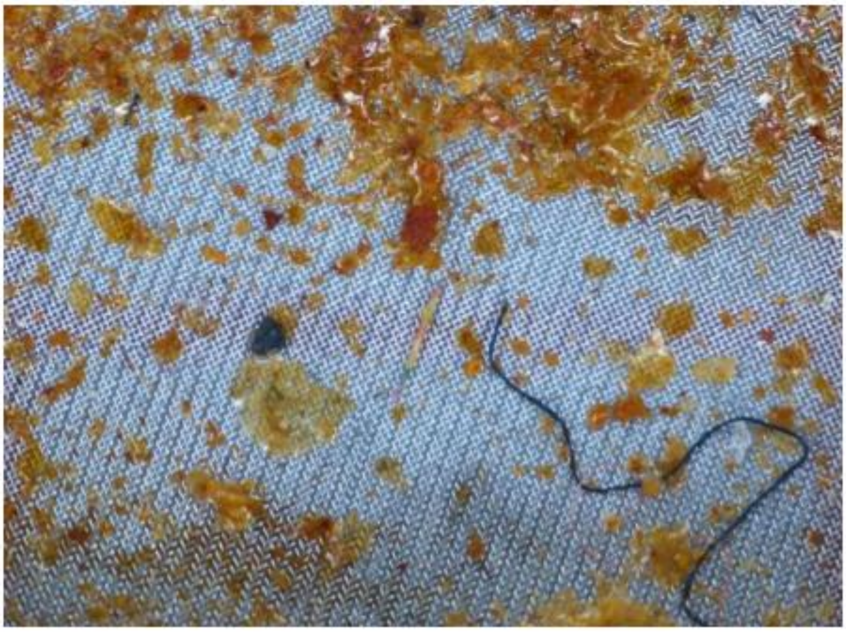

Photo: Bouwman et al. (2018)

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Bachelor thesis, 15 credits, in *Physical Geography and Ecosystem Science*

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Abstract

The aim of this paper was to establish sources for microplastic fibres and fragments based on the presence of specific colours (I) within different land use classes and (II) in relation to the location of wastewater treatment plants. Most of the colours did not result in significantly different counts between the land use classes, except for black fragments, which resulted in significantly higher counts in urban environments compared to natural environments. Since sampling was conducted in the rainy season, run-off from roads that introduces tyre wear in the form of black fragments to the river systems can be assumed to be a source for microplastic pollution that has been previously mentioned in papers, but not yet investigated. Other colours showed a similar pattern as black fragments but did not result in significantly different counts between the land use classes, indicating that with more sampling points these patterns could become statistically significant. The location of wastewater treatment plants did not influence colour counts, most likely due to the spatial distribution of sampling points, which were not positioned adequately for this testing. Further research is needed on the influence of river dynamics on the amounts of microplastics sampled on the sides of the river/at the centre of the channel, as well as investigations into emission of microplastics from sewage sludge, which has been applied to agricultural fields.

Key Words: Microplastic; Colour; Freshwater; Crocodile River; Sources

Acknowledgements

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1. Introduction

Aquatic plastic pollution has been a widely acknowledged as a persisting problem (Seltenrich 2015). Degradation of plastic is a slow process, implying that plastic stays in the environment for hundreds of years (Horton et al. 2017), causing serious damage to ecosystems (Tibbetts et al. 2018). There are a vast number of studies done on plastic pollution in oceans, but the amount of studies conducted on plastic pollution in rivers is relatively small. River systems have been regarded as contributors to plastic pollution in oceans, but are hardly being investigated as polluted environments themselves. This is surprising, as most oceanic plastic contamination is derived from inland waters (Cozar et al. 2015), which provide a direct link to the terrestrial emission sources. The identification of these sources and their relative importance is crucial for the design of any regulatory action on microplastic pollution.

Rivers carry plastic debris of all sizes to the oceans, but they also act as a temporary residence site for those plastics and are themselves affected by pollution, just like oceans (Windsor et al. 2019). But the problem of plastic pollution is not just due to particles visible to the bare eye. Particles tend to break down due to weathering through ultraviolet light and low temperatures (Auta et al. 2017), eventually forming microplastics. Breaking down of plastic debris makes pollution rates less obvious since the particles are smaller, but it does not make the effects of it less harmful and instead poses new challenges in detection processes.

Plastic particles smaller than 5 mm in size are called microplastics (Arthur et al. 2009). They can be mostly found in the form of fragments, fibres or sometimes flakes (Magnusson 2014), although most studies refer to just fibres and fragments (Horton et al. 2017; Ziajahromi et al. 2017). They are of special concern, since they can be ingested more easily due to their small size and in this way travel through the food web more easily than larger particles (Wagner et al. 2014).

Existing literature on microplastics in freshwater suggests mainly two different sources. McCormick et al. (2014), Wagner et al. (2014), Mani et al. (2015), Murphy et al. (2016) and Ziajahromi et al. (2017) all identified wastewater treatment plants filtering household waters as point sources for microplastics, acknowledging however that many wastewater treatment plants have a very high removal capacity. However, even though the retention potentials have been calculated to be relatively high by various studies, wastewater treatment plants can still be considered point sources for microplastic pollution to river systems, as they discharge great amounts of municipal water to recipient rivers each day. The other commonly mentioned source is urban areas, which are a more diffuse source. Urban areas often include wastewater treatment facilities, but the two are differentiated as they have very different, clearly definable processes involved in the emissions of microplastics. In urban environments, microplastic counts fluctuate based on the presence of industrial sites, urban littering (Mintenig et al. 2017) and urban runoff (Klein et al. 2015), with some papers also attributing high population densities to increased microplastic pollution (Mani et al. 2015). Horton et al. (2017) observed the greatest amounts of microplastics in proximity to industrialized areas, which can be attributed to impermeable surfaces of urban areas, increasing run-off rates of industrial waste discharge (Yonkos et al., 2014). As mentioned by Wagner et al. (2014) and Horton et al. (2017), this urban runoff can potentially introduce tyre wear from roads into the river system, although that has so far not been investigated.

One aspect that has been disregarded in most of the papers analysing microplastic is the microplastic colour. None of the identified studies showed usage of colour composites of microplastic fragments and fibres in their analysis. The scoping study on microplastic pollution in South African rivers conducted by Bouwman et al. (2018) mentioned the recording of colour properties of respective plastic particles, yet there are no published results on their findings.

The objective of this paper is to investigate relationships between microplastic structure (colour and fragment vs. fibre) and specific sources releasing plastics, and to further determine the contribution of these sources to pollution. Microplastic colour is a variable not investigated so far in the characterisation/identification of the sources emitting microplastics into river systems. This study is based on a sub-dataset of the data collected in the context of a monitoring exercise in parts of the Crocodile River catchment in South Africa by Bouwman et al. (2018).

1.1 Aim and hypotheses

The overarching aim is to test if there are significant differences between colour of microplastic fibres and fragments found in rivers with sample sites (I) surrounded by different land use classes and (II) being influenced differently by the location of wastewater treatment plants (WWTP). The following hypotheses were tested:

- Hypothesis 1: Microplastic counts are significantly different between urban areas and rural areas due to a higher abundance of sources in urban environments, such as roads, wastewater discharge points and city dump sites.
- Hypothesis 2: Microplastic counts downstream of tributary inflow from WWTPs significantly differ from counts upstream or far away from WWTPs due to the role of wastewater treatment facilities as point sources of microplastic pollution to rivers.
- Hypothesis 3: Black fragment counts are highest in vicinity to urban areas during the wet season because runoff from roads introduces black tyre wear to river systems.

2. Background

2.1 Nature of plastic pollution

Plastic pollution in aquatic environments has become a main point of interest and concern for many scientists. Most of this plastic comes from packaging, which is the biggest contributor to plastic pollution and amounts to a total weight of 141 million tonnes, whereas the second biggest contributor is waste from textiles, which contributes to less than a third as much as packaging, with a respective 38 million tonnes (Geyer et al. 2017). Other relevant industrial sectors generating plastic waste include consumer products (37 million tonnes), transportation (17 million tonnes) and electronics as well as building and construction (both a respective 13 million tonnes) (Geyer et al. 2017). A graphical presentation of the relative contribution of the sources can be found in Figure 1.

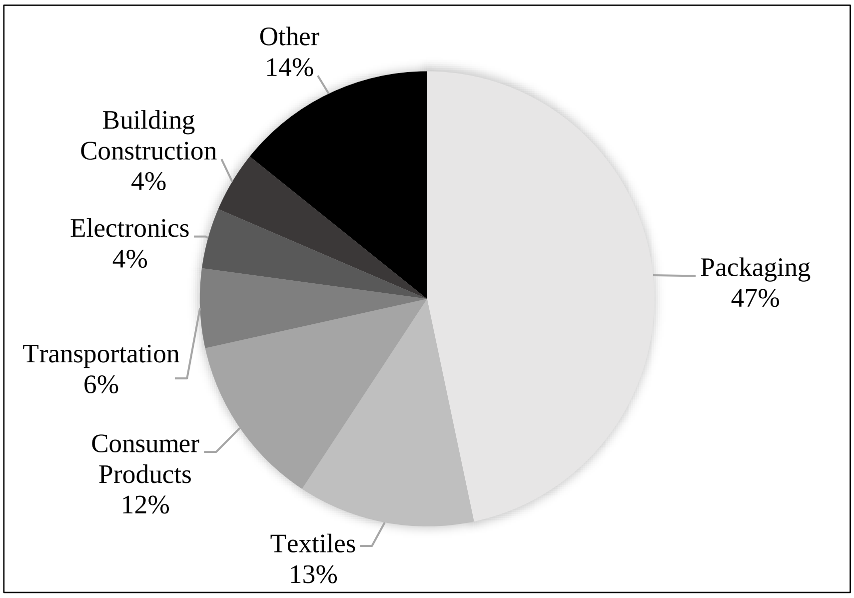


Figure 1: Sources of plastic pollution (Geyer et al. 2017)

However, finding appropriate ways to dispose the produced plastic is a problem that is still being tackled, as the long durability of different plastic types makes it difficult to find an environmentally friendly way of doing so. CO_2 emissions from incineration and leaching from dump sites can have serious impacts on the surrounding ecosystems, which is why it is pressing to find ways that do not incriminate the environment. However, the most common ways plastics are currently still being disposed of are by discarding (55%), through incineration (25.5%) and recycling (19.5%) (Geyer et al. 2017), with the latter being the most environmentally friendly way.

Even though not necessarily environmentally friendly, waste disposal facilities are instrumental in reducing plastic waste. Countries characterized by high-income usually have access to good waste disposal facilities and will dispose of their plastic waste in appropriate ways, keeping contribution to plastic pollution limited (Ritchie and Roser 2018). The countries contributing most to plastic pollution due to inappropriate waste disposal and subsequent high risk of pollution of rivers and oceans are China with a share of 28% of the total global mismanaged waste, Indonesia with 10% and Sri Lanka, accounting for 5% of global mismanaged waste (Jambeck et al. 2015). East Asia and the Pacific are therefore the biggest contributors to mismanaged plastic, with an overall share of 60% (Ritchie and Roser 2018). The relative distribution of the major contributors to plastic pollution can be found in Figure 2.

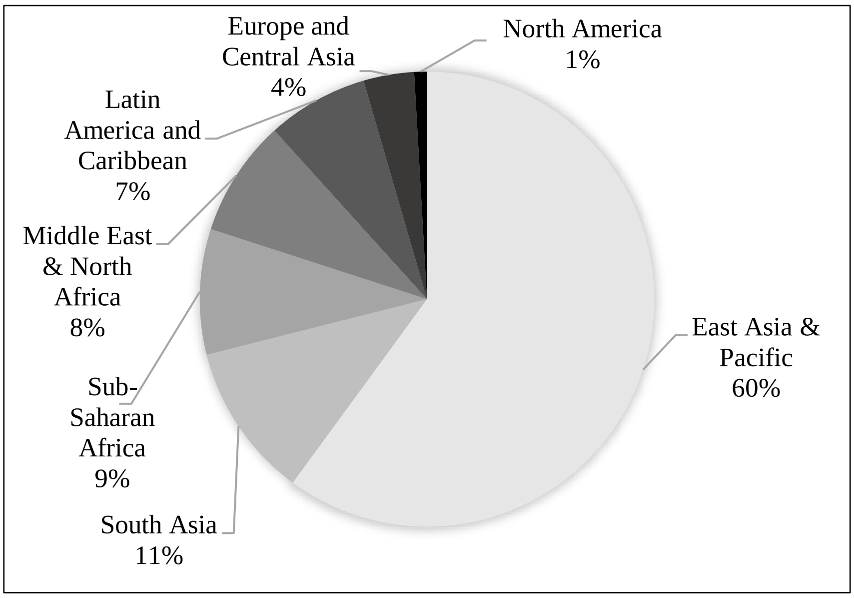


Figure 2: Major contributors to plastic pollution (Ritchie and Roser. 2018)

2.2 Environmental impacts of plastic pollution

There are serious environmental implications that result from incorrect disposal of plastic. Plastic pollution affects especially wildlife and aquatic ecosystems. Wildlife will get entangled or encircled in floating plastic, and marine animals will often get constricted by ropes and netting, causing serious deformations and often a subsequent death (Ritchie and Roser 2018). Plastic debris may also be ingested, leading to an altered sense of satiation and damaging the gut, both with potentially fatal consequences (Ritchie and Roser 2018). Coral reef ecosystems may be damaged by abandoned fishing lines, usually made from nylon, while other aquatic ecosystems are impacted by obstructed light penetration and impacted oxygen exchange in areas where plastic accumulates on the surface of the water (Ritchie and Roser 2018).

2.3 Overview on microplastics

Microplastics are those plastic particles that are smaller than 5 mm in size. They may enter an aquatic system such as a river in two different forms. If they are introduced as primary microplastics, they are produced to be small in size (Mintenig et al. 2017). This may be the case in the production of industrial pellets and scrubbers for personal care products, the latter containing microbeads which are washed down the household drain (Mintenig et al. 2017). Personal care products range from lotions to soaps and face scrubs to toothpaste (Carr et al. 2016). Secondary microplastics on the other hand are also particles smaller than 5 mm, but derived from the breaking down and degradation of bigger plastic debris (Mintenig et al. 2017). This is due to environmental abiotic and biotic processes, as well as the river dynamics and weathering, which result in fragmentation of macroplastic (Mintenig et al. 2017), defined as being bigger than 5 mm (Ziajahromi et al. 2017).

2.4 Sources of microplastics

McCormick et al. (2014), Wagner et al. (2014), Mani et al. (2015), Murphy et al. (2016), Ziajahromi et al. (2017), Kay et al. (2018) stated that wastewater treatment plants, being point sources and filtering water coming from household drains, contribute significantly to microplastic pollution. Wastewater treatment plants filter urban wastewater and then release the water to nearby river systems. The filtering process in wastewater treatment plants does retain some microplastics, but microplastic retention potentials vary according to different sources. Carr et al. (2016) stated that wastewater treatment processes remove microplastics efficiently, without giving any concrete numbers, while Leslie et al. (2017) calculated that an average of 72% of all microplastic was removed by retention sludges in wastewater treatment plants. Estahbanati and Fahrenfeld (2016) discovered a significant increase in primary microplastics at sample locations downstream of wastewater plants, therefore concluding that those plants contribute to plastic pollution in the investigated size categories of 63, 125, and 250 µm. Other retention values calculated in previous studies include 98.41% (Murphy et al. 2016) and 95-99% (Mason et al. 2016). However, even with high retention potentials, there are still considerable amounts of microplastic being released to rivers each day, mainly due to the large amounts of water passing through the plant each day. This was calculated to be about 65 million microplastics per day for specific wastewater treatment plants (Murphy et al. 2016). Nevertheless, Klein et al. (2015) did not find wastewater treatment plants to be significant contributors to plastic pollution, as the data did not show a distinctly larger abundance of particles in the 63-630 µm category. The other commonly mentioned source stems from urban areas (Wagner et al. 2014, Yonkos et al. 2014, Klein et al. 2015, Peters and Bratton 2016, Horton et al. 2017, Mintenig et al. 2017, Nel et al. 2018, Tibbetts et al. 2018). This can be anything from industrial plants, which according to Mintenig et al. (2017) and Nel et al. (2018) are a main source of pollution, as well as urbanized areas. Mani et al. (2015) stated that higher microplastic concentration have been found in proximity to densely populated areas, while Klein et al. (2015) found no significant correlation between population density and microplastic concentrations, as well as no increase in microplastic concentration downstream of a plastic-processing industrial plant.

2.5 Environmental impacts of microplastics

Microplastics have different effects on organisms than big plastic debris, since, due to the small size, they can more easily be ingested. Ingested microplastic can translocate to tissue and even the liver, causing inflammation and lipid accumulation, reduced growth, immobilisation, and eventually death (Tibbetts et al. 2018).

The different kinds of plastics found in the sampling process of the study the present paper is based on (Bouwman et al. 2018) were analysed in an Agilent Cary 660 Fourier Transform Infrared Spectroscopy (FTIR) spectrometer. The FTIR is used to determine polymer composition, which can give information regarding possible sources, rates of decay, as well as predictions on the impacts on the surrounding environments and ecosystems (Bouwman et al. 2018). The polymer types found in the study by Bouwman et al. (2018), where the data for this paper comes from, identified polyethylene and polystyrene as the two polymers present in the sampled water. Naturally weathered polyethylene, a secondary microplastic, has been found to have significant negative effects on endocrine disruption in fish (Jahnke et al. 2017). Polystyrene microplastics have been found to cause metabolism disorder and gut microbial imbalances in larval zebrafish (Wan et al. 2019), which most probably applies to all other organisms with habitats in aquatic environments too. Microplastics tend to bind to organic material and contaminants, increasing their respective density and altering their toxicity (Galloway et al. 2018). Organic material, bacterial growth on the plastic particles and chemical contaminants can cause serious harm to animals ingesting microplastics, since the previously mentioned endocrine disruptors alter the hormone balances of the organisms, with the potential of altering the organisms' metabolism (Galloway et al. 2018). A study by Rochman et al. (2013) showed that fish, ingesting polyethylene particles, accumulate the chemical pollutants absorbed by the plastic particles and subsequently suffer from liver toxicity. In addition to this, ingestion of large amounts of microplastics can lead to an altered sense of satiation (Ritchie and Roser 2018), which affect feeding behaviour and then in turn reproduction.

2.6 Limitations in other studies

It is worth mentioning that older studies on microplastics do not use the same size categories as studies conducted more recently. In a study by Yonkos et al. (2014) as well as Lahens et al. (2018) for example, the presence and concentrations of microplastics were tested according to the occurrence of particles between 300 μm – 5 mm. However, in studies using size classes starting from 30 μm, the biggest proportion of plastic particles was found in the smallest size class (Bouwman et al. 2018). This indicates that studies defining microplastics as being bigger than 300 μm did not take most of the microplastic particles present in the water into consideration (Estahbanati and Fahrenfeld 2016). This is important to know, as the results obtained from such studies may present a large margin of error.

2.7 Overview to this study

Even though most studies lean towards household waters filtered through wastewater treatment plants as being the bigger pollutant (McCormick et al. 2014; Mani et al. 2015; Estahbanati and Fahrenfeld 2016; Murphy et al. 2016; Leslie et al. 2017; Kay et al. 2018), there is still a great proportion of scientists that consider urban areas as the bigger threat (Wagner et al. 2014; Yonkos et al. 2014; Klein et al. 2015; Peters and Bratton 2016; Horton et al. 2017; Mintenig et al. 2017; Nel et al. 2018). Klein et al. (2015) even went on to state that sampling near sewage treatment plants did not result in a distinctly larger abundance of microplastics and excluding wastewater treatment plants as possible sources in their study.

It is important to know if urban areas and wastewater treatment plants contribute to microplastic pollution and if the contribution is significantly larger than those found in rural areas. It is also important to be aware of potential sources within urban areas, and where the different microplastic sizes and colours may come from. Yonkos et al. (2014), as well as Horton et al. (2017) mention that a potential source within urban areas might be particle runoff from roads, resulting in fractions of tyre wear or vehicle parts and road-marking paints being washed into near-by rivers. Wagner et al. (2014) also mention runoff from industrial plastic production sites as runoff, stating however that data is not available yet. With run-off being mentioned by a few papers, it is relevant to investigate whether it contributes significantly to microplastic pollution in freshwater. One way to do this would be by taking into consideration the paper published by Horton et al. (2017) and assuming that particles being washed into the rivers from roads would be represented by black and grey fragments. Run-off from roads most likely represents tyre wear from vehicles (Horton et al. 2017), meaning that in South African wet season, i.e. from November to March, there should be a bigger proportion of black fragments resulting from surface flow into the rivers.

2.8 Importance of this study

Based on the extensive literature review it becomes apparent that there is a general consensus on the effects of microplastics and plastics in general on aquatic ecosystems, however there is still much uncertainty regarding the sources of these plastics. Wastewater treatment plants and urban areas categorised by industrial plants, urban littering and run-off are the sources most commonly mentioned, but there seems to be disagreement amongst scientists as to which of these contributes the most or is the most relevant. It is necessary to try and establish if there is a relationship between different colour fibres and fragments found in vicinity to specific land use areas. By taking into account all particle sizes greater than 20 μm and up to 5 mm, insights into which source contributes the most to microplastic pollution could be given. This is important, as implications resulting from the release of plastics to the environment can be very serious. As stated by Mani et al. (2015), microplastics contain chemical additives, that absorb contaminants from the surroundings. This can be anything from heavy metals to persistent organic pollutants (POPs) (Lahens et al. 2018), which due to the longevity of the plastic may result in long-range transport of those contaminants into non contaminated areas (Bouwman et al. 2018). Additives may also migrate into the food through packaging or even leach into the environment (Bouwman et al. 2018). It is important to understand the nature of plastic and the implications it has for consumers and the environment that is exposed to it.

3. Methodology

3.1 Data source & composition

The data was provided by the Water Research Commission of South Africa (WRC). It was collected for a scoping study by Bouwman et al. (2018) in the Unit for Environmental Sciences and Management at the North-West University, Potchefstroom, South Africa and published in a report by WRC in May 2018. The data comprises 46 sample locations throughout the Gauteng province in South Africa, but this report uses only those sample points that lie in the catchment of the Crocodile River within that province (18 sample points), flowing through Johannesburg and Pretoria.

The data contained detailed information about the amount of fibres and fragments found at each sample location. This was comprised of data on the size distribution and colours of the microplastics. Size categories were 20-300 µm, 301-600 µm, 601-900 µm, 901-1200 µm, 1201-1500 µm and >1500 µm, while colours were classified into seven different categories (blue, black, red, green, yellow/brown, white and purple). Figure 3 and 4 show how the colours of fragments and fibres were distributed amongst the different size classes. The dataset also contained information on which catchment each sample point belongs to, the width of the river at each location, corresponding river order, as well as land use type. Collection of the data happened during the rainy season, in the beginning of 2018. The data represents microplastic particles per volume of 90 litres of water, since that was the amount of water sampled at each location. In Figures 3 and 4, the total fragment and fibre counts were chosen to be displayed as counts per 90 litres of sampled water since this allows for a clearer and better visualisation of the colour and count distribution as opposed to looking at it for particle per litre.

Figure 3 shows the raw data for colour distribution and total amounts of fragments found in each size class for all sample locations. It becomes apparent that total fragment counts are highest in the smallest size class, being around 1500 fragments in the class of 20-300 µm, after which numbers decrease exponentially. In size classes 901-1200 µm, 1201-1500 µm and >1500 µm, counts are lowest and close to 0. The colour distribution on the other hand does not seem to be affected by different size classes, although white appears as the most dominant microplastic colour. Purple fragments are the scarcest, followed by red fragments.

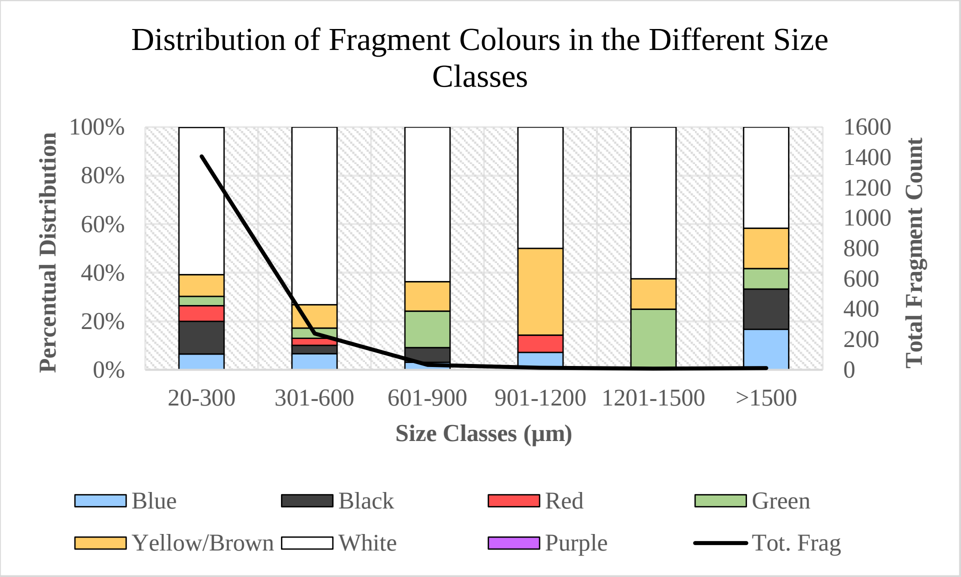


Figure 3: Percental distribution of fragment colours within the different size classes and absolute total fragment counts within the different size classes. Data Source: Bouwman et al. (2018)

The colour distribution also seems to be consistent throughout the size classes with white fibres dominating in all the classes (Figure 4). The rarest fibre colour is green, directly followed by purple. In contrast to fragment counts, total fibre counts are highest in size classes 301-600 µm with about 350 fibres and >1500 µm with around 550 fibres. In contrast to fragment counts, fibre counts never reach 0, for any of the size classes. The presence of microplastic fibres seems to be more consistent throughout the different size classes than for microplastic fragments.

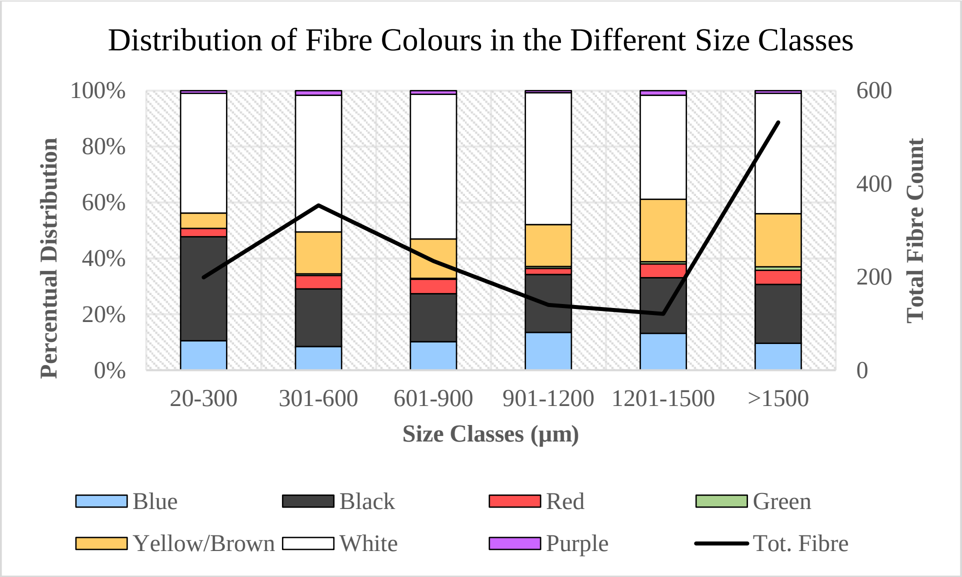


Figure 4: Percental distribution of fibre colours within the different size classes and absolute total fibre counts within the different size classes. Data Source: Bouwman et al. (2018)

The data used to graphically show the study area were collected through different sources, but all projected into the geographic coordinate system of WGS 1984.

- Population density: Raster dataset with 100 metre resolution and population density per pixel from 2015. At time of use, the data was last updated on April 3rd, 2018 and is based on numbers published by world pop (http://www.worldpop.org.uk/). Downloaded from: https://energydata.info/dataset/south-africa-population-density-2015
- Administrative areas: Shapefile by IGIS MAP. Downloaded from: https://www.igismap.com/south-africa-shapefile-download-boundary-line-polygon/
- Water courses: Shapefile by the Department of Water Affairs and Sanitation (Republic of South Africa), with river systems for the Limpopo drainage basin, based on a 1994 river coverage from the Chief Directorate of National Geo-spatial Information. The dataset was improved several times thereafter. Downloaded from: http://www.dwaf.gov.za/iwqs/gis_data/river/rivs500k.aspx
- Underlying areal image: Retrieved in Google Earth Pro and georeferenced in ArcMap 10.5.1

3.2 Data acquisition

The data acquisition has been described thoroughly in the paper by Bouwman et al. (2018). Data collection happened during the late rainy season in the Gauteng Province in the catchment of the Crocodile River. A 90-litre volume of water was sampled through a test sieve with a mesh size of 20 µm and dried in an oven at 90ºC. The dried sample was then digested, a process in which the organic material such as algae is removed. To do this, 20 ml of an aqueous 0.05 M Fe (II) solution (Sulfuric acid (H_2SO_4) + Iron (II) sulphate solution ($FeSO_4$)) was added to the dry sample, followed by 20 ml of 30 % hydrogen peroxide (H_2O_2). This solution was heated up to over 75ºC. Boiling indicates that the reaction is complete, and the organic material is digested. The next step comprises the addition of Sodium Iodide (NaI) to the solution to prepare the sample for density separation. Sodium Iodide is a salt which alters the densities and results in the floating of the plastics in the liquid and the sinking of sediments. For the subsequent density separation, the sample was left to settle in a funnel for a day. The settled sediments could now be poured into a petri dish, while the liquid is poured through a filter with a mesh size of 20 µm. After analysation of the petri dish and consequent transfer of microplastics in the settled sediments to the filter, microplastics on the filter were counted under a Nikon EZ 100 multi-zoom compound binocular microscope.

3.3 Classification of land use types and wastewater treatment plants

The data most relevant to this study was the information on counts of different colour fragments and fibres, as well as the different land use types found at each sample location and information on locations influenced by wastewater treatment plants. Land use types were classified based on the domination of a certain key feature at the corresponding sample location and resulted in:

- Natural: Agricultural fields or green areas
- Informal Settlement: Small villages that do not form part of a city
- Urban/Business: Industrial areas and urban areas, towards the city centre
- Residential: Private housings in a city environment, towards the outer part of the city
- Transition Urban/Rural: Usually on the very outskirts of a city, where residential area changes to natural or rural

Land use classes were determined based on the prevalent land use category around the sample location and not based on the land use types found further upstream. A different upstream land use category may potentially influence microplastic counts differently than the prevalent land use class at the sample location, but more spatial GIS-analysis would have needed to be done to analyse the implications properly. Due to lack of time and data constraints, this was not possible in this paper.

To test whether sample locations were influenced by wastewater treatment plants (WWTP), each location was assigned a category in relation to the location of the nearest wastewater treatment plant:

- Upstream: WWTP can be found within 10 km upstream of the sample location
- Downstream: WWTP is located within 10 km downstream of sample location
- Far away: WWTP is more than 10 km away from sample location

3.4 Statistical analysis and programs used

The data was compiled in a Microsoft Excel 2016 sheet and analysed using the basic functions provided by Microsoft. To do statistical analysis, the program R x64 3.5.3 was used. It provided the necessary commands to test the data for normality using the Shapiro-Wilk normality test, but also to compare the differences among groups of means in a sample through the Kruskal-Wallis H test and to execute a pairwise comparison through the Wilcoxon rank sum test.

- Shapiro-Wilk test for Normality:
  To test for normal distribution of the data, an alpha level of 0.05 was used. The alpha level indicates the probability of incorrectly rejecting the null hypothesis when is actually true (Type I error). The normality test will result in a p-value, which describes

how likely the effect observed in the sample data is if the null hypothesis is true. The p-value must be greater than the alpha level for the data to follow a Gaussian distribution and be normally distributed. If the p-value is less than the alpha level, the data is not normally distributed. Based on the results for the p-values, one of the following hypotheses can be accepted:

H_0 = The sample distribution is normally distributed
H_1 = The sample distribution is not normally distributed

The fragment and fibre count for the different colours were tested for normality, as well as different totals resulting from the summation of diverse counts.

- Kruskal-Wallis H test:

  The one-way Kruskal–Wallis analysis is a non-parametric statistical method that compares the differences among groups of means in a sample. Statistical testing was based on the following hypotheses:

  H_0 = There is no statistically significant difference in colour counts between the different land use classes (null hypothesis)
  H_1 = There is a statistically significant difference in colour counts between the different land use classes (alternative hypothesis)

  The test will result in a p-value, which indicates a ratio of variance between and within sample means. The p-value describes the probability of accepting H_0 and will represent a significant difference in the group of means when below the significance level of 0.05, which accounts for the probability of falsely rejecting the null hypothesis (Type I error). In the present study, the p-values were calculated for differences in the means between the different colours of fibres and fragments falling into different land use categories as well as different wastewater treatment plant categories.
- Wilcoxon rank sum test:

  A pairwise comparison using the Wilcoxon rank sum test between groups was done on the colours that resulted significantly different in the Kruskal-Wallis H test ($p\text{-value} < 0.05$), to find which groups specifically showed significant differences based on the same H_0 and H_1 as in the Kruskal-Wallis H test. P-values below the significance level of 0.05 indicate which classes have colour counts that differ statistically significantly from those found in other classes.

3.5 Study area

The data collection was performed in the drainage basin of the Crocodile River, in the Gauteng Province, South Africa (Figure 5a and 5b). The course of the Crocodile River is indicated in Figure 5c, showing that it forms part of the Limpopo river drainage basin and has its source in the Gauteng Province.

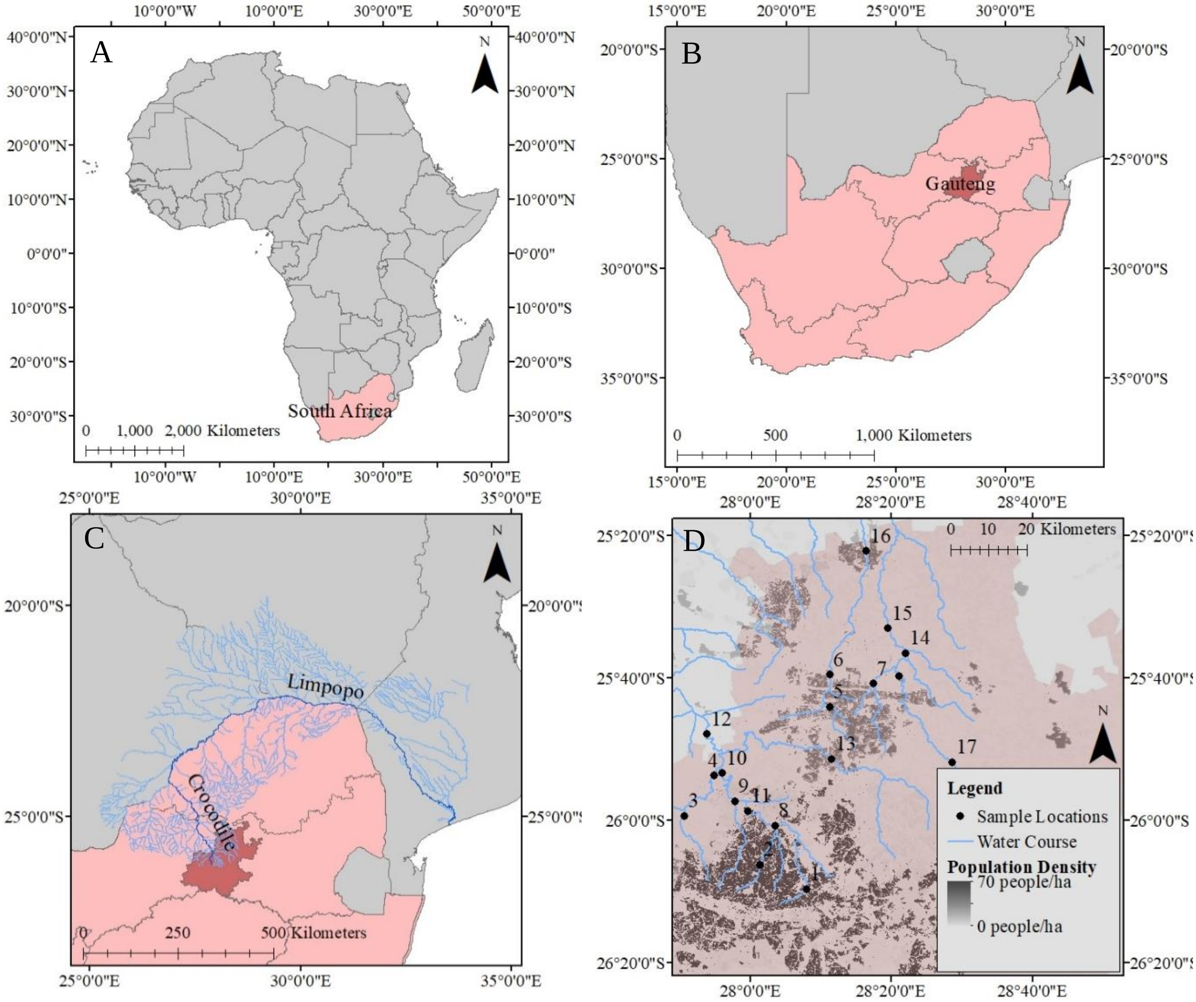


Figure 5(a-d): Location of the Gauteng Province (5b) within South Africa (5a) with the Crocodile river in the Limpopo drainage basin (5c) and the sample locations along the Crocodile river (5d) and underlying population density. Data sources: IGIS MAP (administrative areas), Department of Water Affairs and Sanitation, Republic of South Africa (water courses), Energydata (population density)

The sample points can be observed in Figure 5d, showing the Crocodile river with tributaries, as well as densely populated areas. Sample locations lying downstream of a wastewater treatment plant with the potential of being influenced by the same are sample numbers 6, 7 and 9. All the other sample locations lie either upstream of a wastewater treatment plant or far away.

The study area is extensive and therefore difficult to describe as an entity. One should note however, that the study area comprises two major cities. Sample points 1, 2, 8 and 11 lie within Johannesburg, the largest city in South Africa (Review. 2019a). Johannesburg's 2016 population was estimated at 4.4 million people (Review. 2019a), with an average population density of approx. 29 people per hectare. In Figure 6, the population density is indicated as well, reaching up to 70 people per hectare in some districts. Other sample locations lying in a city environment, but in Pretoria instead of Johannesburg are sample points 5, 6, 7 and 13. As is identifiable in Figure 6, population density is lower, reaching about 11 people per hectare and a total population of 2.125 million people in 2016 (Review. 2019b).

4. Results

Through a visual impression of the average percentages of the colour distribution for fragments (Figure 3) and fibres (Figure 4), it becomes apparent that microplastic particles of the colour white are most commonly found, in both fragments and fibres, while purple and green microplastics are more rare and red microplastics are only found in low quantities compared to other colours. Looking at the colour distribution of fragments (Figure 6a) and fibres (Figure 6b) at each sample site, possible emitters of a specific microplastic colour can be determined.

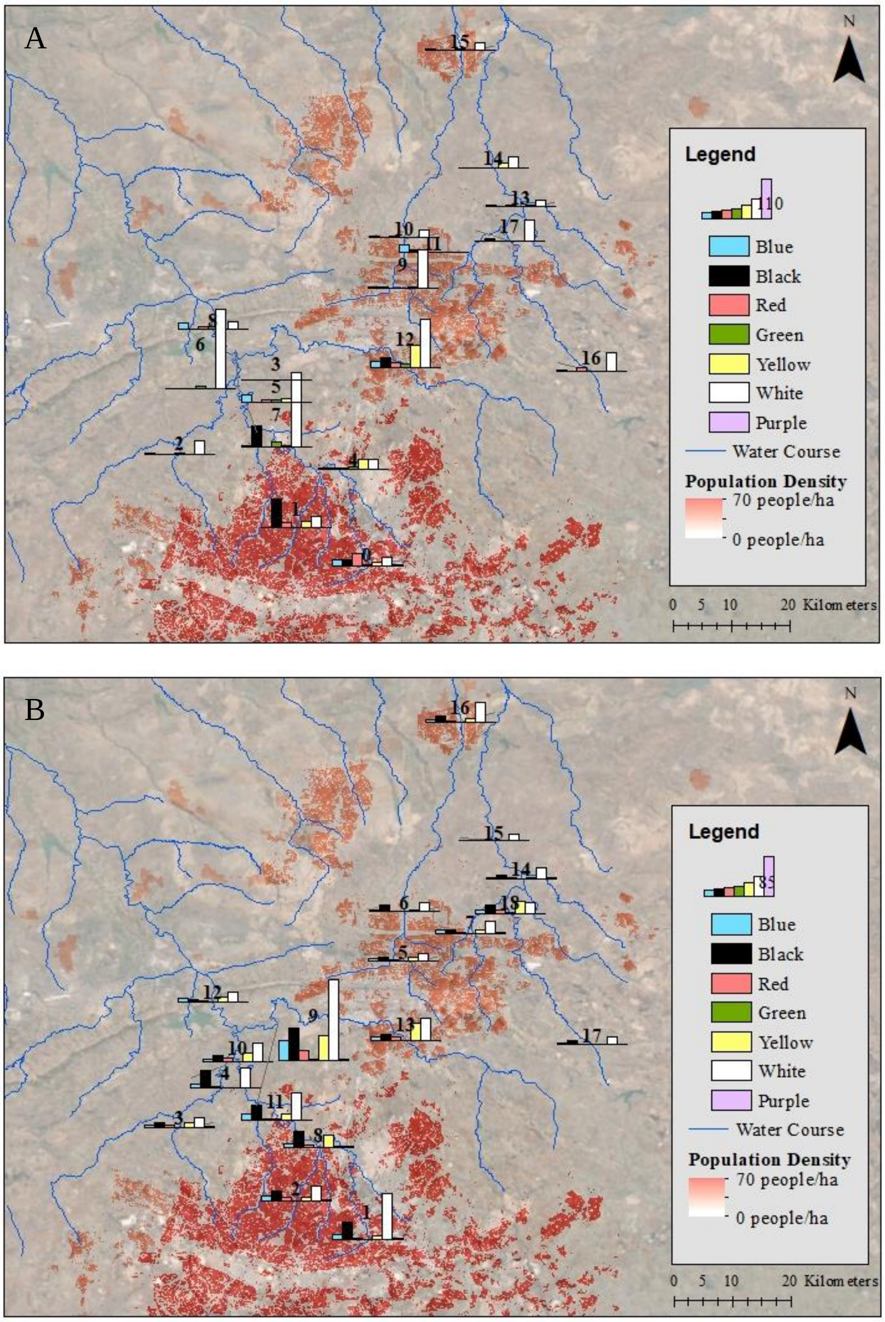


Figure 6: Distribution of absolute numbers (counts per 90 litres of sampled water) for different (a) fragment and (b) fibre colours along the Crocodile River (Gauteng Province, South Africa)). Underlying population density indicates the presence of major cities. Data sources: Department of Water Affairs and Sanitation, Republic of South Africa (water courses), Energydata (population density), Google Earth Pro (underlying image)

4.1 Shapiro-Wilk Test

In order to be able to do any statistical analysis of the data, the data needs to be checked for normal distribution, as that is going to determine whether the subsequent statistical tests must be parametric or non-parametric. This was done through a Shapiro Wilk p-test. As can be seen in Table 1, all p-values resulted lower than $\alpha = 0.05$, meaning that H_0 is rejected and the data is not normally distributed. This indicates the need to continue the data analysis through non-parametric tests.

Table 1: Shapiro-Wilk test for normality and resulting p-values representing the distribution of the different colour fragments and fibres, showing no normal distribution of the data

Fragment Colour	p-value	Fibre Colour	p-value
Blue	0.00022	Blue	6.43e-6
Black	4.47e-6	Black	0.00414
Red	5.04e-5	Red	0.00062
Green	0.00554	Green	0.00014
Yellow/Brown	1.09e-5	Yellow/Brown	0.00223
White	0.00071	White	6.75e-5
Purple	1.06e-8	Purple	0.00112
Total	0.00471	Total	6.24e-5

4.2 Kruskal-Wallis Test

To test the three hypotheses, the Kruskal-Wallis test for significant differences in a group of means had to be conducted. All the results can be found in Table 2.

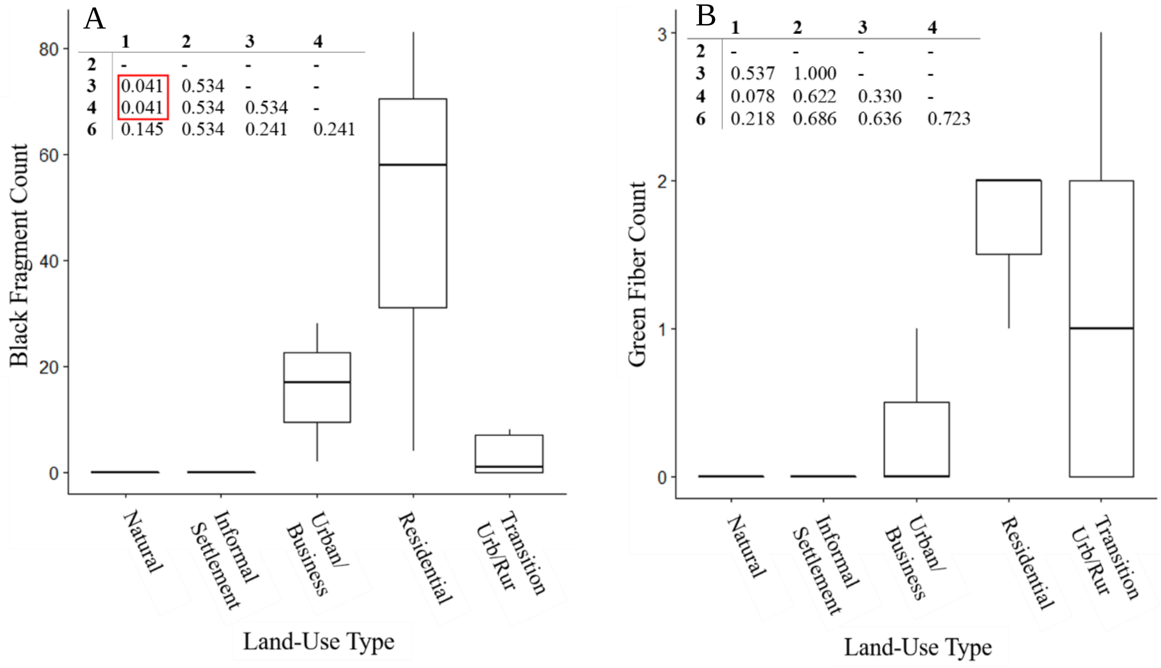
- Hypothesis 1 was tested by running the Kruskal-Wallis test for all the different colour fibres and fragments against the land use classes. Black fragments and green fibres resulted as significantly different for microplastic counts within the various land use classes, rejecting H_0. For all other colour fibres and fragments H_0 was accepted. With a p-value of 0.0130, black fragments were well below the threshold of 0.05 which indicates a significant difference of black fragment counts between the different land use classes. Green fibres had a marginal p-value of 0.0498, implying that they also showed significant differences in counts between the land use classes. Both these p-value are indicated by asterisks in Table 2.
- Hypothesis 2 was tested by running the Kruskal-Wallis test for the different colour fibres and fragments against the locations of wastewater treatment plants. All p-values resulted as greater than 0.05, indicating no statistically significant difference between microplastic counts downstream, upstream or far away from wastewater treatment plants. H_0 was accepted.
- Hypothesis 3 was tested in the same way Hypothesis 1 was tested, by running black fragments against land use types and resulted in a p-value of 0.0130, showing a significant difference between the counts representing the different land use classes. H_0 could therefore be rejected for black fragments, with H_1 being accepted.

Table 2: Kruskal-Wallis test for significant difference between microplastic colour and land use type as well as microplastic colour and location of wastewater treatment plant. P-values showing a significant difference are marked with an asterisk

Fragment Colour	p-value (Land Use)	p-value (WWTP)	Fibre Colour	p-value (Land Use)	p-value (WWTP)
Blue	0.7612	0.0646	Blue	0.0585	0.8971
Black	0.0130*	0.7127	Black	0.0784	0.8371
Red	0.6527	0.1251	Red	0.5426	0.7659
Green	0.8725	0.8317	Green	0.0498*	0.3793
Yellow/Brown	0.1268	0.9669	Yellow/Brown	0.6806	0.9807
White	0.5851	0.4273	White	0.5322	0.7760
Purple	0.2873	0.7788	Purple	0.4464	0.3873

4.3 Wilcoxon Rank Sum Test for multiple pairwise comparison

To find out which individual sample pairs were different among those that resulted in overall significant differences on the Kruskal Wallis H value, a Wilcoxon rank sum test for multiple pairwise comparison between groups was done. This was performed twice, once for black fragments and once for green fibres (Figure 7a and Figure 7b). Black fragments showed a significant difference between microplastic counts in natural environments (1) and urban/business environments (3), as well as natural environments (1) and residential areas (4), observable in Figure 7a and indicated in the red square. Green fibres in Figure 7b do not show any significant difference anymore, now that they are analysed in a pairwise comparison, even though they had a p-value of less than 0.05 in the Kruskal-Wallis H-test.



	1	2	3	4
2	-	-	-	-
3	0.041	0.534	-	-
4	0.041	0.534	0.534	-
6	0.145	0.534	0.241	0.241

	1	2	3	4
2	-	-	-	-
3	0.537	1.000	-	-
4	0.078	0.622	0.330	-
6	0.218	0.686	0.636	0.723

Figure 7: Plastic particle counts in rivers within the Limpopo drainage basin (South Africa) for the particles resulting significantly different in the Kruskal-Wallis test, representing five different land use types. Boxes and whiskers show quartiles of the counts for (a) black fragments and (b) green fibres. The top left insert table of each panel shows p-values from the Wilcoxon rank sum test for pairwise comparison between counts representing different land use types. The numbers 1-6 in the column heading of the insert tables represent natural environments (1), informal settlements (2), urban/business (3), residential (4) and transition urban/rural (6). The data comes from a study by Bouwman et al. (2018) and the number of observations (n) for each land use type is natural (n=6), informal settlements (n=1), urban/business (n=3), residential (n=3) and transition urban/rural (n=5). The red square highlights p-values showing significant differences between land use classes.

4.4 Observed trends

The p-values calculated in the Kruskal-Wallis test showed no significant difference between most of the fragment or fibre colours and the location of wastewater treatment plants or the surrounding land use type. However, looking at the boxplots of the Wilcoxon rank sum test for pairwise comparison between fragment and fibre colours and land use types shows a similar trend for most of the pairwise comparisons as to the one found when comparing black fragments and land use classes. In Figure 8a-f, this trend is highlighted in red, mainly showing that microplastic counts tend to be higher in areas classified as urban/business and residential as opposed to areas classified as natural. The pairwise comparison included in the top left corner of the figure does not show a significant difference, but the trend is very similar to the one found for black fragments, which in turn shows significant differences between those exact classes.

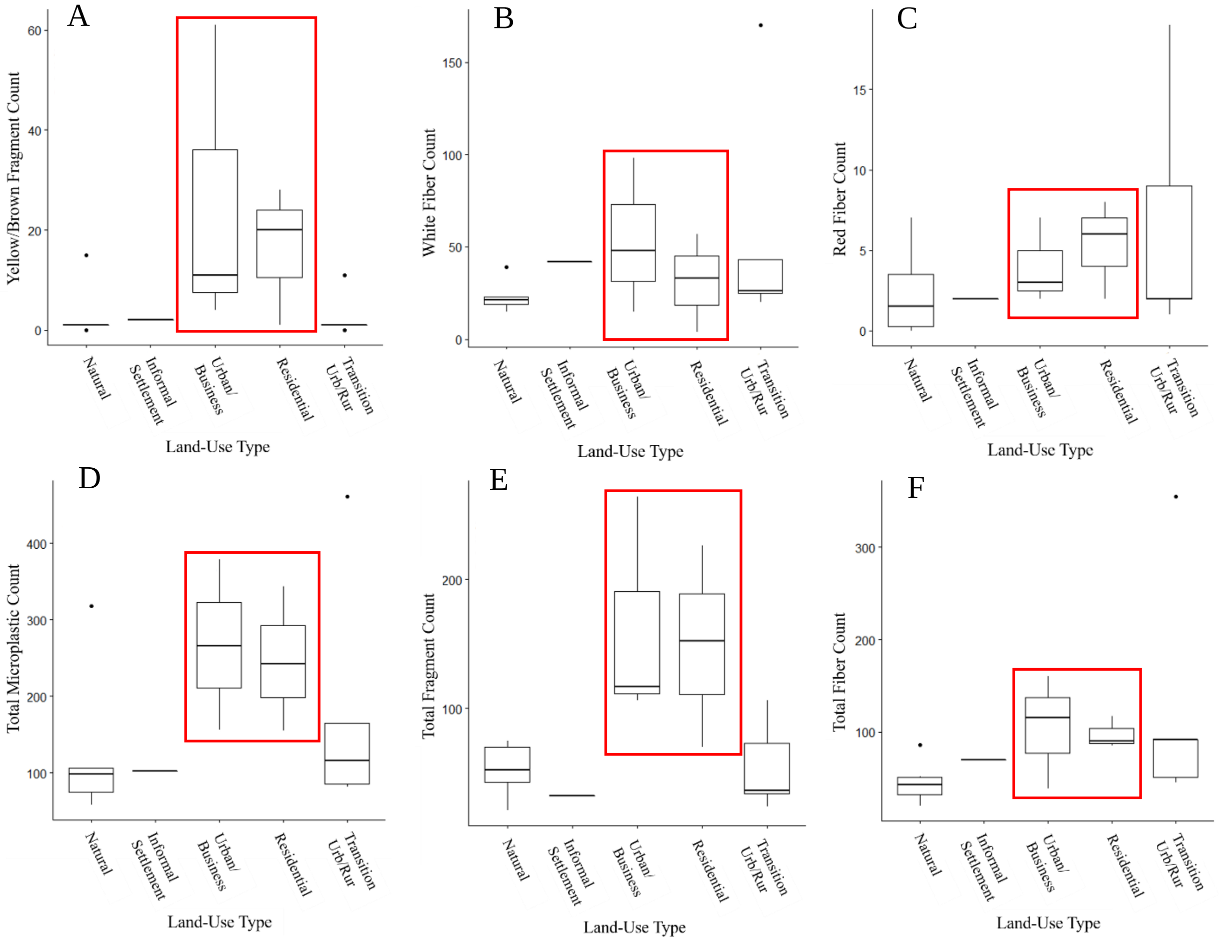


*Figure 8: Plastic particle counts in rivers within the Limpopo drainage basin (South Africa) representing five different land use types. Boxes and whiskers show quartiles of the counts for (a) yellow/brown fragments, (b) white fibres, (c) red fibres, (d) total microplastics, (e) total fragment counts and (f) total fibre counts. Outliers, defined as observations lying outside 1.5*Inter Quartile Range, are shown as dots. The top left insert table of each panel shows p-values from the Wilcoxon rank sum test for pairwise comparison between counts representing different land use types. The numbers 1-6 in the column heading of the insert tables represent natural environments (1), informal settlements (2), urban/business (3), residential (4) and transition urban/rural (6). The data comes from a study by Bouwman et al. (2018) and the number of observations (n) for each land use type is natural (n=6), informal settlements (n=1), urban/business (n=3), residential (n=3) and transition urban/rural (n=5). The red square highlights the trends showing higher counts of microplastics in urban environments (urban/business & residential).*

5. Discussion

This paper has shown how microplastic particle amount, structure and colour differ in a river affected by different terrestrial emission sources. The next few paragraphs will discuss the statistical results and evaluate the hypotheses one by one, as well as discuss limitations of the study. In the final part of the discussion, further development of the sampling method will be suggested, and remaining questions that should be addressed in future studies will be identified.

5.1 Hypothesis 1

Analysing the other p-values calculated in the Kruskal-Wallis test and the subsequent pairwise comparison shows that, except black fragments, none of the other fragment or fibre colours showed any significant difference in microplastic counts between the land use classes. This may indicate that urban areas generally do not result in a significantly different amount of recipient microplastics compared to rural areas, but the lack of significance could also be due to low statistical power. When looking at the boxplots generated by R as an additional step in the pairwise comparison however (Figure 8), it becomes apparent that there is a similar trend for other fragment and fibre colours as the one found for black fragments (Figure 7a), with relatively higher abundance of microplastics in the built-up environments. Interestingly, although not statistically significant, the total number of fragments, fibres and overall microplastic particles were higher in urban than in other environments as well (Figure 8d-f). The reason for these trends not being statistically significant could be due to the rather low number of samples taken in the Crocodile catchment, which amounts to 18 samples. This means that there is a great potential for the colour fragments and fibres to be statistically significantly different throughout the land use classes if more sample points were to be sampled and analysed.

Green fibre counts were found to differ significantly between the given land use classes, as indicated by the p-value retrieved through the Kruskal-Wallis test (Table 2). However, in the subsequent pairwise Wilcoxon rank sum test, aimed at finding the land use classes with significant differences in green fibre count, no such significant difference could be observed (Figure 7b). The p-values of the pairwise comparison do not indicate any significant difference between the land use classes, even though the Kruskal-Wallis test suggested there was. This can potentially be due to issues in the statistical program (R x64 3.5.3), given that the calculated p-value for green fibres in the Kruskal-Wallis test was 0.0498, meaning it just passed the threshold by being lower than 0.05. This may cause problems in the following pairwise comparison, as marginal p-values may show patterns in the data that do not conform with the statistical result obtained. Another possibility is the choice of statistical post hoc test, in this case the Wilcoxon Rank Sum test. Using a non-parametric test allows additional room for error compared to parametric tests and the choice of test has the potential of greatly influencing resulting p-values.

Hypothesis 1 could not be verified. A significant difference between microplastic colour count and the surrounding environment (urban or rural) could not be seen for any of the colours except black fragments. Many of the colours did however show a similar trend of higher counts in urban environments, indicating that, with more data, these trends could result as statistically significant too.

5.2 Hypothesis 2

There was no significant difference in microplastic counts between rivers that were located differently in relation to wastewater treatment plants (downstream, upstream, far away) (Table 2), even though literature suggested there should be a difference (McCormick et al. 2014; Mani et al. 2015; Estahbanati and Fahrenfeld 2016; Murphy et al. 2016; Leslie et al. 2017; Kay et al. 2018). This could be due to the fact that the data was not sampled to test for wastewater treatment plants being a possible point source. Sampling would need to happen upstream and downstream of a wastewater treatment plant to establish if and how much the plant contributes to microplastic pollution. As the data was collected for a scoping study (Bouwman et al. 2018), this was not the case. Only three sample sites were located downstream of a wastewater treatment plant with no respective upstream sample locations along the river, which indicates the need for further sampling. Therefore, it comes as no surprise that the statistical analysis did not show significant differences between microplastic count and respective locations to the closest treatment plants.

Hypothesis 2 could not be verified. For this dataset, the location of the closest wastewater treatment plant did not influence the count of the different colour microplastics, as the data did not comprise upstream and downstream measurements at the treatment facilities present in the study area.

5.3 Hypothesis 3

Black fragments were the only type of microplastic to show significant differences between natural environments and urban/business areas, as well as between natural environments and residential areas (Figure 7a). This indicates the presence of sources within an urban environment that emit substantial amounts of particularly black microplastics to the surrounding rivers. Studies by Horton et al. (2017) and Wagner et al. (2014) suggest that black particles in freshwater systems could be due to run-off from roads, washing tyre wear into the rivers. The effect of this can be estimated to be greatest in the rainy season, as greater amounts of precipitation result in increased run-off on asphalted areas (Miller and Russell 1992). Data collection for this paper was done during the South African rainy season, stretching from November to March. This indicates that the observed results on black fragments might actually come from run-off (Horton et al. (2017), Wagner et al. (2014).

Hypothesis 3 was verified. Black fragment counts were statistically significantly higher in urban environments than in rural environments, indicating the possibility of urban runoff to be a substantial source of microplastics to rivers in the rainy season.

5.4 Bonferroni Correction

Since the statistical analysis did not show a significant difference between the classes of the two variables land use and wastewater treatment plant and most of the fragment and fibre colours, a bonferroni correction was not conducted. In its simplest form, the bonferroni correction adjusts the significance level to reduce the risk of errors when testing multiple hypotheses by dividing the significance level by the number of comparisons, or in this case hypotheses (3). For this paper, the adjusted significance level would have been 0.05 ÷ 3 = 0.0167, since three hypotheses are tested. The bonferroni correction is done for multiple comparisons, since the chances of incorrectly rejecting the null hypothesis (Type I error) for hypothesis 1, 2 or 3 increases with increasing number of hypotheses. However, since the data showed only two values with significant differences (black fragments and green fibres) (Table 2), the bonferroni correction was renounced. Afterall, green fibres would not have passed the bonferroni correction due to the p-value being marginal at 0.0498, and therefore being just under the threshold of 0.05, leaving only black fragments (p-value of 0.0130) as significantly different throughout the land use classes. Even with the adjusted significance level of 0.0167, black fragments would result as significantly different in the Kruskal-Wallis test, therefore not changing the results of this paper.

5.5 Limitations

One limitation that is important to mention is that the numbers of samples taken in the different land use classes were distributed unevenly. There were six samples in natural environments, three for urban/business areas, three for residential areas, five for transitional areas from urban to rural but only one for informal settlements. The single measurement for informal settlement did not allow for a boxplot analysis to find significant differences between microplastic counts in informal settlements and the other land use classes. Informal settlements could therefore not be tested appropriately for significant differences, even though this land use class is present in the study area. Land use classifications in general were a limitation in themselves, as the classification was done based on the predominant land use type around the sample point. Looking at the land use class along the river upstream of the sample point might have given a better indication of possible polluting sources and offer more accurate explanations for microplastic counts found at the respective sample locations. Even with the different methods of classifying land uses however, one thing that has to be kept in mind is that sewage sludge from wastewater treatment facilities is often applied to agricultural areas (Wagner et al. 2014, Leslie et al. 2017, Kay et al. 2018, Tibbetts et al. 2018). Since these facilities do filter some of the plastics out, the filtered plastics will be applied to agricultural fields in the form of biosolids (Leslie et al. 2017), providing a diffuse pathway for rural microplastics into rivers (Tibbetts et al. 2018). This may result in higher counts of microplastics downstream of natural environments, which is the exact opposite of what has been tested in this paper.

Something that has been disregarded in this paper are river dynamics and catchment properties influencing river flow, microplastic paths in the river as well as potential sinks for microplastics residing in the rivers. Since the water flowing close to the riverbed tends to flow landward, microplastics suspended in the water of the river may be retained within estuarine sediments (Horton et al. 2017). Microplastics have the potential of accumulating in sediments at an order of magnitude higher than in the water column (Horton et al. 2017), which is a sink, caused by the river dynamics, not accounted for in this paper. Other potential sinks that have not been accounted for in this study are microplastic uptake by biota, dilution as well as settling or skimming of particles during transport (Estahbanati and Fahrenfeld 2016). To fully understand the importance of the sinks however, the data collection would have needed to be done more systematically, in each tributary that feeds into the main channel. This has not been done, which makes current data analysis harder too, as it is unknown if the counts measured at a specific sample location are actually those that have been introduced directly to the main channel or if the plastic particles have their source in one of the many tributaries and are therefore introduced into the main channel by a first or second degree stream. Also, the fact that the sampling technique involved sampling 90 litres of water instead of sampling sediments might have reduced the amounts of plastics found, as microplastics tend to accumulate in the sediments (Horton et al. 2017).

Laboratory limitations focus mainly on the technique used to digest the organic material in the sample and the subsequent counting. The methodology to this process has been adapted from Masura et al. (2015) and executed by Bouwman et al. (2018). There is more than one option on how to treat the samples in the laboratory, but the chosen method by Masura et al. (2015) is described as cost-effective, developed to provide simple, reproductible techniques, without requiring extensive equipment (Masura et al. 2015). This leaves room for error in the subsequent identification and counting of microplastics, as microplastics are small in size and therefore sometimes hard to distinguish from other particles that have not been digested properly in the laboratory. Other ways, maybe more expensive ways, of digesting the organic material might eliminate the problem.

Avoiding potential error sources such as the sampling technique used to obtain the data would most likely yield different results for hypothesis 1 and 2. Hypothesis 1, stating that a higher abundance of different colour microplastic particles would be found in urban areas, could most probably be verified if more samples would have been taken for each land use class in the crocodile river, providing a higher and more equal number of samples throughout the land use classes. The same goes for hypothesis 2, stating that a higher count of different colour microplastic particles would be found at sampling locations downstream of wastewater treatment plants compared to sample locations upstream of a wastewater treatment plant inlet. By sampling in a way that provides data for respective upstream and downstream locations along tributaries with wastewater inlets, the results would have most likely verified the hypothesis.

5.6 Proposed sampling model

To execute the aim properly and analyse the hypothesis accordingly, a different sample collection would have needed to be done. A proposed sampling model would need to measure the counts of the different colour fibres and fragments in all the tributaries joining the main channel of the Crocodile river, as well as measuring upstream and downstream of the different wastewater plants and include measurements that are representative for the different land use classes. When looking for potential sources of microplastics, any information about tributaries and possible incoming microplastic loads within those tributaries is relevant. A sampling strategy like this would also allow for the identification of the tributaries contributing the most to microplastic pollution which may indicate that a shift of focus from the main channel to polluting tributaries is necessary. Also useful in such an analysis is measuring microplastic counts along the main channel at defined intervals. This might give an idea of possible sinks within the river as well as indicate sources along the course of the channel, through the spiking of counts for example. This could allow for a better distinction of where the plastics come from and allows for the testing of the influence wastewater treatment plants as well as different land use practices have on microplastic pollution.

As far as land use classification goes, looking at the land use upstream of the sample locations might give a better idea of pollution rates. This can be either analysed in field but looking at it in Google Earth Pro for example might give an even better indication of the land use along the river channel. To fully understand the contribution of the different land use classes however, a more complicated catchment analysis would need to be done, looking at flow direction and soil properties to best estimate the microplastic input influencing counts at the different sample locations.

5.7 Remaining questions

There are a few questions that remain after having worked with the data. Plastics in general and microplastics in particular have the capability of absorbing trace metals and persistent organic pollutants (Lahens et al. 2018) and potentially carry them to areas into non contaminated areas (Bouwman et al. 2018). What remains unclear however is, if due to the longevity these plastics, the absorbed pollutants are ever released to the environment after the plastic degrades. This is important to know, as these pollutants may travel up the food web if ingested by organisms and affect many different species and ecosystems.

There is no available data on the pollutants associated with the different colours of microplastics, as this study is one of the first to analyse microplastics by colour and not just by size or environmental impact. Knowing that black fragments can be found in significantly larger counts in urban areas than in rural areas, it might be relevant to know whether the different colours absorb different kinds of pollutants.

Something mentioned in papers (Horton et al. 2017; Leslie et al. 2017; Kay et al. 2018) is sewage sludge, usually retained in wastewater treatment plants, with the potential of being a significant diffuse source when applied to agricultural fields. So far, little to no investigations have been made into the release of microplastics from sewage sludge, which often contains those microplastics that have been filtered out of the city's wastewaters. A re-application to natural areas can alter microplastic counts found at the respective sample locations and more in-depth knowledge about this process is highly needed.

Lastly, microplastic counts measured at specific sample locations are most likely influenced by the river hydrology and catchment properties of the river. This can be anything from different flow velocities and depths across the river channel, to specific mixing conditions of water flowing in the main channel and water joining the main channel through a tributary. It is fairly difficult to take all these factors into consideration when evaluating results as information about all these processes is needed for each sample location, but a model could perhaps help understand the sampling conditions better. The same goes for microplastics deposited through atmospheric deposition (Kay et al. 2018), which, when taken into consideration, could explain observed results and trends in data, such as presence of microplastics in rural areas, more accurately.

This shows that there is a great need for further investigations into the nature of microplastics and their sources, as well as their environmental impact and durability.

6. Conclusion

Looking at microplastic colour provides a new way of identifying possible sources, which is the first step in limiting plastic pollution and in implementing regulatory actions. This is why the aim of the paper was to analyse the colour distribution and the corresponding microplastic count throughout different land use categories for the Crocodile River in South Africa, as well as looking at microplastic counts in relation to locations of wastewater treatment plants. This was achieved by the three hypotheses:

- Hypothesis 1 could not be verified. A significant difference between microplastic colour count and the surrounding environment (urban or rural) could be seen for black fragments and green fibres but the other colours did not result in significant differences. They did however show a similar trend of higher counts in urban environments, indicating that, with more data, these trends could result as statistically significant too.
- Hypothesis 2 could not be verified. For this dataset, the location of the closest wastewater treatment plant did not influence the count of the different colour microplastics, as the data did not comprise upstream and downstream measurements at the treatment facilities present in the study area.
- Hypothesis 3 was verified. Black fragment counts were statistically significantly higher in urban environments than in rural environments, indicating the possibility of urban runoff to be a substantial source of microplastics to rivers in the rainy season. This could potentially result from tyre wear washing into rivers through runoff during precipitation events.

By determining the exact polymer types of the black fragments it will be possible to make accurate statements as to where they actually origin from, allowing for mitigation practices to be implemented directly at the source. This is of utmost importance for the other colours as well. Future investigations on runoff data and possible other sources and environmental effects for microplastic particles should be conducted, to fully understand the contribution of runoff to microplastic pollution. By investigating river dynamics and atmospheric deposition, possible patterns as well as trends and additional sources can be determined, completing .

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