Spatial distribution and abundance of microplastics particles in the bed sediment of Zeekoevlei Lake, Cape Town

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A mini-thesis submitted in partial fulfilment of the requirements for the degree
Master of Philosophy in Integrated Water Resources Management

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ABSTRACT

Microplastics (MPs) are an emerging micro-pollutant that pose a threat to the ecological integrity of freshwater rivers and wetlands. Most previous work on MPs pollution has focused on marine environments. This study aimed to investigate the spatial distribution and abundance of MPs particles in the bed sediment of a freshwater coastal lake in the Zeekoevlei Nature Reserve, Cape Town, which is fed by rivers and stormwater outlets draining a heavily urbanized environment. The first objective was to characterize the spatial distribution of MPs across the lakebed environment, in relation to possible point and non-point sources of contamination. The second objective was to provide insight into local fluvial and lacustrine dispersal mechanisms as a mode of transport for MPs throughout the Lake. Geospatial mapping was used to illustrate the configuration of points of inflow from rivers and stormwater outlets, as well as any potential macro-plastics pollution sources (e.g., picnic sites, walkways). A boat lakebed sediment sampling strategy was devised in order to balance spatial coverage and sampling efficiency. MPs particles were extracted from sediment in the laboratory using a density separation method and counted under a microscope following standardised MPs identification protocols. The fine particle size of lakebed sediment (mud) made extraction difficult, and some contamination of filter extracts by silt-sized quartz material was noted during microscopy and quantified under a polarising microscope. As a result of this contamination, no irregular-shaped particles were counted as plastics, and counting was restricted to particles that passed a set of published criteria. The concentration of spherical MP particles (e.g., primary MP abrasive agents, likely to be from sewage spills and stormwater inflows), and synthetic fibres was found to be high by international standards, likely a consequence of the heavily urbanised setting and regular incidence of sewage spills. Spatial variation across the lakebed was shown to vary to some degree by distance from possible inflow sources, but this relationship is complex and may be influenced by variation in wind intensity and direction, and local currents within the lake during material transport, as well as possible re-suspension of settled particles through these processes. It is hoped that the findings will contribute new understanding of controls on the spatial distribution of MPs pollution at Zeekoevlei and in coastal lake and wetland sediments in general and will highlight a need for national policies and legislation to include and recognize the importance of protecting these ecosystems from MPs contamination.

Key words: Plastic pollution; sediment-associated; lacustrine ecosystems; urban rivers; wetlands

DECLARATION

I Kyle Wade Kennedy, hereby declare that an investigation done on the SPATIAL DISTRIBUTION AND ABUNDANCE OF MICROPLASTICS PARTICLES IN THE BED SEDIMENT OF ZEEKOEVLEI LAKE, CAPE TOWN is my own work, that has not been submitted before for any degree or examination in any other university, and that all sources I have used or quoted have been indicated and acknowledged by complete references.

Signed: Kennedy

Kyle Kennedy

Date: July 2021

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1. Introduction

1.1. Background and Rationale

Microplastic particles (MPs) (5mm - 1µm) has become a ubiquitous constituent of water and sediment in various water bodies throughout the world. MPs are vastly a form of pollution that has become a widespread cause for concern in aquatic environments with an evident increase in its negative biological effects. MPs are essentially considered to be a micro-pollutant and have been split into Primary and Secondary MPs based on their sources and characteristics (Avio *et al*., 2017, Townsend *et al*., 2019). MPs are a growing concern for ecosystem health in water and sediment environments (He *et al*., 2018). The presence of MPs has become very well established amongst various water bodies and benthic sediments, which ultimately has an impact on environments throughout the world (Avio *et al*., 2017). Approximately 10 % of the global annual plastic production is deposited into aquatic ecosystems, which adversely threatens the well-being of the ecology and its ability to function effectively (Avio *et al*., 2017; LeBreton *et al*., 2017). It is thus of great importance that the distribution and accumulation of MPs within the bed sediment of aquatic ecosystems are understood. Although the importance of this is ever present in majority of the research having been done in marine environments, there remains a lack in microplastic research being done in terrestrial ecosystems (He *et al*., 2020). There has, however, been studies done on the occurrence and distribution of MPs within freshwater ecosystems (Su L *et al*., 2016). The study provided information that will assist in creating a better understanding of the occurrence and distribution of MPs within freshwater ecosystems but despite this, there remains a great lack in descriptive comprehension of this within freshwater ecosystems as opposed to marine environments.

1.2. Problem Statement

The Zeekoevlei Lake is therefore the perfect study area to provide information on the above-mentioned concern due to it being surrounded by an urban area and urban activities. There is no data at present on MPs within the lake, despite it being surrounded by urban activity. There are many households and communities in general situated directly on the boundary of the lake, as well as many industrial areas and households located along the two rivers (largely urban canals) that feed the lake. These rivers are susceptible to pollution (including reported incidents of sewage spill) and may therefore act as a transporting media for MPs into the lake. There is a risk involved in the absence of this study which is quite simply a lack of awareness of a pollutant that cannot be detected with the naked eye, as well as this pollutant being allowed to accumulate and further distribute throughout the Zeekoevlei Lake and the surrounding ecosystem thus having detrimental impacts on the health of the environment and humans as well.

1.3. Research Questions

- How are MPs spatially distributed in the bed sediment of the Lake?
- In which areas of the Lake are MPs most concentrated?
- How are MPs dispersed and what causes their dispersal within the Lake?

1.4. Aim and Objectives.

This study aims to investigate the spatial distribution and abundance of MPs particles in the bed sediment of a freshwater coastal lake in the Zeekoevlei Nature Reserve, Cape Town, which is fed by rivers and stormwater outlets draining a heavily urbanized environment. Two objectives were established to achieve this aim:

- The first objective is to characterize the spatial distribution of MPs across the lakebed environment, in relation to possible point and non-point sources of contamination.
- The second objective is to provide insight into local fluvial and lacustrine dispersal mechanisms as a mode of transport for MPs throughout the Lake.

1.5. Overview of Research Approach

The research approach applied in this study consists of 3 fundamental parts. These are geospatial analysis, fieldwork including a lakebed sediment sampling strategy by boat, and laboratory analysis using a density separation method followed by counting under microscopy.

1.5.1. Geospatial Analysis

Geospatial analysis made use of ArcGIS to provide maps for the visual representation of the spatial distribution and abundance of MPs. MP concentrations was shown on these maps in the form of circles, with each circle being a size that represents the mean concentration for that particular sampling site in order to show variation.

1.5.2. Field work and Laboratory Analysis

Fieldwork included the collection of a total of 40 sediment samples. This took place over 6 hours of sampling within one day. Bed sediment samples were collected using a grab sampler whilst navigating on the lake in a boat. Thirty Samples were collected around the lake boundary whilst ten were collected down the centre of the lake. These samples were then collected in a labelled glass container. The collected sediment samples were then taken to a laboratory at the University of the Western Cape (UWC) for laboratory analysis. A density separation technique was used to separate MPs from sediment after the samples were dried. Density separation was carried out using a separating funnel with Zinc Chloride ($ZnCl_2$) as the floatation media. Once density separation occurred, the solution with the floating particles were transferred to a Millipore Filtration System (MFS) where the particles were separated from the $ZnCl_2$ solution. Some samples that were organically rich was treated with Hydrogen Peroxide in order to eradicate the organic material.

1.6. Thesis Overview

This thesis is comprised of seven chapters including which includes the introductory chapter. Chapter two provides an overview of the research study area. Chapter three provides a critical review of literature. Chapter four gives a description of the specific methods used in this investigation. Chapter five describes the results for the spatial distribution and abundance of MP particles in the bed sediment of the Zeekoevlei Lake. Chapter six interprets and discusses the results and chapter seven concludes and suggests recommendations for future research.

2. Study Area

2.1. Description of Study Area

The study area was the Zeekoevlei Lake located in the Western Cape, specifically in Cape Town as seen in Figure 2.1 below.

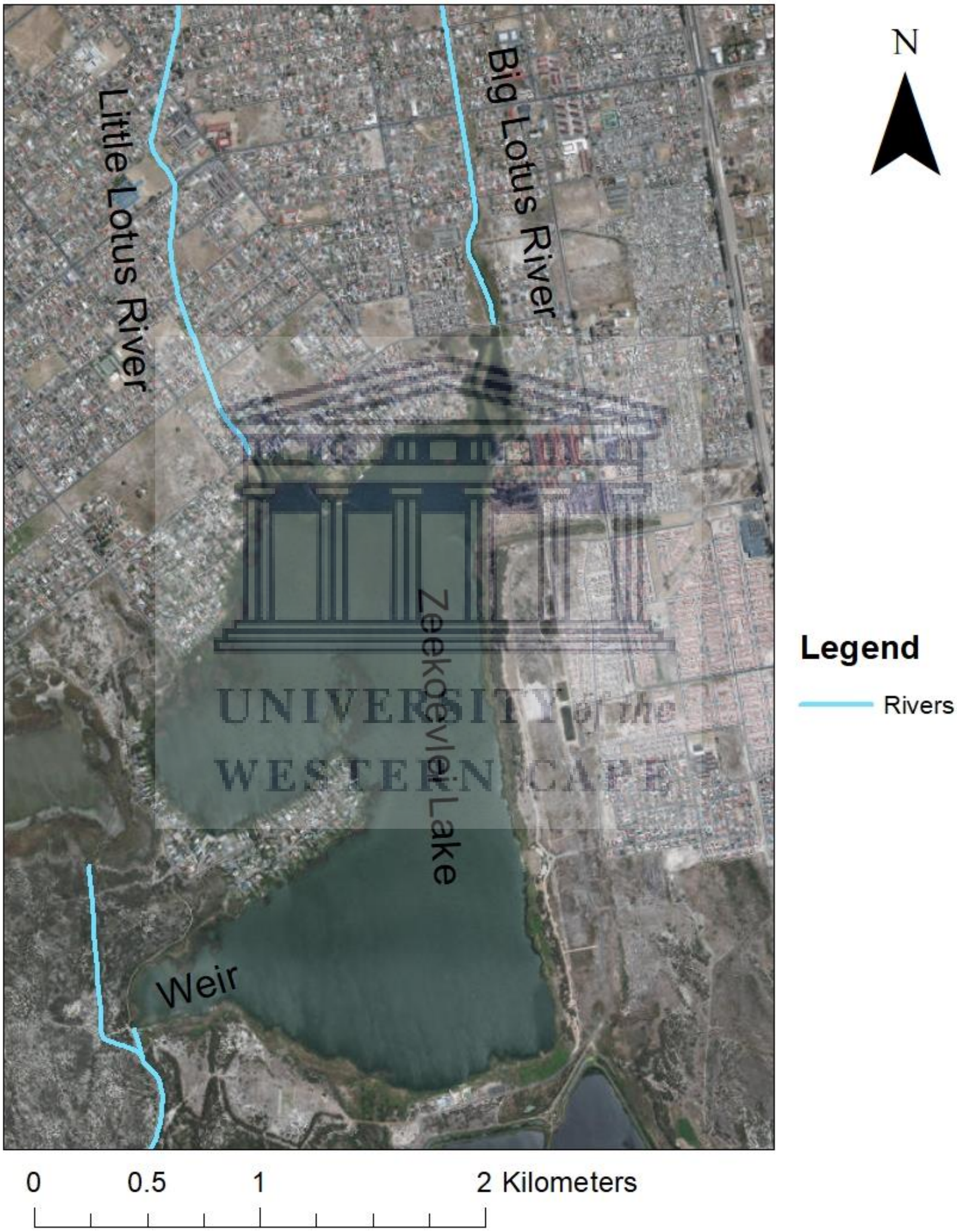

Figure 2.1: Map of Study Area

The Zeekoevlei Lake has an area of approximately 284 hectares which comfortably makes it one of the largest Lakes in the Western Cape. The Lake covers a total area of approximately 1,200 hectares of land on the southern edge of Cape Town and forms part of the False Bay Ecology Park (FBEP) (City of Cape Town, 2011). The FBEP includes the Zeekoevlei Nature Reserve, the Rondevlei Nature Reserve, the Cape Flats Wastewater Treatment Works (CFWWTW), a Coastal Park Landfill site, and a section of coastal strip.

Zeekoevlei is surrounded by at least 700 hectares of wetland and urban residential areas such as Grassy Park, Pelican Park, Retreat and Zeekoevlei which are believed have an immediate contribution in the concentration of MPs in the Lake. Zeekoevlei has been recognized as part of Zeekoevlei Nature Reserve, which is managed and owned by the City of Cape Town. The reserve is open for the public to access, with recreational activities available for people to indulge in; the reserve is also shared with private homeowners of which some live on the boundary of the Zeekoevlei Lake (City of Cape Town, 2011).

Figure 2.1 shows an aerial view of the Zeekoevlei Lake as well as the 2 main rivers (i.e., Big and Small Lotus River) that feeds into the lake. As seen in Figure 2.1, the rivers are surrounded by human activity and can be believed to be immediately affected by this. A weir can also be seen, which serves the function of reducing continuous flow downstream. Once a year the weir is opened in order to prevent the lake from flooding and also to reduce the levels of nutrients within the lake.

The reserve has many recreational activities as mentioned before and primarily serves the community as a recreational site with picnic facilities, ablution facilities and fishing spaces. Zeekoevlei is also popular for its water sports and homes a canoeing club as well. More than twenty-three registered clubs make use of Zeekoevlei for various water sports (Tapela *et al.*, 2015). Fishing is widely practised at Zeekoevlei, with fish species consisting mainly of alien species such as carp (*Cyprinus Carpio*). Due to a recent sewage spill as reported by locals as well as an increase in nutrients, fishing has become a risk, especially with aquatic life being affected by high levels of pollution and possibly being reduced in population as a response. The reserve also has an abundance of reeds especially towards the centre of the lake, which assists in nutrient uptake and the reduction in the levels of nutrients present in the lake.

Figure 2.2: Nutrient rich water of Zeekoevlei Lake due to sewerage spill (20 October 2020)

Figure 2.3: Dense stands of *Schoenoplectus scirpoides* in the center of the Zeekoevlei Lake (20 October 2020)

3. Literature Review

This chapter will provide a background on MPs as a micro-pollutant, their sources and how they are deposited and their impact on the health of the environment. It will also look at the relevance of MPs in sediments.

3.1.MPs as a Micro-pollutant

MPs can be referred to as the broken down or weathered products of macro-plastics and act as a micro-pollutant that diminishes the quality of the environment and leads to the development of various socio-economic concerns (Cole *et al*., 2011). The presence of MPs has become very well established amongst various water bodies and benthic sediments which ultimately have an impact on the environment. According to various articles, there is approximately 10 % of the global annual plastic production that gets deposited into aquatic ecosystems, which adversely threatens the well-being of the ecology and its ability to function effectively (Avio *et al*., 2017; Lebreton *et al*., 2017). It has also been determined that the closer urbanized areas are to coastal regions or wetlands, the more plastics will be distributed and thus have very negative impacts on aquatic ecosystems (Eriksen *et al*., 2013).

Plastics are considered to be unique pollutants within aquatic ecosystems, since they exist in water as a solid that can be characterized based on various shapes, sizes, colors and even densities (Zhang *et al*., 2018). These plastics also display the ability to attach themselves to various other pollutants, which increases the threat posed by macro-plastics and MPs to aquatic ecosystems (Lahens *et al*., 2018). As mentioned, plastics exist in different sizes which is supported by various studies, with most studies stating that the diameter of MPs are less than 5 mm (Andrady., 2017; Nel and Froneman., 2015). Regardless of the size of MPs, they are all extremely resistant to threats from microbes, thus creating significant extensions in their lifespans (Revel *et al*., 2018).

MPs are vastly becoming an increasing threat towards the functionality of various ecosystems to provide ecosystem services such as provisioning services including the provision of food, fresh water, fuel, Fibre, and other goods; regulating services such as climate, water, and disease regulation as well as pollination; supporting services such as soil formation and nutrient cycling; and cultural services such as educational, aesthetic, and cultural heritage values as well as recreation and tourism (Andrady., 2017). The ability to integrate aquatic and terrestrial food chains as well as being able to bio-accumulate across these food chains (e.g., water birds on land eating aquatic organisms and then being eaten by land-based mammal predators) is also a very interesting feat that MPs possess (Carbery *et al*., 2018). In order to comprehend the abundance of MPs, and the risks they pose to life on land and in aquatic ecosystems, which includes the well-being of benthic communities, various ecological processes, and food security, we must first obtain accurate measures of the origin and the abundance of MPs that are present in sediments (Coppock *et al*., 2017).

3.1.1. Sources of MPs

Although all MPs possess very similar characteristics, they also share a very distinctive and obvious difference, in which they can be differentiated based on their place of origin. MPs can thus ultimately be separated based on (i) primary MPs and (ii) secondary MPs, which are extensively determined by the various sources of MPs (Peng *et al*., 2020).

(i) Primary MPs

Primary MPs are plastics that has been manufactured at a microscopic level and at a microscopic size (Cole *et al*., 2011). These MPs are usually classified as granulated particles, which are incorporated in various industrial and a variety of household products (Boucher and Friot., 2017; Nel and Froneman., 2015). These granulated particles are also called microbeads, micro-pellets or micro-Fibres which varies in size, shape and composition based on the product. They are usually used for human care in products such as facial cleaners/ scrubs and exfoliating hand cleansers that enter waterways via domestic or industrial drainage systems (Cole *et al*., 2011; Peng *et al*., 2020). What is arguably most concerning about the use of exfoliating hand cleaners that contains plastics, is that the use of these products has been experiencing a dramatic increase annually since it first became available for household usage (Fendall and Sewell, 2009). Other sources for MPs also include industrial scrubbers that are used to blast clean surfaces, the use of plastic powders in molding and the use of nano-particle plastics used in various industrial processes. There are also virgin resin pellets that are cylindrical in shape and are extensively used during the manufacture process of plastic and the transport of resin ‘feedstock’ before being used to produce plastic products (Kershaw and Rochman., 2015).

(ii) Secondary MPs

Secondary MPs are MPs that are produced from the degradation, or the fragmentation of larger products or debris items that contain plastics during the usage of various products or weathering degradation of those products being littered (Andrady., 2017; Kershaw and Rochman., 2015). The fragmentation of plastics is believed to be initiated by polymer chain backbone weathering due to the exposure of the plastics to sunlight (UV), oxidants, hydrolysis and physical shearing caused by currents, waves, or friction with sand particles (Gerritse *et al*., 2020). There has also been evidence that suggests the occurrence of microplastic pollution in riverbeds due to the presence of secondary MPs such as microplastic fragments (Andrady., 2017). This is largely due to the degradation or weathering of larger plastics from terrestrial sources such as landfill or litter from various industries and surrounding households (Andrady., 2017; Tibbets *et al*., 2018). Secondary MPs also derive from sources such as synthetic fibres that comes from the washing of clothing that are mostly made from polyester or acrylic, which is then discharged in large quantities within household sewage discharges into rivers or the ocean (Reynolds and Ryan., 2018).

When MPs are transported from their initial place of origin and into the environment or aquatic ecosystems, they tend to increase in concentration through physical sorting processes (Kleinhans, 2010) environments that favor their accumulation.

3.1.2. Accumulation of MPs in sediments

Once MPs are deposited into aquatic ecosystems from various sources, either as primary MPs or secondary MPs, they undergo accumulation. The deposition and ultimately accumulation of MPs has, to a large extent, been proven to be due to erosion and surface run-off of larger plastics that are degraded over a period (Cole *et al*., 2011). Although there are many large plastics that undergo degradation and break down into MPs within aquatic ecosystems such as wetlands, many of those MPs have been proven to have accumulated in wetland sediment (Townsend *et al*., 2019). There are various studies which has shown that MPs has been sourced by various urban activities and has ultimately entered the environment via storm water runoff (Townsend *et al*., 2019). Studies have also shown that majority of plastic fragments found consist of a dominant type of microplastic that is found in sediments that have been affected by storm water runoff within urban areas (Townsend *et al*., 2019). Catchments that naturally undergo the water cycle, such as still water bodies (for e.g., lakes) also acts as storage basins for MPs that lack the extensive time required to accumulate thus resulting in an increase in concentration of MPs within these areas (Fischer *et al*., 2016).

Once these MPs are deposited and accumulates, they have the ability to further accumulate a diverse range of toxic and persistent organic contaminants, including pesticides, flame retardants and polychlorinated biphenyls (PCBs). This is due to the ability of microbeads to absorb and concentrate toxic hydrophobic substances within local water and wastewater which are also prone to extensive transport, which may result in the development of efficient pollutant delivery systems (Wu *et al*., 2017). Once MPs have accumulated, the next process it undergoes, is the fundamental process of degradation.

3.1.3. Breaking down/Degradation of MPs in the environment

The presence of Microplastic fragments within the environment is a very clear indication that the degradation of plastics being littered is a definitive source of MPs in industrial regions and ultimately in urban environments. (Townsend *et al*., 2019). Once MPs have accumulated within water or benthic sediments, they undergo degradation which is the process in which polymers are converted into smaller molecular units and have the possibility to become completely mineralized (Klein *et al*., 2018). Plastics are synthetic polymers which are very difficult to break down in the environment since they have very low degradation capabilities and long residence times especially within sediments (Klein *et al*., 2018). The degradation of these synthetic polymers in the environment are susceptible to five main processes, as seen in Figure 3.1 below, such as (i) Physical degradation by abrasive forces, heating, or cooling, freezing, or thawing and wetting or drying; (ii) Photo-degradation which occurs usually by UV light; (iii) Chemical degradation due to oxidation or hydrolysis and (iii) Biodegradation by organisms such as bacteria, fungi, or algae (Klein *et al*., 2018).

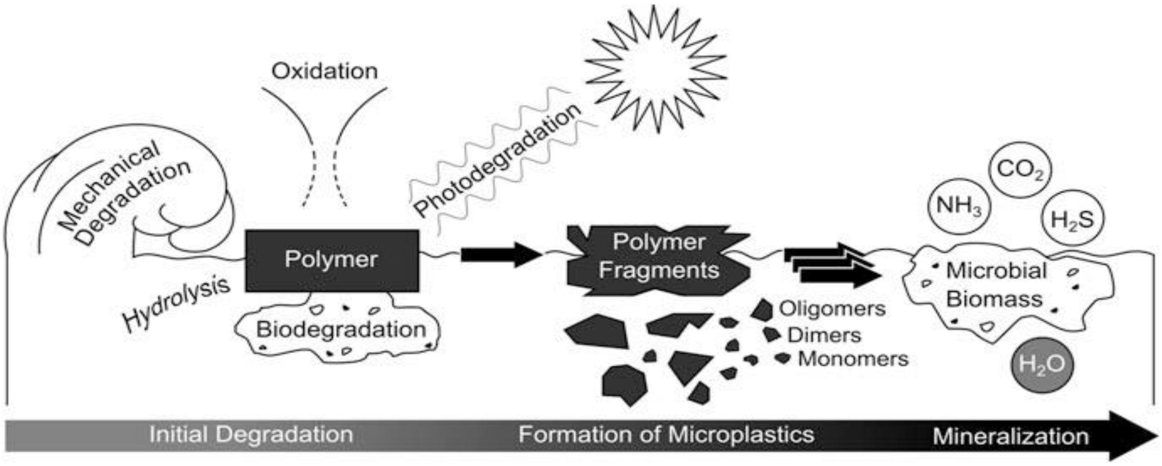

Figure 3.1: Degradation pathways of synthetic polymers in the aquatic environment with degradation processes involved and intermediate steps until complete mineralization (Klein *et al.*, 2018).

(i) Physical degradation

Physical degradation refers to synthetic polymers such as plastics, experiencing extensive exposure to various environmental factors that includes moisture (wetting or drying), freezing or thawing, heating, or cooling, or abrasive forces that ultimately causes polymers to be eroded thus becoming MPs. These factors however had different effects on different types of plastics, such as the rates of degradation on some synthetic polymers being lower in plastics found in marine environments, as opposed to those found in landfills (Chamas *et al*., 2020).

(ii) Photo-degradation

Photo-degradation of plastics occurs due to extended periods of synthetic polymers being exposed to sunlight which may ultimately result in UV radiation in sunlight causing the polymer matrix to oxidize and thus causing bonds within the polymers to break into polymer fragments as seen in Figure 3.1 (Andrady, 2011; Cole *et al*., 2011;). The process of photo-degradation will continue until bigger plastic fragments decrease in size over time, until they eventually become MPs (Cole *et al*., 2011). Materials that are buried and thus hindered from receiving any or limited amounts of light penetration, often experience reduced solar UV radiation, and affects the rate or effectiveness of photo-degradation on synthetic polymers. The rate of photo-degradation can also be hindered by biofouling which decreases the penetration of sunlight and may be time dependent due to many plastic debris that continuously sink and float. This is because after many biofouled plastic debris have sunk in various water bodies, it has the tendency to undergo defouling due to the absence of sunlight which causes the density of the water film to decrease and the plastics then to resurface (Chamas *et al*., 2020).

(iii) Chemical degradation

Chemical degradation occurs at outdoor temperatures within the environment which involves the process of either hydrolysis which requires the presence of water or oxidation which requires the presence of oxygen as seen in Figure 3.1. Both processes can also be accelerated by microbial activity, heat, light, or a mixture of these (Chamas *et al*., 2020). Hydrolysis results in the degradation of synthetic polymers within the environment on a molecular basis which then eventually leads to the synthetic polymers undergoing chemical oxidation. The predominant mechanisms. The effect of chemical oxidation on the synthetic polymers strongly depends on the type of polymer undergoing the reaction and once the reaction has occurred, the molecular weight of the polymer is decreased, thus leaving the end products susceptible to microbial degradation. The process of photo-oxidation usually occurs at a slower rate in aquatic ecosystems as opposed to more terrestrial areas, thus many plastics can stay within the aquatic environment for decades or even hundreds of years (Klein *et al*., 2018).

(iv) Biodegradation

Biodegradation of synthetic polymers can occur in a biotic or abiotic environment to which the extent of the plastics being degraded into H_2, CH_4, salts, minerals, and biomass may either be full or partial. In order for biodegradation to occur, micro-organisms such as bacteria, fungi or algae must be present in order to depolymerize the synthetic polymer and mineralize the monomeric compounds with enzymes of an appropriate metabolic pathway; the environmental parameters that encompasses temperature, pH, moisture, and salinity has to provide the necessary conditions for degradation to occur; and the polymers morphological traits has to attract micro-organisms as well as the formation of a biofilm, whilst the structure of the polymer must not hinder microbial activities (Klein *et al*., 2018).

Once the degradation process has concluded, the MPs may then reside, within the sediments or sediments upon which it has accumulated and degraded in, for a very long time, thus affecting the health and structure of the sediments or sediments. In this paper, however, sediments will take priority.

3.1.4. Effects of residual time of MPs in sediments on the ecosystem

Once synthetic polymers such as plastics enter the environment and are biodegraded into MPs which then enters the sediments, they are subjected to long residence times (Klein *et al*., 2018). There has however been attempts at developing "biodegradable" plastics such as Oxo-degradable plastics that can be industrialized and that most importantly might have a much shorter residence time in the environment. These types of plastics contain additives that accelerates the oxidation process, however, under natural conditions in the environment, the MPs produced by Oxo-degradable plastics, still take a long time to biodegrade completely and thus still have a long residence time that persists in threatening the health of the environment (Kubowicz and Booth *et al*., 2017).

Aquatic ecosystems consisting of freshwater such as lakes and reservoirs often act as sinks for contamination by MPs due their high residence times (Anderson *et al*., 2016; Nel *et al*., 2018). This is supported in Eerkes-Medrano *et al*, 2015 which states that MPs particles with a size of <5 mm, have succumbed to high densities (e.g., 100 000 items per m^3) in waters and sediments, and are interacting with organisms and the environment in various ways (Eerkes-Medrano *et al*., 2015). Environments that are lotic such as streams, rivers and estuaries experience a much lower residency time than still-water environments (Nel *et al*., 2018).

It has also been determined that Microplastic fibres from the washing of synthetic clothing that are abundant at sewage disposal sites have long residence times, which allows a large number of MPs to accumulate in freshwater ecosystems (Browne *et al*., 2011; Ryan and Reynolds., 2018). This results in the formation of not only threats to a biodiversity but also as a source for estuarine and marine ecosystems that receive water from ecological areas to become contaminated (Ryan and Reynolds., 2018).

Ultimately, the residence times for MPs in the environment could last from hundreds to thousands of years which in all likelihood is detrimental to aquatic ecosystems (Tibbetts *et al*., 2018).

3.2. MPs in sediments

With urbanization comes many households and industrial areas that will inevitably pollute the environment, and one can therefore presume that urbanization is strongly associated with Microplastic pollution (Townsend *et al*., 2019). As one can generally hypothesize, it was also both predicted and proven during a study done by Nel *et al* in 2018, that there was a temporal difference in sediment Microplastic densities in different seasons. During the winter seasons there was an increase in Microplastic abundance due to reduced rates of river flow (Nel *et al*., 2018).

Urban wetlands are ultimately the type of aquatic ecosystems that are most likely to be a source for Microplastic pollution especially towards downstream ecosystems. Without the necessary removal of day-to-day littering, macro-plastics present in wetlands are vulnerable to degradation and thus resulting in the formation of MPs. The accumulation of MPs within wetland sediments risk being suspended once again if not removed or treated and thus may be distributed during a period of disturbance such as the occurrence of large floods (Townsend *et al*., 2019). According to findings in Tibbetts *et al* (2018), the presence of a still water body such as a lake in particularly alongside a river will result in a big change in the abundance of MPs, which may be due to reduced velocities of flow upon entering the lake. This then allows for the deposition of finer sediments and for the potential for MPs (Tibbetts *et al*., 2018).

It is therefore of utmost importance that those in charge of maintaining wetlands or any other type of aquatic ecosystem should seek to ensure that it does not become a source of MPs. Some methods in order to do so includes Pollutant traps could be installed and consistently maintained within wetlands that are prone to high loads of litter, the protection of ecosystems located downstream and whom has high ecological values in order to minimize the formation of MPs from litter degradation (Townsend *et al*., 2019).

3.3. Global, national, and regional context of spatial distribution and abundance of MPs particles in bed sediment

MPs has vast become a concern that only continues to persist as a contaminant not only in aquatic ecosystems, but also in sediment ecosystems. Although there has been extensive research done on its occurrence in aquatic ecosystems, its occurrence in sediment ecosystems continue to be largely unexplored in a global, national, and regional context of the proposed study (He *et al*., 2018). The presence of MPs has become very well established amongst various water bodies and benthic sediments which ultimately have an impact on environments throughout the world. According to various articles, there is approximately close to 10 % of the annual plastic production that gets deposited into aquatic ecosystems, which adversely threatens the well-being of the ecology and its ability to function effectively (Avio *et al*., 2017; LeBreton *et al*., 2017). In order to further understand the presence of MPs, at least three case studies will be reviewed for each context, with regards to what has been outlined in the introduction.

3.3.1. Global context

There has been limited studies done on the abundance and distribution of MPs, especially in terms of their presence in the bed sediments of lakes. One study that has been conducted by Huffman *et al* (2017), had based their research on determining the abundance and distribution of plastics within Lake Macatawa sediments. Lake Macatawa is located in the USA and has served as the sampling site for the research that has been done. There were ten sampling sites that were chosen around the boundary of the lake. The sampling sites were based on locations related to stream influx as well as adjacent areas that is characterized by high human traffic (Huffman *et al*., 2017). The aim of their investigation was to determine whether there were plastics present in the sediments of Lake Macatawa. They also aimed to determine the nature of the abundance and distribution of MPs within the sediment of the Lake, and whether the Microplastic concentrations were related to land use activities, to hydrodynamic processes that occur within the lake or whether the distributions were a correlation of both (Huffman *et al*., 2017). The study found that there were indeed microplastics present within the bed sediments of Lake Macatawa.

MPs has become a significant and persistent threat towards the health of various ecosystems. It is thus imperative to understand the likelihood of the accumulation of MPs in areas within rivers that may be prone to Microplastic pollutants and can thus be considered to be hotspot areas (Tibbetts *et al.*, 2018). In order to provide an understanding of this, Tibbetts *et al* (2018) has conducted their research on the abundance, distribution, and drivers of Microplastic contamination in urban river environments. The study was conducted through a Microplastic survey done on a catchment that is heavily urbanized, as well as on the River Tame including four of its tributaries, which flows through the city of Birmingham, UK which also ultimately served as the researcher's study area. The study found that all sediment samples contained microplastics and that while urban areas generally have a greater abundance of microplastics as compared to rural, there is no simple relationship between the number of microplastics and population density or proximity to wastewater treatment sites. The occurrence and distribution of MPs within freshwater ecosystems continues to lack a descriptive comprehension as opposed to marine environments (Su L *et al.*, 2016). In order to provide information that will assist in creating a better understanding of the occurrence and distribution of MPs within freshwater ecosystems, Su L *et al* (2016) conducted a study on MPs in the Taihu Lake of China. They essentially investigated the pollution levels of MPs in the water, sediments, and an organism during the year of 2015 in Lake Taihu, which is also the third largest lake in China and is situated in one of the most urbanized areas in China (Su L *et al.*, 2016). The aim of their investigation was to determine the extent of the pollution of MPs within a lake located in an area with significant urban activities which resembles the aim of the proposed study especially in terms of the type of area the study area is located in. The study found that high levels of microplastics occurred not only in water but also in organisms in the Taihu Lake.

Although there has been a significant amount of research related to MPs done, there remains a somewhat lack of insight and information on the accumulation and distribution of MPs in bed sediment of lakes. This seems to be the case for not only global studies but also particularly for national and regional studies.

3.3.2. National Context

MPs has become an emerging threat to the health of ecosystems at a global scale and has become a threat that has been recently identified and studied on a minimal basis especially in developing countries such as South Africa. Although there are very few studies that has been done on MPs in freshwater ecosystems in South Africa, it is still very much being actively pursued. In South Africa, plastics are a crucial aspect within the manufacturing industry, and it can thus be assumed that the natural environment suffers as a consequence to this (Verster *et al.*, 2017). Although information on the pollution of MPs within South Africa is limited with very few research to vouch for its occurrence and distribution, it remains essential to improve the understanding of Microplastic pollution in South Africa. It is important especially since South Africa is ranked within the top 20 countries in the world with a high mass of mismanaged plastic debris (Nel and Froneman., 2015).

MPs within the freshwater ecosystems of South Africa lack thorough understanding as majority of research are focused on its pollution in marine environments. Many studies have focused on quantifying MPs in different areas situated along the coastline of South Africa (Verster C *et al*., 2017). Majority of these studies shows a high concentration in the density levels of MPs around areas with multiple activities occurring at the coastlines such as in major coastal metropolitan areas located in Cape Town and Durban (Nel and Froneman 2015). This ultimately suggests that majority of the studies have been and continue to be conducted within those areas of South Africa. Some of these studies include a study done by Naidoo *et al* (2015) along the KwaZulu-Natal coastline in which the aim of the study was to determine the concentration values of MPs in the sediment of the Durban harbour. Another study would be the investigation performed by Nel and Froneman (2015) in which the aim of their investigation was to determine whether bays were possible Microplastic sinks along the south-eastern coastline.

3.3.3. Regional context

On a global and national scale, there has been minimal but significant research done on MPs as a pollutant, especially in marine environments. In a regional context, research done will be focused on previous literature on MPs within Cape Town, as it is ultimately the region in which the proposed study area is located. In a regional context however, there has been insufficient research done on the occurrence and distribution on MPs within Cape Town.

The only piece of literature that been encountered thus far is that of Lamprecht (2013), which focuses on the abundance, distribution, and accumulation of plastic debris in Table Bay, Cape Town, South Africa. This study consisted of two aims. The first aim of the study was to assess how the abundance, composition and accumulation rates of marine debris have changed on Milnerton and Koeberg beaches since 1994. The second aim was to sample meso- and Microplastic debris to establish a method and improve the understanding of its vertical distribution, abundance, and composition. The study ultimately took place across two beaches in Table Bay.

Based on the lack of findings on a regional scale, it is obvious that more studies based on MPs have to be conducted especially within Cape Town. The study mentioned above took place in 2013 which also suggests that more awareness on the threats of MPs should be raised.

The lack of studies in MPs not only in freshwater ecosystems but also coastally in the City of Cape Town, requires serious attention before it becomes an even more concerning environmental issue.

4. Methods

This chapter will provide an in-depth description of the various methods used to evaluate the spatial distribution and abundance of MPs particles in the bed sediment of Zeekoevlei Lake. These methods include geospatial analysis, fieldwork including a boat lakebed sediment sampling strategy, and laboratory analysis using a density separation method followed by microscopy. Fieldwork encompassed the determination of physical parameters such as the collection of sediment samples, whilst laboratory analysis determined the number of MPs per sample. The density separation method is used in a laboratory to extract MPs from sediments, by using the principle of the difference in specific density of plastics and sediments.

4.1.Research Approach

This study required a quantitative research design in order to achieve its aim and objectives in an effective manner. A quantitative research design had been identified as the desired design, based on the required data sets which were collected for analysis. These data sets were collected in the field and then measured after treatment, such as the Microplastics that were separated from the sediment samples through density separation. This is ultimately referred to as descriptive research, which is a type of quantitative research design, which has been used to provide information on the accumulation and abundance of Microplastics in bed sediments, in a systematic manner.

Sampling took place at regular intervals, starting at the main points of entry and moving around the lake margin in an anti-clockwise direction, with approximately 10 points having been sampled within the lake. The goal of this approach was to sample in a way that was relatively even and would cover the lakebed within the amount of time spent on sampling (1 Day). This type of sampling is referred to as a stratified random approach in which there were relatively regular spacing of samples around the margin and across the area of the lake, but with a random selection of sampling points in each general site. Duplicate samples were taken at each sampling site; one sample from each side of the boat to avoid re-sampling the same area of the lakebed twice. Approximately 15 sampling sites were sampled along the lake margin whilst 5 were sampled further away from the lake margin. Only 20 sites were sampled with approximately 40 samples being collected and analyzed, due to time constraints. The co-ordinates of each sampling point was collected and recorded using a Garmin eTrex GPS with 3 to 5 m accuracy.

A boat lakebed sediment sampling strategy was devised to balance spatial coverage and sampling efficiency. This was done by taking bed sediment samples within the lake with the aid of a boat.

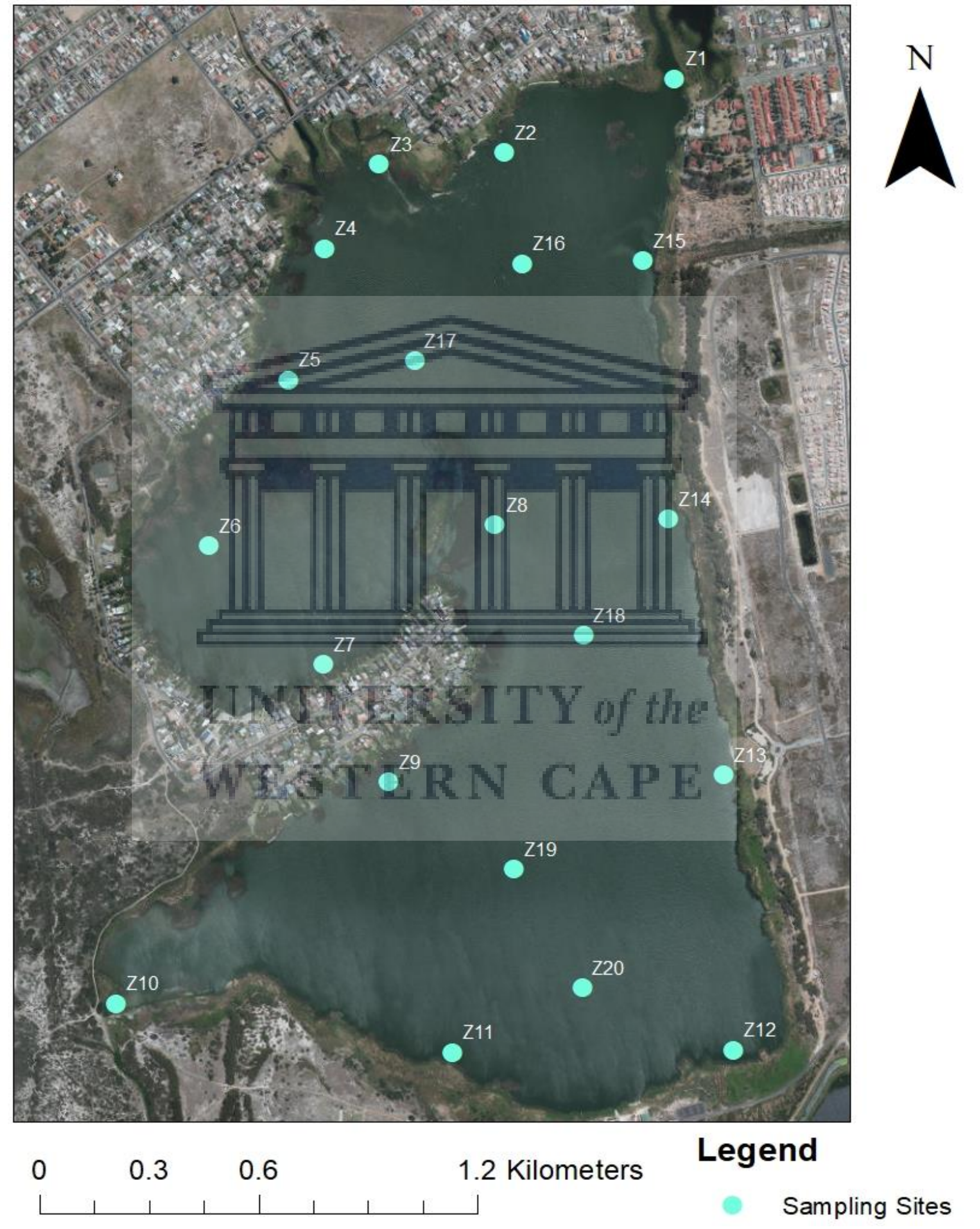

Figure 4.1: A map generated in ArcGIS of the location of sampling sites with regular intervals, recorded with a GPS on a boat in the Zeekoevlei Lake, Cape Town

4.2. Data Collection

4.2.1. Parameters collected

This study did not determine any physical, chemical, or biological parameters, due to a limited amount of time that was allocated for the completion of the study, however acknowledges the impact that these parameters may have on the distribution and abundance within the bed sediment of the Zeekoevlei Lake. The study therefore only focussed on determining the Microplastic concentration of the samples that was collected during fieldwork and processed during laboratory analysis. A fixed amount of sediment was collected (at least 10g of sediment mass) at each sampling location. Once the sediment samples were collected and stored in glass jars that were first cleaned with deionized water in order to prevent contamination, they were transported to the laboratory, the number of Microplastics from each sample was them counted during microscopic analysis.

4.2.2. Data type collected

The type of data that was measured in this paper was the concentration of Microplastics in the lakebed sediment, which can also be referred to as quantitative data. The concentration of Microplastics in the lakebed sediment was expected to provide valuable insight on characterization of the spatial distribution of Microplastics across the lakebed environment, in relation to possible point and non-point sources of contamination which essentially addresses the first objective of the study.

The recorded data was then used for geospatial mapping in order to illustrate the configuration of points of inflow from rivers and storm-water outlets, as well as any potential macro-plastics pollution sources (e.g., picnic sites, walkways). The main types of quantitative data that was collected in this study was therefore the sediment samples as well as the concentration of Microplastics residing within the sediments that was extracted via density separation and the data sets for geospatial mapping.

4.2.3. Data sources

The sediment samples were collected onsite and stored in glass jars at the Zeekoevlei Lake and then transported to the laboratory at the University of the Western Cape, where the Microplastics residing in it was extracted and recorded. These recorded data sets were then compared to additional data sets obtained from the results of studies that had already been performed in order to better understand the distribution and accumulation of MPs. These studies were accessed from various search engines (for e.g., Google Scholars, Elserivier etc). The data sets for geospatial mapping in relation to stormwater outlets and other potential MPs sources, and field sampling, were obtained from the City of Cape Town and was generated via ArcMap 10.8 which was provided by ESRI. The stormwater spatial data were provided by the City of Cape Town with a base image that was a 0.5 m resolution Worldview 2 image.

4.2.4. Instruments used for data collection

A GPS was used to collect the co-ordinates for each sampling site. These co-ordinates were then used in ArcMaps 10.8 to provide a visual representation of the locations of the sites in the lake. Sediment samples were collected using a Lamotte grab sampler with a capacity of 1.097. The grab sampler was of stainless steel in order to avoid contamination. The sediments that were being sampled was grabbed from the bed of the lake with the grab sampler and placed into an aluminium sampling trap that was rinsed before transferring the next batch of sediment into it. The sediment was then transferred from the trap and into clean glass jars that were labelled with the respective sampling sites. A book and pen were used to record the place (such as inflow or non-inflow region) and the exact GPS location including any other visible information. In the laboratory, the extracted Microplastics was then viewed underneath a microscope at a suitable magnification for each sample after processing. The number of Microplastics counted under the microscope was then recorded in a data log sheet.

4.3. Fieldwork and Laboratory analysis

Once the delineation of the sampling points had been completed, fieldwork took place. Upon arrival at the lake, the boat was setup along with ensuring that fuel and sampling equipment had be loaded and checked before departure. Sampling began at the mouth of the big Lotus River. This project focuses strictly on the spatial distribution and abundance of MPs in lakebed sediment and therefore, samples were comprised of a collection of sediment samples only. These samples were collected by using a grab sampler. Upon arrival at each sampling point, an anchor was lowered to keep the boat stationary. Two samples were taken, one on either side of the boat. The grab sampler was lowered into the water whilst attached to a rope, once the grab sampler reached the bed sediment, a weight was sent down to cause the grab sampler to essentially grab sediment. At least 5 cm of sediment from the top of the lake boundary and lakebed was captured by the grab sampler and pulled up with the rope. The grab sampler was opened onto a tray to release the captured sediment. The sediment was then scooped into a rinsed sampling jar and stored for sampling. The sampling jars were labelled with the site I'D. The co-ordinates of each site were recorded using a GPS as well as any observations. All 40 samples were collected within one day of sampling. These samples were then transported to the University of the Western Cape and stored for further processing.

4.4. Quality assurance and Quality control during sampling

Quality assurance (QA) and Quality control (QC) measures were adopted to prevent potential background contamination from occurring during sampling and the processing of samples in the laboratory (Jiang *et al.*, 2019). Since there is a high potential of a sampler, the air or sample equipment to contaminate the sample, especially in the Microplastics field, a very high level of QA/QC was required for laboratory or microscopy analysis. In order to reduce contamination, certain QA/QC steps were followed, such as limiting sampling equipment made of plastic due to an increase in the potential for the equipment to contaminate samples over time (for e.g., material that the sampling container is made of) (Barrows., 2017). Cotton lab coats and nitrile gloves were worn at all times during all of the processes that occur in the field and the laboratory (Coppock *et al.*, 2017, Jiang *et al.*, 2019). Other steps that were taken included triple rinsing all sampling equipment as well as taking a control sample of water used for rinsing for laboratory analysis. Procedural blanks were also used throughout field sampling and sample processing as a contamination control in order to reduce or prevent equipment contamination (Coppock *et al.*, 2017). In total, ten procedural blanks were taken (one procedural blank was taken after the processing of every 5 samples). The blanks were treated with $ZnCl_2$ solution of at least 400 ml through the vacuum filter and analyzed under the zircon microscope for possible handling errors and airborne contamination (Horton *et al.*, 2017). Microscopes were also cleaned before and after usage, especially when filtered samples were processed. Samples were exposed for minimal periods of time in order to further reduce the possibility of contamination occurring (Coppock *et al.*, 2017).

4.5. Preparation of samples

Once samples along the lake-boundary and away from the lake boundary were collected, they were prepared for processing. This was done before the samples underwent extraction through visual inspection, flotation through density separation and ultimately extraction.

4.5.1. Drying

The drying of the collected samples was based on a method outlined in Masura *et al*. (2015). A clean and dry 45 mL jar was first weighed in grams and the weight recorded. Once the jars were filled with the sediment sample during field work, they were transferred to the laboratory at the University and dried in a drying oven at 60°C overnight until the samples were completely dried. This temperature was chosen due to it being below the melting point of all common polymers and would not be expected to cause any change in the shape of particles for the analysis (Horton *et al*., 2017). Once the samples were dried, the dried beaker and material were weighed to determine the dry sample weight. The mass of the dry beaker measured at the start was then subtracted from the dry sample weight to determine the mass of total solids. The result is referred to as the mass of the sample matrix.

Once this was completed and the samples were prepared, the samples were covered to prevent any airborne contamination and then stored for sorting, through an extraction method protocol, and analysis.

4.6. Processing of Samples

The processing of the dried sediment samples followed an outline of methods similar Horton *et al*. (2017) and Coppock *et al*. (2017). There will be 4 parts which will be used to find and separate Microplastic particles from sediments. The first step will involve the makeup of the flotation media; the second step will involve the Separating Funnel method for separation and the third step will involve the cleaning, purging, and priming of the separating funnel (Coppock *et al*., 2017, Horton *et al*., 2017, Nguyen *et al*., 2018).

- **Step 1: Makeup of Flotation media**

To separate plastics from sediment that are generally denser, flotation is used (Nguyen *et al*., 2019). Flotation is thus basically a process which results in materials floating in a liquid substance. To achieve flotation, a density separation method was implemented through a flotation media. Density separations for plastics (MPs in particular) most commonly makes use of salt solutions in order to cause the plastics to become buoyant (Nguyen *et al*., 2019). To achieve this, a flotation media was first developed. This was done by preparing a zinc chloride ($ZnCl_2$) solution with a recommended density of 1.5 g/cm³ in order to allow fine grained sediments to settle down and thus enabling the denser plastics or MPs of interest to float through the process of density separation (Coppock *et al*., 2017). The solution was then first filtered through a 10 mm nucleopore membrane (i.e., filter paper) in order to ensure the removal of any contaminants before it is used for the extraction process. The solution was ultimately filtered through filter paper and stored in a tall beaker before usage.

- **Step 2: Separating Funnel method**

To extract MPs from various sediment types in a rapid, simple, and efficient manner, a separating funnel method was used. There is very limited prior research that were performed on the extraction of MPs using this method. The separating funnel can be cleaned rapidly to ensure that cross-contamination does not occur. There were approximately 8 steps that were developed for this method:

- **Step 1:** 10g of sediment was weighed in a 200ml beaker. The mass was then recorded once the sample was weighed.

- **Step 2:** The 10g sample within the beaker was then topped up to 250ml in the beaker with the prepared Zinc Chloride ($ZnCl_2$) solution.

- **Step 3:** The solution of $ZnCl_2$ and sediment was then slowly and carefully poured into the separating funnel in order to avoid any spilling. Once the entire solution was transferred, the bottom and sides of the beaker was rinsed out with more $ZnCl_2$ to ensure that all the sample has been transferred over into the separating funnel. It was important to ensure that the tap of the separating funnel was closed tightly to prevent any leaking. Once all of the sample had been transferred into the funnel, the funnel was closed and turned onto its side and rotated slowly for approximately 10 seconds. This was done to allow sample to mix with the $ZnCl_2$ and to become suspended. Once this was completed, the funnel was put back onto the stand. The solution was left to settle for at least 30 minutes in order to allow separation to occur.

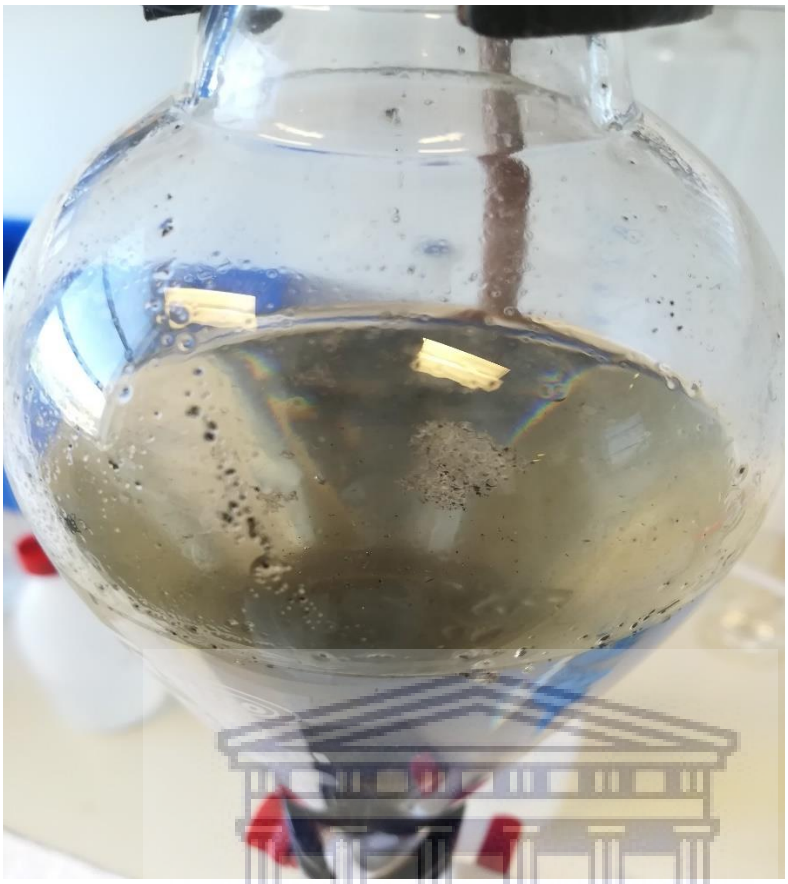

Figure 4.2: Transferred mixture in separating funnel with particles floating at surface (density separation occurring effectively). Particles on the side of the funnel was washed down with $ZnCl_2$.

- **Step 4:** Once the solution had settled and separation had occurred, the tap of the funnel was opened in order to remove the sediment that had settled at the bottom of the funnel. The sediment was removed and stored in a separate beaker. Once all of the sediment had been drained, the tap was quickly closed, with only the $ZnCl_2$ solution with the MPs floating on the surface left behind. The beaker containing the sediment was then rinsed with $ZnCl_2$ and filter through filtering paper.

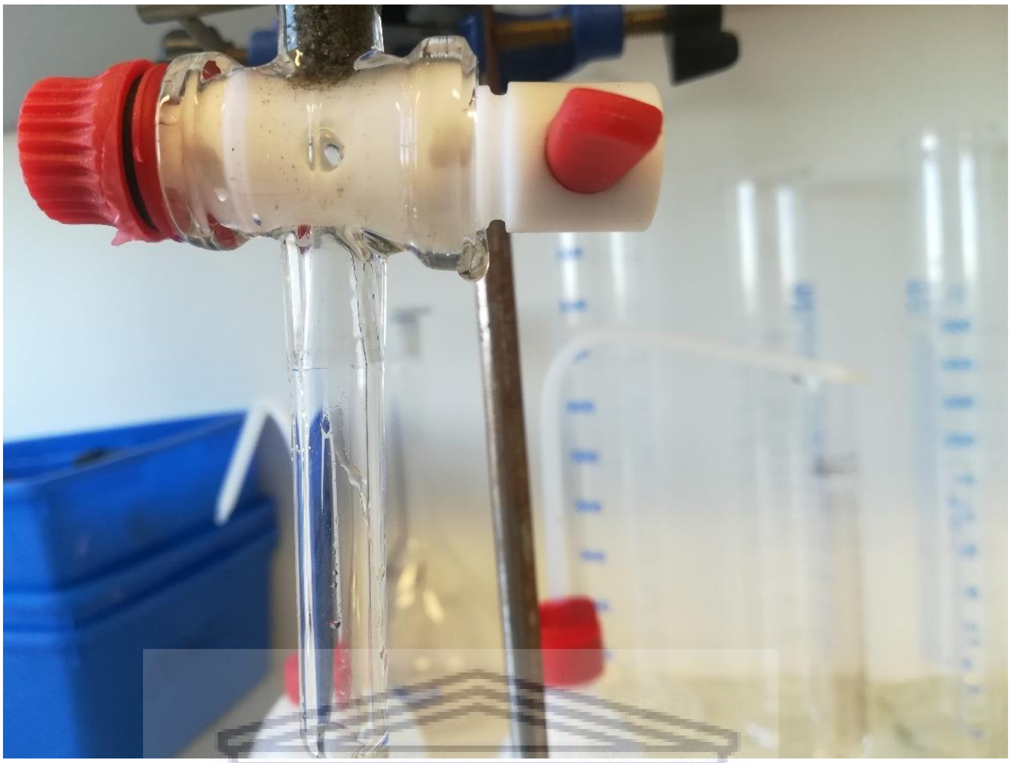

Figure 4.3: Tap of separating funnel which was opened to remove settled sediment at the bottom of the funnel and closed to leave behind the $ZnCl_2$ solution with separated particles floating at the surface.

- **Step 5:** The filter paper containing the sediment was then transferred to a silver container for particle size analysis.

- **Step 6:** The $ZnCl_2$ with the floating MPs was then drained into a Millipore Filtration System (MFS). Prior to this, white filter paper was first placed onto the MFS to capture the floating particles whilst allowing the $ZnCl_2$ to be collected. Once transferred to the MFS, the solution was filtered using a motor.

- **Step 7:** Once the particles were collected on the filter paper, it was removed and stored in a closed storage bottle to avoid contamination whilst awaiting microscope analysis.

- **Step 8:** The drained $ZnCl_2$ that had been collected during the MFS method, was then filtered into the flask containing the rest of the $ZnCl_2$ solution for further usage.

The flasks containing $ZnCl_2$ solutions were only rinsed with $ZnCl_2$ in order to prevent any contamination of the $ZnCl_2$ solution that may impede its effectivity. When $ZnCl_2$ solutions are transferred into the flask containing the rest of the solution, it was done by filtering it through filtering paper. All other equipment such as the separating funnel and MFS components were rinsed using Deionized water and left to dry before re-usage to avoid contamination.

- **Step 4: Cleaning, purging, and priming of separating funnel.**

Once the separating funnel components were obtained, they underwent cleaning, purging, and priming like that of Coppock *et al*. (2017), in order to further ensure that the impacts of contamination on the quality of the results that will be obtained.

- **Cleaning**

  Once the separating funnel and MFS had been assembled, it was cleaned thoroughly with distilled water in order to remove any contaminants that may have accumulated during its assemblage and then prepared for extraction.

- **Purging and priming**

  After cleaning has taken place, purging and priming took place before each sediment sample underwent extraction for MPs. During these processes, at least 250 mL of $ZnCl_2$ which also amounts to 1.5 g/cm^3 of solution, was poured into the separating funnel until the half of it had been filled. Priming then took place, in which the tap of the separating funnel was open and closed continuously. This was done in order to ensure that the internal pathway of the funnel had been filled in order to make sure that there was no hindrance that may occur once sample processing took place when the tap was used. The solution was then refilled above the tap of the funnel, in which case the funnel was left for a set period (i.e., 5 minutes as advised in Coppock *et al*) in order for contaminants that may have originated externally, to float to the surface in a process called flotation. Once the set period had elapsed, the tap was openly positioned. The $ZnCl_2$ solution was then filtered through filter paper and into a clean flask so that it can be used once more. In order to ensure that all internal sides of the separating funnel were free of any sorts of contamination, the unit was rotated.

- **Step 5: Density separation and Extraction of MPs from sediments through a Millipore Filtration System (MFS)**
  Once cleaning, purging, and priming of the separating funnel had taken place, Density separation along with the extraction of MPs from sediment samples through a Millipore filtration system occurred. This was done by assembling the MFS and transferring the solution with the floating particles from the separating funnel into the MFS which had filter paper added to it in order to capture the particles and allow the $ZnCl_2$ to pass through it. The Millipore Filtration system played a crucial role in the recording of Microplastic (MP) particles. The Millipore Filtration system was used in order to have the MPs on filter paper for analysis. Although leaving the mixture of sediment and zinc chloride to settle for 30 minutes before having it subjected to Millipore Filtration, some organic materials were still present and observed after density separation had occurred. The method ultimately proved to be successful as many Microplastic particles on the filter paper were identified and counted when viewed underneath the microscope.

Figure 4.4: Millipore Filtration System (MFS) component

4.7. Density separation

Density separation is the process in which less dense material (i.e., MPs) floats and separates from material that sinks and are denser such as sediments, in a salt solution (Nguyen *et al.*, 2019, Quinn *et al.*, 2016). In this project a $ZnCl_2$ solution was used as a separating liquid to extract MPs from sediment samples. The solution $ZnCl_2$ was used instead of a NaCl solution due to the latter being considered as less dense than some Microplastic particles (Tibbetts *et al.*, 2018). This had been supported by Gimiliani *et al*, in which a NaCl solution was used as a separating liquid for the extraction of MPs in which multiple plastic particles were lost within the phase of separation, thus resulting in an underestimation in the quantity of MPs (Gimiliani *et al.*, 2020). The $ZnCl_2$ solution, was therefore the most reliable separating solution for density separation, as it is effective at extracting high-density MPs from sediment samples, and since higher density particles generally have a lower density than the $ZnCl_2$ solution (Gimiliani *et al.*, 2020, Tibbetts *et al.*, 2018).

4.7.1. Treatment of organic rich samples

During the density separation in the separating funnel, some samples resulted in clogging the tap whilst draining. These samples were then treated with Hydrogen Peroxide along with samples that were collected and remained organically rich. These samples were added to a 500 ml beaker and filled with Hydrogen Peroxide to about the 100 ml mark of the beaker. The solution was left to react underneath a fume hood overnight and collected the following day. Once this had occurred, and the organic material had been destroyed, the samples successfully underwent density separation and was successfully processed through the MFS.

4.8. Microscopic Analysis

Once particles were separated from the sediment and isolated onto the filter paper during the Millipore filtration step, they were stored in sealed containers and then analyzed using a microscope. A zircon microscope with a magnification of 40x was used to view samples of 5mm and less, as well as to count the particles that were certain to be MPs based on a predetermined list of requirements that would qualify particles as MPs. In order for particles to qualify as MPs, they had to be unnaturally colored compared to the majority of other particles in the sample (e.g., bright blue, yellow etc.) and appeared to be a homogenous material or texture, they should also have had an unnatural shape (e.g. perfectly spherical) and an unnaturally bright color coating. They should also have been shiny/glassy, and flexible or able to have been compressed without being brittle (Horton *et al.*, 2017).

Counting of particles took at least two weeks for the full 40-sample set in order to ensure an accurate counting of each sample. The number of particles counted was then recorded for further analysis and classified based on whether they were a bead, Fibre, or fragment. A component of fine-grained quartz sand contamination was noted during counting, due to this material adhering to the side of the separating funnel outlet stem. These particles were excluded from the plastics count due to their irregular shape. The magnitude of quartz contamination of each sample extract was assessed using a polarizing microscope.

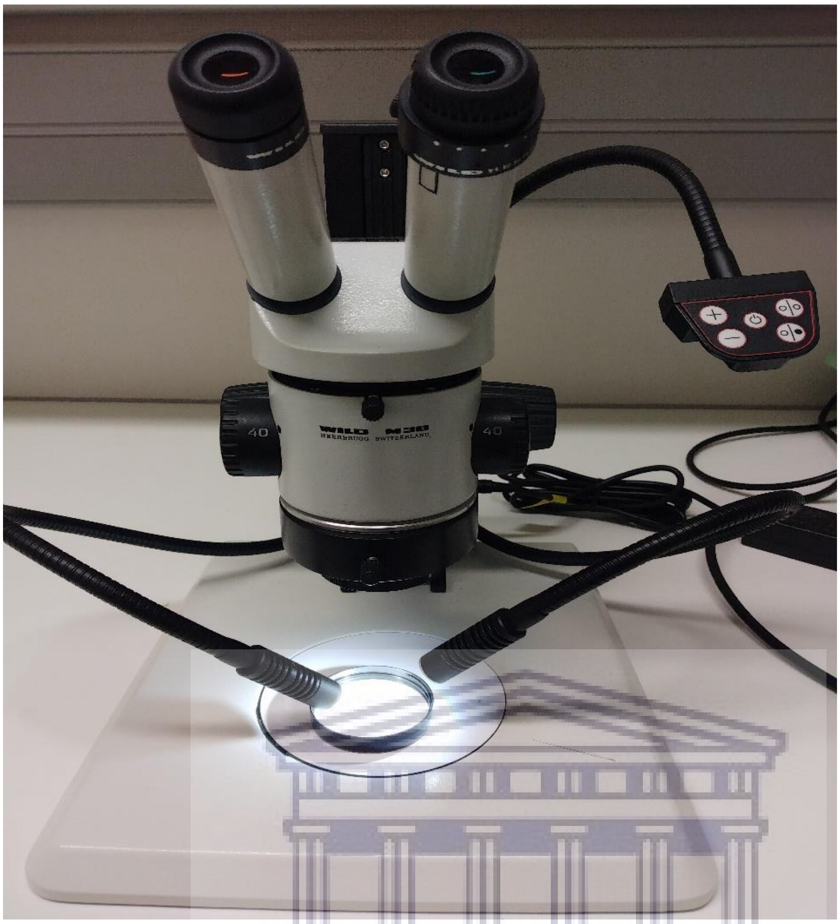

Figure 4.5: Zircon Microscope used for counting of MP particles

5. Results

The following chapter describes the spatial distribution and accumulation of MPs as it pertains to the results obtained from the bed sediment samples collected in the Zeekoevlei Lake. This chapter seeks to satisfy the aim of the project as well as the objective of the study which is to characterize the spatial distribution of MPs across the lakebed environment, in relation to possible point and non-point sources of contamination.

5.1. Microscopic Analysis results

A large number of MPs were identified, especially micro-fibres and micro-beads. The ten control filters that were analyzed to assess possible contamination during extraction, contained no MPs thus suggesting that no contamination took place. Concentrations of total MPs (fibres plus beads) in samples analyzed varied from 1483 total counted MPs to 14795 total MPs/100 g. Concentrations of beads varied from 475 counted beads to 4732 beads /100 g, and concentrations of fibres varied from 1008 counted fibres to 10063 fibres /100 g. In addition, some organic matter along with unidentifiable organic material were observed during counting. Every sample contained microfibres and beads of varying colors and sizes. MPs were identified under 40X magnification in order to ensure a level of confidence in identification. Numerous micro-fragments were identified; however, many could easily be mistaken for quartz as they possessed similar features in terms of shape, color, and size. Some fragments weren’t unnaturally colored or unnaturally bright, however, they were unnaturally shaped and not brittle. Some microbeads also appeared to be organic and when poked with a charge needle, they burst open to release fluid. All other particles possessed the requirements mentioned in the methods section. The only way to clearly distinguish between the two would be identifying them underneath a polarizing microscope, in which quartz possessed interference colors and MPs did not.

5.2. Microplastic distribution and accumulation analysis

A large number of MPs was counted in all samples. As seen in Table 5.1, there are several MP Fibres and beads that were identified. The distribution and accumulation of the mean concentrations/100g (of the two samples taken at each sampling site) in relation to their distance from the various sources is represented in terms of graphs and maps in order to provide visual analysis. The mean concentration was determined through the calculation involving the mass (g) of each sample and the total number of MPs counted as seen in Table

5.1 below. Sample Z15-1 has the highest Total MP (n/100g) and is situated quite close to the big Lotus River, whilst sample Z4-2 has the least despite having been sampled for close to the small Lotus River and close to other nearby inlet points.

Table 5.1: Sediment mass (g) per sample taken during sampling with the total number of beads and Fibres along with the number of MPs per 100g for beads, Fibres, and total MPs.

Sample	Beads n/100g	Fibres n/100g	Total MP n/100g
Z1-1	100	309	408
Z1-2	310	430	740
Z2-1	10	219	229
Z2-2	0	150	150
Z3-1	40	110	150
Z3-2	0	199	199
Z4-1	30	100	130
Z4-2	0	30	30
Z5-1	100	190	290
Z5-2	0	230	230
Z6-1	0	220	220
Z6-2	10	109	119
Z7-1	290	379	668
Z7-2	149	80	229
Z8-1	70	368	437
Z8-2	149	239	388
Z9-1	99	527	627
Z9-2	80	289	368
Z10-1	110	229	339
Z10-2	30	139	169
Z11-1	349	269	618
Z11-2	199	249	447
Z12-1	99	199	298

Z12-2	208	346	554
Z13-1	119	229	348
Z13-2	159	568	727
Z14-1	198	536	734
Z14-2	149	219	368
Z15-1	50	387	436
Z15-2	140	609	748
Z16-1	79	169	248
Z16-2	40	189	229
Z17-1	40	369	409
Z17-2	50	149	199
Z18-1	30	249	279
Z18-2	79	218	298
Z19-1	276	237	513
Z19-2	351	185	537
Z20-1	340	58	398
Z20-2	200	86	286

5.2.1. Micro-Fibres

The spatial distribution and accumulation of MP Fibres appear to have no clear pattern as seen in Figure 5.1 below. As seen in Figure 5.1, there are a higher MPs concentration along the east margin of the lake, which is located closer to the big Lotus River which is believed to be the main source of contamination due to its origin and flow path. There are, however, much lower concentrations in the northwest part of the Lake, which is located close to the small Lotus River which appear not to be as contaminated as the big Lotus River based on observation. Sample Z13 has a large concentration of Fibres as well and can be seen to be situated close to a stormwater conduit in Figure 5.1.

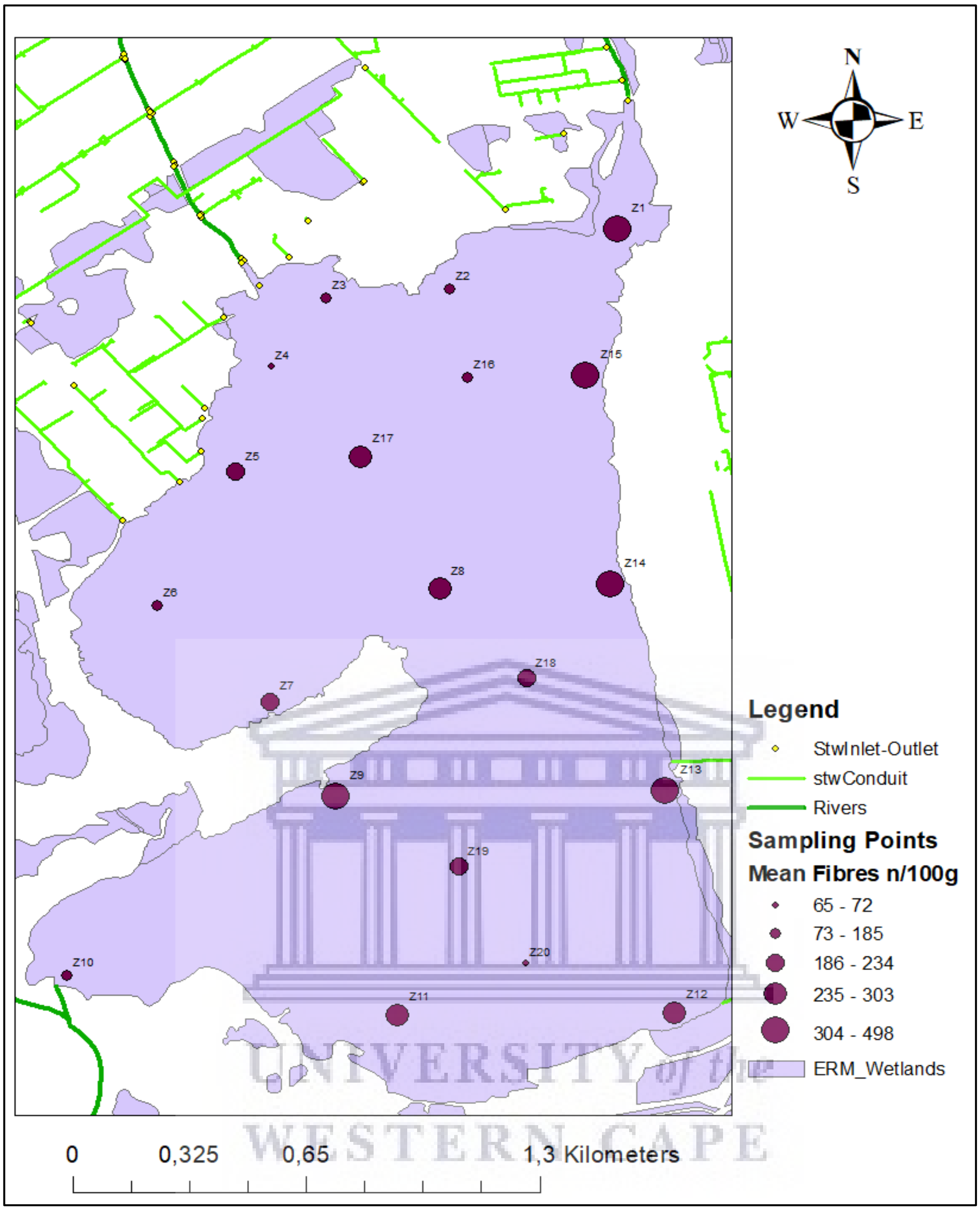

Figure 5.1: Spatial distribution and accumulation of total MP Fibres within the lake-bed sediment of the Zeekoevlei Lake for the 40 samples collected.

5.2.2. Micro-beads

The spatial distribution and accumulation of MP beads appear to have no clear pattern as seen in Figure 5.2 below. This is a very similar representation as seen in Figure 5.1. There are higher concentrations of Microplastic beads towards the south margin of the Lake as well as along the east margin of the Lake. There is a clear increase in MP concentrations eastwards. The concentration of MPs is much less towards the west margin of the Lake, especially near to the Small Lotus River.

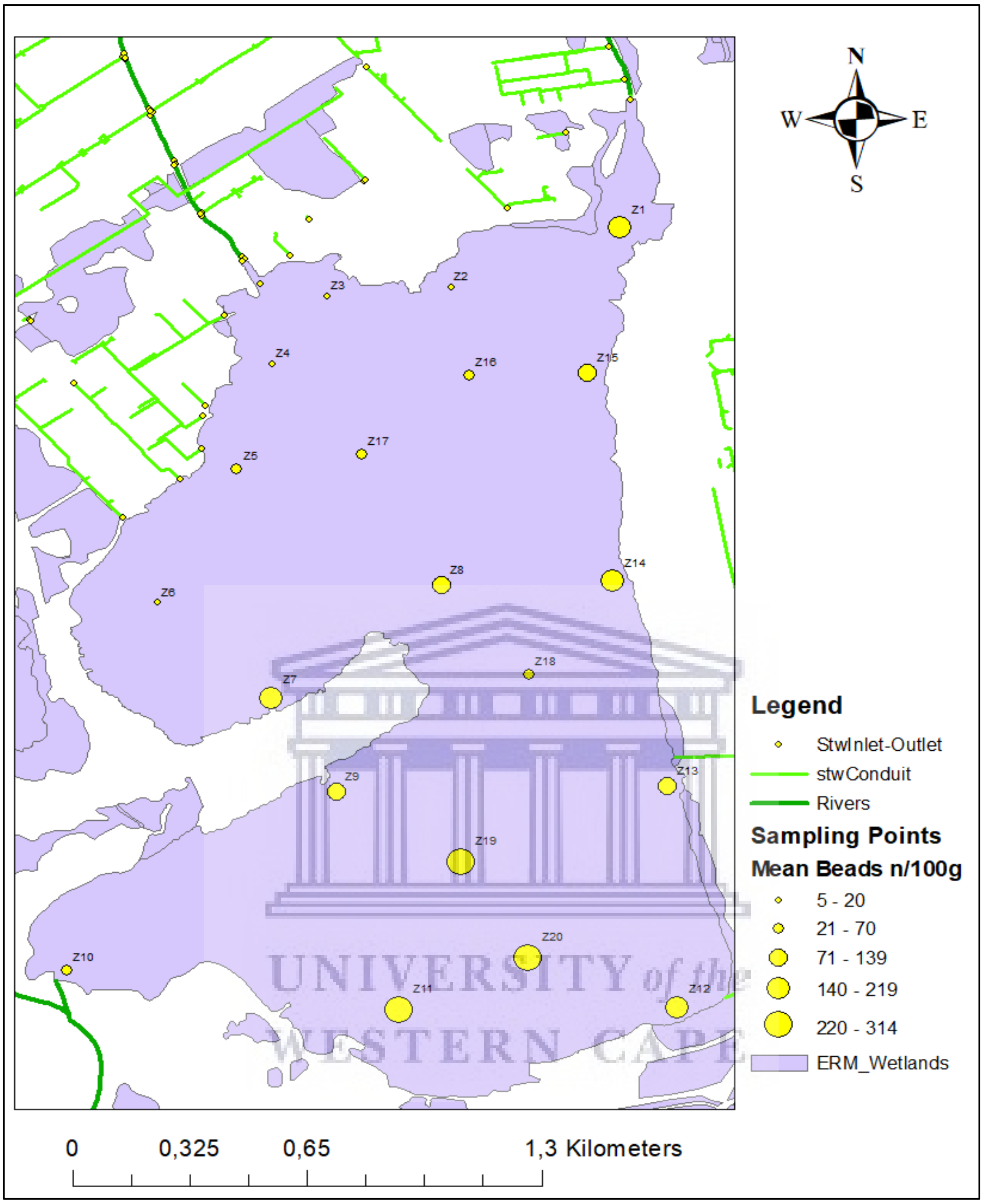

Figure 5.2: Spatial distribution and accumulation of total MP beads within the lakebed sediment of the Zeekoevlei Lake.

5.2.3. Total Micro-beads and Micro-Fibres

As seen in Figure 5.3, there are clearly more Fibres detected than beads apart from a few areas containing more beads. There are most notably more beads than Fibres in the Northern section of the lake which is also situated closest to the big Lotus River. In the southern region of the lake there is more beads than Fibres with distance from the sources.

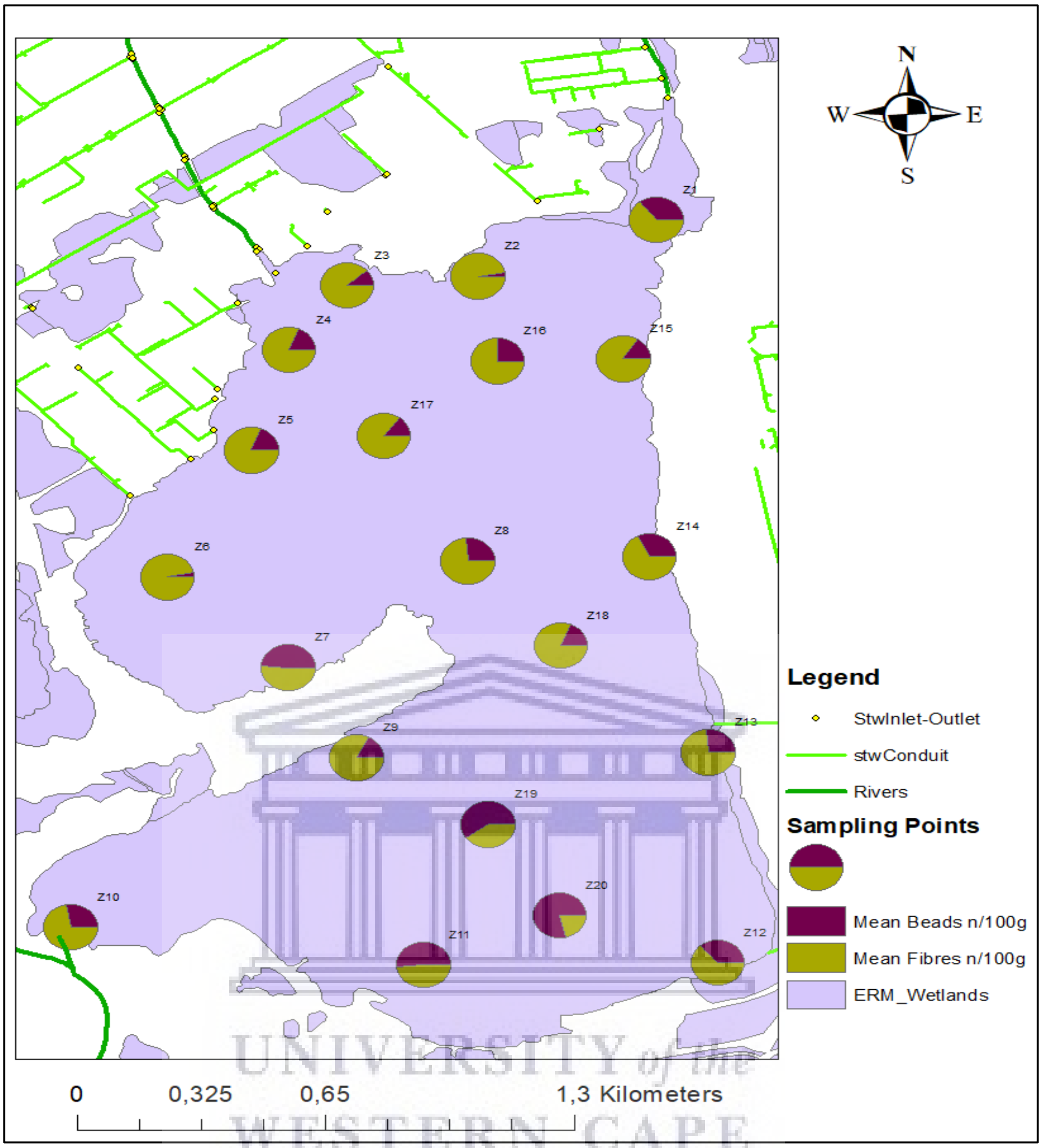

Figure 5.3: Spatial distribution and accumulation of total MPs within the lakebed sediment of the Zeekoevlei Lake also showing sources of MPs through stormwater inlet and outlet points as represented by the yellow dots

5.3. Spatial distribution of MPs in relation to potential sources

A form exploratory data analysis was used to investigate the relationships between MPs concentrations and factors potentially controlling their spatial distribution. As seen in Table 5.2 sample Z15 possesses the highest mean concentration of particles/100g whilst sample Z4 possesses the lowest. Sample Z15 is situated quite close to the Lotus River whilst sample Z6 is situated closer to the small Lotus River and nearby inlets. The correlation between micro-beads and micro-Fibres are R= 0.15, which suggests that although this is a positive correlation, the relationship between the two variables is weak, as the value is closer to 0 than to one. This ultimately means that there is no clear correlation between the two in terms of their distribution within the bed sediment of the lake and that their distribution may be due to other environmental factors.

Table 5.2: Mean beads, Fibres, and total MPs per 100g for each sampling site

Site	Mean Beads n/100g	Mean Fibres n/100g	Mean Total MPp n/100g
Z1	205	369	574
Z2	5	185	190
Z3	20	154	174
Z	15	65	80
Z5	50	210	260
Z6	5	164	169
Z7	219	229	449
Z8	109	303	413
Z9	90	408	498
Z10	70	184	254
Z11	274	259	533
Z12	154	273	426
Z13	139	398	538
Z14	174	377	551
Z15	95	498	592
Z16	60	179	238
Z17	45	259	304
Z18	55	234	288
Z19	314	211	525
Z20	270	72	342

The distribution and accumulation of MPs throughout the Lake can thus also be represented through distance. This is done by determining the distance of the samples to the Lotus River and their nearby sources, as seen in the following section. The distance is then plotted onto a graph in excel along with the mean total MP, beads, and Fibres per 100g.

5.4.Exploratory analysis for total MPs per 100g with distance from source

In order to further analyze the distribution and accumulation of MPs in the Zeekoevlei Lake, the mean concentration of MP particles/100g as shown in 5.4, is represented as distance from the Lotus River and their nearby sources, respectively. This will be represented as mean beads, Fibres, and the total of these per 100g of sample with distance. The Figures below were generated using Excel with the use of the data analysis tool. The data analysis tool was used in order to determine the descriptive statistics for the results as well as the Pearson's correlation coefficient between the MPs as well as distance from sources.

Table 5.3: Co-efficient of determination for the relationship between mean MPs (n/100g) and distance from the Lotus River (m).

Relationship	**R^2**
Distance from Lotus (m) versus Mean Total MPs (n/100g)	0.038
Distance from Lotus (m) versus Mean Bead MPs (n/100g)	0.18
Distance from Lotus (m) versus Mean Fibre MPs (n/100g)	0.051

Table 5.4: Co-efficient of determination for the relationship between mean MPs (n/100g) and distance from the nearest source (m).

Relationship	**R²**
Distance from nearest source (m) versus Mean Total MPs (n/100g)	0.213
Distance from nearest source (m) versus Mean Bead MPs (n/100g)	0.281
Distance from nearest source (m) versus Mean Fibre MPs (n/100g)	0.11

5.4.1. Total MPs

As seen in Table 5.3, an R^2 value of 0.038 is relatively low and suggests that at least 4% of the variation in total MP concentrations can be explained by distance to the Lotus River.

As seen in Figure 5.4 and Table 5.2, an R^2 value of 0.213 is relatively highly for the type of data and suggests that at least 21% of the variation in total MP concentrations can be explained by distance to the nearest source. There is a weak 2nd order polynomial relationship between the mean total MPs and distance from the nearest source ($R^2 = 0.213$). Concentrations increase with distance from the source, and then decrease, but there is considerable variation around this trend. Figure 5.4 shows a variation in MPs from the nearest source with no clear increase in particles close to or away from the nearest source.

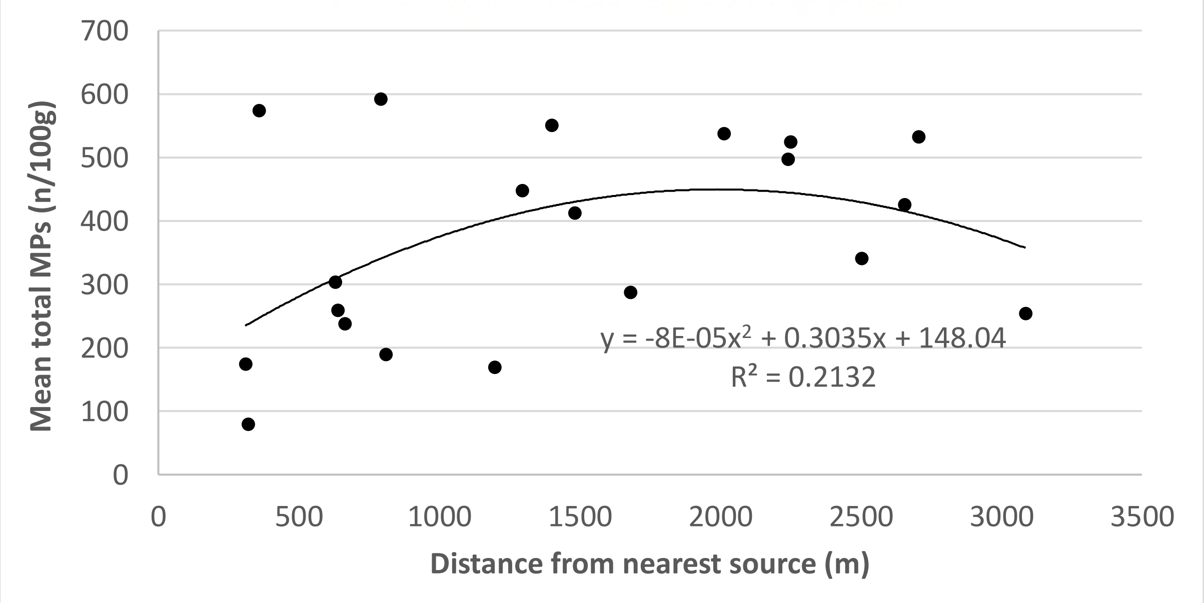

Figure 5.4: **Mean concentration of MP particles/100g with distance from Large Lotus River (m), with a second-order polynomial to depict the trend of the data, with R² representing the correlation between the mean total MPs (n/100g) and distance from nearest source.**

The mean concentration of MPs away from and towards the nearest source and Lotus River, shows a relationship suggesting that the total MP concentrations ultimately increase away from the source and Lotus in a non-linear way (based on the trend depicted by the second-order polynomial line).

5.4.2. Micro-beads

As seen in Figure 5.5 and Table 5.3, an R² value of 0.18 is relatively low and suggests that only 18% of the variation in total micro-bead concentrations can be explained by distance to the nearest source. There is a weak 2nd order polynomial relationship between the mean of micro-beads and distance to the Lotus River (R² = 0.18). Concentrations of beads increase with distance from the source but shows considerable variation.

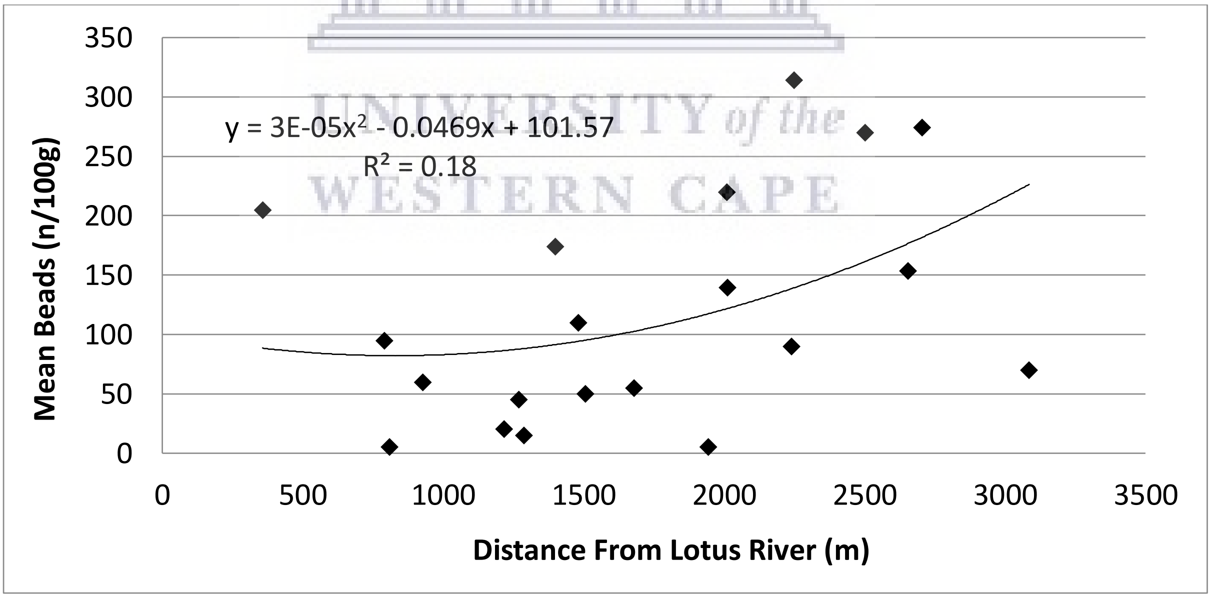

Figure 5.5: Mean concentration of MP beads particles/100g with distance from Lotus River (m), with a second-order polynomial to depict the trend of the data, with R² representing the correlation between the mean beads (n/100g) and distance from the Lotus River (m).

As seen in Figure 5.6 and Table 5.4, an R^2 value of 0.281 suggests that at least 28% of the variation in total micro-bead concentrations can be explained by distance to the nearest source There is a weak 2nd order polynomial relationship between the mean beads and distance from the nearest source ($R^2 = 0.281$). Concentrations increase with distance from the source, and then shows a slight decrease. Concentrations do, however, show a considerable variation.

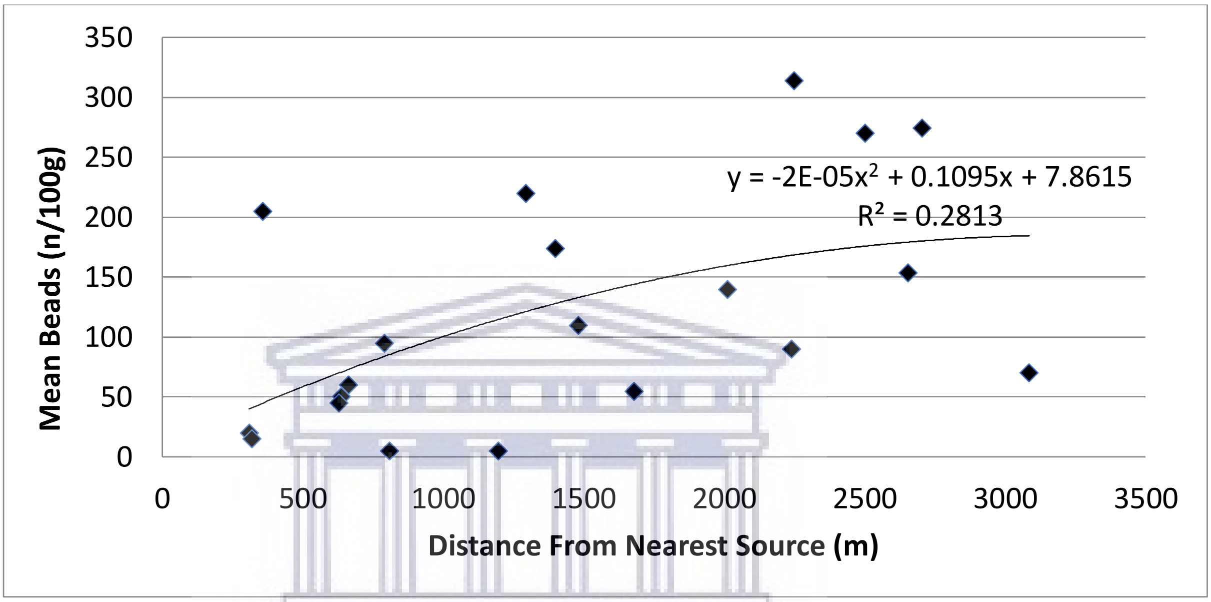

Figure 5.6: Mean concentration of MP beads particles/100g with distance from the nearest source (m), with a second-order polynomial to depict the trend of the data, with R^2 representing the correlation between the mean beads (n/100g) and distance from the nearest source(m).

The mean concentration of MP beads tends to primarily increase away from the nearest source and Lotus River, and ultimately shows a relationship suggesting that the beads concentrations ultimately increase in a non-linear way (based on the trend depicted by the second-order polynomial line). This suggests that bead particles travel quite a distance into the lake before accumulating. Based on the correlations determined for bead concentrations and distance to the Lotus River and nearest source, it can be noted that there is a relatively weak relationship between them, thus suggesting that there are other factors influencing the distribution and accumulation of bead particles.

5.4.3. Micro-fibres

As seen in Table 5.3, an R² value of 0.05 is relatively high for the type of data set and suggests that only 5% of the variation in total micro-Fibre concentrations can be explained by distance to the Lotus River. There appears to be no meaningful relationship between micro-Fibre concentrations and distance from the Lotus River.

As seen in Table 5.4, an R² value of 0.11 which is relatively weak suggests that 11% of the variation in total micro-Fibre concentrations can be explained by distance to the nearest source.

The mean concentration of MP Fibres seems to decrease away from the Lotus River and nearest source as deduced from the R² values seen in the Tables above. The data ultimately shows a relationship suggesting that the beads concentration decreases in a somewhat non-linear way (based on the trend depicted by the second-order polynomial line). This suggests that Fibre particles accumulate closer to the source and travels a shorter distance than bead particles. Based on the correlations determined for Fibre concentrations and distance to the Lotus River and nearest source, it can be noted that there is a relatively weak relationship between them, thus suggesting that there are other factors influencing the distribution and accumulation of Fibre particles as well.

5.5.Micro-fragments

A large number of micro-fragments were counted throughout the microscopic process as seen in Table 5.5 These micro-fragments, however, closely resembled quartz particles (silt) as mentioned earlier in the chapter. Underneath the Zircon microscope, these particles appear to be identical, however, underneath a polarizing microscope, micro-fragments possessed a clear surface whilst quartz possess interference colors. As seen in Figure 5-8 below, majority of the particles were identified as fragments rather than as quartz. Fragments appeared to be clear with no unnatural colors, they appeared not to have unnaturally brightly colored coating on other particles but were unnaturally shaped, appeared to be shiny/glassy and were not brittle when compressed.

Table 5.5: Total number of fragments counted during the microscopic process.

Sample	Fragments
Z1	3323
Z2	2711
Z3	3367
Z4	2116
Z5	2280
Z6	821
Z7	340
Z8	1174
Z9	1231
Z10	1523
Z11	1415
Z12	740
Z13	2758
Z14	2115
Z15	1512
Z16	613
Z17	951
Z18	982
Z19	307
Z20	775

As seen in Table 5.5, there are much more MP fragments situated along the lake boundaries opposed to the center of the Lake, especially in the samples taken close to the Lotus River and nearest sources.

In order to account for quartz contamination, 30 particles from each sample were randomly selected and analyzed under the polarizing micro-scope and counted as either a fragment or a quartz particle based on how the polarized light reflected from their surfaces (i.e., MP fragments shows a clear surface whilst quartz shows interference colors). As seen in Figure 5.8 below, there were much more fragments of Microplastics identified based on the criteria outlined in the methods section than quartz particles. Micro-fragments are, however, ultimately excluded from analysis due to a lack of confidence in the differentiation between MP fragments and some quartz particles in particular.

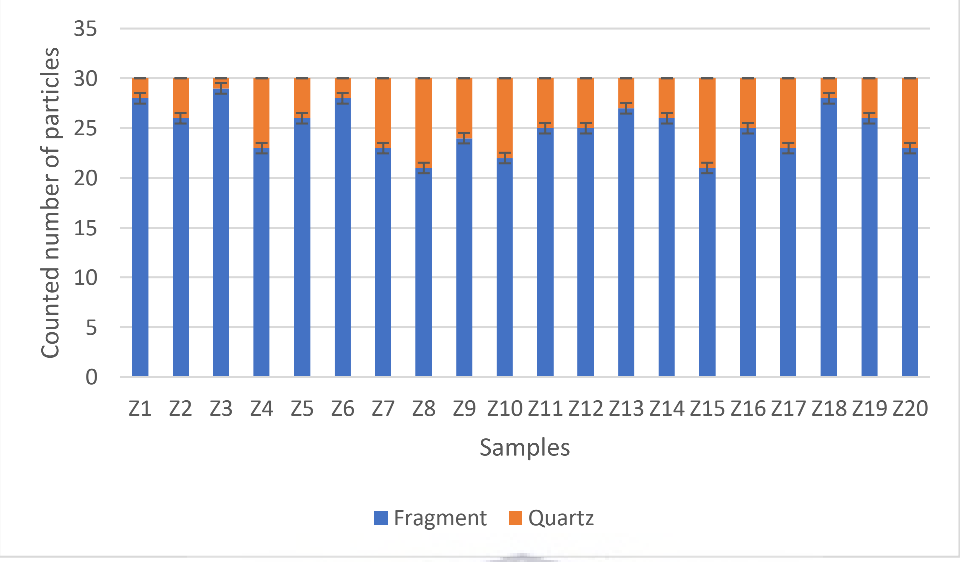

Figure 5.8: Counted number of MP fragments represented by blue bars and quartz represented by orange bars underneath a polarizing microscope

6. Discussion

The following chapter will discuss and interpret the results presented in the previous chapter. This chapter will primarily seek to satisfy the aim and objectives of the study by thoroughly analyzing and discussing the information presented in the results.

6.1. Overall results obtained.

Due to the presence of quartz and its resemblance to MP fragments, it has been decided that fragments will not be discussed as a means for satisfying the aim and objective of the study. This is largely an outcome of uncertainty, as it compromised the overall identification of this type of MP. In terms of the 2 MPs that will be discussed (i.e., Beads and Fibres), the floatation step successfully removed 474 beads and as many as 987 fibres in total of all samples. Density separation by means of floatation has ultimately proven to be an effective method for removing MPs (dense particles) from the bed sediment of aquatic ecosystems such as lakes. Restricting the analysis to these particle types of results in an underestimate of overall MP concentrations but has the advantage of focusing on particles that should respond to transport within the aquatic environment in a consistent way (all micro-Fibres would have broadly similar transport modes, and all micro-beads would have broadly similar transport modes).

Evidently there are more Fibres that were counted as opposed to beads as seen in Figure 6.1 below. The results clearly shows that there are MPs present within the bed sediment of the Zeekoevlei Lake and indicates where the number of MPs is higher within the Lake. The results ultimately identify the spatial distribution of MPs at various points within the wetland and their degradation. The results show a relationship for distance from any source as a second-order polynomial for the entirety of the datasets. Concentrations has been noted to increase with distance up to a certain point away from the source, and then decrease with further distance from the source. This suggests that particles are conveyed some distance into the lake before settling in bed sediment. This ultimately suggests that the distribution and accumulation of MP particles in the lakebed sediment is affected by various factors (e.g., wind and wave activity) other than by distance from sources (Wang *et al*., 2018). As seen in the results, majority of the MP particles appear to settle down between 1000 – 2000 m away from the source, thus allowing for remediation processes to be performed in order to reduce the concentrations of MPs within the lake, and ultimately enhancing the overall health of the ecosystem.

Based on the non-linear way (the second-order polynomial relationship that best fits the data) in which the MP particles increase or decrease away or towards their sources, it can be further substantiated that MP particles especially beads are conveyed further into the lake before accumulating in the bed sediment. This particularly makes sense for particles that are relatively buoyant such as both Fibres and beads. The variation in distribution and accumulation of these particles can also be accounted for by variable processes such as, variation in wind speed and direction, wave action, biofilm formation and variation in inlet flow discharges located close to particles along the lake boundary.

Figure 6.1: Percentage (%) of beads to that of Fibres for all 40 bed sediment samples collected.

6.2. Beads and Fibres

The total MPs are accounted for based on the combined results in concentrations of beads and fibres, which amounted to 14795 MP particles/100g. This concentration is much more than that obtained in the Thames River in the United Kingdom which reported a concentration of 66 MP particles/100g. The main sources of MP pollutants for the Zeekoevlei Lake comes from nearby inlet points as well as most probably the main sources being the big and Small Lotus River. They are the main contributors for MP pollution due to the rivers flowing through heavily urbanized areas, such as industrial and residential areas which are susceptible to pollution from their point of origin right up until their point of flow, into the Zeekoevlei Lake (Zhang *et al*., 2015). It has thus been hypothesized that where the rivers and nearby inlets flow into the lake, is where the highest concentrations of MP particles will be found. This, however, has not entirely been the case as seen in the Figures and stats presented in the results chapter. Fibres and beads alike are deposited in great quantity in regions located downstream. As mentioned, this could be due to various environmental factors and due to the buoyant nature of these plastics. With correlations ranging between 0 and 0,5, primarily much lower than 0,5, for MP means/100g with distance from the Lotus River and nearby sources; it is suggested that distance has a minimum influence on the distribution and accumulation within the Lake. The distribution and accumulation within the lake can, however, be described and accounted for based on wind speed and wave activity within the lake as observed during field day upon collection of samples as well as stated in previous studies (Davaasuren *et al*., 2018; Sedlak., 2017).

6.3. Lotus River and nearby inlets

Rivers are broadly viewed as major pathways for microplastic transportation from terrestrial areas to aquatic ecosystems (Horton *et al*., 2017). Rivers with high water flows especially during winter would transport more microplastics from potential sources, as well as high flow velocity in bottom water layers of rivers, are suggested to facilitate the transport of microplastics within sediments (Corcoran *et al*., 2015). The sediments of rivers, however, act as a sink for microplastic pollutants instead of being a transport pathway (Coppock *et al*., 2017). This suggests that majority of the MPs transported into aquatic ecosystems, comes from those suspended in the water column or floating on the surface of the water, and is alternatively affected by winds and wave activity upon entering the lake. Whilst suspended in the water column of rivers and travelling downstream towards the lake, MPs are also susceptible to biofouling as rivers pick up various micro-organisms such as bacteria on their way downstream (Wu *et al*., 2020). Based on this information, it can be understood to a great degree as to why MP beads and Fibres are not accumulating at high concentrations at or near the main source points.

6.4. Effect of winds

The most common mode for dispersal for any micro-particle, is winds. Winds can transport light particles such as MPs from one place to another without altering the state of the particles. Winds directly affect the surface of any water body by causing a type of rippling effect and often wave activity. These winds thus cause material entering a lake or a river that feeds into a lake, to have particles being distributed and concentrated more towards the region of the water body in which direction the wind will be blowing. This wind-driven effect distributes the Microplastic particles throughout the upper water column of the lake, resulting in the abundance and distribution of the MPs to be concentrated in areas that were not predicted to have the highest concentration of MPs (Bullard *et al*.,2021).

Wind speed plays a crucial role in MP dispersal and can be seen as an influence in the distribution and accumulation of MPs throughout the bed sediment of the Zeekoevlei Lake despite having not been measured. The elevated wind periods such as wind speeds of >2m/s and interspersed periods of calm wind speeds of <0.5m/s, may assist in the conveyance and deposition of the MPs throughout the lake (Allen *et al*.,2019). This is supported by the results obtained throughout this project. According to the results obtained, the highest MP concentrations occurred towards the east margin of the lake. The reason for this, is due to the wind direction and speed as seen in Figure 6.2 below. Figure 6.2 shows the wind blowing in an easterly direction at speeds >2m/s which suggests that MPs on the land surface surrounding the lake especially those situated on the southwest margin of the Lake, being blown towards the east margin of the Lake, where it will ultimately be deposited and thus accumulate in the bed sediment. This is particularly relevant in terms of the concentration of Fibres being dominant in the east and north margin of the Lake. Fibres are easily the most buoyant type of MPs and are thus easier to be transported by calm winds <0.5m/s than any other MP. The diagram below also substantiates the large concentrations of MP Fibres located in the North and East margin of the Lake. In terms of the beads concentrations, according to the results, there are higher concentrations along the east margin of the lake, this could be due to the winds blowing beads on the land surface as well as surface of the lake, towards the east margin of the Lake.

With wind speeds of >5.7m/s, the results can be accounted for in a sense that such a high speed of wind, could certainly transport buoyant MPs on the surface of the water, as well as those entering the lake via inlets, further away from those sources, where they will eventually be deposited in the bed sediment in the lower regions of the lake.

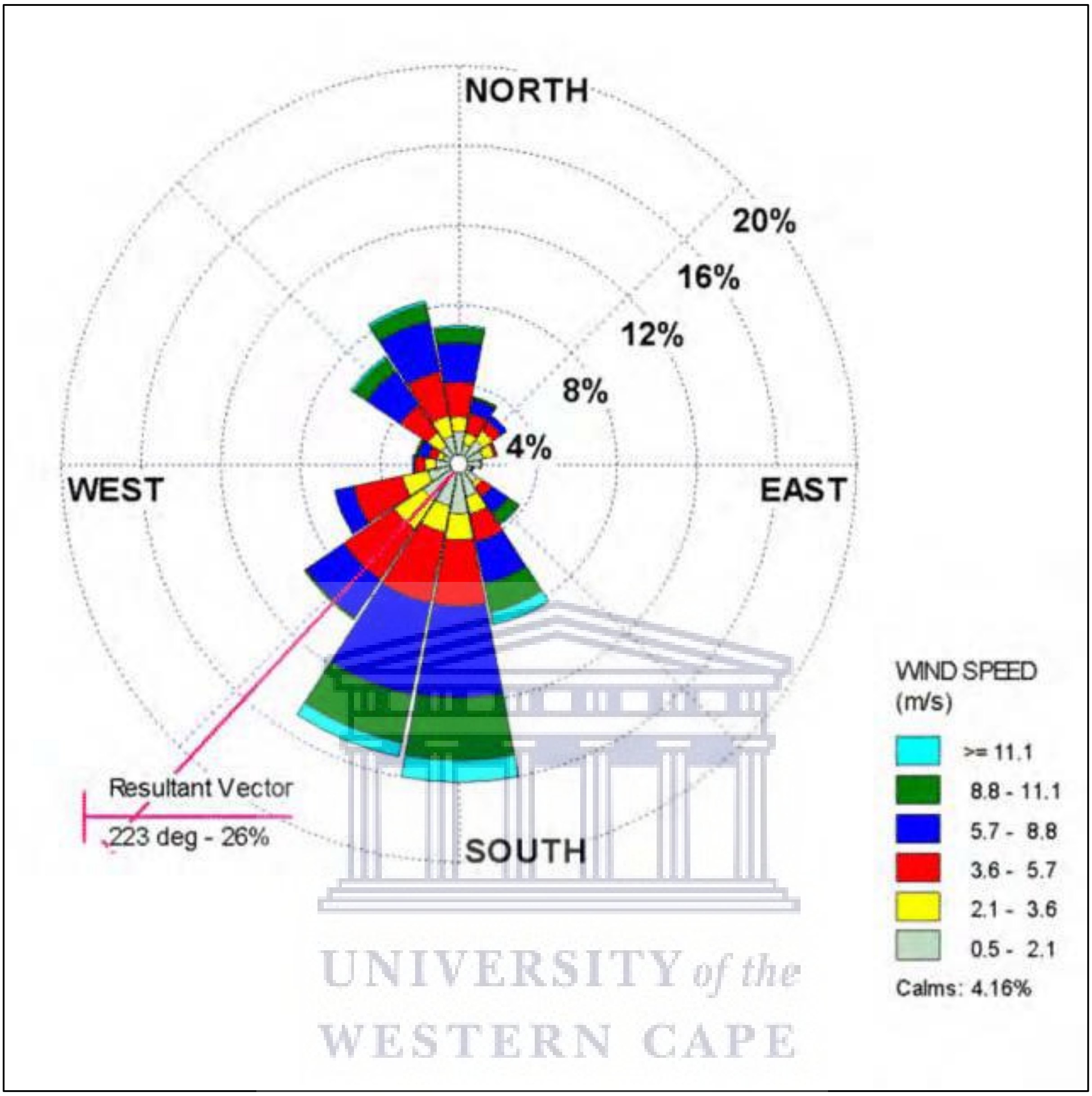

Figure 6.1: Wind Rose diagram for Cape Town (Weston., 2014)

6.5. Effects of Waves

As mentioned in 5.5, winds are a major driving force in the formation of waves within various water bodies, especially lakes. When winds carry MPs from surrounding areas and into the water of the Lake, the settling and accumulation of these MPs in the lake are then directly affected by not only the direction of the wind but also by the waves caused by the wind. These waves keep the MPs in suspension within the water column.

When wind blows over a relatively calm lake surface, it first creates an effect that may be perceived as varying and fluctuating ruffling of the surface. The first wave primarily consists of capillary waves which are small, uniformly developed waves. These waves are transient and can end rather abruptly if the wind comes to a standstill. If the winds, however, keep on developing, they cause a much more frequently observed gravity wave (Rafferty., 2010). Whilst on a boat collecting the bed sediment samples, waves were encountered the longer the day went by. These waves were ultimately caused by an increase in wind speed. The surface of the lake quickly went from being calm to being rough with continuous wave activity. These waves caused the boat to take on some water, which was alarming due to wind speed not being as rapid as the waves made it out to be. Waves are primarily known for preventing material that is in suspension from sinking and accumulating in the bed sediment of aquatic ecosystems. The near shore of lakes often experiences sediment transportation due to wave activity. These waves generate turbidity currents which are responsible for the carrying and cycling of suspended sediment into and within lakes, reservoirs, and the deep sea (Garcia., 2008). This principle also applies to the carrying and cycling of MPs within aquatic ecosystems. Due to the density of MPs, it is possible that the turbulence caused by wave activity within the lake, caused the MPs to be carried whilst in suspension to various parts of the lake, away from the sources and then deposited once the waves dissipate.

Waves also cause sediment that has already settled at the bottom of the lake (bed sediment) to become suspended. The suspended sediment underneath the wave is referred to sediment that has been picked up and been entrained from the lakebed by the waves and is then kept entrained by the activity of the waves. This suspension occurs due to the movement of the waves being entraining loosely consolidated sediments containing MPs such as sand and then transporting them into the water column away from the lakebed. When the waves essentially become inactive, the sediments eventually settle back down to the lakebed due to gravity (Soulsby., 1994).

During periods where there are almost no wind and consequently no wave activity, a variation in MP dispersal across the Lake can be accounted for due to the effect of biofilm formation on the surface of the MPs.

6.6. Effects of biofilm formation

Biofilms essentially forms when certain microorganisms such as various types of bacteria, adhere to the surface of some objects (i.e., MPs) in moist environments such as lakes and begin to reproduce. These microorganisms then essentially form an attachment to the surface of the object by secreting a slimy, glue-like substance. This formation of biofilm has a direct effect on the distribution and accumulation of MPs in bed sediment. Biofilm formation occurs on the surface of MPs and could result in the changing of the density of MP particles that alters the specific gravity for the mass of Microplastic pollutants. This essentially means that pieces of minerals become incorporated in Microplastic debris and thus increase the density of the MP particles which causes them to sink. The Zeekoevlei Lake experienced a sewage spill at the mouth of the Big Lotus River according to locals, which would have increased the number of bacteria present within the Lake, thus making the environment rich in bacteria. This would only have had a major contribution towards biofilm formation and essentially MP concentrations within the lake. This accumulation of bacteria (i.e., microorganisms) which is most referred to as biofouling, results in significant changes within the buoyancy of MPs. As mentioned, this increases the specific gravity of the MPs, and leads to MP particles descending in the water column to a depth of comparable density where they accumulate. This could also be why the concentrations of MPs are not as high as predicted at the sources and were highly variable across the twenty sampling points.

7. Conclusion and Recommendations

In conclusion the objectives were addressed through the implementation of the steps and procedure covered in Chapter 4 of this study. Fieldwork and laboratory analysis addressed the first objective which was to characterize the spatial distribution of MPs across the lakebed environment, in relation to possible point and non-point sources of contamination and the second objective which was to provide insight into local fluvial and lacustrine dispersal mechanisms as a mode of transport for MPs throughout the Lake. These objectives were also thoroughly discussed in Chapter 6 of this study. Fieldwork included the selection of 40 samples across the study area, with 20 samples around the lake boundary and 10 down the center of the lake. Each sampling site consisted of 2 samples taken on either side of the boat. At each site, at least 5 cm of bed sediment was collected with a grab sampler and stored in a glass jar for laboratory analysis. These sediment samples were than taken to the laboratory for the separation of MPs from the sediment through a process called density separation. This was done over the course of only one field trip.

The results of this investigation concluded that the Zeekoevlei Lake is most certainly possess MPs within its bed sediment which shines new light on the ever-present threat presented by ongoing pollution in and around the lake. This conclusion was based on the number of Microplastic particles counted during microscope analysis as well as the statistical calculation performed for the mean concentration of MPs per 100g of sample. This ultimately provided a broader understanding of the distribution and occurrence of MPs throughout the Lake. Theses MPs appeared to be concentrated in areas of the lake that were not predicted to have high concentrations prior to counting. This was ultimately discussed and understood to be due to various environmental factors such as the effects of wind, waves, and biofilm formation. Additional factors that influenced the distribution and accumulation of MPs were deemed to be surface runoff carrying Microplastic pollutants to various parts of the lake (especially those parts situated away from the inlet points). The lake was also believed to have high concentrations of *Escherichia coli* which indicates the presence of pathogens and essentially bacteria in the water. This could not only be a health risk for people living on the lake boundary, aquatic life and bird species using the water for recreation or consumption, but also contributes to the biofouling of MP particles resulting in them sinking throughout various parts of the lake.

Despite the numerous numbers of MP particles identified, there was still difficulties encountered in the differentiation between micro-fragments and quartz particles based on appearance. This ultimately led to fragments being disregarded due to lack of confidence despite having proven that majority of particles were most likely to be fragments. Only Fibres and beads were used to depict the distribution and accumulation of MPs in the bed sediment of the lake. It is thus recommended that an FTIR analysis be performed on samples post microscopic analysis in order to provide clarity on what portion of the samples are indeed MPs and what portion are organic. Although differentiating between MPs and other non-plastic particles are relatively easy to identify based on a clear and homogeneous color, and the presence of cellular or organic structures, an FTIR analysis would provide much more information in not just the identification of MPs but also n what their sources are most likely to be. It is also recommended, to make use of a method that completely separates MPs from any organic material or to treat organically rich samples with hydrogen peroxide before performing density separation. In addition to this, it is recommended that awareness of the presence of MPs especially in Zeekoevlei as a whole and surrounding areas, be raised in order to encourage surrounding communities to reduce their contribution to pollution. Micro-pollutants are extremely difficult to detect with the naked eye and have already been proven to be present in the air we breathe as well as the fish we eat. It is recommended that necessary national policies and legislations be geared towards protecting aquatic ecosystems and the environment as a whole from not only macro-pollutants, but also micro-pollutants.

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