Environmental concentrations and risk assessment of microplastics in selected echinoderms in rocky shores of the Western Cape, South Africa.

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Marine Science

In the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

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District Six, Cape Town
Date submitted: March 2023

DECLARATION

I, Danielle Renee Julius, declare that the contents of this thesis represents my own work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed: Date: 31 October 2022

ABSTRACT

Plastic debris is accumulating in all environments globally and South Africa's poor waste management plan has led to an increase in plastic contamination throughout the country. Microplastics (MPs) are defined as plastic particles smaller than 5 mm . Information about MPs in coastal environments and biota in South Africa is poor. The aim of this study was to determine coastal MP concentrations in water (MPs/L) and sediment (MPs/kg) and ingested MP in echinoderm species (MPs/g and MPs/I). Sampling took place during in summer 2020 during low at 14 sites along the coast of the Western Cape, South Africa. Water (n= 5 per site), sediment (n= 5 per site) and echinoderms (n= 20 per site) were sampled at each site. Sampling efforts for echinoderms were subject to availability, but at least two echinoderm species were analysed. Environmental and biological samples were digested in 10% KOH at 60 ºC (24 hours). MPs were extracted and analysed based on visual type, colour, size and polymer type (using an FTIR-ATR). A risk assessment was done to assess the risks posed by MPs in all sample types. The results showed a higher mean concentration in sediment (185.07 MPs/kg; $\pm$ 15.25 SE) samples followed by echinoderms (1.44 $\pm$ 0.12 MPs/g) and water (1.33 $\pm$ 0.15 MPs/L) samples, suggesting sediment is a MP sink. Gordan's Bay (site 12) had the highest concentration in sediment samples (360 $\pm$ 36.74 MPs.kg), identifying harbours as a source for MP contamination. Kalk Bay (site 9) displayed the highest concentration in both water and echinoderm samples (4.97 $\pm$ 0.18 MPs/L and 2.90 $\pm$ 0.38 MPs/g respectively), suggesting the source of MPs are from stormwater outfall pipes. In addition MPs present in the water column are ingested directly by echinoderms based on feeding strategy. Filaments were the most dominant MP type (89.33%) with black/grey being the most dominant colour (41.12%). PET was the most dominant polymer type (41.33%). Based on the risk assessment, MPs recorded at Mouille Point (site 6) poses the greatest risk associated with polymers. MP concentrations reported in this study provide a baseline for future studies, with a

need for investigations to focus on the effects of MPs on echinoderms in rocky shores environments along the Western Cape coastline, South Africa.

ACKNOWLEDGEMENTS

I wish to thank:

CPUT: Resources and facilities

Dr. C. Sparks and Dr. A. Awe: Supervisors

Liam Ferguson: Field and lab assistance and support

Juan and Naomi Julius: Encouragement and support

The financial assistance of National Research Foundation (NRF) towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the National Research Foundation.

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GLOSSARY

Biodegradable	The ability for a material or substance to be broken down naturally by the organisms in an ecosystem.
Contamination	The presence of a foreign material or substance having no harmful effects.
Invertebrates	A cold-blooded animal with no backbone
Keystone species	An organism that helps define an entire ecosystem.
Microplastic	Plastic particles less than 0.5 mm in diameter, varying in type, colour and shape.
Plastic	Synthetic or semi-synthetic polymers.
Pollution	The presence of a substance or material that may cause harmful effects.
Ubiquitous	Present or found everywhere
Upwelling	A process in which deep, cold water rises toward the surface.
Rocky shore	The interface of land and sea, forming a narrow border around the coastline of a country.

ABBREVIATIONS/ACRONYM

ABS	Acrylonitrile-butadiene- styrene
APP	Antifouling paint particles
Natural	Cellulose/protein based polymer
CF	Contamination factor
CS	Coarse sand
EVA	Ethylene vinyl acetate
FTIR	Fourier-transform infrared spectroscopy
FS	Fine sand
KOH	Potassium Hydroxide
KW	Kruskal-Wallis
MP	Microplastic
MPA	Marine Protected Area
MS	Medium sand
PA6	Polyamide 6
PAA	Polyacrylic acid
PBMA	Poly-butyl methacrylate
PE	Polyethylene
PEPP	Polyethylene propylene
PET	Polyethylene terephthalate
PLI	Pollution Load Index
PMMA	Polymethyl methacrylate
PP	Polypropylene
PRI	Polymer Risk Index
PS	Polystyrene
PUR	Polyurethane
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
RO	Reverse Osmosis
SA	South Africa
SOP	Standard Operating Procedure
SR	Synthetic Rubber
SSR	Semi-synthetic rubber
VCS	Very Coarse Sand
VFS	Very Fine Sand

WWTW	Waste Water Treatment Works

Chapter 1

Introduction

Since the 1960s, the demand for plastic has drastically increased and production has exceeded 322 million tonnes (GESAMP, 2019; A. Lusher, 2015a; Wright, 2015). Global plastic production increases by approximately 8% per year of which at least 8 million tonnes end up in the marine environment and make up 80% of all marine debris from surface waters to deep-sea sediments (Ašmonaitė & Carney Almroth, 2019; Ryan, 2018). Due to its lightweight and durability, plastic debris is found accumulating in all environmental compartments at an alarming rate, from beaches and surface water in the oceans, deep sea and sediments, artic ice, fresh water systems, soil and terrestrial niches, to indoor environments as well as food and drinking (Ašmonaitė & Carney Almroth, 2019; Williamson, 2015; Wright, 2015). Africa, as a continent, is responsible for producing 1% of the world's single-use plastics (Ryan, 2018). In 2015 South Africa was listed as the 11th worst country for poor solid-waste management, of which more than half is mismanaged (Ryan, 2018). Due to South Africa's poor waste management plan, since 2010 there has been an increase of 4.8 million tonnes to 12.7 million tonnes of plastic waste discharge into freshwater, estuarine and marine environments (A. Lusher et al., 2017).

Plastics that break into pieces < 5 mm are classified as secondary microplastics (MPs) and plastics specifically manufactured at < 5 mm are classified as primary MPs (GESAMP, 2019). MP types vary and are characterised based on shape (i.e fragments, filaments/fibres, films and spheres) polymer type and even colour (GESAMP, 2019). MPs enter the marine environment in various ways and are emerging marine contaminants and potential pollutants (A. Lusher, 2015a; Wright, 2015). The wide range of properties (size, shape, density) MPs possess influences their distribution in the marine environment i.e sink or float. In addition MP properties change over time as a result of degradation (Lusher, 2015a; Wright, 2015). The problem is that plastic products have a long durability and often outlive their utility, become waste and enter the marine environment. Rocky shore ecosystem harbour plastic debris differently to other marine ecosystems. In addition they serve as "grinding mills" facilitating the acceleration of MPs being formed (Debrot et al., 1999; Eriksson & Burton, 2003). Rocky shore ecosystems are located all along South Africa's coastline, some areas being more densely populated than other. Densely populated coastal areas have been reported to have a significant amount of MPs present in sediment due to currents, wave and wind action, river outflows and direct littering, thus making the relationship between MPs found in these areas and the increasing human populations directly proportional (Wright, 2015).

Plastic waste entering the marine environment has accumulated substantially over the last 70 years (Browne, 2007; Wright, 2015). Due to its characteristics, marine organisms are unable to distinguish between food and non-food particles and unintentionally ingest plastic. Ingested plastics have physical and physiological effects on marine organisms, including coastal invertebrates such as echinoderms (Williamson, 2015). Echinoderms occur in rocky shore ecosystems (Howell et al., 2003; Branch and Branch, 2018; White et al., 2011), providing economic and ecological importance (Ambrose et al., 2001; Castelló y Tickell et al., 2022; Chenelot et al., 2006; Cossi et al., 2021; Day & Branch, 2002). Invertebrates are a vital link between primary producers and nekton, with plastic-induced changes in their population structure potentially having detrimental effects on the ambient ecosystem (Golstein et al., 2012). The effects of ingested MPs by echinoderms have been monitored and assessed in laboratory experiments (Graham & Thompson, 2009; Iwalaye et al., 2020a; Nobre et al., 2015; Richardson, 2020), however environmental based studies are still scanty (A. Lusher, 2015a; A. Lusher et al., 2017).

This research will serve as a baseline study for MPs concentrations and abundance along the Western Cape coastline, South Africa and will 1) Identify possible sources of MP contamination along the Western Cape coastline; 2) Identify factors influencing spatial distribution of MPs and 3) Identify MPs based on polymer type using Fourier Transform Infrared Spectroscopy (FTIR). Methods used will assist in developing a standard operating procedure (SOP). A risk assessment will be done to assess the risk posed by MPs in water, sediment and echinoderms. The results will also determine whether echinoderms can be used as indicator species for MP contamination.

Chapter 2

Literature review

2.1 Introduction

The increase in global population has led to the increase in the demand for disposable plastic. Since the 1950s approximately 8.3 billion tonnes of plastic has been produced (Andrady, 2011; Geyer et al., 2017; Stephanie, 2017). Plastics are synthetic materials made of organic polymers. Plastics can be classified as thermoplastics or thermoset plastics. Thermoplastic is meltable and is shaped when soft and retains its structure when hard whereas thermoset plastic is not meltable (Zaman & Newman, 2021). Plastic production is inexpensive and additives, such as fillers, plasticizers, colorants, stabilizers and processing aids are used to enhance the performance of the plastic (Andrady & Neal, 2009). These additives allow for plastics to be durable, non-degradable and versatile and have numerous functions and applications. Applications include packaging, automotive, building and construction, electrical, electronic, textile, fishing gear, the list is endless (Andrady & Neal, 2009; GESAMP, 2016). Plastic is dominated by 6 classes of plastics namely polyethylene (PE, high and low density), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS, including expanded EPS), polyurethane (PUR) and polyethylene terephthalate (PET) (GESAMP, 2019; Plastics Europe, 2016). PP and PE are the most commonly used thermoplastic as it is inexpensive and can be moulded into a variety of products. Products produced range from packaging products, household and personal goods to horticulture and dielectric insulator (Andrady & Neal, 2009). Compared to other plastics that are carbon and hydrogen based, PVC in addition contains chlorine. PVC is in the form of white powder and mixed with other ingredients to produce products used in buildings and furniture (Andrady & Neal, 2009). PS is available in two grade forms that are then modified to produce insulating material for buildings and mouldable packaging. Compared to other polymers, PET is only plastic with balanced properties suitable for producing bottles. Its lightweight and resistance has resulted in the complete replacement of glass (Andrady & Neal, 2009).

Microplastic (MP) (< 5 mm) is formed when larger plastic products breakdown or are manufactured. Their properties vary in sizes, densities, chemical compositions and shape. MPs are classified as being either primary MPs or secondary MPs. Primary MPs are MPs specifically manufactured at < 5 mm for industrial or domestic use. Applications include facial cleansers, shower gels, scrubs, tooth paste and resin pellets, synthetic clothing, abrasives found in drilling fluids, cleaning products (Cole et al., 2011; Costa et al., 2010). Secondary MPs formed from larger plastics through a combination of photo-degradation, mechanical transformation from wave and wind action and biological degradation by organisms (Andrady & Neal, 2009; Browne, 2007; Cole et al., 2011). These processes reduce the structural integrity of large plastic, resulting in MPs (Cole et al., 2011).

Between 60% – 80% of anthropogenic debris in marine environment is in the form of plastic (Andrady, 2011; Barnes et al., 2009; Napper & Thompson, 2020). Plastic debris is being released into the marine environment at an alarming rate, raising global concerns regarding the health of the environment (GESAMP, 2019; A. Lusher, 2015a; Rochman et al., 2016; UNEP, 2016; Williamson, 2015). Sources of release encompass land- and sea-based. Land-based sources include, urban and stormwater runoff, sewer overflows, littering and illegal dumping and trading, inadequate waste management, industrial activities, tyre abrasion and construction. Sea-based sources include activity from the fishing industry, oil drilling, rivers, aquaculture and nautical activities (Gall & Thompson, 2015; GESAMP, 2019; J. Li et al., 2016; Thushari & Senevirathna, 2020). Studies have identified microfibres as being the most predominant type of secondary MP (Browne et al., 2011; Martin et al., 2017; Naidoo et al., 2015) and is linked to domestic waste and sewage-sludge disposal sites (Browne et al., 2011). Other studies have identified harbours and marinas as potential sources of MP contamination due to harbour dredging, accidental discharge of oil and chemical spills, shipping paint and repair works from boating maintenance, uncontrolled disposal and leakage of industrial and urban waste (Paradas & Amado-Filho, 2007) .

There is a global concern with regards to how marine plastic interacts in the environment as it could pose a threat to marine ecosystems and their function. Once present in the marine environment, environmental conditions have the potential to alter marine plastic contamination and its properties. This change could facilitate in MP production and affect the distribution and accumulation of plastic in the environment. MPs have been recorded in the most remote and pristine marine environments such as Marine Protected Areas (MPAs). MPs have been found in sediment and organisms from MPAs in the Adriatic, Argentine and Mediterranean Seas and the Sea of the Hebrides (Alomar et al., 2016; Arias et al., 2019; Cossi et al., 2021; Fossi et al., 2017; la Beur et al., 2019). Furthermore, biofouling assists in the sinking of MP particles (Fazey & Ryan, 2016; Kaiser et al., 2017). Biofouling is a successive build-up of organic matter and organisms. Due to plastic being hydrophobic, organic matter is absorbed and provides the ideal conditions for bacteria and other microalgae to grow (Kaiser, 2017). As a result MPs have the potential to introduce invasive species to the environment and be consumed my various marine organisms (Anderson et al., 2016). Studies have also observed a link between these invasive species and an increase in diseases in coral reefs (Lamb et al., 2018) .

Plastic contamination is prominent in developing Asian, African and South American countries (UNEP, 2016). This is due to these countries having limited resources (Kaza et al., 2018) to manage infrastructure and not prioritising social and economic needs regarding environmental issues. South Africa is a developing African country ranked as 20th country in the world for producing the most waste per year (Jambeck et al., 2015; Kaza et al., 2018). South Africa is faced with poverty, high unemployment and HIV/AIDS and prioritises these issues over ecological issues such as plastic contamination (Verster et al., 2017).

2.2 Microplastics in coastal environments

MPs enter the marine environment via freshwater inputs from rivers (Ryan & Perold, 2021), tides, stormwater run-off and effluent, winds and currents (Cózar et al., 2014; Kukulka et al., 2012; Murphy et al., 2016; Song et al., 2018; Zalasiewicz et al., 2016), anthropogenic and maritime related activities (Sparks & Awe, 2022). Sources of MP contamination and plastic composition (polymer type) are responsible for the spatial distribution of MPs in coastal areas. Other sources include the degradation of macroplastic debris and sewage sludge (Alomar et al., 2016; Leslie et al., 2013). Poor waste management has led to the occurrence of MPs in the most pristine oceans, offshore and marine coastal environments (Andry and Neal, 2009). Reports show that 1% of plastic that is transported offshore is significantly lower compared to the 37 % of plastic that is released from coastal areas (Lebreton et al., 2019; Ryan, 2020a). Studies have linked high MP concentrations to industrial sites, harbours and coastal areas (Nel et al., 2017; Sparks & Awe, 2022). Half of the world's population lives near the coast, making the coastal zone an area of concern for MP contamination (Cole et al., 2011). Studies suggest that there is a direct relationship between MP concentrations and coastal population density (de Villiers, 2018; McCormick et al., 2014; Murphy et al., 2016; Naidoo & Glassom, 2019; Song et al., 2018).

2.2.1. Spatial distribution of microplastics in coastal water

The vertical distribution of MPs in the water column is dependent on the buoyancy (density and biofouling level) of the MP. Whereas the horizontal distribution is dependent on hydrodynamic forces such as wind, tides, waves and thermohaline gradient. Other factors include the size-selective removal of MPs by biota through ingestion and egestion (Ryan, 2020b). Song et al. (2018) investigated the vertical and horizontal distribution of MPs in Korean coastal waters and found that the middle and bottom waters had higher levels of MPs than predicted by physical mixing of water. Suggesting that the downward movement of low-density MPs are influenced by biological interactions.

Studies have shown a strong correlation between the concentration of MPs in coastal water and population density. (Kwon et al., 2020) investigated spatial distribution of MPs in surface waters along the coast of Korea and found that higher levels of MPs were observed in urban areas compared to rural areas, suggesting that the concentration of MPs is directly proportional to human activity within an area. Similar results was observed in the coastal water of Korea (Song et al., 2018). However, a study conducted by de Villiers (2018) found that rural areas presented higher concentrations of microfibres in rivers close to rural communities than urban areas. This is due to the fact that rural communities lack the infrastructure for piped water and greatly rely on rivers as a primary source of water, one of which is washing clothes directly into the river (de Villiers, 2018). Suggesting that underdeveloped areas contribute to high concentrations of microfibres.

Due to the various factor affecting the distribution of MP, concentrations will continue to increase resulting in the significant accumulation in coastal environments. In addition due to the large surface area to volume ratio, MPs are susceptible to absorbing waterborne organic pollutants and have the potential to release toxic plasticisers from polymer matrices into the environment (Lusher et al., 2015; (Egbeocha et al., 2018).

2.2.2 Spatial distribution of microplastics in coastal sediment

Various studies have been conducted to investigate the source, factors and occurrence of MPs in marine sediment. MPs from various sources (Browne et al., 2011; Pagter et al., 2020) tend to accumulate in coastal sediment in high concentrations (Alomar et al., 2016; Nuelle et al., 2014; Woodall et al., 2014). Regardless of the MP property (e.g. type, colour, size, density), MPs will eventually settle on marine sediment (Alomar et al., 2016; Pham et al., 2014).

Therefore sediment is considered a long-term sink for MPs (Cozar et al., 2014), with the top 5 cm possessing the highest concentration (Martin et al., 2017).

Beach shorelines have the ideal environmental conditions to harbour and degrade macroplastics into MPs (Browne et al., 2007). Conditions include wave and wind action and high irradiation and temperature, facilitating in changing the structural integrity and spatial distribution of MPs. Bayo et al (2020) investigated MP pollution on the strandline of urban and natural beaches of southeast Spain and found that plastic degradation formed film as it is prone to cracking under environmental stress. The study also noted that sources of MPs particles are a result of littering and runoff (Bayo et al., 2020). Other factors such as morphodynamics is responsible for the distribution of stranded MPs on beach strandlines (Pinheiro et al., 2019). This is because as MPs wash up in a linear front and accumulates in a series of strandlines ranging from most recent wave front to last high tide line and a succession of older strandlines to most extreme storm strandline (Browne, 2007; GESAMP, 2019; Ryan, 2020b). The abundance of MPs in strandline sediment range between a few particles to thousands of particles per kg of dry weight sediment (Nguyen et al., 2020).

Enclosed marine environments with shallow depths, which are low-energy and receiving significant amounts of land-based inputs retain more MPs than open marine environments with deep depths, with higher-energy and are further from potential sources of MPs (GESAMP, 2019; P. T. Harris, 2020; van Cauwenberghe et al., 2015). Sun et al (2021) investigated factors influencing the occurrence and distribution of MPs in coastal sediment in China and found higher concentrations of MPs in semi-enclosed coastal areas than in open coastal areas. Martin et al (2017) investigated MPs in marine sediment and found that 97% of MPs reside within shallower sediment depths than deeper depths and the highest concentration being observed at the water-sediment interface. Other studies (Y. Li et al., 2020; Matsuguma et al., 2017; Näkki et al., 2017) have investigated the vertical distribution of MPs and suggested that MP concentrations increase with sediment depth, as MP particles accumulate as they get covered with sand.

Studies suggest that there is a direct relationship between population density, anthropogenic activity and the concentration of MPs in coastal areas (Andrady, 2011; Browne et al., 2011; de Villiers, 2018; Jambeck et al., 2015). Abidli et al. (2018) investigated MPs in sediment from the littoral zone of the north Tunisian coast and found that higher concentrations of MPs was observed in sediment close to densely populated areas with high industrial activity.

MPs are solid, transportable forms of matter and therefore principles used to explain physical sedimentology can be used to understand the fate of MPs in the marine sediment and its interaction in the environment. Hydraulic equivalence is a concept used to quantify the "plastic as sediment" analogy (Enders et al., 2019; P. T. Harris, 2020; Kane & Clare, 2019). Hydraulic equivalence postulates that plastic particles of a particular shape, size and density will behave similarly to naturally occurring sediment particles of similar shape, size and density in the environment (Enders et al., 2019; P. T. Harris, 2020). This is because natural grains range in shape, the same as plastic particles. Furthermore, sediment is classified and categorised based on the grade and composition of grain size. Classification class of sediment include gravel, sand and mud which is classified further into silt and clay. Studies conducted have shown a direct relationship between grain size and MP distribution in the marine environment (Falahudin et al., 2020; Wang et al., 2020). This is due to MP deposition being directly proportional to total organic carbon (TOC) composition in sediment (Maes et al., 2017; Mendes et al., 2021) and TOC is directly proportional to the decrease in grain size (Bergamaschi et al., 1997; Mendes et al., 2021). This suggests that particles have a greater potential to be trapped by finer grains (Green & Johnson, 2020; Mendes et al., 2021) than more coarser grains. It is also theorised that sand, silt and clay-sized particles have hydraulic equivalence with larger-sized plastic particles despite the difference in density (Enders et al., 2019; P. T. Harris, 2020; Ling et al., 2017). Mendes et al (2021) investigated whether the distribution and abundance of MPs in the coastal sediment of Ireland is dependent on grain size and distance from source. The study found that higher concentrations of MPs was recorded in fine-grain sized sediment compared to more coarse sediment. This is because coarse sediment is more loose compared to fine sediment which is tightly packed. Therefore coarse sediment has a higher holding capacity for MPs compared to finer sediment (Mendes et al., 2021; Vermeiren et al., 2021).

Once MPs are present in marine sediment they have the ability to be resuspended and transported via sediment trawling, bioturbation (Näkki et al., 2017; Wu & Wang, 2022), weather and currents (Martin et al., 2017)Mu *et al*., 2018). These events act as pathways for previously deposited MPs to be repeatedly exposed and become bioavailable to marine organisms such as deposit- and filter-feeders (Martin et al., 2017; Rist et al., 2016). Factors responsible the resuspension of MPs could influence the fluctuation in the spatial distribution and concentration of MPs in sediment. Studies have shown that MPs form aggregates with phytoplankton (Long et al., 2017) and marine snow (Porter et al., 2018; Zhao et al., 2017), flocculate and adhere to transparent exoploymers (Passow et al., 2001); Engel, 2004; Summer et al., 2018) or are present in faecal pellets (Cole et al., 2016; Katija et al., 2017). These biological interaction have the ability to reduce the buoyancy of MPs and increase sinking rates, redistributing MPs from surface water to the ocean floor. The distribution and characteristics of MPs pose a threat to the health of marine ecosystems and has the potential to cause adverse effects on organisms.

2.2.3 Microplastics in marine biota

The spatial distribution of MPs in the marine environment poses risks to marine organisms. Studies have shown that most marine organisms ingest MPs from sea birds (Acampora, 2017; Ryan, 1987, 1988) , fish (Adika et al., 2020; A. L. Lusher et al., 2013; Nelms et al., 2018), marine mammals (Alexiadou et al., 2019; de Stephanis et al., 2013; Eriksson & Burton, 2003; Kühn & van Franeker, 2020; Nelms et al., 2018)and marine invertebrates (Graham & Thompson, 2009; J. Li et al., 2016; A. Lusher, 2015b; Pagter et al., 2021; Renzi et al., 2018). MPs have been associated with primary producers. Primary producers interacting with MPs have the potential to change algal photosynthesis (Sjollema et al., 2016), growth (Besseling et al., 2014; Lagarde et al., 2016), gene expression and colony size and morphology via adhesion and/or transfer of pollutants associated with MPs (Yokota et al., 2017). Studies have shown that primary producers serve as MP net autotrophic "hot spots" (Bryant et al., 2016). MPs ingested by marine organisms are dependent on the feeding mode, habitat, diet and age (Ryan, 1987; Ryan et al., 2020) and their interaction with water and sediment (Pinheiro *et al*., 2020). The direct ingestion of MP particles is a result of accidental consumption due to non-selective feeding strategies (e.g. filter-feeding) or MPs being mistakenly identified as food through more active-selection (Hall et al., 2015; de Sá et al., 2018; Nelms et al., 2018). Filter-feeding organisms, e.g. mussels and sea cucumbers are susceptible to ingesting MPs as they are non-selective to particle filtration and ingestion is dependent on size of MP. This suggest that feeding strategies are not only responsible for the ingestion of MPs, but the abundance and size thereof (Fang et al., 2018). Studies have shown that filter-feeding marine invertebrates tend to ingest more MPs than grazers, predators and deposit-feeders (Setälä et al., 2016; Taylor et al., 2016).

The benthic environment has been identified as sink and source of MPs (Browne et al., 2011) with particularly high concentrations near developed coastal areas (Browne et al., 2011; Vikas & Dwarakish, 2015). Factors that influence MP concentrations in the benthos include MP density, weathering, biofouling, egestion and gravity (Peng *et al*., 2018; Woodall et al., 2014). The benthic zone has the potential to facilitate the uptake of MPs in organisms residing in these areas (Martin et al., 2017; Pagter et al., 2021; Taylor et al., 2016). A study by Pagter et al (2020) investigated the difference in MP abundance within demersal communities and found that MP abundance varies depending on the factors influencing bioavailability. The study also suggested that the bioavailability of MPs in species is influenced by several factors and that more research needs to be done on MP abundance in epifauna and infauna.

MPs have the ability to be ingested indirectly as a result of trophic transfer (Eriksson & Burton, 2003; Murray & Cowie, 2011; Nelms et al., 2018). This is due to predators consuming prey already contaminated with MPs (Farrell & Nelson, 2013). Studies conducted on the fur seals have found MPs ingested by lantern fish was present in seal scat, suggesting that MPs present in fish is transferred to seals (Eriksson & Burton, 2003). An experimental study by Murray & Cowie (2011) exposed fish to polypropylene (PP) and were fed to lobsters. The results showed the polypropylene was found 24 hours later in stomachs of the lobster. This suggests that MPs are not only transferred from one trophic to the next, but has the potential to bioaccumulate and bio-magnify (Farrell & Nelson, 2013; Teuten et al., 2007). (Braid et al., 2012) found plastic pellets in the stomachs of mass stranded Humboldt squids. It is important to note that these predators feed at depths between 200 and 700 m. The study was not able to point out the exact route of uptake, but suggested that the pellets were consumed directly due to it sinking or indirectly through organisms with pellets already present in their digestive system (Braid et al., 2012; A. Lusher, 2015a).

Ingested MPs have the potential to pass through the gut or may be retained in the digestive tract (Browne et al., 2008; Iwalaye et al., 2020a). Iwalaye et al (2020) examined the pathways of MP uptake in sea cucumbers and found that all samples ingested microfibres via their tentacles. However traces of microfibres were also found in the coelomic fluid and respiratory trees. Another study by (Watts et al., 2014) observed that crabs do not only ingest MPs along with food but accidently draw plastics particles into the gill cavity via their respiratory mechanism. The adverse effects of the bioaccumulation of MPs in the intestines has the potential to clog the digestive system causing a false sense of satiation that results in a decrease in the consumption of food (Gregory, 2009; Iwalaye et al., 2020a). This highlights the importance of potential routes of exposure and translocation of MPs in marine invertebrates. Exposure studies have recorded MPs concentrations to exceed the expectation of concentrations observed in the field (A. Lusher, 2015a). This raises the concern for studies to be conducted on the retention and long-term effects of MPs in marine organisms.

Investigating the factors that affect MP ingestion could assist in not only understanding MP abundance in marine biota (Pagter et al., 2021) but how MPs are transmitted throughout the marine environment (Moore, 2008; Pinheiro et al., 2020; Tanaka et al., 2013).

2.3 The fate of plastic in Rocky Shores

When plastic enters the marine environment it has the ability to migrate between shoreline and open sea via waves, run off and winds (Nagelkerken et al., 2001; Thushari et al., 2017a). Plastic litter on sandy beaches have been monitored and documented as it is easily accessible and have been useful in identifying sources of litter (Ryan, 1990; Ryan et al., 2018). However, there are fewer efforts to monitor and assess the distribution and accumulation of plastic litter and MPs in rocky shores (Thiel et al., 2013).

Rocky shores occur at the interface of land and sea, forming a narrow border around the coastline of a country (Branch et al., 2008a; Satyam & Thiruchitrambalam, 2018; Thompson et al., 2002; Underwood, 2000). The structure of rocky shores differ depending on region, wave exposure, rock type and sand cover (Hill et al., 1998; Branch and Branch, 2018; Sunamura, 2015). As a results Organisms inhabiting these ecosystems experience daily and seasonal fluctuations in their living environment. Organisms are therefore diverse and well-adapted to tolerating extreme changes in temperature, salinity, moisture and wave action to survive (Satyam & Thiruchitrambalam, 2018). In addition, organisms are under increasing threat from anthropogenic activities.

Studies have shown a great difference between plastic litter present on sandy beaches compared to plastic litter found in rocky shores. This is due to fact that rocky shores harbour debris differently to other marine ecosystems. Once plastic is introduced to the rocky shore environment, plastics are subject to being caught in rocks, prolonging its bioavailability in rocky areas compared to sandy beaches (Crowe et al., 2000; Eriksson et al., 2013). Environmental factors such as wind and wave action has the potential to bury plastic debris in rocks and sand (Kusui & Noda, 2003; S. D. A. Smith & Markic, 2013). In Taiwan, plastic litter showed to be significantly higher in rocky shores than on sandy beaches indicating greater efforts are required to cleaning rocky shore areas (Kuo & Huang, 2014; Weideman, Perold, Omardien, et al., 2020). Thiel et al. (2013) reported higher concentrations of polystyrene in rocky shores than sandy beaches in northern-central Chile.

Monitoring plastic debris in rocky shores is crucial for understanding marine debris trends not only these areas but also how they affect plastic concentrations found in other marine ecosystems. Plastic litter protocols on sandy beaches have been well developed and standardised, whereas protocols for rocky shores are not (McWilliams et al., 2018).

2.3.1 Microplastics in Rocky shores

Rocky shores serve as "grinding mills" that break larger debris and has the ability to accelerate the rate at which MPs are formed (Debrot et al., 1999; Eriksson & Burton, 2003) which is why environmental dynamics of rocky shores are important for understanding marine MPs contamination. Studies have shown that while macroplastics have the ability to work their way up to the surface and MPs remain buried in sediment. However, rocky shore ecosystem have very little to no sediment, and MPs are constantly being resuspended in the water coloumn, increasing the interaction between MPs and he environment. This poses a potential threat to the ecosystem and could further aggravate the potential impact of contaminates and the effects on ecosystem communities.

Nel & Froneman (2018a) investigated MPs in the tube structure of intertidal polychaetes. These reef-building sabellariid polychaete construct intertidal habitats by cementing suspended sand grains and particles from the water coloumn. From this study, it was suggested that MPs present in the water coloumn has the potential to be incorporated in the structure of the polychaetes (Nel & Froneman, 2018a).

McWilliams et al (2018) investigated protocols for MPs in rocky shores of the Fogo Islands. Although they were not able to state the factors contributing to the horizontal and vertical distribution of MPs, the results however did report on the type, size and location of MPs from the source. The study was also adapted to allow trends to be examined in rocky shores that were overlooked in shoreline protocols. (Pinheiro et al., 2019) investigated the effects of beach rocks on MP deposition on the strandline of sandy beaches and found that beach rocks influence the accumulation of MPs on the beach face.

Rocky shore ecosystems are situated all along the South Africa's coastline, experiencing tremendous environmental pressure from densely populated areas and related activities. Anthropogenic activities pose a threat to these marine ecosystems through plastic contamination and potentially polluting these areas (Branch et al., 2008b; A. Lusher, 2015b). In South Africa studies have surveyed macro- and MP litter on sandy beaches (Ryan, 1990, 2020b; Ryan et al., 2018) and great efforts are being made for investigating MP contamination in rocky shore organisms (Nel & Froneman, 2018b; Sparks, 2020; Weideman, Perold, Arnold, et al., 2020; Weideman, Perold, Omardien, et al., 2020).

2.4 The fate of plastic pollution along South Africa's coastline

South Africa's coastline stretches approximately 3400 km and is known for its rich biodiversity (Harrison, 2004; Naidoo, 2018). The coastline possess natural bays, coves and estuaries (Rust, 1991), harbours and marinas. In 2011 approximately 40% of the country's population lived within 100 km (Wigley, 2011) of the coast and rely on the coast for various services, one of which being food (Atkinson & Clark, 2005). South Africa is a developing country with a slow growing economy. As a consequence socio-economic issues are prioritised over environmental issues, such as plastic pollution. South Africa's plastic industry contributed approximately 1.6 % to the gross domestic product (GDP) in 2014 (Steyn, 2016). The plastic industry has employed over 60 000 people and government has recognised promoting the sector to secure sustainable economic growth and employment creation. In 2017 South Africa produced 42 tonnes of waste, of which only 11 % was recycled (Department of Forestry Fisheries and Environment, 2018). Even though legislation encourages recycling and the sustainable use of natural resources, efforts are primarily implemented through corporate initiatives, non-profit organisations, domestic recycling and informal collectors.

South Africa's poor management plan and lack of infrastructure has led to the alarmingly large number of plastic entering the marine environment. Research is important to monitor sources and understand spatial distribution of plastic contamination as it will aid in determining the health of the environment. The Western Cape Province, located in the south west part of South Africa, is responsible for 20 % of the total waste produced in South Africa. Cape Town accounts for 64 % of the total population and possess the major metropole of this region. In addition the Western Cape has the highest number of waste water treatment plants compared to other provinces (de Villiers, 2018). The Western Cape is an infamous tourist destination due its pristine beaches and highly aesthetic coastal environment (Sparks, 2020). The tourism sector contributes significantly to the country's economic growth and therefore the health of the environment is important for visiting tourists (Rust, 1991).

There are approximately 300 river outlets that are seasonally connected to the ocean and linked to MP contamination in surrounding areas. (Ryan & Perold (2021) investigated stranding litter around a river mouth in Cape Town, South Africa, and found that litter is carried down by smaller rivers and shortly wash ashore after entering the ocean.

In addition, four of the largest cities in the country is located along the coastline where waste water treatment facilities discharge waste directly into the coastal zone (de Villiers, 2018). Waste water discharged into South Africa's marine environment include municipal wastewater (often also including trade effluent), effluent from fish processing operations, wastewater from chemical works, refineries and other industries, and cooling water (Sink et al., 2012). It is reported that only a fraction of South Africa's sewage is treated before being discharged (Brown, 1987), and with increase in urbanisation and poor management of waste water treatment works (WWTW) (Mema, 2010), has led to plastic contamination in the marine environment.

Oceanographic models predict that depending on the location of source and density, plastic entering the marine environment from South Africa has the ability to be exported to the South Atlantic and Indian Ocean (Collins & Hermes, 2019). (Ryan, 1988) investigated plastic particle distribution at the sea-surface off the southwestern Cape Province of South Africa and found plastic particles enter via land-based sources or ships. The study also noted that circulation patterns within the southwest Cape Province region is responsible for the spatial distribution of plastic.

Another factor contributing MPs entering the ocean is a result of urban-industrialisation along South Africa's coastlines. These MP particles tend to accumulate close to coastal urban-industrialised areas (Collins & Hermes, 2019). Naidoo & Glassom (2019) investigated plastic concentrations at sea-surfaces along the coastal shelf of KwaZulu-Natal and found significantly higher plastic concentrations near urbanised areas. Other studies investigated plastic debris on

South Africa's beaches and found that higher concentrations of plastic is found on beach close to urban-industrial areas (Ryan et al., 2018). P. G. Ryan et al (2018) found that the mean concentration of pellets decrease in size the further away from local urban centres, suggesting that meso- and MP contamination is a result of local, land-based sources.

2.4.1 Microplastic research along South Africa's coastline

The first study investigating MPs along the coast of South Africa was conducted by P. G. Ryan (1987) investigating plastic particles ingested by seabirds. Since then there has been numerous investigations and studies towards understanding the factors and sources contributing towards the distribution and concentration of MPs along South Africa's coastline.

Nel & Froneman (2015) investigated MP concentrations in water and sediment along the coastline of South Africa and found significantly higher concentration of MPs in sediment than in water samples. Another study by de Villiers (2018) investigated microfibre concentrations along South Africa's beach sediment and found that the highest level of microfibres were recorded from sites close to large coastal waste water treatment discharge points. The study suggests that waste water treatment discharge points are sources of MP contamination in beach sediment.

Naidoo et al (2015) investigated MP levels within five estuaries long Durban's coastline and intervening beaches. The results showed that high MP concentrations were observed in the harbour. The study also found that concentrations of MPs decrease the further away sampling sites were from the harbour (Naidoo et al., 2015). Sparks & Awe (2022) investigated MP in the coastal sediment of a marina in Simon's Town, South Africa and found that MP filaments was highest close to stormwater outfall pipes. Preston-Whyte et al (2021) monitored MPs in the Port of Durban and found that concentrations were significantly high within in the harbour, particularly at sites closer to stormwater drain inputs. All three studies suggest that the storwater/outfall pipes are sources of MP contamination in coastal areas of South Africa.

Studies investigating MPs in harbours found that harbour sediment acts as sinks for contamination from surrounding industries and urbanisation (Knott et al., 2009; Su et al., 2019). Sparks & Awe (2022) investigated MP concentrations from antifouling paint particles (APP) in the coastal sediment of a marina in Simon's Town and found that contamination is a result of vessel maintenance.

Sparks (2020) investigated MPs in mussels along the coast of Cape Town, and found that 98% of mussels analysed contained MPs ranging in type, colour and size. Iwalaye et al. (2020b) investigated MP occurrence in marine invertebrates from KwaZulu-Natal and found MP in more than 95% of samples, of which fibres were the predominant MP. The study also suggested that feeding methods affect the accessibility of MP ingestion in marine invertebrates (Iwalaye et al., 2020b). Sparks et al. (2021) investigated the presence of MPs in retail mussels sold in supermarkets and wholesalers in Cape Town, South Africa and although concentrations were low compared to other studies, routine monitoring of seafood was suggested. Mussels as a resource is consumed by humans, and with the increase in MPs found in coastal environment, there is great concern regarding the indirect consumption of MPs found in mussels and their potential health risks (Seltenrich, 2015; Sparks et al., 2021).

2.4.2 Factors and sources affecting MPs into the marine environment along the Western Cape coastline.

The Western Cape has the longest coastline in South Africa. The coastline stretches over 1000 km from north of the Olifants River on the Atlantic Ocean coast, to the mouth of the Blaaukrantz River on the southeast coast. The coastal environment is diverse and dynamic and is influenced by both the cold, northward-flowing Benguela Current and warm, southward-flowing Agulhas Current. The coastline possess various environments ranging from sandy beaches and rocky shores, to estuaries and wetlands. The coastal zone is relatively narrow and is directly influenced by the interaction between land and sea. Most people live within 25 km of the coast and as a result there is a higher risk of pollution in and around the coastal areas. Populated coastal areas have been associated with high MP concentrations as a result of more point sources such as WWTW, potentially contaminating nearby marine environments (Murphy et al., 2016; Nel et al., 2017; Nelson & Hutchings, 1983) .

There are various environmental factors and sources that contributes towards the potential contamination of MPs in the marine environment along the coastline of the Western Cape. Sources include a combination of land- and marine based sources from river systems, streams, stormwater and sewerage outfalls, WWTW, dredging and dumping and shipping activity (Ryan, 2020b; Verster & Bouwman, 2020; Weideman, Munro, et al., 2020; Weideman, Perold, Omardien, et al., 2020). Between 60 – 90% of plastic waste is land-based and is stranded along the coastline (Ryan, 2020b). One of the major contributors towards urban litter entering the sea is run-off from stormwater drainage systems. Stormwater systems transport large amounts of rainwater from the streets and have the potential to move waste from gardens, roofs, footpaths, streets and parking lots. In South Africa stormwater run-off is not processed before it is discharged into the marine environment. There are approximately 124 authorised outfall pipes in the Western Cape is approximately 124, 44 of which are not (Department of Forestry Fisheries and Environment, 2018). It is reported that the highest waste water contributor in the Western Cape is from aquaculture facilities.

The West Coast region of South Africa stretches from Cape Town to the Northern Cape at Touws River. The West coast is located within the northern Benguela upwelling system. The Benguela current is slow flowing current and water circulation is driven by large-scale winds resulting in an anticyclonic motion and thermohaline forcing (Fennel, 1999; Garzoli & Gordon, 1996). Longshore equatorial winds result in coastal upwelling (Shannon & O'toole, 1999). Upwelling is a process whereby cold, bottom water is brought to the surface. The coastal route from Cape Town to Lambert's Bay is 270 km and includes coastal towns Langebaan, Saldanha Bay, Velddrif and Paternoster. River systems that are potential sources of MP contamination include the Berg River, Verlorenvlei and Jakkalsrivier (City of Cape Town, 2019). The Berg River is the dominant perennial river of this region and drains into the Atlantic Ocean at St. Helena Bay. The coastal zone is a mixed-used area, where fishing, aquaculture and agriculture is the dominant activity. In addition, the Berg River is the main source of water for farms located along the river banks and where a major marina is located at Port Owen. Studies have shown that MP tend to accumulate around areas close to aquaculture farms and fishing grounds and are regarded as MP pollution "hotspots" (Xu et al., 2018). The various activities, such as fishing, sea-side resorts and popular lagoons, taking place in and around this region has made the West Coast susceptible MP contamination.

Table Bay is located within the southern Benguela upwelling system. Water circulation within the bay is primarily drive by wind, shelf and offshore currents and tides. Wave-driven flows have an effect on the nearshore. During summer, south-easterly winds cause currents to flow northwards in an anti-clockwise direction within in the bay (Lamprecht et al., 2013; Quick & Roberts, 1993). Cold upwelling water enters Table Bay from Oudekraal, located at the south of Table Bay. This upwelling event results in shoreward bottom flows. Surface currents are generally weak in this area, with very little to no influence from outside currents (Quick & Roberts, 1993). The coastline stretches 19 km from Melkbosstrand to Cape Town. The coastline is consists of 13 km of sandy beach (between Blouberg and Table Bay harbour, 3 km of rocky shore (between Blouberg and Mouille Point) and 4 km of artificial shore protection and breakwaters (Port of Cape Town) (Lamprecht et al., 2013).

Cape Town is the largest coastal city in South Africa with a population of 4.5 millions people (World Population Review, 2020; Weideman, Perold, Arnold, et al., 2020) and is responsible for approximately 40% of the plastic entering the marine environment (Collins & Hermes, 2019; Weideman, Perold, Arnold, et al., 2020). In Addition, Cape Town harbour forms part of Table Bay which is a potential MP pollution hotspot (Xu et al., 2018). There are a number of river systems and streams that are potential sources MP contamination within Table Bay include Salt River, Liesbeek River, Black River, Elsieskraal River, Camps Bay stream, Diepsloot River, Blinkwater River, Kasteelpoort River, Platpoort River and Lekkerwater River (City of Cape Town, 2019). The Salt River system joins the Liesbeek and Black River and drains straight into Table Bay, whereas as the other river systems drain straight into the Atlantic ocean (City of Cape Town, 2019). Studies have identified the Black River as a source of pollution due to effluents from industrial and residential areas in and around Cape Town (Scarfe *et al*., 1985). The Table Bay district includes major metropolitan nodes, including the Cape Town CBD (City of Cape Town, 2019). The coastal zone from Mouille Point to Camps Bay is a mixed-use area where commercial, residential and recreational activities take place. A notable feature of this region is the large amount of WWTW dedicated to processing domestic waste. Effluent pipelines discharge directly into near shore environments and contaminate coastal surface waters (City of Cape Town, 2019) (de Villiers, 2018). In addition there are various storm water pipes that also directly discharge into coastal surf zone (de Villiers, 2018). Other factors that have the potential to influence MP contamination and quality of coastal waters around Table Bay include discharge and spills from activities in Cape Town harbour.

False Bay is located on the south side of the Cape Peninsula. The area extends from Cape Hangklip on the east to Cape Point on the west (City of Cape Town, 2019). Prevailing cyclonic, southerly winds is responsible for the clockwise circulation in False Bay (Pfaff *et al*., 2019). Near the mouth of the bay, surface currents flow in a westward direction and are controlled by weather, shelf waves, the warm Agulhas rings and eddies (Griindlingh & Largier, 1988; Lutjeharms, 2006; Pfaff et al., 2019). During summer south-easterly winds cause off-shore transport and upwelling at Cape Hangklip, which enters False Bay. There are 11 small estuaries and river systems that discharge directly into False Bay, the largest being Zandlvei (O'callaghan, 1990; Pfaff et al., 2019). River systems within False Bay include Lourens River, Sir Lowry's River and the Steenbras River (O'callaghan, 1990). All the mouths of the estuaries are seasonally closed naturally, with Steenbras River being an exception as it is permanently open to the sea. However, abstraction, flow diversions and waste-water discharges interfere with the seasonal patterns, increasing the time smaller systems are closed and cause permanently open-mouth conditions (Pfaff et al., 2019; van Niekerk et al., 2015). Pressures surrounding the 11 estuaries within False Bay are a result of highly urbanised areas, WWTW and industrial effluent, urban and agricultural runoff, degrade catchments, infilling of open water areas, development of marinas, harbours, mouth manipulation and recreational activities (Pfaff et al., 2019; van Niekerk et al., 2015). In addition, False Bay is impacted by beach goers, invertebrate harvesting for bait and coastal development (L. Harris et al., 2015; Pfaff et al., 2019). Potential sources of high MP contamination along the northern coastline include leakage from sewers and contaminated stormwater, with localised contamination hot spots from Kalk Bay harbour (Pfaff et al., 2019; Rundgren, 1992) and The Sir Lowry's River receiving waste water from Gordan's Bay WWTW (Pfaff et al., 2019; Rundgren, 1992).

False Bay possess a variety of intertidal rocky shores varying in wave exposure, temperature and rock type, all of which impact community structure and functioning (Pfaff et al., 2019). Most of the MP contamination is found near these intertidal rocky shores, where it is trapped between rocks or has the potential to be ingested by benthic organisms. Factors that impact the distribution and amount of plastic waste within False Bay is caused by northwest winds driven by upwelling events. The highest concentration of plastic waste was observed near the mouths of Zeekoei, Eerste and Lourens rivers, suggesting that the major source of pollution is a results of water-borne run-off (Jury, 2020; Pfaff et al., 2019). There is little efforts towards management and monitoring along the False Bay coast, with the exception of Zandlvei which has an active Estuary Management Forum (Zandvlei Trust) and regular monitoring. Other monitoring programmes that focus on water quality occur at Silvermine, Zeekoei, Eerste and Lourens estuary (Pfaff *et al*., 2019).

2.5 Echinoderms

Echinoderms are amongst the most conspicuous marine invertebrates. There are five echinoderm classes comprising of Holothruoidea (sea cucumbers), Echinoidea (sea urchin and sand dollar), Asteroidea (sea stars), Crinoidea (feather star and sea lilies) and Ophiuroidea (brittle star). Their radially symmetrical bodies are made up of a water vascular system unique to this phylum. The water vascular system connects with tube feet which are extensions of the body wall that protrude through holes in the skeleton. The tube feet possess suction cups on the tips and are used for locomotion, feeding, respiration and sensory reception.

The water vascular system consists of a ring canal, radial canal and ampullae. The ring canal surrounds the digestive tract and five radial canals radiate from the ring canal like spokes of a wheel. The ring canal is connected to a porous plate called the madreporite, by a lime-walled tube called the stone canal. The madreporite varies in different groups. Waters enters the vascular system through the madreporite. Short lateral canals made up of valves connect from the radial canals into the tube feet. A muscular, water-filled bulb called the ampulla is connected to each foot. As the valve closes the ampulla contracts, water is squeezed into the tube foot, resulting the foot being extended. The foot is retracted when the attached muscle contracts, forcing the water back into the ampulla. Sea cucumbers, sea urchins and sea stars move by extending and retracting groups of tube feet, using suction cups to grip and pull them along. The tube feet are thinly-walled with a surface that aids in the inward diffusion of oxygen into the body cavity and outward diffusion of carbon dioxide and wastes.

The tube feet are responsible for respiratory function in most echinoderms; however, some groups have developed auxiliary respiratory structures. Echinoderms have no excretory organs and possess an open circulation system. The body cavity, containing the coelomic fluid, possess flagellated cells creates an internal current. Amoebocytes, found in the coelomic fluid, is responsible for transporting food and storing insoluble waste. Echinoderms have a simple, underdeveloped nervous system that is sensitive to light, temperature and vibrations. Some sea star and sea urchin species are able to perceive light and dark, some even showing some level of vision.

They are only found living in the marine environment inhabiting a variety of habitats, from the poles to the equator and from the intertidal zone to the deep sea, with greater abundance and diversity in shallower shelf areas (Ahmed et al., 2022; de Moura Barboza et al., 2011; Filander & Griffiths, n.d.; Gomes & Madeira, 2019; L. C. Smith et al., 2010). Sea cucumber are found inhabiting all marine environments, from intertidal zones to deep benthic areas. Sea cucumbers prefer inhabiting muddy sand as it possess high levels of organic material and easy to consume. However, they found living in coarse sand and rocky environments. Sea urchins are found aggregating in the crevices of rocks by day and emerge at night (Day & Branch, 2002;

Hammond et al., 2013; Underwood, 2000). Sea stars have been found areas from the lower intertidal zones to sandy benthic areas of up to 650 m (Garrido et al., 2021).

Despite sharing the same niche with various other marine species, echinoderms have various different feeding modes ranging from filter- and deposit feeding (such as sea cucumbers), grazers (such as sea urchins and cushions stars) to active hunters and scavengers (such as sea stars) (Ahmed et al., 2022). Most echinoderms have straight forward digestive system whereby food enters the mouth and exists through the anus. Sea cucumbers burrow in the sand using their mouth and directly swallow surrounding sediment using their tentacles. The characteristics of the sand particle size consumed is a reflection of its preferred substrate (Sabilu et al., 2021). Sabilu et al. (2021) investigated the relationship between sand particle size in the natural habitat and digestive tract of sea cucumbers and found that smaller sea cucumbers tend to consumer higher amounts of coarser sand compared to larger sea cucumbers consuming a finer sand. Sea star species are predatory feeders, some possessing a two-part digestive system, composed of a cardiac stomach and an internal pyloric stomach. The cardiac stomach has the ability to extend out through the mouth, digest the tissue of the captured prey, all while outside the body. The internal pyloric stomach is observed during feeding, appearing as an amorphous translucent blob, digesting food and processing nutrients throughout the body.

2.5.1 The role of echinoderms in the environment

Keystone species are species that play a vital role in the maintaining and functioning of the structure of an ecological community. Echinoderms are particularly resilient and due to their high abundance, biomass and diverse feeding strategy, they play an important role in energy transfer among trophic levels and structuring nearshore ecosystems within coastal communities (Ambrose et al., 2001; Chenelot et al., 2006). Sea cucumbers are bioturbators and are responsible for the distributing bacteria, exchange of nutrients and dissolved oxygen in marine sediment (Hammond2, 1981; Kroh & Smith, 2010; Purcell et al., 2016). Sea urchins forage on macroalgae and control kelp forest density, affecting community function and structure. Mcclintock & Robnett (1986) found that sea stars in rocky shores of the Pacific Northwest maintain the diverse community and prevent mussels from out-competing other species occupying the same habitat. Sea stars (Asteroidea) are one of the most diverse classes of echinoderm species. They are top predators and are considered keystone species as they influence community structure by regulating the abundance and distribution of other marine invertebrates (Castelló y Tickell et al., 2022; Cossi et al., 2021; Mah & Blake, 2017).

2.5.2 MPs in echinoderms

Given the ecological importance of echinoderms in the marine environment, there is dearth data available on the ingestion of MPs in echinoderms (Bour et al., 2018; Cossi et al., 2021). Some of the earliest attempts to investigating MPs in echinoderms reported synthetic polymers in benthic sea stars, namely in *Hymenaster pellucidus, Asterias rubens, Ctenodiscus crispatus and Leptasterias polaris* species (Cossi et al., 2021; Fang et al., 2018). Gerber & Robertson-Andersson (2017) investigated the ingestion of MPs in *Stomopneustes variolaris* and *Tripneustes gratilla* and found that the size of the madreporite in sea urchins are large enough for MPs to enter the water vascular system, allowing for the uptake of MPs. This shows dual mechanisms of MPs in echinoids (Gerber & Robertson-Andersson, 2017). Fendall & Sewell (2009) investigated MPs in facial cleaners and found that filter-feeding echinoderms ingested MPs of up to < 2 mm in size. Graham & Thompson (2009) studied deposit- and suspension-feeding sea cucumbers and found that echinoderms are more likely to encounter MPs that are more dense than water. Cossi et al. (2021) investigated MPs in sea star samples collected from an MPA in Argentina and found fibres to be the predominant MP in 61% of samples.

Studies have shown that deposit-feeding sea cucumbers inhabit low-energy environments with rich organic sediment (Pawson et al., 2010; Plee & Pomory, 2020). Sediment is known to be a MP sink and sea cucumbers inhabiting these areas are susceptible to ingesting MPs due to availability and feeding strategy (Plee & Pomory, 2020; Vianello et al., 2013). Graham & Thompson (2009) conducted a laboratory experiment and found that sea cucumbers preferred ingesting plastic fragments to sediment. Sea stars are amongst the top marine predators in benthic communities. Studies have shown higher contamination levels in sea stars than in other organisms, as they directly consume MPs present in water and sediment and/or indirectly through trophic transfer as they consume prey (Cossi et al., 2021). Courtene-Jones et al. (2017) and Fang et al. (2021) investigated MPs in various benthic organisms and found higher MP concentrations in sea star species. This is due to sea stars having the ability to consume macroscopic organisms (> 1 cm in length) which are much greater than the average MP size (Cossi et al., 2021; Howell et al., 2003).

2.6 Risk assessment

Plastic is synthetic or natural organic polymers mixed with additives to enhance, reinforce and improve its function and durability (Andrady & Neal, 2009; Bouwman et al., 2018). These additives allow for plastic products to range from, coatings, adhesives, sealants, synthetic fibres and foams (Andrady & Neal, 2009; Bouwman et al., 2018; Lithner et al., 2011). Plastic polymers are biochemically inactive due to its large molecular size and is therefore considered not hazardous to human health or the environment. However, polymerisation reactions are rarely completed and unreacted residual monomers are found in polymeric material, several of which are hazardous to human health and the environment (Andrady & Neal, 2009; Lithner et al., 2011). Depending on the type of the polymer, polymerisation technique and techniques used to reduce residual monomer content, will cause residual monomers to vary. In addition, other polymerisation impurities such as oligomers, low molecular weight polymer fragments, catalyst remnants and polymerisation solvents, could be present in plastic products (Lithner et al., 2011). These non-polymeric elements are of low molecular weight and have the ability to move from plastic products to air, water and other media (Crompton, 2007; Lithner et al., 2011). Lithner et al. (2011) developed a hazard ranking model of plastic polymers by incorporating the chemical hazard of additives, monomers, polymers and polymerisation. This hazard ranking model can be used to assess the hazard effects of plastic polymer on human health and the environment. This hazard ranking model is then used to conduct a risk assessment associated with MPs present in the environment. Xu et al. (2018) investigated the potential risk of MPs in surface waters of the Yangtze River Estuary and found that the score assigned to polymers affected the results. Laboratory exposure experiments have attempted to determine the risks associated with MPs but was unsuccessful as exposure concentrations are comparable to environmental thresholds (Koelmans et al., 2016; Xu et al., 2018). In order to accurately assess and determine the risks associated with MPs comprehensive data, such as abundance, potential sources, environmentally parameters and biological effects, needs to be collected (Zhang et al., 2022). Ambiguous terms such as "potential" and "could" are often used to describe the risks and policy makers are misled when developing or improving policies regarding pollution management (Koelmans et al., 2017; Xu et al., 2018).

Due to the lack of research done on MP concentrations in echinoderms in South Africa, the aim of this study is to 1) compare MP concentration and abundance in water (MPs/L), sediment (MPs/kg) and echinoderm species (MPs/g and MPs/I), 2) compare MP concentrations in echinoderm feeding-strategies, 3) examine the spatial distribution of MP contamination in rocky shores along Western Cape coastline, South Africa. A risk assessment was done to assess the risk posed by MPs in water, sediment and echinoderms.

Chapter 3

Materials and Methods

3.1 Study site

Sampling took place at low tide during summer 2020 at 14 sites along the coastline of the Western Cape, South Africa (**Error! Reference source not found.**). For the purpose of this study, sites were chosen based on distance from outfall pipes, stormwater drains (SWOD), river mouths, harbours/marinas and waste water treatment works (WWTW) as it is known to be potential sources of MP contamination.

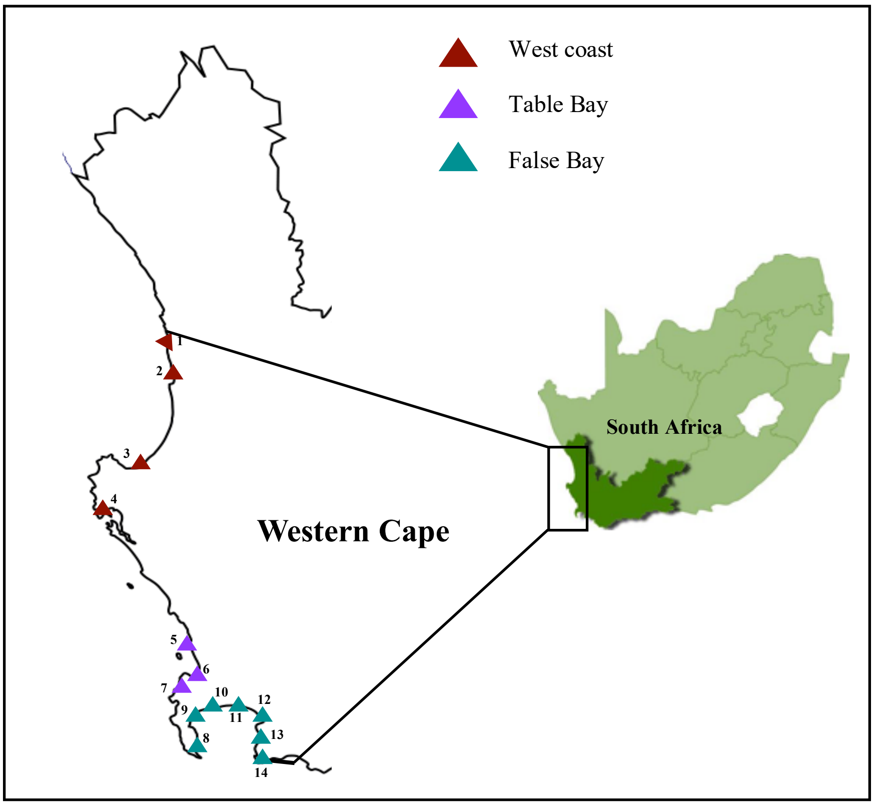

Figure 3.1: Study site including sampling stations 1: Lambert's Bay (- 32.085°S, 18.313°E), 2: Eland's Bay (- 32.316°S, 18.335°E), 3: Velddrif (- 32.771°S, 18.143°E), 4: Saldanha Bay (- 33.007°S, 17.946°E), 5: Blouberg (- 33.805°S, 18.463°E), 6. Mouille Point (- 33.899°S, 18.364°E), 7: Maiden's Cove (- 33.945°S, 18.374°E), 8: Simon's Town (- 34.190°S, 18.430°E), 9: Kalk Bay (- 34.127°S, 18.450°E), 10. Strandfontein (- 34.089°S, 18.553°E), 11: Strand (- 34.117°S, 18.825°E), 12: Gordan's Bay (- 34.166°S, 18.858°E), 13: Rooi Els (- 34.298°S, 18.820°E) and 14: Pringle Bay (- 34.343°S, 18.829°E).

South Africa's continental shelf varies with width and length. The Western Cape has a narrow continental shelf with a coastline stretching over 1 000 km (**Error! Reference source not found.**). The continental shelf along the west coast varies in width and depth. The Benguela is an eastern boundary current located on the west coast of South Africa and is one of four major currents systems in the world. The current is relatively slow (0.25 – 0.5 m.s^{-1}) flowing along the eastern Atlantic (Roberts, 2014). The oceanography of the Benguela region is influenced by winds at various times and spatial scales, ranging from basin-wide seasonal and longer period processes to local inshore events of a few hours (Hutchings et al., 2009; Shannon & O'toole, 1999). Coastal upwelling is a processes whereby cold nutrient-rich subsurface water is brought to the surface under the influence of longshore winds headed towards the equator (Hutchings et al., 2009; Shannon & O'toole, 1999). Both the West Coast and Table Bay region experience upwelling events during summer (Hutchings et al., 2009; Quick & Roberts, 1993; Shannon & O'toole, 1999). In addition, there are two current configurations within Table Bay, one in the central bay water and the other a bimodal long-shore current system (Quick & Roberts, 1993). The current generated by the central bay water circulation is wind driven and is either clockwise or anti-clockwise. Whereas long-shore currents are driven by swell direction. The water generally flows in a northerly direction (Quick & Roberts, 1993).

False Bay is a semi enclosed bay surrounded by mountains and comprised of linear beaches. False Bay is southward facing covering approximately 10 000 km^2 with Cape Peninsula on the west and Cape Hangklip on the east (Jury, 2020; Pfaff et al., 2019). Ocean circulation is driven by wind dynamics. Wave dynamics are controlled by south-easterly winds during summer and by north-westerly winds in winter (Jury, 1985). In summer the high-pressure cells of the South Atlantic and South Indian Ocean join and produce dry weather aiding in sea surface temperature (SST) and upwelling. Cyclonically sheared southerly winds, moving surface currents westwards in the bay (Jury, 2020) is responsible for the clockwise circulation within the bay.

Sources and factors influencing microplastic input and concentration along the Western Cape coastline include seasonal rainfall, tidal changes, sediment and rock type, extent of catchment areas, riverine inputs to coastal areas, population density, poor waste management, harbour related activities, waste water treatment works and outfall and stormwater pipes.

3.2 Field sampling

A permit granting permission to sample at various sites in the western Cape was obtained from the Department of Environment, Forestry and Fisheries (DEFF). In addition, ethical clearance was granted by the university.

Sampling for water, sediment and echinoderms took place at 14 different sites along the Western Cape, South Africa. Sites were chosen based on closeness to outfall pipe, river mouth, harbours and waste water treatment facilities (Table 0.1). Sites 1 and 2 were located 150 m from a closed river mouth (Table 0.1). Site 3 was located within in the Bergrivier. Sites 4, 5, 6, and 7 was located within 80 m of an outfall pipe. Site 8 was located within Simon's Town marina and sites 9 and 11 was located within 100 m of a stormwater outfall drain. Site 10 was located 100 m from a waste water treatment works. Site 12 was located 100 m outside a harbour. Sites 13 and 4 was located within 200 m from an open river mouth (Rooi Els Rivier and Buffels Rivier respectively).

Table 0.1: Site location and description

Site no.	Region	Site	Site type	Co-ordinates		Population	Urban/Rural	Potential MP sources	Distance from nearest water source (m)*
1	West Coast	Lambert's Bay	Rocky Shore	18.313	- 32.085	6 120	Rural	River (closed)	150
2	West Coast	Eland's Bay	Rocky Shore	18.335	- 32.316	1 525	Rural	River (closed)	150
3	West Coast	Velddrif	Harbor	18.143	- 32.771	11 017	Rural	River	0
4	West Coast	Saldanha Bay	Rocky Shore	17.946	- 33.007	111 173	Urban	Outfall pipe	80
5	Table Bay	Blouberg	Rocky Shore	18.463	- 33.805	172 601	Urban	Outfall pipe	30
6	Table Bay	Mouille Point	Rocky Shore	18.364	- 33.899	253 301	Urban	Outfall pipe	10
7	Table Bay	Maiden's Cove	Rocky Shore	18.374	- 33.945	253 301	Urban	Outfall pipe	30
8	False Bay	Simon's Town	Marina	18.430	- 34.190	6 700	Urban	Marina	0
9	False Bay	Kalk Bay	Rocky Shore	18.450	- 34.127	700	Urban	SWOP**	10
10	False Bay	Strandfontein	Rocky Shore	18.553	- 34.089	37 911	Urban	WWTW***	100
11	False Bay	Strand	Rocky Shore	18.825	- 34.117	55 558	Urban	SWOP	50
12	False Bay	Gordan's Bay	Rocky Shore	18.858	- 34.166	16 776	Urban	Harbour	100

13	False Bay	Rooi Els	Rocky Shore	18.820	- 34.298	125	Rural	River (open)	200
14	False Bay	Pringle Bay	Rocky Shore	18.829	- 34.343	801	Rural	River (open)	5

*Outfall pipe, stormwater outfall pipe, river mouth, harbour/marina and waste water treatment facility.

** Stormwater outfall pipes

***Waste Water Treatment Works

Five replicates of 20 litres of water was collected using a metal bucket per site. Water samples were filtered on site with a 250 µm metal sieve, where the remaining MP particles on the mesh were transferred to a (pre-cleaned) Falcon tube, stored on ice and taken to the laboratory freezer until further analysis. Sediment samples was collected at the high tide strandline parallel to the coastline. Collection took place downwind of the sampling area to minimis MP contamination from clothes worn out in the field. Five random replicates (5 m apart) along the strandline were sampled at each site using a 0.25 m x 0.25 m quadrate and collected at a depth of 5 cm using a metal spoon. Sediment samples were stored in Ziploc bags and taken to laboratory until further analysis.

Echinoderms collected was subject to availability (Table 0.2) and at least two echinoderms types (sea cucumber, sea urchin, sea star or cushion star) was collected from each site. Sites 13 and 14 possessed no echinoderm species and at site 3 only 10 sea urchins were collected without affecting the ecosystem. Samples were placed in Ziploc bags and stored in a cooler box with ice bricks to reduce metabolism and prevent loss of MPs through depuration (A. L. Lusher et al., 2017). Organisms were transported to the laboratory and kept in the freezer for further processing and analysis.

Table 0.2: Number of echinoderms collected per site along Western Cape coastline, South Africa.

Site no.	**Site**		**n**			
			Sea cucumber[a]	**Sea urchin**[b]	**Sea star**[c]	**Cushion star**[d]
		Feeding strategy	Suspension/deposit	Grazer	Predator	Predator
1	Lambert's Bay		30	-	-	30
2	Eland's Bay		27	-	-	30
3	Velddrif		-	-	-	-
4	Saldanha Bay		-	-	-	-
5	Blouberg		-	10	-	-
6	Mouille Point		24	21	-	-
7	Maiden's Cove		20	18	-	-
8	Simon's Town		-	-	30	30
9	Kalk Bay		21	20	-	-
10	Strandfontein		-	20	-	30
11	Strand		20	-	-	19
12	Gordan's Bay		-	29	21	-
13	Rooi Els		22	21	-	-
14	Pringle Bay		-	27	-	24

n = Total number of biota

[a] *Roweia frauenfeldii* and *Roweia stephenson*

[b] *Parenchinus angulosus*

[c] *Marthasterias africana*

[d] *Parvulastra exigua*

3.3 Laboratory analysis

3.3.1 Water processing

After samples were collected it was processed based on the method by (Sparks, 2020). All glass wear were autoclaved to minimise. Water samples were removed from freezer and left to thaw at room temperature. Samples were then transferred to a glass jar and digested in 10% potassium hydroxide (KOH) (ratio of 1:2 water sample: acid) for 24 hours at 50 ^{o}C to digest organic material. To make 1 L solution of 10% KOH, 100 g of KOH is added to 900 ml of filtered reverse osmosis (RO) water and stored in a dark bottle. Samples were then filtered through a 20 µm mesh using a Buchner Funnel system and vacuum pump. The jar was rinsed 3 times with 10 µm filtered RO water and filtered through same 20 µm mesh. The mesh was removed using a tweezer and placed in a covered labelled petri-dish to prevent and minimise air born contamination. The samples were then air-dried before analysing for MPs under a stereo microscope.

3.3.2 Sediment processing

Sediment samples were removed from the Ziploc bags and separated into 2 (sample A and sample B) aluminium containers for each site. Sample A was used for MP analysis and sample B was used for grain size analysis. Samples were then covered with aluminium foil and put in the oven to dry at 60 °C (24 hours). Once dried, 100 g of dry sediment from sample A (MP analysis) was weighed into a glass jar and digested in 10% KOH (ratio of 1 : 2 ; sediment sample : acid) for 24 hours at 50 °C. A hypersaline solution using NaCl was prepared by adding 360 g NaCl to 1 L filtered RO water. Once salt was dissolved, the hypersaline solution was filtered through a 10 µm filter to account for potential contamination from salt manufacturer. To extract MPs from sediment samples, the hypersaline solution was added to digested sediment sample (ratio 1 : 2 ; sediment : hypersaline solution) and stirred vigorously with a metal spoon for 2 minutes. The sample was then left to settle for 15 minutes. Once the sample was settled, the supernatant was filtered through a 20 µm mesh using a Buchner funnel system and vacuum pump. This process was repeated 3 times for the same sediment sample. The mesh was removed using a tweezer and placed in a covered labelled petri-dish to prevent and minimise airborne contamination. The samples were then air-dried before analysing for MPs under a stereo microscope. To conduct grain size analysis, 150 g of dry sediment of sample B was sieved through 1180 µm, 500 µm, 250 µm, 125 µm, 63 µm and < 63 µm mesh using a sediment shaker for 5 minutes . Sieves were then weighed with captured dry sediment.

The sediment weight obtained from each sieves category was processed through GRADISTATv9.1 to classify the sediment type.

3.3.3 Echinoderm processing.

Samples were removed from the freezer and left to thaw at room temperature. The body length (mm), weight (g), body diameter (mm) and mouth opening (mm) were recorded. Echinoderms were placed in individual glass jars and digested in 10% KOH for at least 24 hours at 60 °C. Once digested the samples were then filtered through 20 µm mesh using a Buchner funnel system and vacuum pump. The glass jar was rinsed 3 times with 10 µm RO water and filtered through the same 20 µm mesh. Sea urchin (*P. angulosus*) samples were first filtered through 125 µm mesh then through 20 µm. This is due to the fact that indigestible debris clogging the when filtering. The 20 µm mesh was removed using a tweezer and placed in a covered, labelled petri-dish to prevent and minimise air born contamination. The samples were then air-dried before analysing for MPs under a stereo microscope.

3.4 Microplastic visual identification using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

Identifying and recording MPs was done according to methods in GESAMP (2019). Samples were visually sorted under a Zeis DV 4 dissecting microscope (100x) and MPs were categorised according to type (filament, film, fragment, sphere), colour (white, transparent, yellow, red/pink, blue/green, black/grey) and size (< 100 µm, 100 – 500 µm, 500 – 1000 µm, 1000 – 2000 µm, 2000 – 5000 µm, > 5000 µm). The size category was measured using 1 mm x 1 mm graph paper placed at the bottom of petri-dish. MP particles were identified by possessing unnatural shape/type, colouration and size. MP count was peer reviewed to ensure all MP are accounted for. Once identified, 10% of the total number of MPs (> 500 µm) were identified according to their polymer type using a spectroscopy (Perkin Elmer Two ATR-FTIR spectrometer) following the methods of Sparks et al. (2021). Spectral wave numbers ranged from 4000 – 450 cm^{-1} , resolution set to 4 cm^{-1}, data interval set to 1 cm^{-1} and scans set to 10. A background scan was done before starting FTIR scans and the ATR crystal was cleaned with ethanol between scans. Polymer identification was done by comparing spectral scans with the ST Japan Library and a Perkin spectral library provided by the supplier (Perkin Elmer).

3.5 Quality control

In order to eliminate plastic contamination within field sampling and laboratory analysis the use of plastic was minimised in favour of metal and glass equipment and instruments. Lab doors and windows was closed to minimise airborne contamination as well as the cleaning of of work benches. Metal and glass wear was autoclaved, then rinsed with MilliQ ultra-pure water. The same clothes were worn so that it was easy to identify and eliminate MPs from samples collected in the field and when processing samples in the lab. Water, 10% KOH and hypersaline solution was filtered through 20 µm mesh to minimise and eliminate possible MP contamination. Samples and solutions in lab were covered with foil to prevent and minimise air born contamination. Extraction efficiency was done by filtering a known number of filaments and were treated and processed the same as water processing procedure. MPs were then counted and recovery percentage was calculated (at least 80% was recovered). Blanks (negative controls) were filtered to eliminate possible contamination from the filtration system. Positive controls (petri-dish with damp filter paper) were placed at the processing station in the lab to capture air born contamination for the duration of laboratory work. The controls are checked at the start and end of each day and contamination was recorded. Recorded contamination was then subtracted from analysed samples. Petri dishes were kept closed at all times and only opened when being anaylsed under the microscope to record MPs.

3.6 Risk Assessment Calculations

Microplastic indices was applied to all samples to provide comparative assessments of the potential risks of MPs in the environment, based on risk categories. The concentration of MPs ($C_{microplastic}$) compared to background concentrations is assessed using a MPs contamination factor (MPCF).

Equation 3.1 $$MPCF_i = \left(\frac{C_{microplastic}}{C_{baseline}}\right)$$

Where the $C_{baseline}$ value is the lowest average number of MPs at a particular site for water (Rooi Els), sediment (Simon's Town) and echinoderm (Rooi Els) samples, as there is no available historical data for the area and this method is considered acceptable (Kabir et al., 2021). MP pollution index (MPPLI) was calculated as follows

Equation 3.2 $$MPPLI_{site} = \sqrt[2]{MPCFil \times MPCFilm}$$

Where MPCFil and MPCFilm were MPCFs for filaments and film, respectively. Filament and film particles were the most abundant MP type across all samples types. The chemical toxicity of polymers was analysed based on the method used by Lithner et al. (2011), where hazard scores are assigned to polymer types to assess the risk of polymers

Equation 3.3 $$H_i = \sum P_n \ X \ S_n$$

Where H_i is the calculated polymer risk index, P_n is the ratio of a polymer type recorded at a site and S_n is the polymer hazard score assigned by Lithner et al. (2011). The pollution risk index (PRI) was calculated

Equation 3.4 $$PRI = \Sigma \ H_i \times MPPLI_{site}$$

Where PRI_i is the ecological hazard of polymers associated with polymer risk index (H_i).

3.7 Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics v28 software. MP data was expressed as counts per L, dry weight kg, weight wet (g) and per individual for water, sediment and echinoderm samples respectively. Descriptive statistics was calculated for sample type (water, sediment, echinoderms), region, urban/rural, site, echinoderm type and feeding strategy. Data was tested for normality using the Kolomogorove-Smirnov test in conjunction with reviewing the skewness and kurtosis values and histograms. Data was not normally distributed and non-parametric analyses was conducted using Kruskal-Wallis H tests for analysing significant differences (the significant level is $p < 0.05$) and H represents degrees of freedom between more than 2 groups (region, site, echinoderm type and feeding-strategy) and a Mann-Whitney U test for two groups (urban/rural) for different sample types (water, sediment and echinoderms). Post-hoc tests with pairwise comparisons using the Kruskal-Wallis H method was conducted to show significant different between two categories (the significant level is $p < 0.05$). Variance of data for statistical analysis was presented using standard error of the mean (SE) and presented as error bars on graphs. Significance of parameters was set at $p < 0.05$. Spearman rank correlation (r-value) was done to determine the relationship between water (MPs/L) and sediment (MPs/kg) concentrations, the relationship between MP concentration in echinoderms (MPs/g), water and sediment, the relationship between the total number of MPs and echinoderm weight (g) and the relationship MP concentration in sediment and grain size.

Chapter 4

Results

Extraction efficiency results showed 80% of MPs was recovered from the processing system. A total of 9252 MP particles was identified in all samples, of which 2.74% (261 particles) being airborne contamination (Appendices A). There was no contamination recorded from negative controls.

4.1 Water samples

4.1.1 MP concentration in water samples

Of the 70 water samples, 69 (98.57%) contained MP particles. A total of 1840 MP particles were recorded, with a mean concentration of 1.33 MPs/L (± 0.15 SE) (Figure 4.2). At a regional scale (Figure 4.2a) the mean MP concentration was highest in water samples collected from the West Coast (1.52 ± 0.20 MPs/L). There was a significant difference between MP concentrations in water samples across the regions ($H(2) = 7.376$, $p = 0.025$), with the pairwise comparison showing a significant difference only between the West Coast and False Bay samples ($H(2) = -2.493$, $p = 0.013$). The mean MP concentration in urban areas (Figure 4.2b) were significantly higher (1.52 ± 0.20 MPs/L) compared to concentrations observed in rural areas (0.98 ± 0.20 MPs/L) ($U = 705$, $p = 0.038$). The mean MP concentration varied across water samples collected from the different sites (Figure 4.2c) with Kalk Bay (site 9) displaying the highest concentration (4.97 ± 0.18 MPs/L) and Rooi Els (site 13) the lowest (0.13 ± 0.05 MPs/L). There was a significant difference in MP concentrations between sites ($H(13) = 62.891$, $p = 0.000$), with Kalk Bay having MP concentrations that varied significantly compared to all the other sites, except for Eland's Bay (site 2) ($H(13) = -0.710$, $p = 0.478$) and Blouberg (site 5) ($H(13) = -0.923$, $p = 0.356$) as shown by the pairwise comparison.

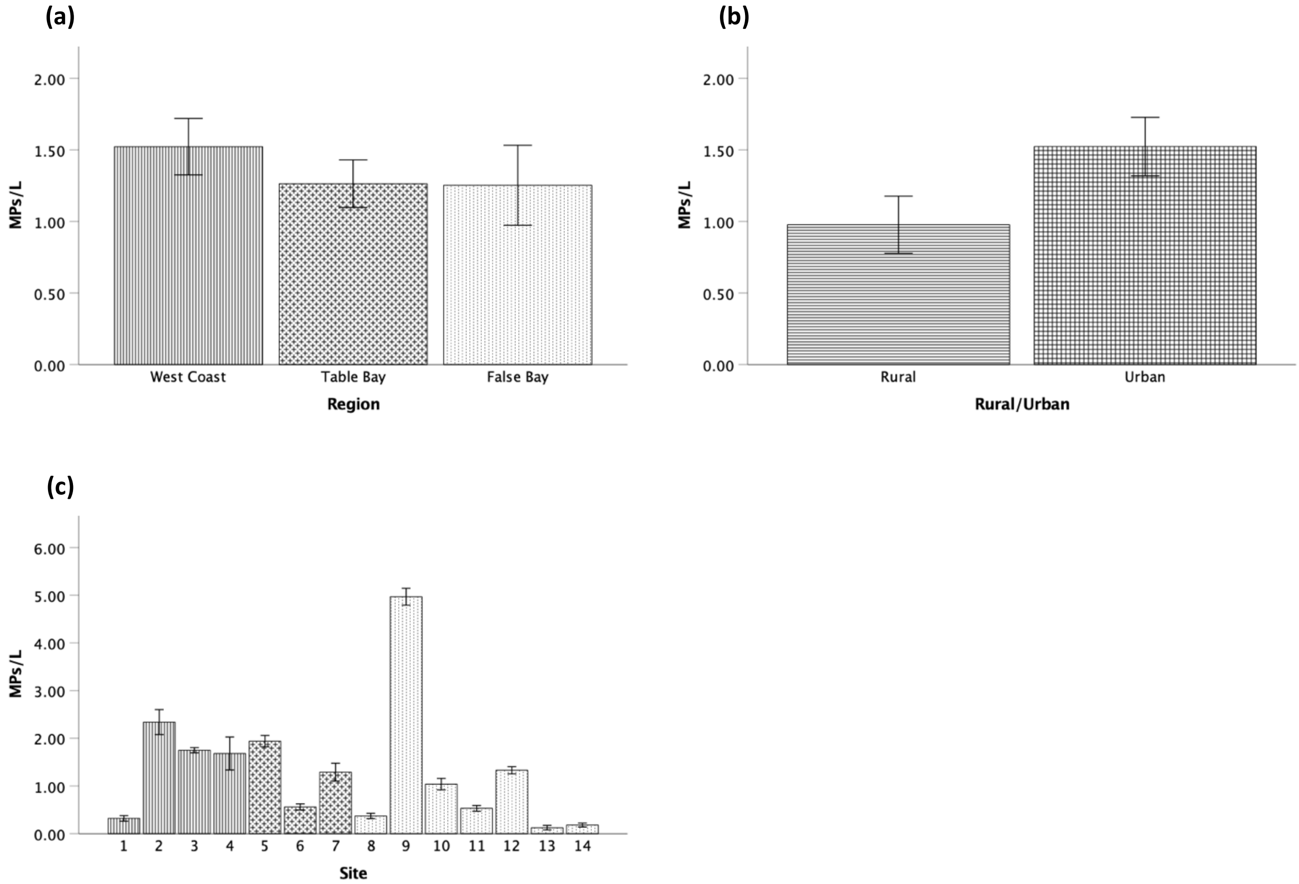

Figure 4.2: Microplastic concentration in water samples a) in each region, b) in urban and rural areas and c) at each sites along the Western Cape coastline, South Africa.

4.1.2 Type, colour and size of microplastics in water samples

Microplastic type

MP type varied in water samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.3). The most dominant MP type recorded in all water samples combined (Figure 4.3a) was filaments and film (73.29% and 25.72% respectively), where fragments was the least abundant MP type (0.99%). At a regional scale (Figure 4.3b) the percentage of filaments was highest in Table Bay (80.17%), whereas the percentage of film was highest along the West Coast (35.53%). The most dominant MP type recorded in water samples collected from urban and rural areas (Figure 4.3c) was filaments (75.10%) and film (29.02%) respectively. MP type varied in water samples across the sites (Figure 4.3d) with the most dominant type being filament and film. The highest percentage of filaments and film was observed at Simon’s Town (site 8) (97.50%) and Saldanha Bay (site 4) (54.79%) respectively.

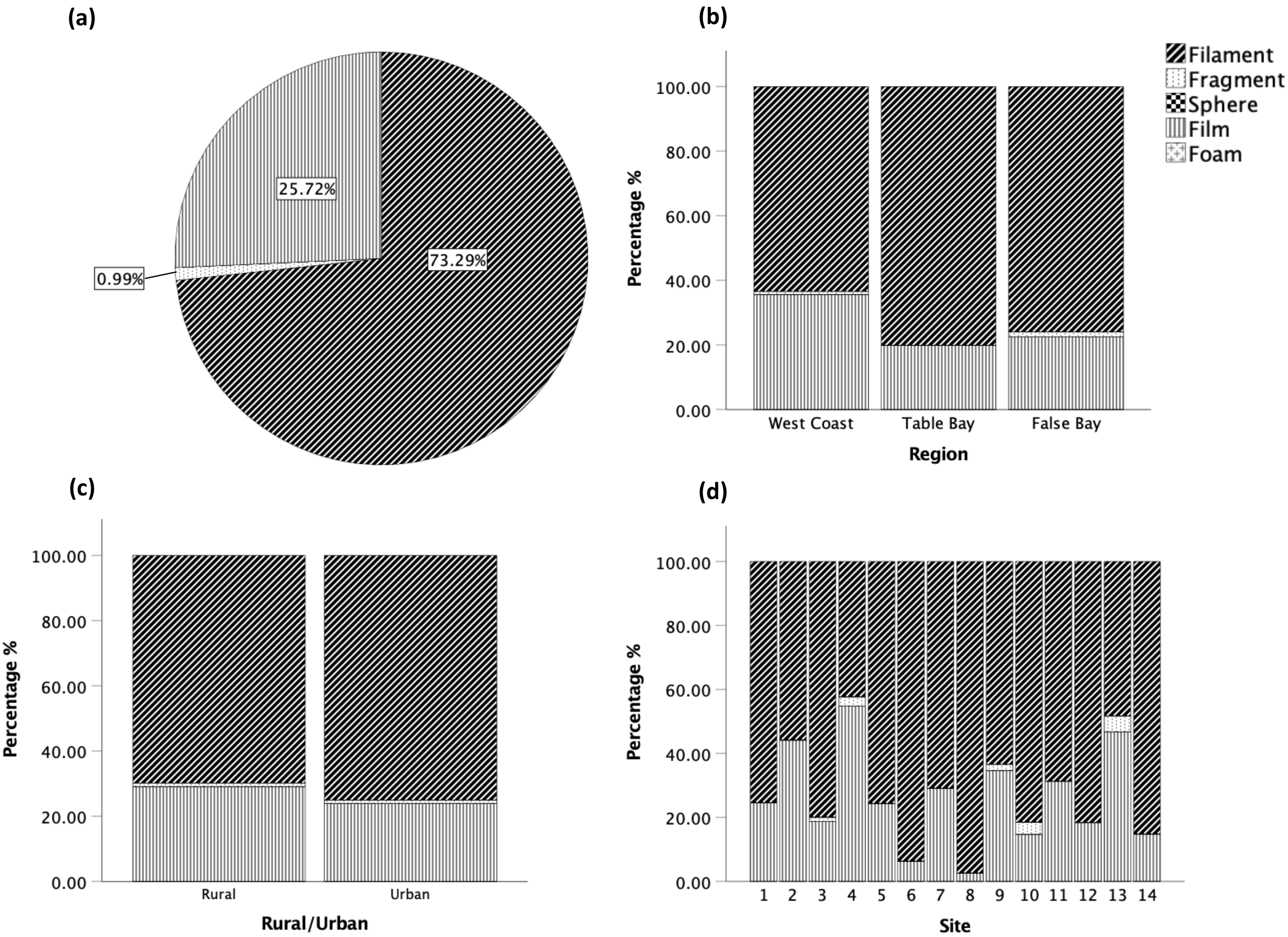

Figure 4.3: Percentage (%) of microplastic type in water samples a) combined b) region, c) rural versus urban area and d) sites along the Western Cape coastline, South Africa.

Microplastic colour

The colour of MP particles varied in water across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.4). The most dominant MP color recorded in all water samples combined (Figure 4.4a) was black/grey, white and transparent (30.69%, 17.36% and 17.36% respectively). At a regional scale (Figure 4.4b) the percentage of black/grey MPs was highest in False Bay (37.09%), where the percentage of white and transparent MPs was highest along the West Coast (39.21%) and in Table Bay (23.18%) respectively. The most dominant MP colour in urban and rural areas (Figure 4.4c) was white, transparent, black/grey and blue/green MPs. The percentage of white, transparent and black/grey MPs was highest in rural areas (32.81%, 18.28% and 32.11% respectively), whereas the percentage of blue/green MPs was highest in urban areas (16.72%). MP colours varied in water samples across the sites (Figure 4.4d) with the most dominant colours being white, transparent, red and black/grey particles. The percentage of white and transparent MPs was highest at Blouberg (site 5) (52.89%) and Maiden's Cove (site 7) (37.98%) respectively, where the percentage of red/pink and black/grey MPs was highest at Simon's Town (site 8) (53.23%) and Pringle Bay (site 14) (58.67%) respectively.

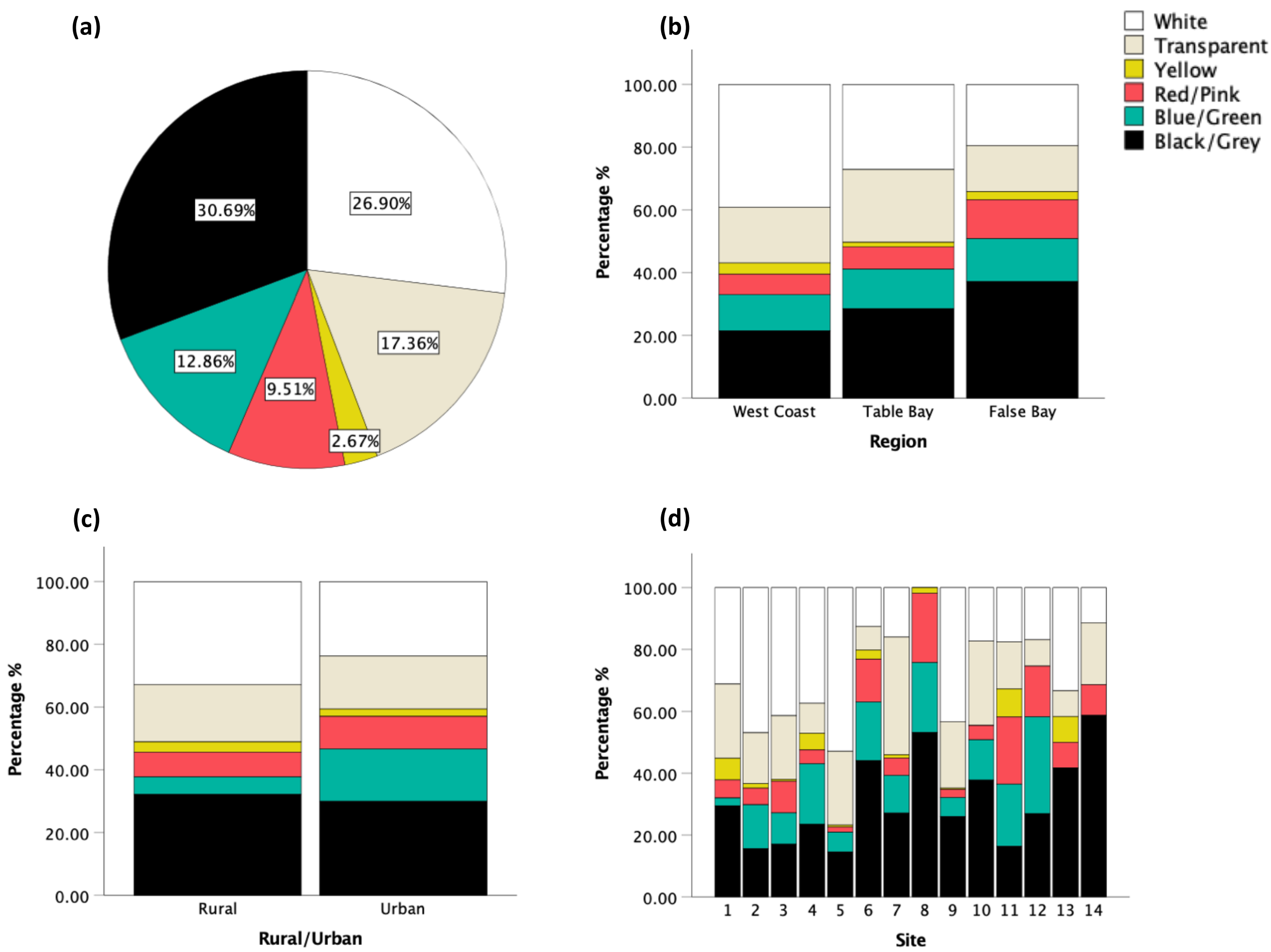

Figure 4.4: Percentage (%) of microplastic colour in water samples a) combined, b) region, c) rural versus urban area and d) sites along the Western Cape coastline, South Africa.

Microplastic size

MP size varied water samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.5). The most dominant MP size recorded in all water samples combined (Figure 4.5a) was between 1000 – 2000 μm (42.02%) and 2000 – 5000 μm (32.88%). At a regional scale (Figure 4.5b) the percentage of MP particles between 1000 – 2000 μm and between 2000 – 5000 μm was highest along the West Coast (47.81%) and in Table Bay (48.17%) respectively. The most dominant MP size in rural and urban areas (Figure 4.5c) was between 1000 – 2000 μm (49.63%) and between 2000 – 5000 μm (35.86%) respectively. The size of MPs varied in water samples across the sites (Figure 4.5d) with the most dominant size being between 1000 – 2000 µm, 2000 – 5000 µm and > 5000 µm in samples collected from Lambert's Bay (site 1) (74.50%), Eland's Bay (52.72%) and Kalk Bay (site 9) (62.55%) respectively.

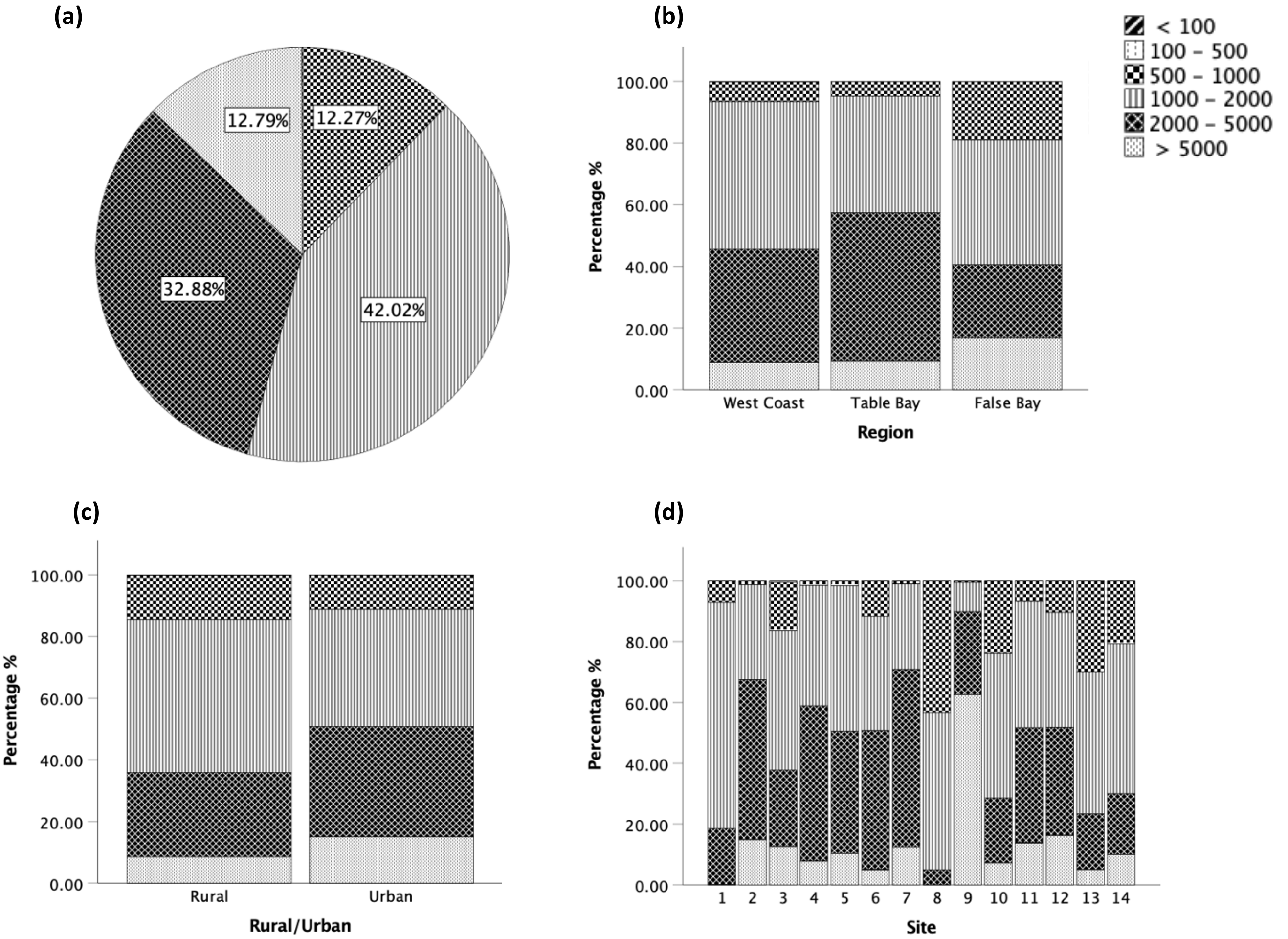

Figure 4.5: Percentage (%) of microplastic size (µm) in water samples a) combined b) region, c) rural versus urban area and d) sites along the Western Cape coastline, South Africa.

4.1.3 Microplastics polymer identification in water samples

The percentage of MP polymers was identified in all water samples combined (**Error! Reference source not found.**a) where PET and PE was the most abundant polymer type (29.33% and 21.63% respectively). Polymer identification varied in MP type (**Error! Reference source not found.**b) with PET and PE being the most abundant polymer type in filament (42.26%) and film (53.85%) particles, respectively. The most abundant polymer type of foam and fragment particles was identified as PS (100%) and PAA (50%) respectively.

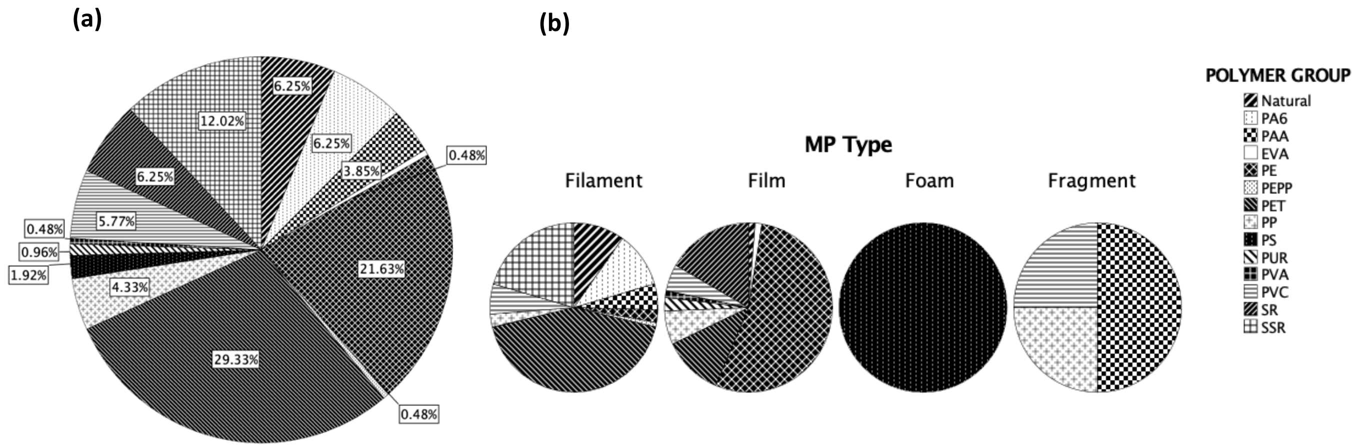

Figure 4.6: Polymer identification of a) overall MPs and b) different MP types in water samples collected along Western Cape coastline, South Africa. Natural: Cellulose/protein based polymers; EVA: Ethylene vinyl acetate; PA6: Polyamide 6; PAA: Polyacrylic acid; PE: Polyethylene; PEPP: Polyethylene propylene; PET: Polyethylene terephthalate; PP: Polypropylene; PS: Polystyrene; PUR: Polyurethane; PVA: Poly vinyl acetate; PVC: Poly vinyl chloride; SR: Synthetic rubber; SSR: Semi-synthetic rubber.

The MP polymer type varied in each region (Figure 4.7). False Bay displayed the most variability in polymer type, with PET and PE being the most abundant polymer type (28.49% and 25.14% respectively). The West Coast region displayed the least variability in polymer type however had the highest percentage of SSR (38.89%) was recorded.

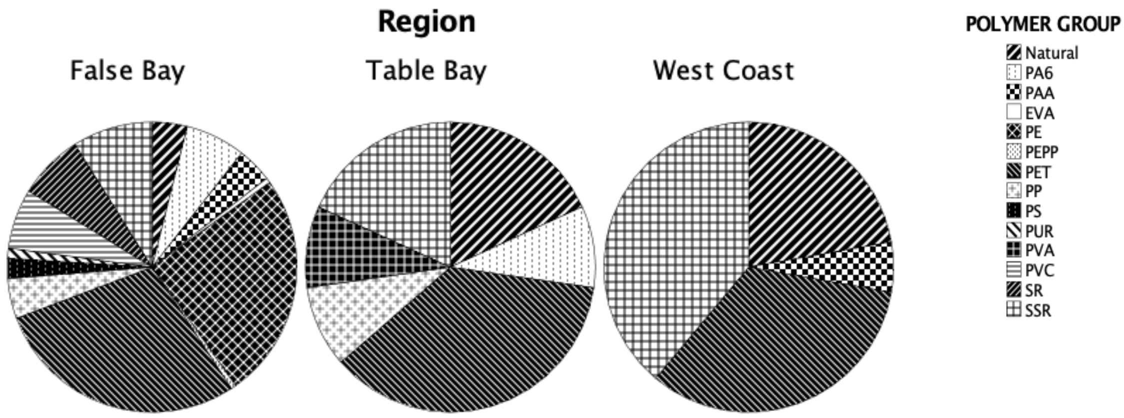

Figure 4.7: Microplastic polymer identification in water samples collected in each region along the Western Cape coastline, South Africa.

MP polymer type varied between urban and rural areas (Figure 4.8). Urban areas displayed the most variability in polymer type, with PET and PE being the most abundant polymer type (28.57% and 23.81% respectively). Rural areas displayed the highest abundance of PET (36.84%), SSR (26.32%) and Natural (26.32%) polymer types.

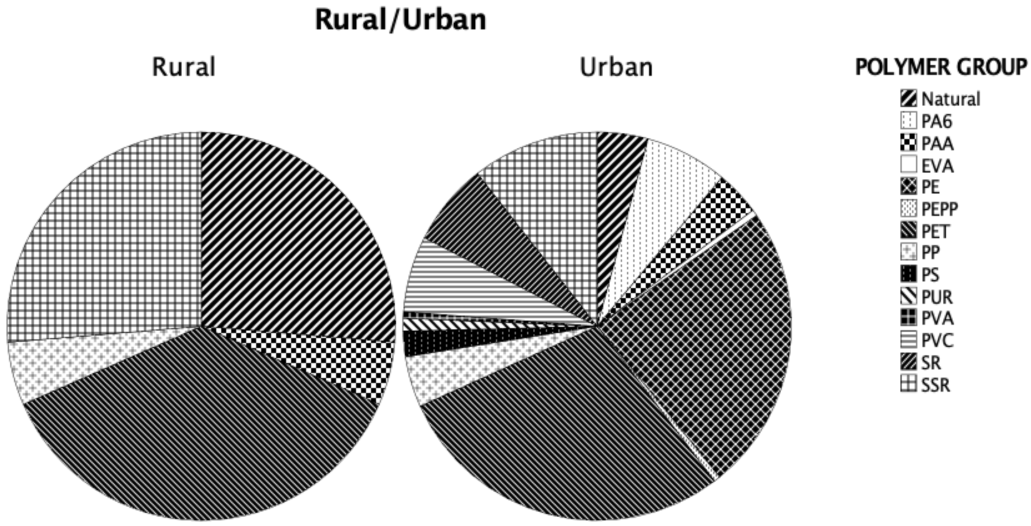

Figure 4.8: Microplastic polymer identification in water samples collected in rural and urban areas along the Western Cape coastline, South Africa.

4.2 Sediment samples

4.2.1 MP concentration in sediment samples

Of the 70 sediment samples, 69 (98.57%) contained MP particles. A total of 1277 particles were recorded, with a mean concentration of 185.07 MPs/kg (± 15.25 SE) (Figure 4.9). At a regional scale (Figure 4.9a) the mean MP concentration was highest in sediment samples collected from Table Bay (234.67 ± 31.42 MPs/kg) and lowest along the West Coast (211.05 ± 27.05 MPs/kg) and False Bay (149.71 ± 21.17 MPs/kg). There was a significant difference between MP concentrations in sediment samples across the regions ($H(2) = 6.646$, $p = 0.036$), with the pairwise comparison showing a significant difference only between Table Bay and False Bay samples ($H(2) = -2.273$, $p = 0.023$). The mean MP concentration in urban areas (Figure 4.9b) (199.11 ± 20.69 MPs/kg) were significantly higher compared to concentrations observed in rural areas (158.75 ± 19.83 MPs/kg) ($U = 614.500$, $p = 0.348$). The mean MP concentration varied across sediment samples collected from the different sites (Figure 4.9c) with Gordan's Bay (site 12) displaying the highest concentration (360.00 ± 36.74 MPs/kg) and Simon's Town the lowest (38.00 ± 2.00 MPs/kg). There were significant differences in MP concentrations between sites ($H(13) = 48.438$, $p = 0.000$), with Gordan's Bay having MP concentrations that varied significantly compared to all the other sites, except for Veldriff ($p = 0.320$), Saldanha Bay (site 4) ($p = 0.298$), Blouberg (site 5) ($p = 0.962$), Mouille Point (site 6) (0.270) and Kalk Bay (site 9) ($p = 0.302$) as shown by the pairwise comparison.

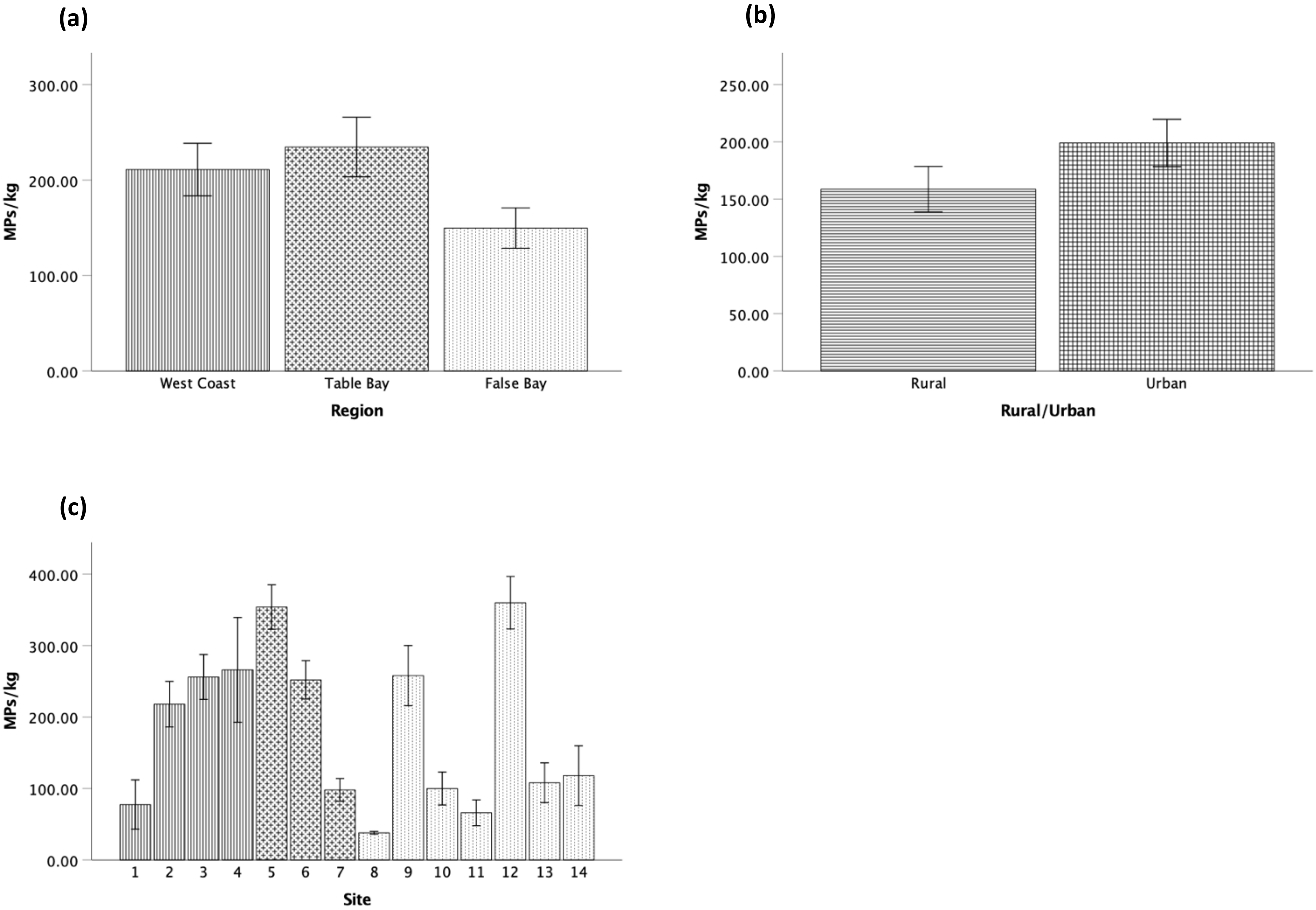

Figure 4.9: Microplastic concentration in sediment samples a) at each region, b) in urban and rural areas and c) at each site along the Western Cape coastline, South Africa.

4.2.2 Grain size analysis and sediment classification

Grain size analysis was conducted to determine grain size distribution at sampling sites using GRADISTATv9.1 (Figure 4.10a). All sediment was classified as sand, with coarse sand (1180 – 500 µm) and medium sand (500 – 250 µm) being the most dominant sediment type for all sites (Figure 4.10b). Coarse sand (CS) was the most dominant grain size in Saldanha Bay (site 4), Rooi Els (site 13) and Eland's Bay (site 2) (62%, 61% and 40% respectively), whereas medium sand (MS) was the most dominant grain size in Kalk Bay (site 9), Gordan's Bay (site 12), Strand (site 11) and Lambert's Bay (site 1) (83%, 79%, 66%, 66% and 64% respectively). There is a noticeably high amount of Fine sand (FS) in Strandfontein (site 10) and Simon's Town (site 8) (77/97% and 53.73% respectively).

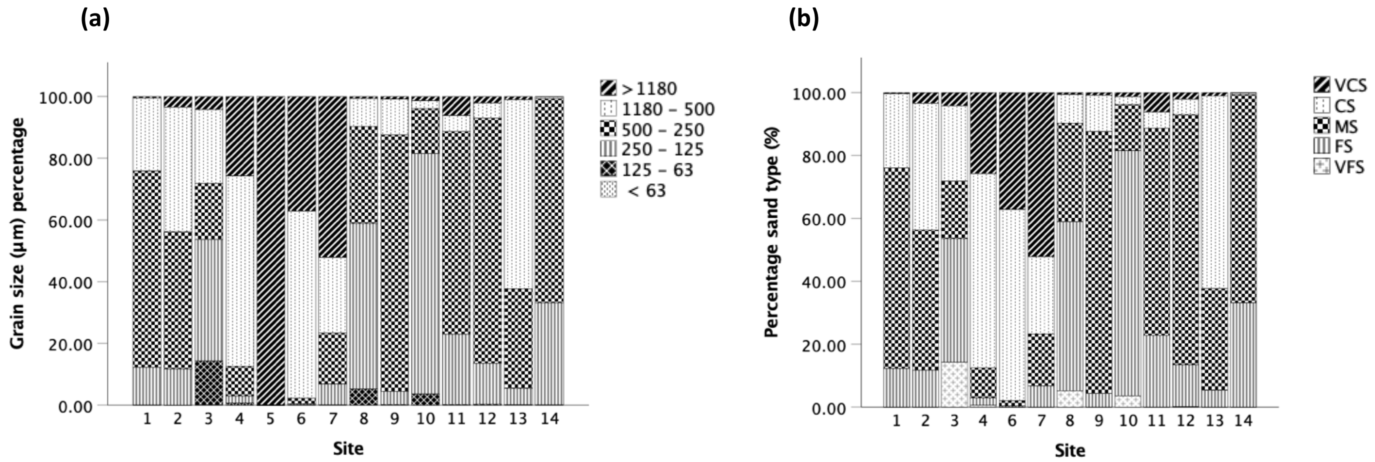

Figure 4.10: a) Grain size (µm) percentage and b) sediment type percentage of samples collected at each site along Western Cape coastline, South Africa.
VCS: Very Coarse Sand (1 - 2 mm); CS: Coarse Sand (500– 1000 µm) ; MS: Medium Sand (250 – 500 µm); FS: Fine Sand (125 – 250 µm); VFS: Very Fine Sand (63 – 125 µm). Note Blouberg (site 5) sediment was not classified due insufficient data.

The Spearman rank correlation showed no relationship ($r = < 0.1$) and no significant difference ($p > 0.05$) between MP concentrations and grain size for all grain sizes, expect for fine sand (FS) showing a weak inversely proportional relationship with significant difference ($r = - 0.460$; $p = 0.001$) (Figure 4.11d).

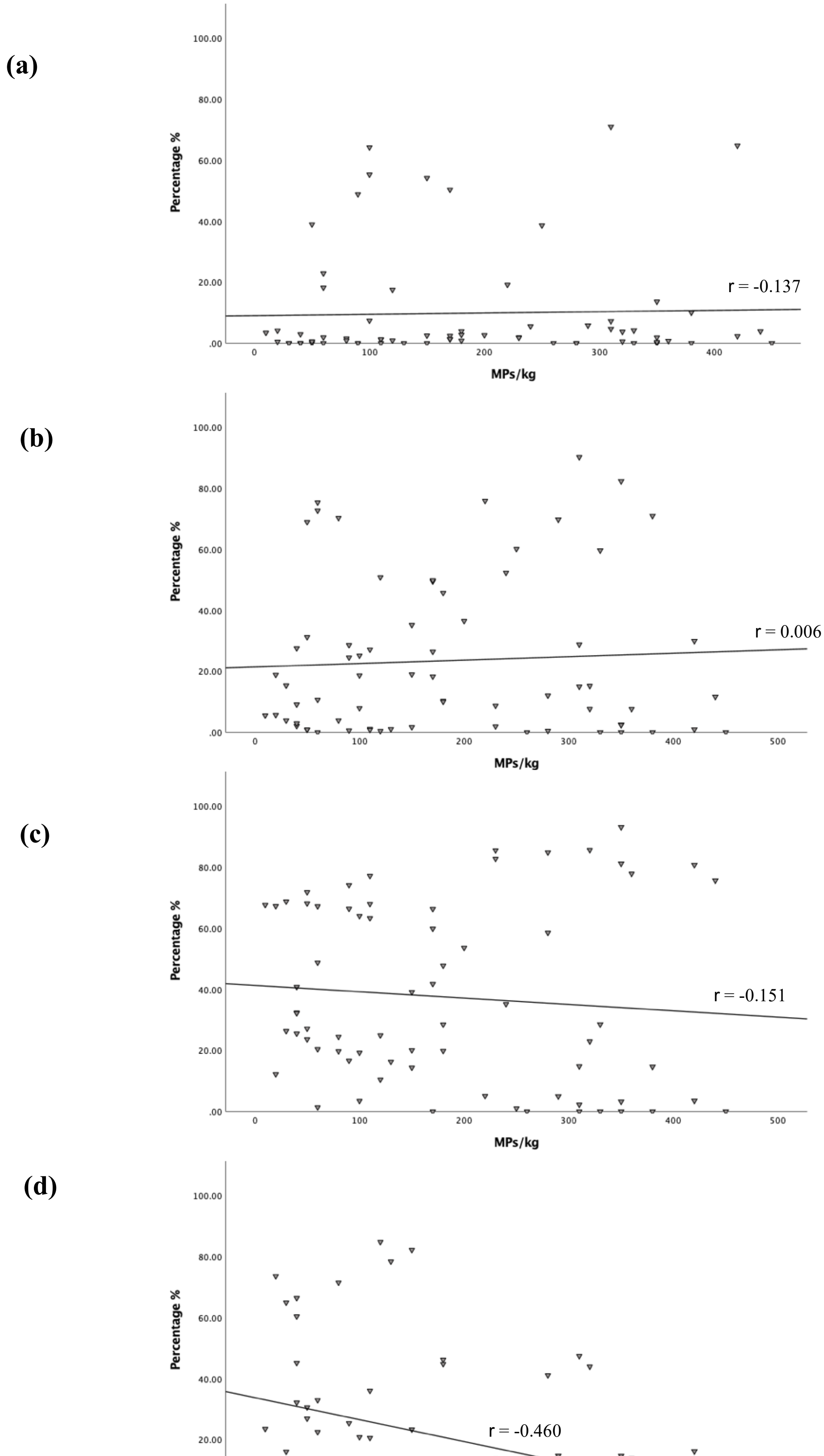

Figure 4.11: Correlation between microplastic concentration (MPS/kg) and percentage of sediment type along the Western Cape coastline, South Africa. a) VCS: Very Coarse Sand (1 - 2 mm), b) CS: Coarse Sand (500– 1000 µm), c)MS: Medium Sand (250 – 500 µm) and d) FS: Fine Sand (125 – 250 µm).

4.2.3 Type, colour and size of MP in sediment samples

Microplastic type

MP type varied in sediment samples across regions, urban and rural areas and sites (Figure 4.12). The most dominant MP type recorded in all sediment samples combined (Figure 4.12a) was filaments and film (66.96% and 28.13% respectively). At a regional scale (Figure 4.12b) the percentage of filaments and film was highest at Table Bay (72.49%) and along the West Coast (36.50%) respectively. The most dominant MP type recorded in sediment samples collected from rural and urban areas (Figure 4.12c) was filament (67.53%) and film (29.02%) respectively. MP type varied in sediment samples across the sites (Figure 4.12d) with the most dominant type being filament and film. The highest percentage of filament and film was observed at Kalk Bay (97.56%) and Saldanha Bay and (57.21%) respectively.

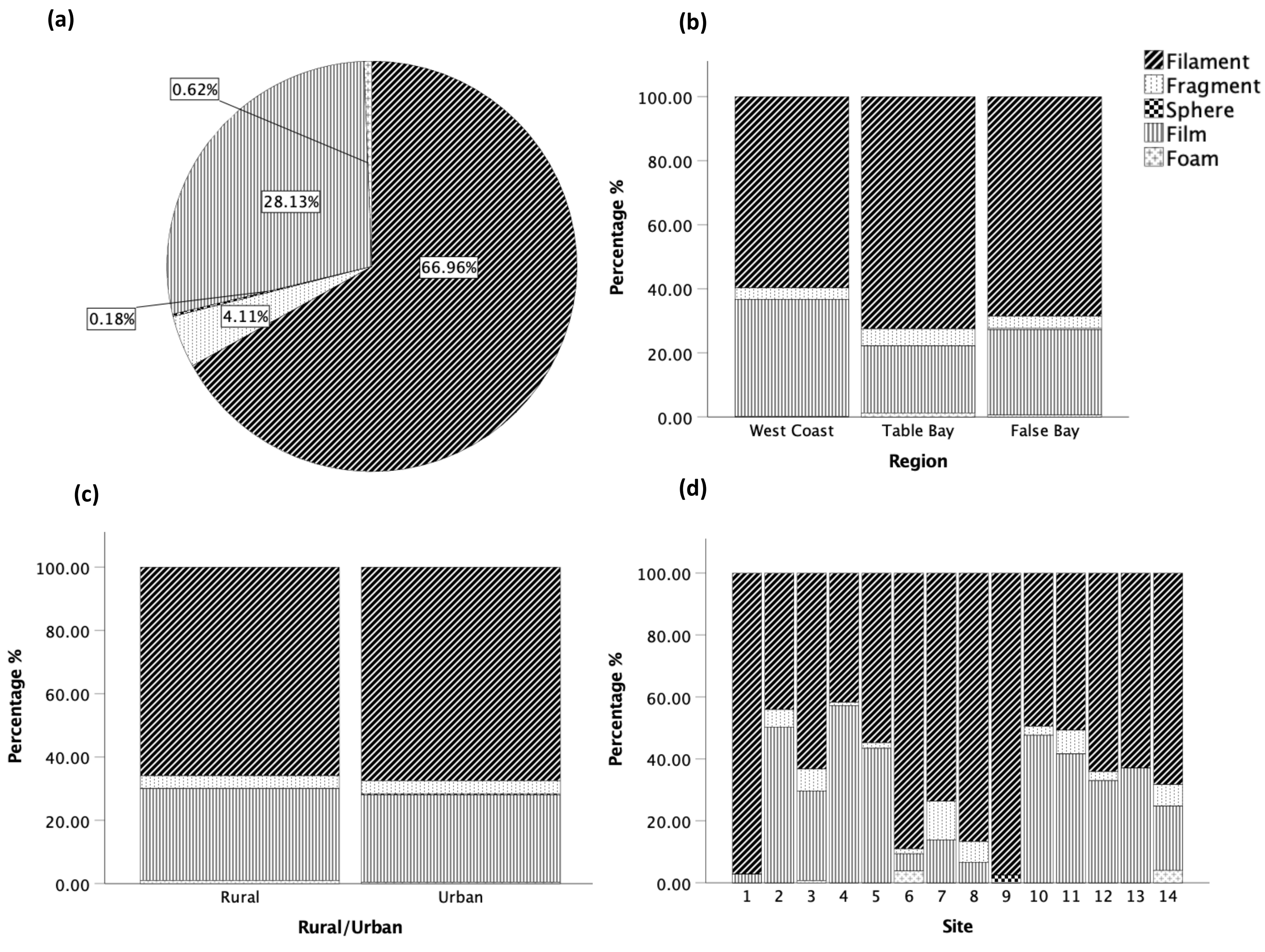

Figure 4.12: Percentage (%) of microplastic type in sediment samples a) combined, b) in each region, c) in rural versus urban area and d) at each site along the Western Cape coastline, South Africa

Microplastic colour

The colour of MP particles varied across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.13). The most dominant MP colour recorded in all sediment samples combined (Figure 4.13a) was white, transparent, blue/green and black/grey (29.64%, 30.80%, 13.90% and 18.32% respectively). At a regional scale (Figure 4.13b) the percentage of white and transparent MP particles was highest along the West Coast (36.51% and 32.72% respectively), whereas the percentage of blue/green and black/grey MP particles was highest at False Bay (17.16% and 21.74% respectively). The most dominant MP colour in urban and rural areas (Figure 4.13c) was white, transparent, blue/green and black/grey MP particles. The percentage of the transparent, blue/green and black/grey MP particles was highest in rural areas (32.28%, 14.66% and 19.44% respectively), whereas white MP particles was highest in urban areas (20.77%). MP colurs varied in sediment samples across the sites (Figure 4.13d) with the most dominant colours being white, transparent, blue/green and black/grey. The percentage of white and transparent MPs was at Saldanha Bay (site 4) (57.17%) and Velddrif (site 3) (57.41%) respectively, where the percentage of blue/green and black/grey MPs was highest at Simon's Town (site 8) (35.00%) and Kalk Bay (site 9) (35.35%) respectively.

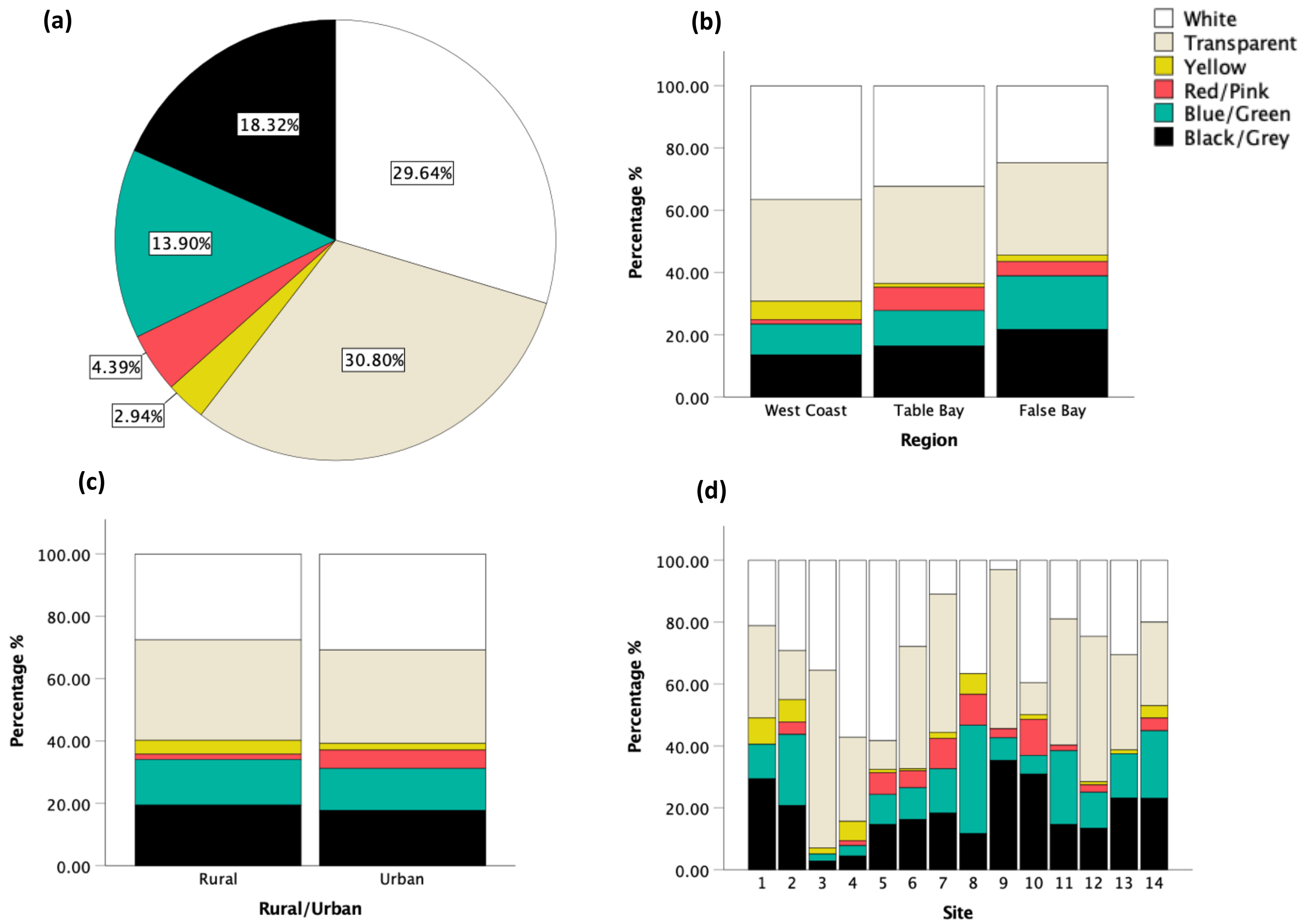

Figure 4.13: Percentage (%) of microplastic colour in sediment samples a) combined, b) in each region, c) in rural versus urban area and d) at each site along the Western Cape coastline, South Africa.

Microplastic size

MP size varied sediment samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.14). The most dominant MP size recorded in all sediment samples combined (Figure 4.14a) was between 1000 – 2000 µm and 2000 – 5000 µm (33.25% and 45.28% respectively). At a regional scale (Figure 4.14b) the percentage of MP particles between 1000 – 2000 µm and 2000 – 5000 µm was highest along the West Coast (41.09%) and in False Bay (48.70%) respectively. The most dominant MP size in rural and urban areas (Figure 4.14c) was between 1000 – 2000 µm (33.98%) and 2000 – 5000 µm (45.74%) respectively. The size of MPs varied in sediment samples across the sites (Figure 4.14d) with the dominant size being between 1000 – 2000 µm and 2000 – 5000 µm in samples collected from Velddrif (site 3) (57.51%) and Strand (site 11) (39.24%) respectively. Pringle Bay (site 14) displayed the highest percentage of MP particles > 5000 µm (25.75%).

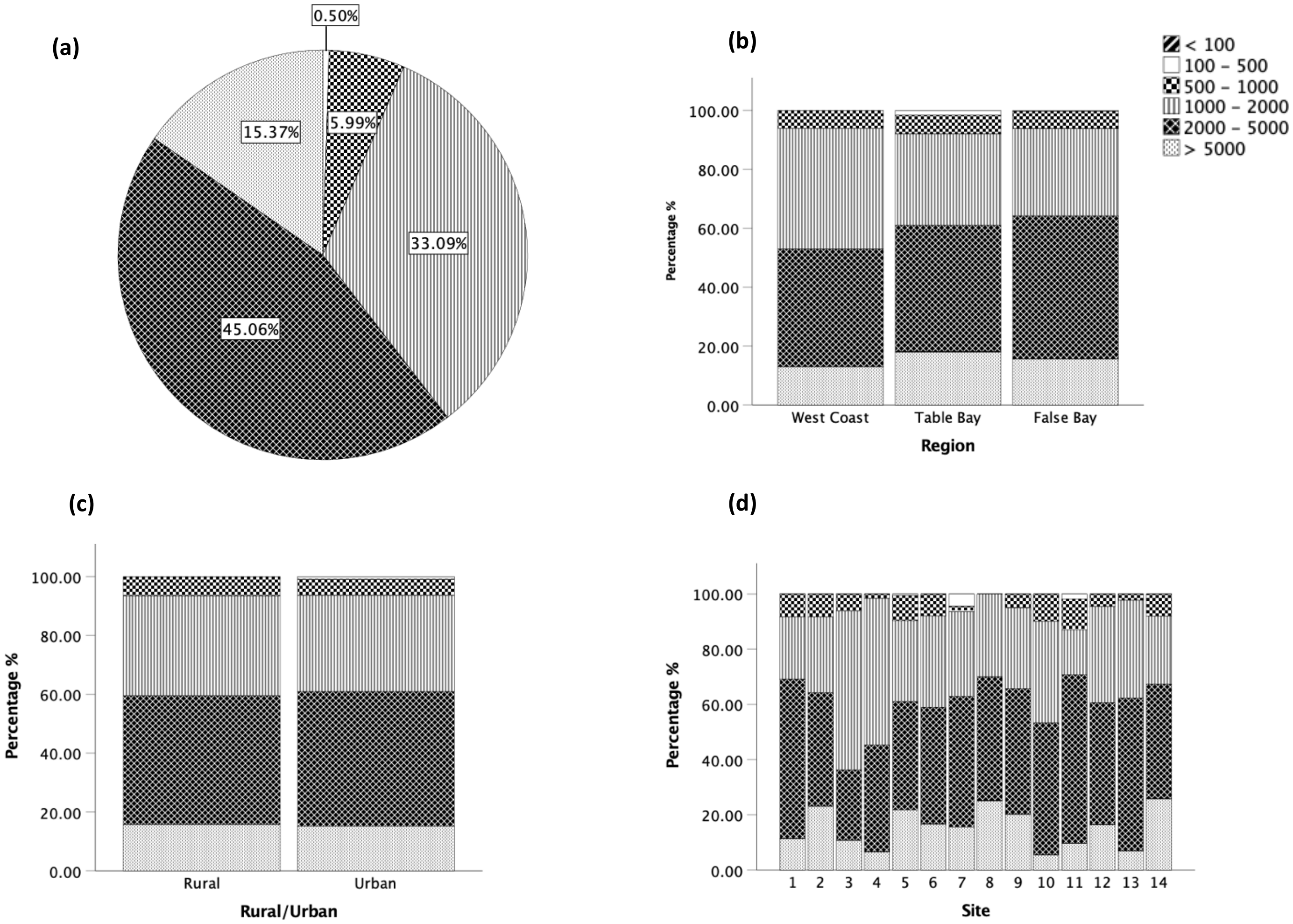

Figure 4.14: Percentage (%) of microplastic size (µm) in sediment samples a) combined b) in each region, c) in rural versus urban area and d) at each site along the Western Cape coastline, South Africa

4.2.4 Microplastics polymer identification in sediment samples

The overall percentage of MP polymers was identified in all sediment samples combined (Figure 4.15a) where Natural (cotton), PS and PET was the most dominant polymer type (32%, 24% and 16% respectively). Polymer identification varied in MP type (Figure 4.15b) with Natural and PET displaying the dominant polymer type in filaments (50% and 25% respectively). The dominant polymer type of film and foam particles were was identified as PE (66.67%) and PS (100%) respectively.

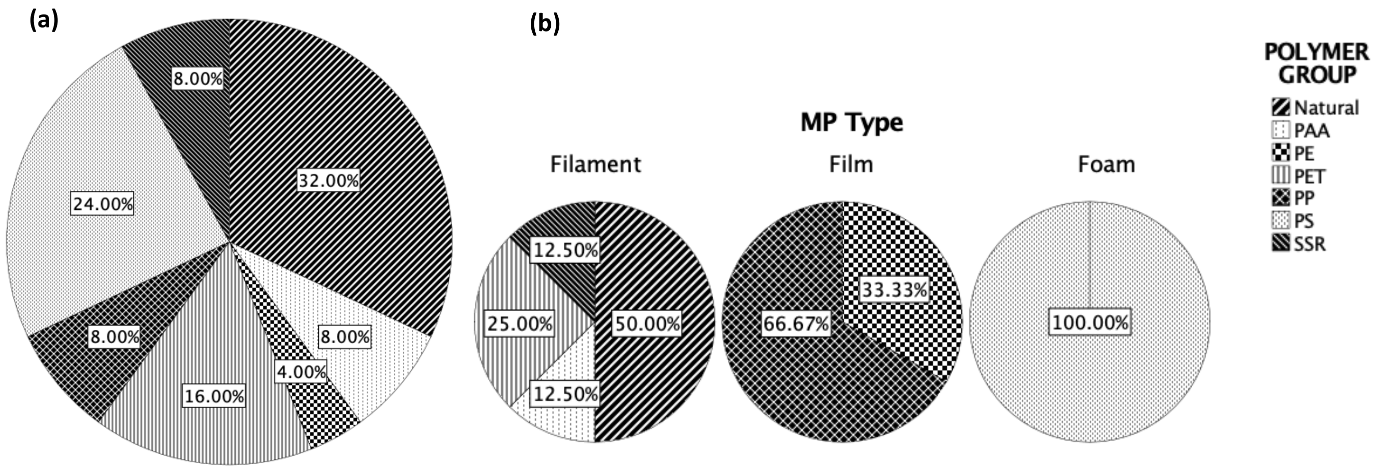

Figure 4.15: Polymer identification of a) overall MPs and b) different MP types in sediment samples collected along the Western Cape coastline, South Africa.
Natural: Cellulose/protein based polymers; PAA: Polyacrylic acid; PE: Polyethylene; PET: Polyethylene Terephthalate; PP: Polypropylene; PS: Polystyrene; SSR: Semi-synthetic rubber.

MP polymer type was identified sediment samples and varied in each region (Figure 4.16). West Coast displayed the most variability in polymer type, with Natural (cotton) and PS being the most dominant polymer type (38.89% and 22.22% respectively). The False Bay region displayed the highest percentage of PET (50%) and Table Bay displayed the highest percentage of PS (40%).

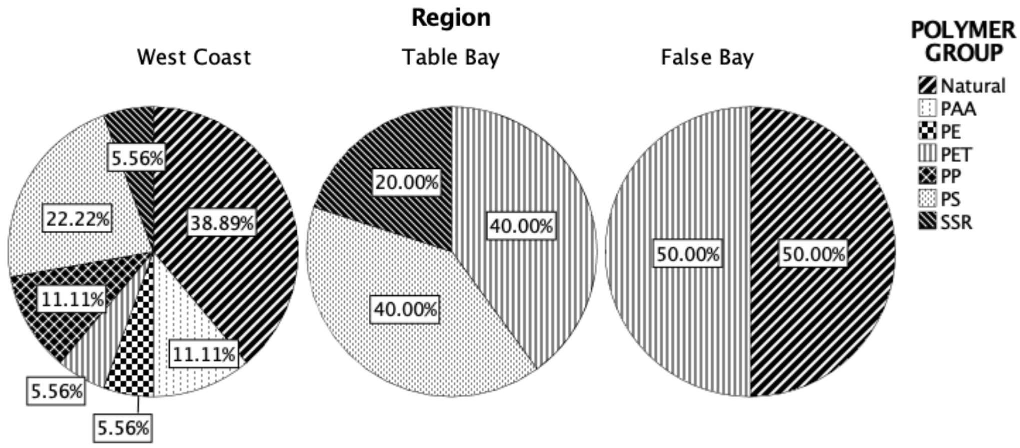

Figure 4.16: Microplastic polymer identification in sediment samples collected in each region along the Western Cape coastline, South Africa.
Natural: Cellulose/protein based polymers; PAA: Polyacrylic acid; PE: Polyethylene; PET: Polyethylene Terephthalate; PP: Polypropylene; PS: Polystyrene; SSR: Semi-synthetic rubber.

MP polymer type was identified in sediment samples collected from urban and rural areas (Figure 4.17). Rural areas had the highest percentage of Natural, PAA and PET (45.45%, 18.18% and 18.18% respectively), whereas urban areas displayed the highest percentage of PS and SSR (35.71% 14.29% respectively) polymer type.

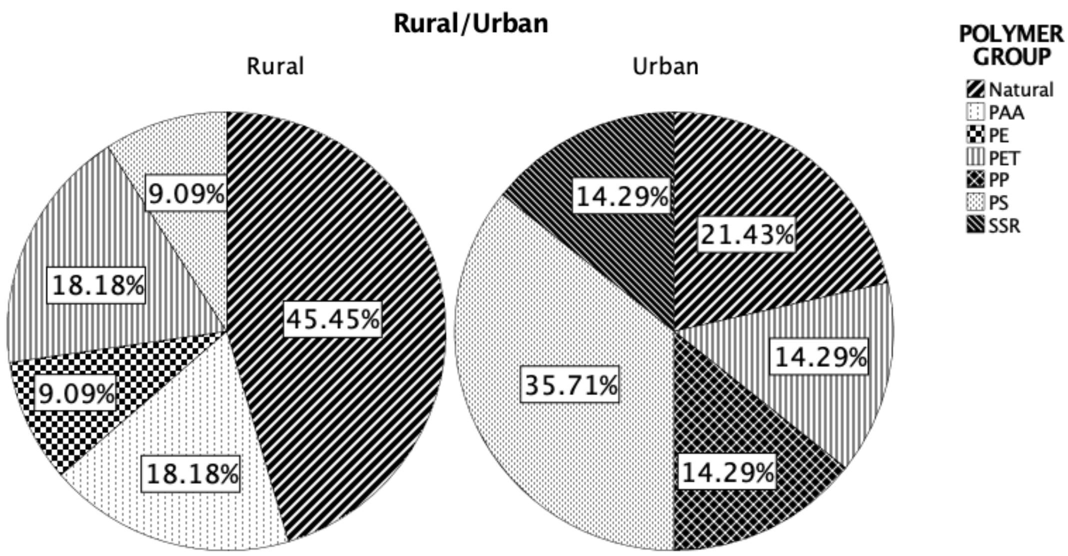

Figure 4.17: Microplastic polymer identification in sediment samples collected in rural and urban areas along the Western Cape coastline, South Africa
Natural: Cellulose/protein based polymers; PAA: Polyacrylic acid; PE: Polyethylene; PET: Polyethylene Terephthalate; PP: Polypropylene; PS: Polystyrene; SSR: Semi-synthetic rubber.

4.3 Correlation between MP concentration in water (MPs/L) and sediment (MPs/kg) samples

There is a strong (positive) correlation ($r = 0.618$; $p < 0.001$) between MP concentrations in water and sediment samples collected along the coastline of the Western Cape, South Africa (Figure 4.18a). There is a strong (positive) correlation between MP concentrations recorded in water and sediment samples collected from rural areas ($r = 0.632$; $p < 0.001$) compared to urban areas ($r = 0.579$; $p < 0.001$) (Figure 4.18b). The results show a directly proportional relationship between MP concentrations in water and sediment samples with the exception of a few outliers.

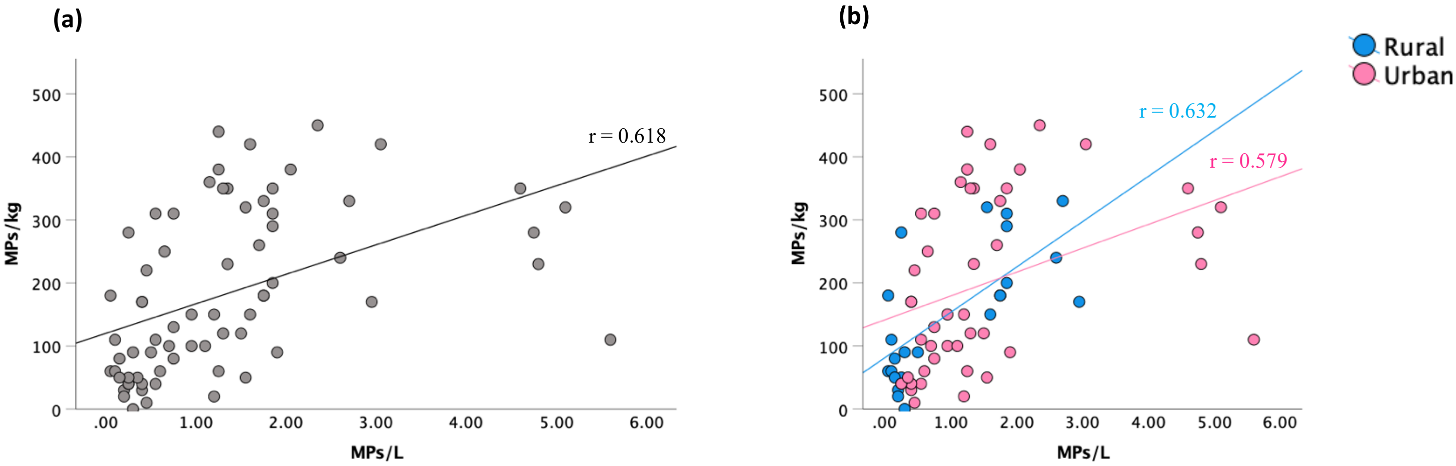

Figure 4.18: a) Correlation between microplastic concentrations in water (MPs/L) and sediment (MPs/kg) and b) difference in urban and rural areas samples collected along the Western Cape coastline, South Africa.

4.4 Echinoderm samples

4.4.1 MP concentration in echinoderm samples

Sampling for echinoderms was subject to availability at each site (Table 3.2). Of the 539 echinoderms analysed, 523 (97.03%) contained MP particles. A total of 6135 MP particles was recorded, with a mean MP concentration of 1.44 MPs/g (± 0.12 SE) (Figure 4.19). At a regional scale (Figure 4.19a) the mean MP concentration was highest in echinoderm samples collected along the West Coast (1.86 ± 0.34 MPs/g) compared samples collected from False Bay (1.65 ± 0.13 MPs/g) and Table Bay (0.23 ± 0.02 MPs/g). There was a significant difference between MP concentrations in echinoderm samples across the regions ($H(2) = 81.342$, $p = 0.000$), with the pairwise comparison showing significant differences between Table Bay and False Bay ($H(2) = 7.676$, $p = 0.000$), Table Bay and the West Coast ($H(2) = -8.551$, $p = 0.000$) and False Bay and the West Coast ($H(2) = -2.850$, $p = 0.004$) samples. There was no significant difference in the mean MP concentration in echinoderm samples collected from urban and rural (1.46 ± 0.13 MPs/g and 1.41 ± 0.20 MPs/g respectively) ($U = 32280.00$, $p = 0.987$) (Figure 4.19b). The mean MP concentration varied across echinoderm samples collected from the different sites (Figure 4.19c) with Kalk Bay (site 9) displaying the highest concentration (2.90 ± 0.38 MPs/g) and Rooi Els (site 13) the lowest (0.16 ± 0.02 MPs/g). There were significant differences in MP concentrations between sites ($H(11) = 239.320$, $p = 0.000$), with Strand (site 11) having MP concentrations that varied significantly compared to all the other sites, except for Lambert's Bay (site 1) (($H(11) = -0.701$, $p = 0.483$), Kalk Bay ($H(11) = -0.019$, $p = 0.985$) and Strandfontein (site 10) ($H(11) = -1.651$, $p = 0.099$) as shown by the pairwise comparison.

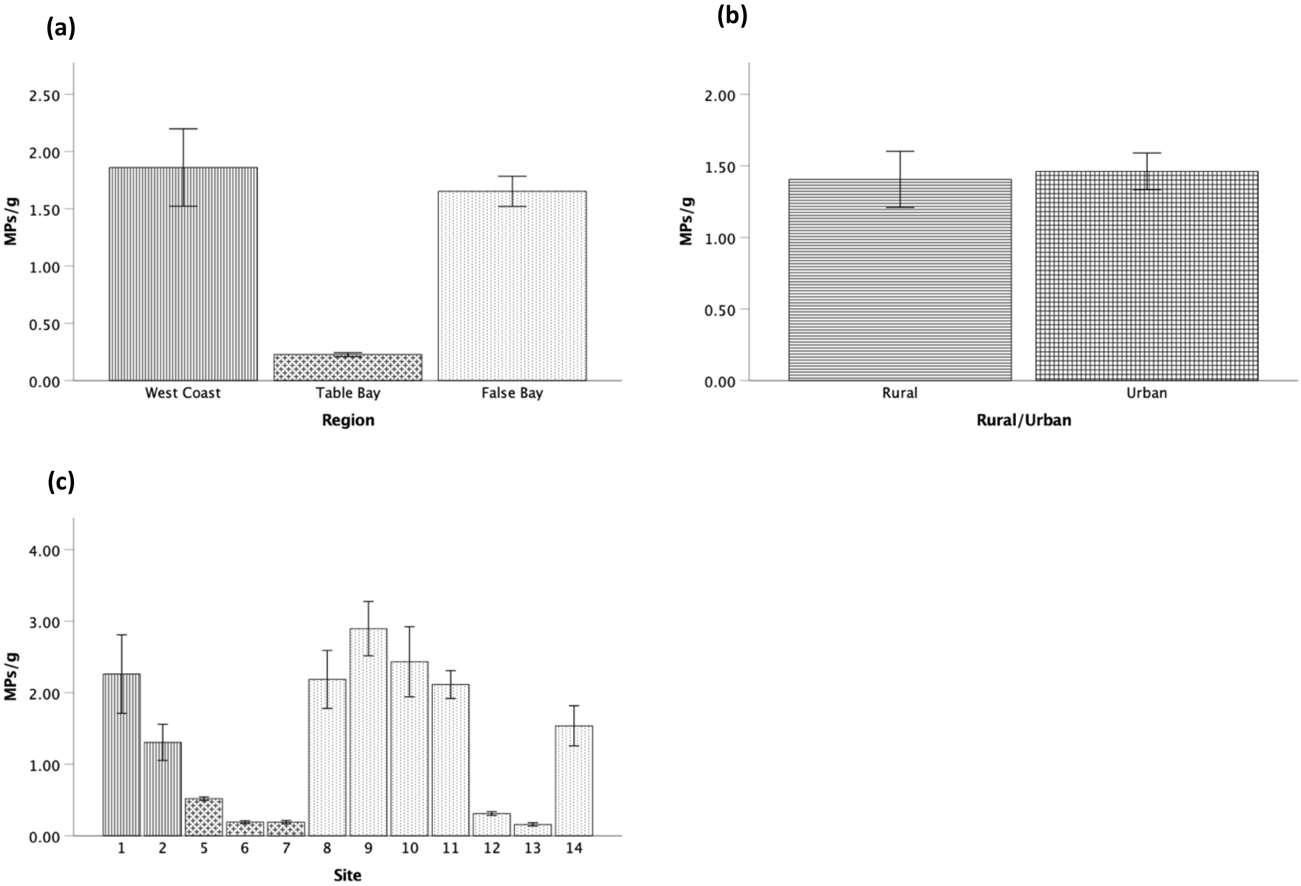

Figure 4.19: Microplastic concentration in echinoderm samples a) in each region, b) in urban and rural areas and c) at each site along the Western Cape coastline, South Africa.

4.4.2 MP abundance in echinoderm samples

Sampling for echinoderms was subject to availability at each site (Table 3.2). Of the 539 echinoderms analysed, 523 (97.03%) contained MP particles. A total of 6135.40 MP particles was recorded, with a mean MP abundance of 11.68 MPs/I (± 0.76 SE) (Figure 4.20). At a regional scale (Figure 4.20a) the mean MP abundance per individual was highest in echinoderm samples collected from False Bay (14.30 ± 1.16 MPs/I) and lowest in samples collected from the West Coast (5.40 ± 0.64 MPs/I). There was a significant difference between MP abundance in echinoderm samples across the regions ($H(2) = 32.006$, $p < 0.001$), with the pairwise comparison showing significant differences between Table Bay and West Coast ($H(2) = 3.984$, $p < 0.001$), False Bay and West Coast ($H(2) = 5.611$, $p < 0.001$) samples. The mean MP abundance in echinoderm samples collected from urban (Figure 4.20b) (15.22 ± 1.15 MPs/I) and rural (5.81 ± 0.45 MPs/I) areas were significantly different ($U = 45438.500$, $p < 0.001$). The mean MP abundance between echinoderm samples collected from the different sites

(Figure 4.20c) was highest in samples collected from Kalk Bay (site 9) (59.95 ± 4.68 MPs/I) and lowest in samples collected from Eland's Bay (site 2) (1.40 ± 0.16 MPs/I). There were a significant differences in MP abundance between sites (H(11) = 206.737, p = 0.000), with Kalk Bay having MP abundance that varied significantly compared to all the other sites, except Blouberg (site 5) (H(11) = -0.486, p = 0.627) as shown by the pairwise comparison.

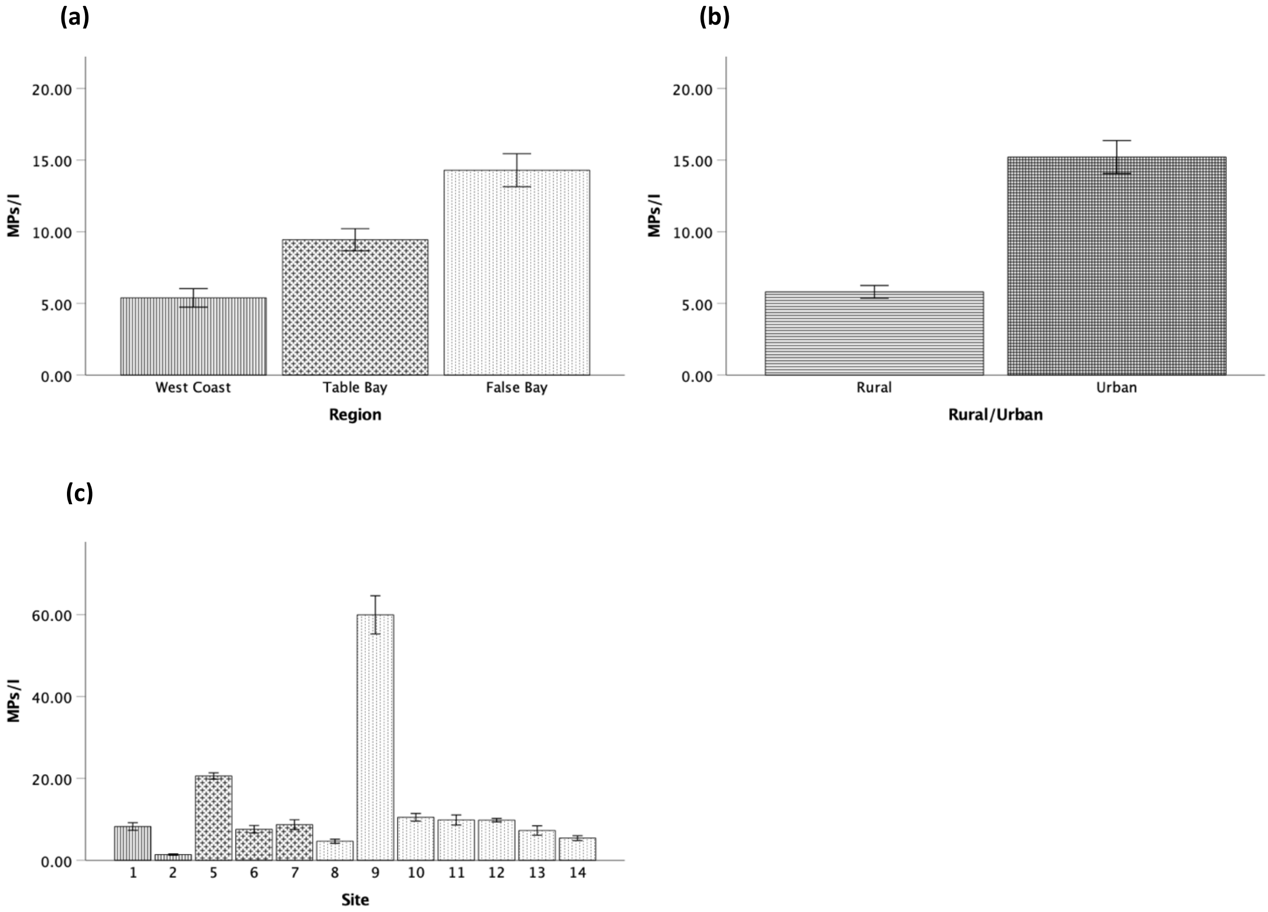

Figure 4.20: Microplastic abundance per individual echinoderm withing a) in each region, b) in urban and rural and c) at each site along the Western Cape coastline, South Africa.

The mean MP concentration (MPs/g) and abundance (MPs/I) was analysed in echinoderm type and feeding type in samples collected along the Western Cape coastline (Figure 4.21). The mean MP concentration in echinoderm species (Figure 4.21a) was highest in cushion star species (3.13 MPs/g) (± 0.28 SE) and lowest in sea star species (0.14 ± 0.03 MPs/g). There was a significant difference between MP concentrations in echinoderm species (H(3) = 211.804, p = 0.000), however the pairwise comparison showed no significant difference between MP concentrations in sea cucumber and sea urchin species (H(3) = -0.647, p = 0.518). The MP concentration in different feeding types (Figure 4.21b) was highest in predator species

(2.47 ± 0.23 MPs/g) and lowest in grazer species (0.42 ± 0.02 MPs/g). There was a significant difference between MP concentrations in different feeding types ($H(2) = 74.941$, $p = 0.000$), however the pairwise comparison showed no significant difference in MP concentrations between suspension/deposit feeders and grazers ($H(2) = -0.647$, $p = 0.518$). The mean MP abundance in echinoderm species (Figure 4.21c) was highest in sea cucumber species (17.57 ± 2.34 MPs/I) and lowest in cushion star species (3.32 ± 0.33 MPs/I). There was a significant difference in MP abundance between echinoderm species ($H(3) = 194.849$, $p = 0.000$), however the pairwise comparison showed no significant difference in abundance between sea cucumbers and sea star species ($H(3) = -1.025$, $p = 0.305$). The mean MP abundance in feeding type (Figure 4.21d) was highest in suspension/deposit-feeders (17.57 ± 2.34 MPs/I) and lowest in predators (4.29 ± 0.31 MPs/I). There was a significant difference between MP abundance in different feeding types ($H(2) = 170.134$, $p = 0.000$).

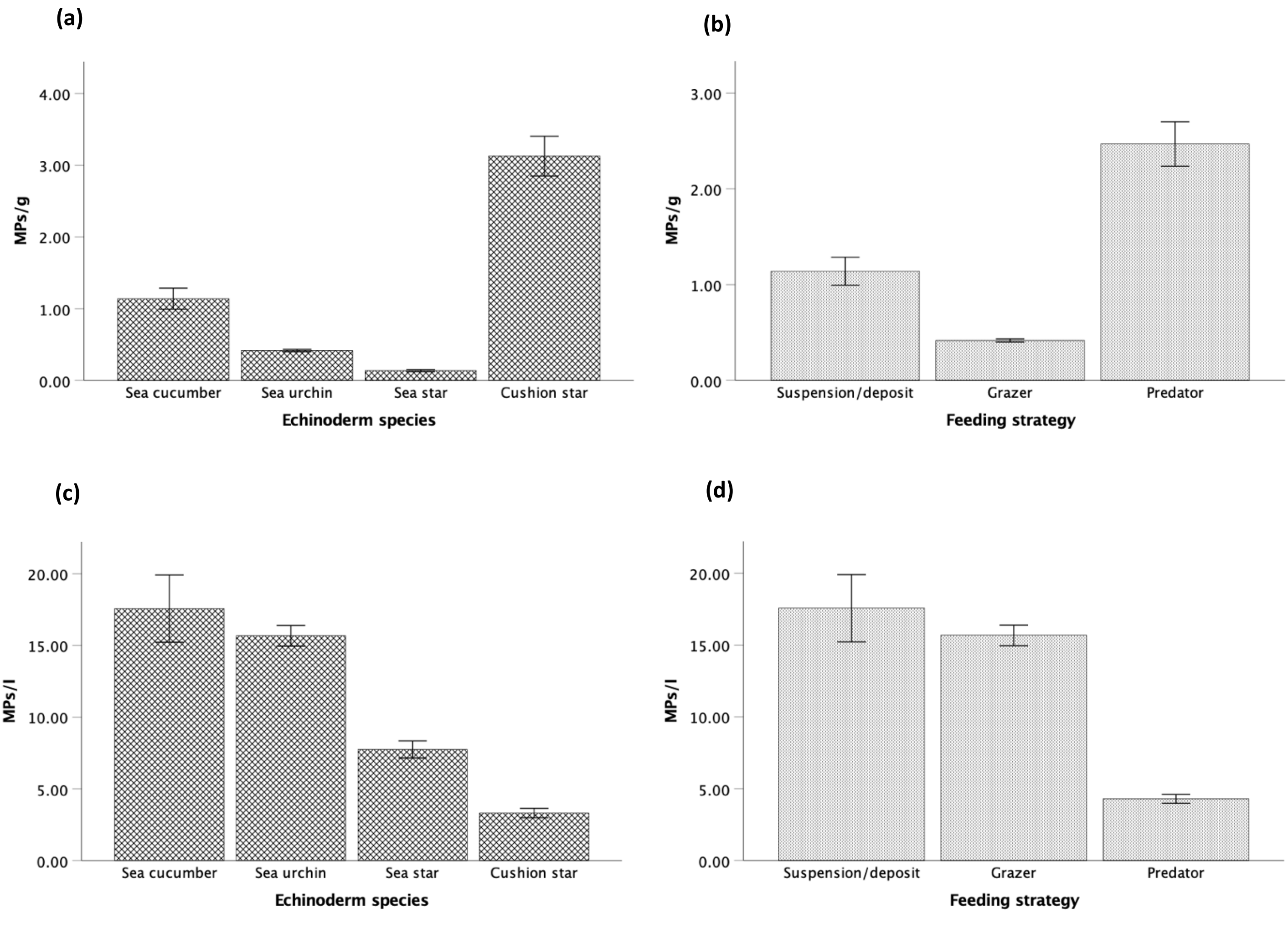

Figure 4.21: Microplastic concentration in a) echinoderm species, b) feeding strategy (MPs/g) and abundance in c) echinoderm species and d) feeding strategy (MPs/I) along the Western Cape coastline, South Africa.

4.4.3 Type, colour and size of MP in echinoderm samples

Microplastic type

MP type varied in echinoderm samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.22). The most dominant MP type recorded in all echinoderm samples combined (Figure 4.22a) was filaments and film (94.48% and 4.75% respectively). There were no spheres recorded in of the echinoderm samples. At a regional scale (Figure 4.22b) the percentage of filaments was highest in along the West Coast (99.29%), whereas the percentage of film was highest in Table Bay (5.78%). The most dominant MP type recorded in echinoderm samples collected from rural and urban areas (Figure 4.22c) was filaments (97.84%) and film (6.41%) respectively. MP type varied in echinoderm samples across the sites (Figure 4.22d) with the most dominant MP type being filaments and film. The highest percentage of filament and film was observed at Eland's Bay (site 2) (100%) and Gordan's Bay (site 12) (10.29%) respectively.

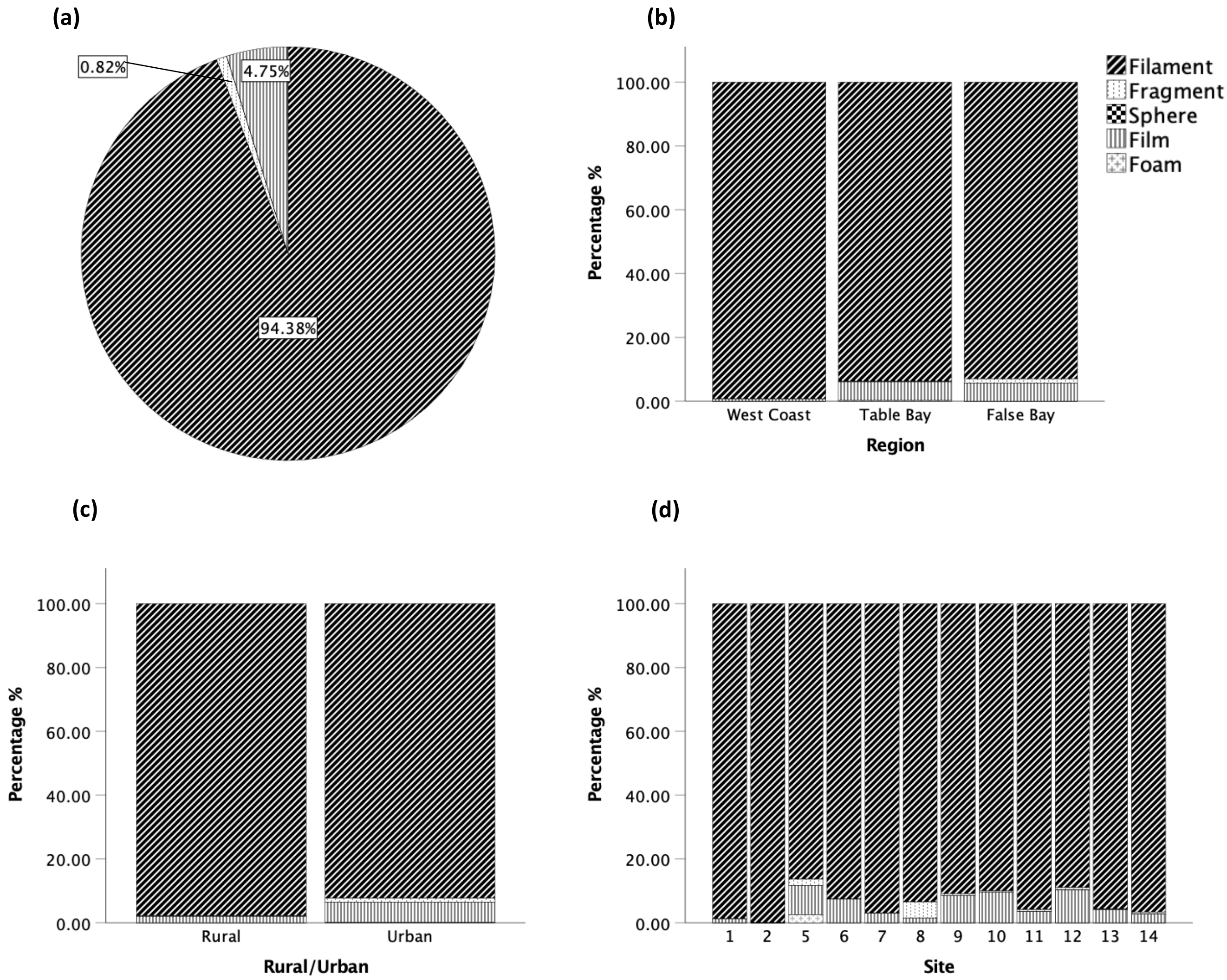

Figure 4.22: Percentage (%) of microplastic type in echinoderm samples a) combined, b) in each region, c) in rural versus urban area and d) at each site along the Western Cape coastline, South Africa

MP type varied in echinoderm species and feeding type (Figure 4.23). The dominant MP type observed in echinoderm species (Figure 4.23a) was filaments, where the percentage highest was recorded in sea cucumbers samples (97.37%) and fragments were only observed in sea star samples (6.46%). The dominant MP type observed in feeding type (Figure 4.23b) was filaments, where the highest percentage was recorded in suspension/deposit-feeding samples (97.37%).

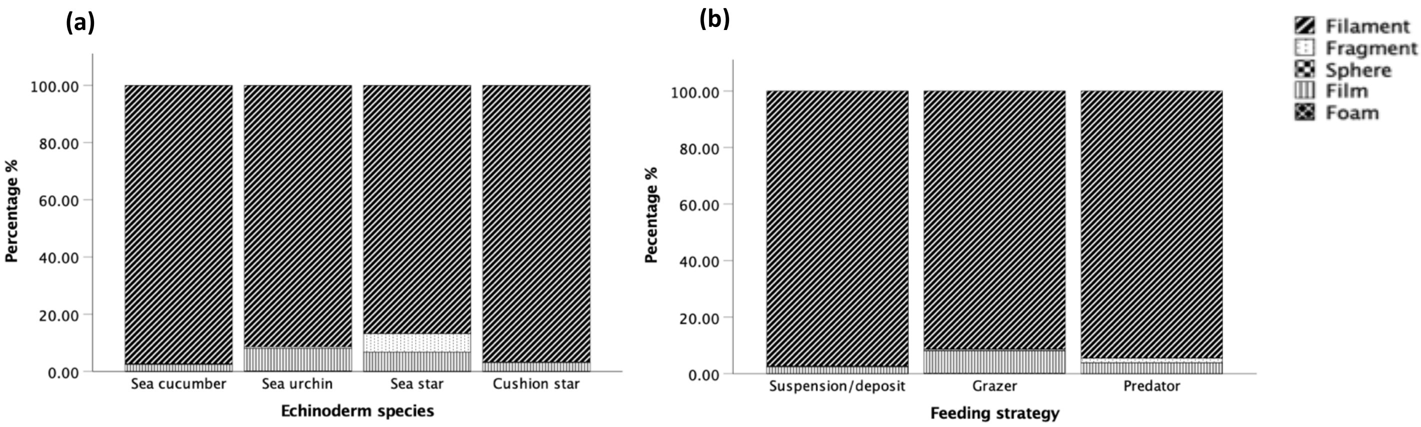

Figure 4.23: Percentage (%) of microplastic type in a) Echinoderm species and b) Feeding strategy samples collected along the Western Cape coastline, South Africa

Microplastic colour

MP size varied echinoderm samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.24). The most dominant MP colours recorded in all echinoderm samples combined (Figure 4.24a) was black/grey, transparent, blue/green and white (45.48%, 19.99%, 11.92% and 11.75% respectively). At a regional scale (Figure 4.24b) the percentage of white MPs was along the West coast (47.66%), where the percentage of transparent and black/grey MPs was highest in Table Bay (21.86% and 20.44% respectively) and the percentage of blue/green MPs was highest in False Bay (13.08%). The most dominant MP colour in urban and rural areas (Figure 4.24c) was transparent (21.45%) and black/grey (49.84%), respectively. MP colours varied in echinoderm samples across the sites (Figure 4.24d), where white, transparent, blue/green and black/grey MPs was the most abundant MP colour. The percentage of white and transparent MPs was highest in Mouille Point (site 6) (21.90%) and Blouberg (site 5) (35.77%) respectively. The percentage of blue/green and black/grey MPs was highest in Pringle Bay (site 14) (20.01%) and Eland’s Bay (site 2) (64.01%) respectively.

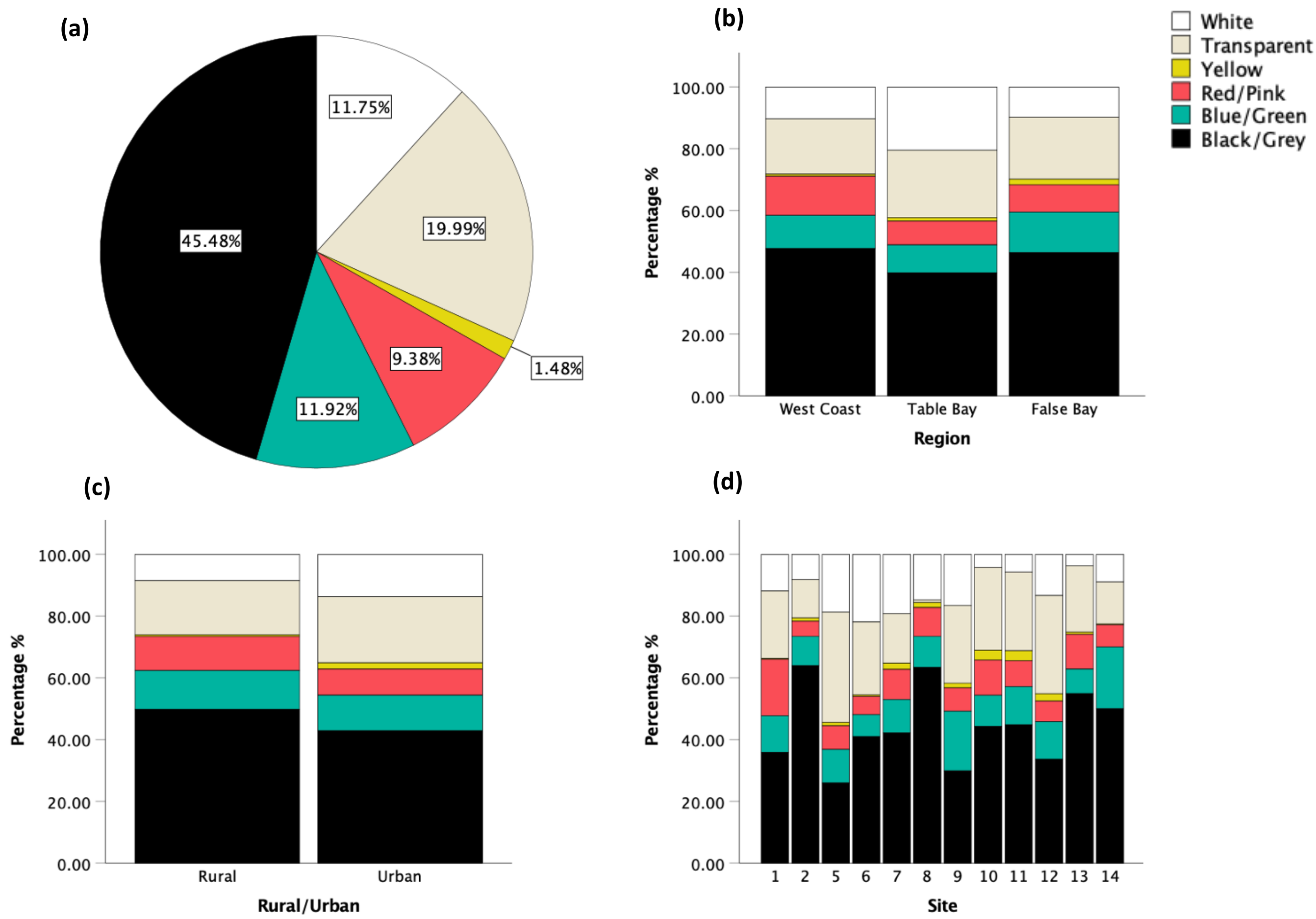

Figure 4.24: Percentage (%) of microplastic colour in echinoderm samples a) combined, a) in each region, c) in rural versus urban area and d) at each site along the Western Cape coastline, South Africa.

MP colour varied in echinoderm species and feeding type (Figure 4.25). The most dominant MP colour in echinoderm species (Figure 4.25a) was black/grey, transparent and blue/green. The percentage of black/grey and blue/green MPs was highest in cushion star species (59.26% and 10.68% respectively), whereas transparent MPs was highest in sea urchin species (31.07%). The most dominant MP colour between feeding types was black/grey and transparent (Figure 4.25b). The percentage of black/grey MPs was highest in predator species (56.82%) and transparent MPs was highest in grazer species (31.07%).

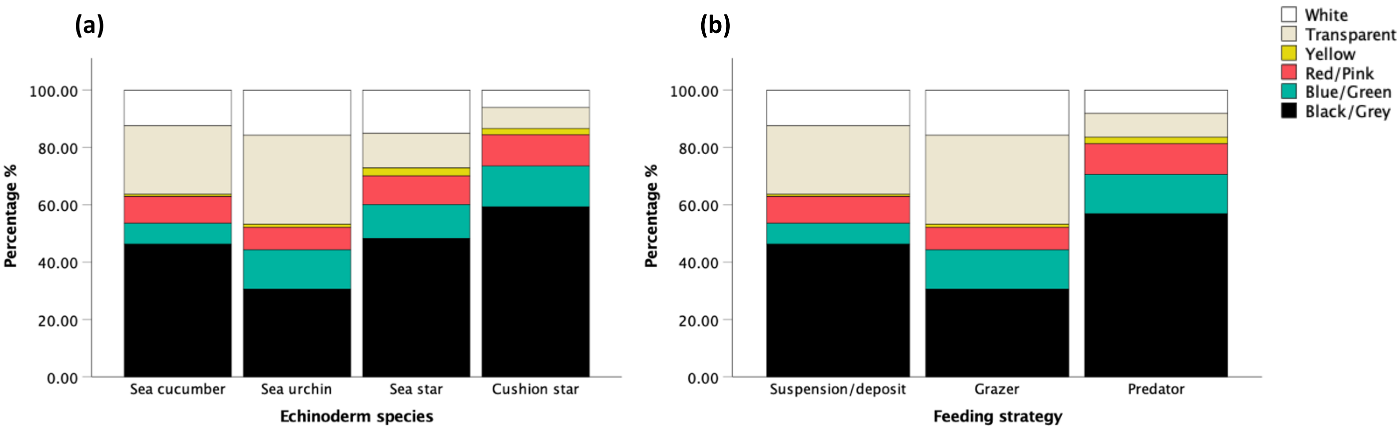

Figure 4.25: Percentage (%) of microplastic colour in a) Echinoderm species and b) Feeding type samples collected along the Western Cape coastline, South Africa

Microplastic size

MP size varied echinoderm samples across regions, urban and rural areas and sites along the Western Cape coastline (Figure 4.26). The most dominant MP size recorded in all echinoderm samples combined (Figure 4.26a) was between 1000 – 2000 µm, 2000 – 5000 µm and > 5000 µm (31.86%, 25.86% and 20.24% respectively). At a regional scale (Figure 4.26b) the percentage of MP particles between 1000 – 2000 µm was highest in False Bay (37.04%), whereas the percentage of MP particles between 2000 – 5000 µm and > 5000 µm was highest along the West Coast (30.71%) and in Table Bay (41.95%) respectively. The most dominant MP size in urban and rural areas (Figure 4.26c) was between 1000 - 2000 µm (35.32%) and between 2000 – 5000 µm (27.30%) respectively. The size of MPs varied in echinoderm samples across the sites (Figure 4.26d) with the most dominant size being between 500 - 1000 µm, 1000 – 2000 µm, 2000 – 5000 µm and > 5000 µm in samples collected from Eland's Bay (site 2) (33.37%), Kalk Bay (site 9) (45.14%), Strand (site 11) (33.96%) and Maiden's Cove (site 7) (55.46%) respectively.

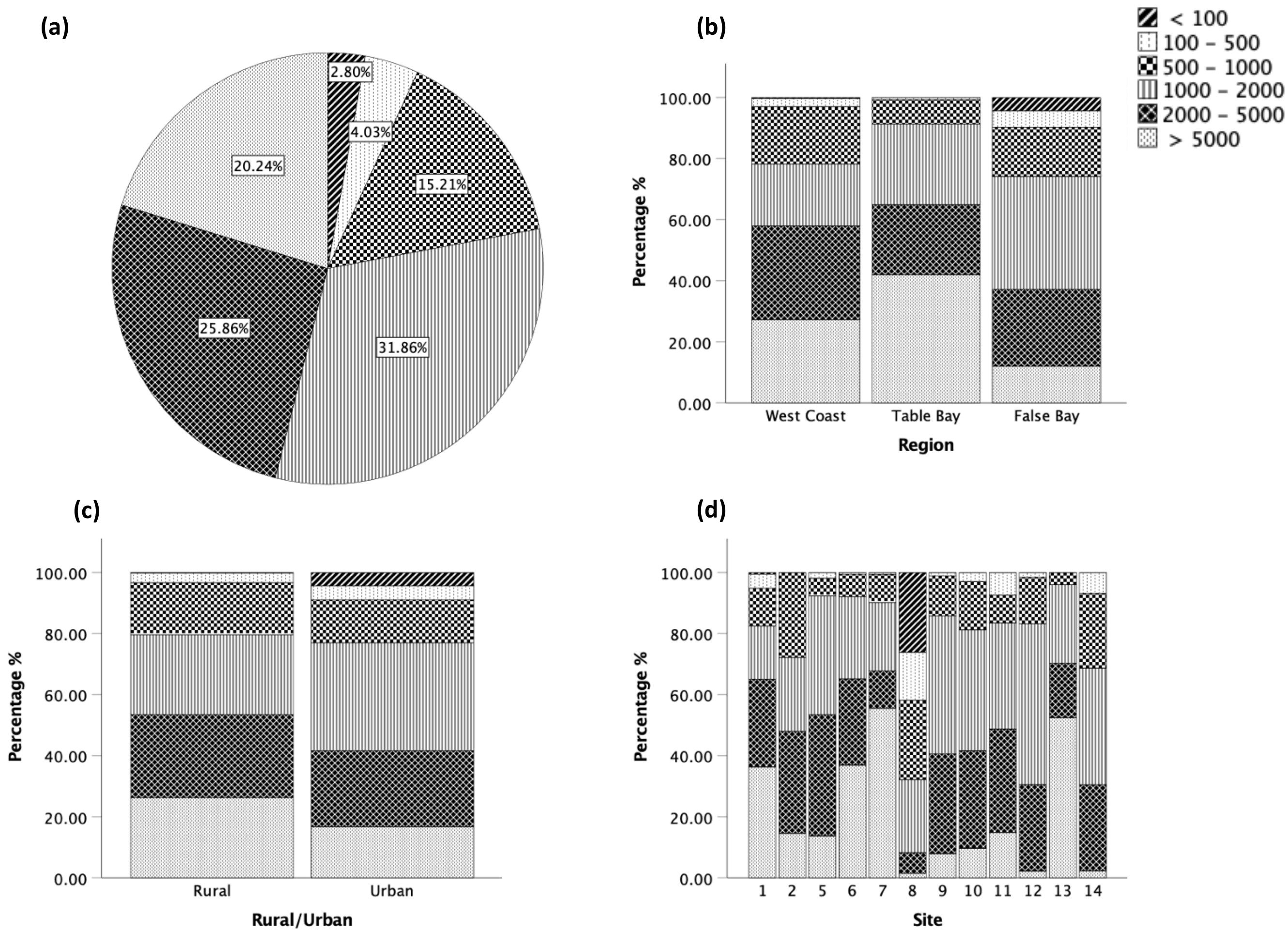

Figure 4.26: Percentage (%) of microplastic size (μm) in echinoderm samples a) combined b) in each region, c) in rural versus urban areas and d) at each site along the Western Cape coastline, South Africa

MP size varied in echinoderm species and feeding type (Figure 4.27). The most dominant MP size between echinoderm type (Figure 4.27a) was between 500 – 1000 μm, 1000 – 2000 μm and 2000 – 5000 μm. Sea urchin samples displayed the highest percentage of MP particles between 1000 – 2000 μm and 2000 – 5000 μm (45.13% and 32.39% respectively), whereas cushion stars displayed the highest percentage of MP particles between 500 – 1000 μm (28.15%). There is a noticeably high percentage of MP particles < 100 μm in sea star species (58.25%). The most dominant MP size across feeding type (Figure 4.27b) was between 500 – 1000 μm, 1000 – 2000 μm and 2000 - 5000 μm. Grazers displayed the highest percentage of MP particles between 1000 – 2000 μm and 2000 – 5000 μm (14.33% and 32.39% respectively) whereas predators had the highest percentage of MP particles between 100 – 500 μm (23.65%).

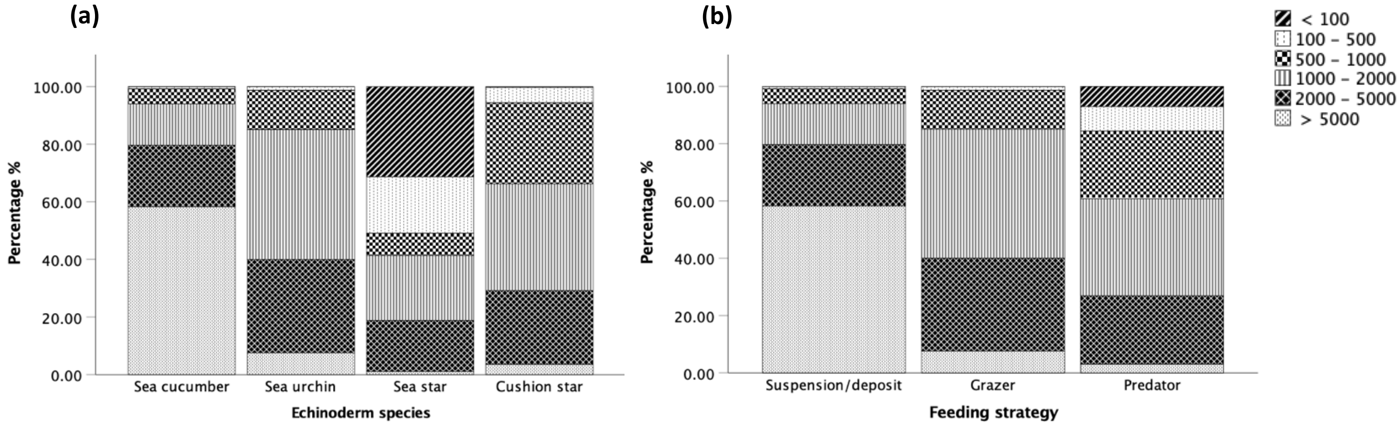

Figure 4.27: Percentage (%) of microplastic colour in a) Echinoderm type and b) Feeding type samples collected along the Western Cape coastline, South Africa

4.4.4 Microplastics polymer identification in echinoderm samples

The percentage of MP polymers was identified in all echinoderm samples (Figure 4.28a) where PET was the dominant polymer type (49.48%). Polymer identification varied in MP type (Figure 4.28b) with PET, PP and PE being the most dominant polymer type across all MP types. Filaments showed the most variation, with the PET being the most abundant polymer type (57.89%). The most abundant polymer type of film and fragment particles were identified as PP (30.26%) and PE (50%) respectively.

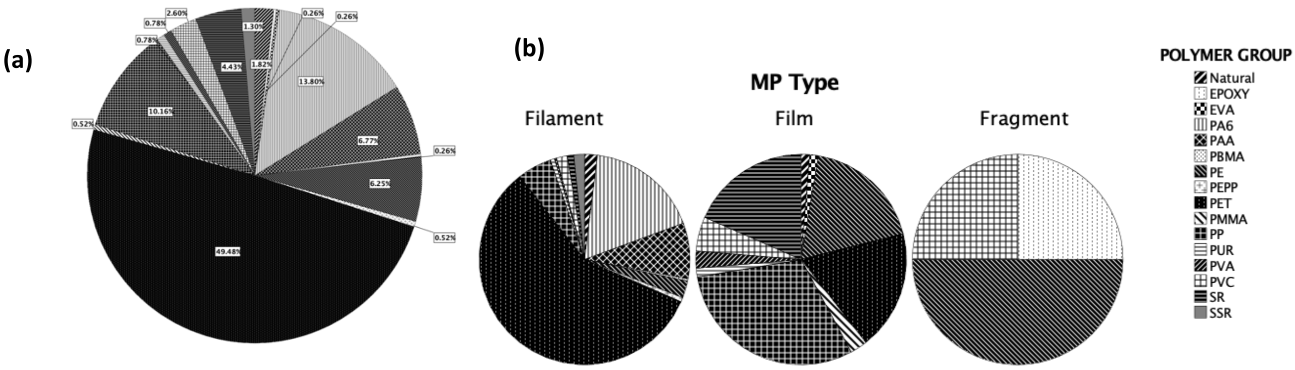

Figure 4.28: Polymer identification of a) overall MPs and b) different MP types in echinoderm samples collected along the Western Cape coastline, South Africa.
Natural: Cellulose/protein based polymers; EPOXY: Epoxy resin; EVA: Ethylene vinyl acetate; PA6: Polyamide 6; PAA: Polyacrylic acetate; PBMA: Poly butyl methacrylate; PE: Polyethylene; PEPP: Polyethylene polypropylene; PET: Polyethylene Terephthalate; PMMA: Polymethyl methacrylate; PP: Polypropylene; PUR: Polyurethane; PVA: Poly vinyl acetate; PVC: Polyvinyl chloride; SR: Synthetic rubber; SSR: Semi-synthetic rubber.

The MP polymer type varied in each region (Figure 4.29). False Bay displayed the most variability in polymer type, with PET, PA6 and PP being the most abundant polymer type

(49.19%, 13.98% and 10.48% respectively). Table Bay displayed the highest percentage of PET (60%) and the West Coast displayed the highest percentage of Natural based MPs (50%).

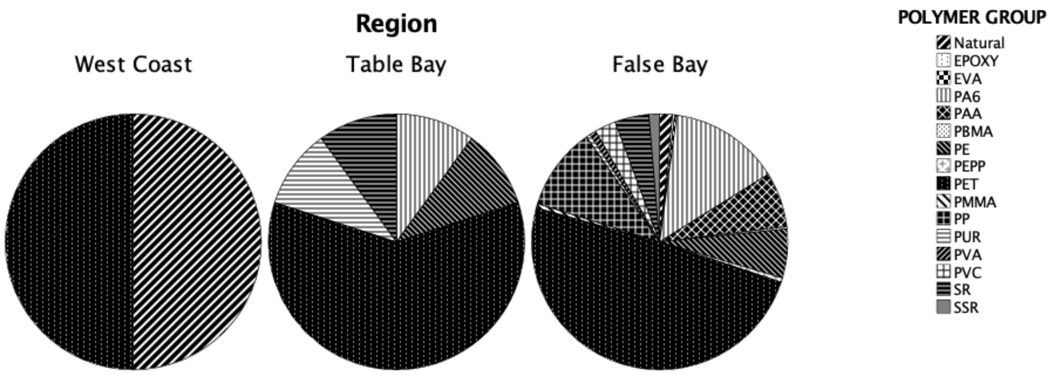

Figure 4.29: Microplastic identification in echinoderm samples collected along the Western Cape coastline, South Africa.

MP polymer type varied in urban and rural areas (Figure 4.30). Urban areas displayed the most variability in polymer type with PET, PA6, and PP being the most abundant polymer type (49.61%, 13.91%, 10.24% respectively). Rural areas displayed equal amounts of PET, PAA and Natural (33.33%).

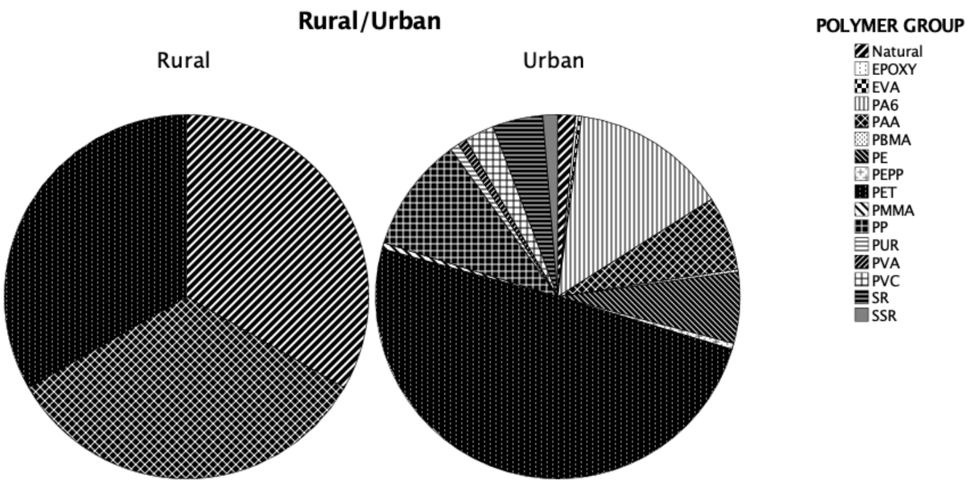

Figure 4.30: Microplastic identification in echinoderms samples collected from urban and rural areas along the Western Cape coastline, South Africa

4.4 Correlation between echinoderm, water and sediment

4.4.1 Correlation between echinoderm weight (g) and total number of MPs (n).

A correlation between the total number of MPs recorded in echinoderm type and echinoderm weight (g) was conducted (Figure 4.31). The results show no relationship between total number of MPs and total weight, however, sea cucumbers and sea urchins showed to have more MPs in smaller organisms than in bigger organisms.

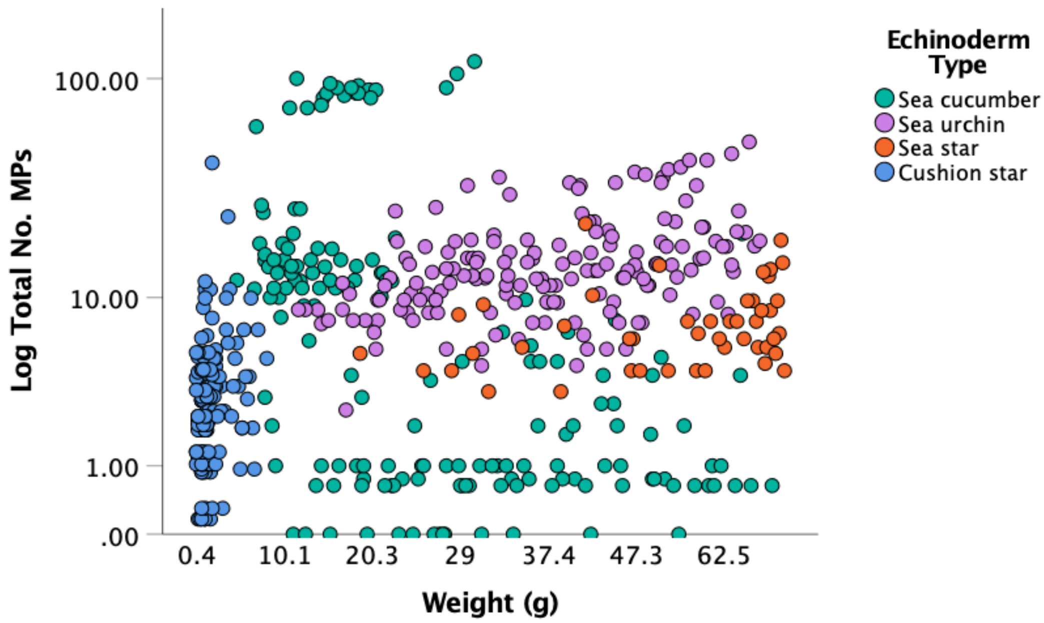

Figure 4.31: Correlation between total number of microplastics in echinoderm type and echinoderm total weight (g)

4.4.2. Correlation of MP concentrations in echinoderm (MPs/g), water (MPs/L) and sediment (MPs/kg).

A correlation between MP concentrations at each site was conducted on water (MPs/L) and echinoderm (MPs/g) samples (Figure 4.32a). There was a weak (negative) correlation ($r = -0.131$; $p = 0.282$) between water and echinoderm concentrations. The results show an inversely proportional relationship between MP concentrations with the exception of a few outliers, namely Kalk Bay (site 9). A correlation between MP concentrations at each site was conducted on sediment (MPs/kg) and echinoderm (MPs/g) samples (Figure 4.32b). There was a moderate (negative) correlation ($r = -0.408$; $p < 0.001$) between sediment and echinoderm concentrations. The results show an inversely proportional relationship between MP concentrations with the exception of a few outliers, namely Blouberg (site 5), Kalk Bay (site 9) and Gordan's Bay (site 12).

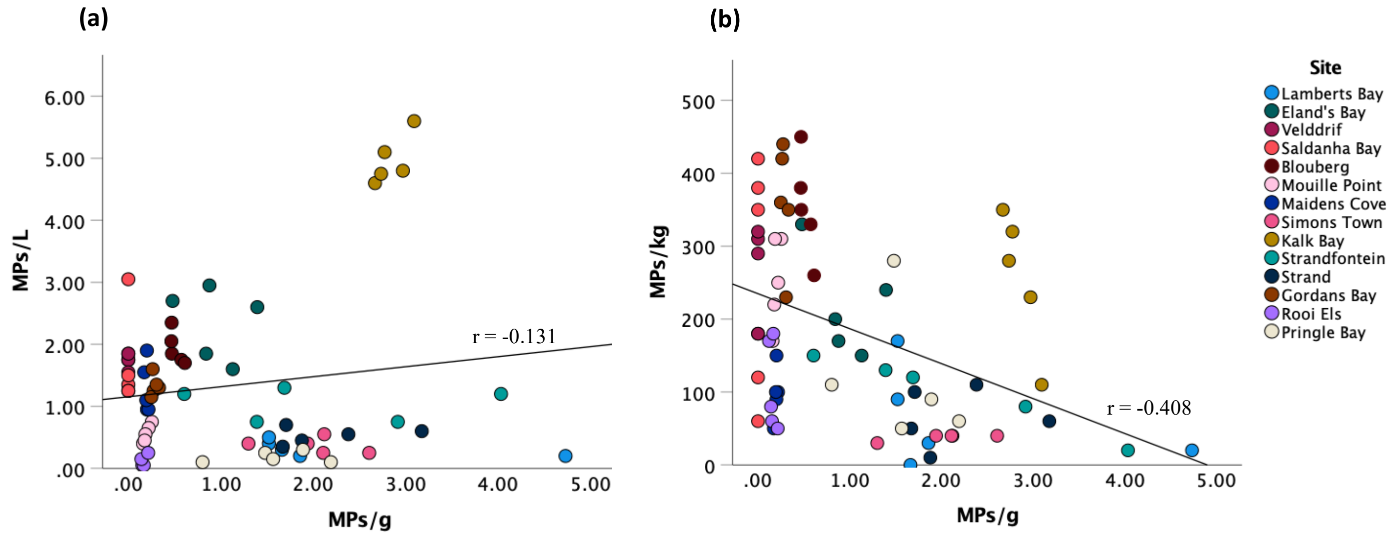

Figure 4.32: Correlation between microplastic concentrations in a) water (MPs/L) and echinoderm (MPs/g) samples and b) sediment (MPs/kg) and echinoderm (MPs/g) samples collected along the Western Cape coastline, South Africa.

4.5 Risk assessment of microplastics in water, sediment and echinoderm samples

A risk assessment was conducted to assess the risk posed by MPs in samples collected along the coastline of the Western Cape, South Africa. Risk categories of indices for MP contamination is displayed in Table 0.1.

Table 0.1: Risk categories of indices for microplastic contamination in samples collected along the Western Cape coastline, South Africa

Risk Category	**Low (I)**	**Moderate (II)**	**High (III)**	**Very high (IV)**	**Dangerous (V)**
Contamination Factor (CF)	< 1	1 - 3	3 - 6	> 6	
Pollution Load Index (PLI)	< 1	1 - 3	3 - 4	4 – 5	> 5
Polymer Risk Index (H)	< 10	10 - 100	101 - 1000	1000 – 10000	> 10000
Pollution Risk Index (PRI)	< 150	150 - 300	300 - 600	600 - 1200	> 1200

The MP Pollution Load Index (PLI) generally showed low contamination levels in water samples collected at each site (Figure 4.33a), however Kalk Bay (site 9) displayed moderate contamination levels (1.55). The MP PLI in sediment samples (Figure 4.33b) varied across all sites, with dangerous contamination levels at Eland's Bay (site 2) (5.20), Velddrif (site 3) (5.75), Saldanha Bay (site 4) (6.20), Blouberg (site 5) (8.75), Mouille Point (site 6) (5.87), Kalk Bay (7.53) and Gordan's Bay (site 12) (8.73). The MP PLI in echinoderm samples (Figure 4.33c) displayed generally low to moderate contamination levels across the sites, however dangerous levels of contamination was seen at Kalk Bay (18.47), Strand (8.90) and Strandfontein (site 10) (5.67).

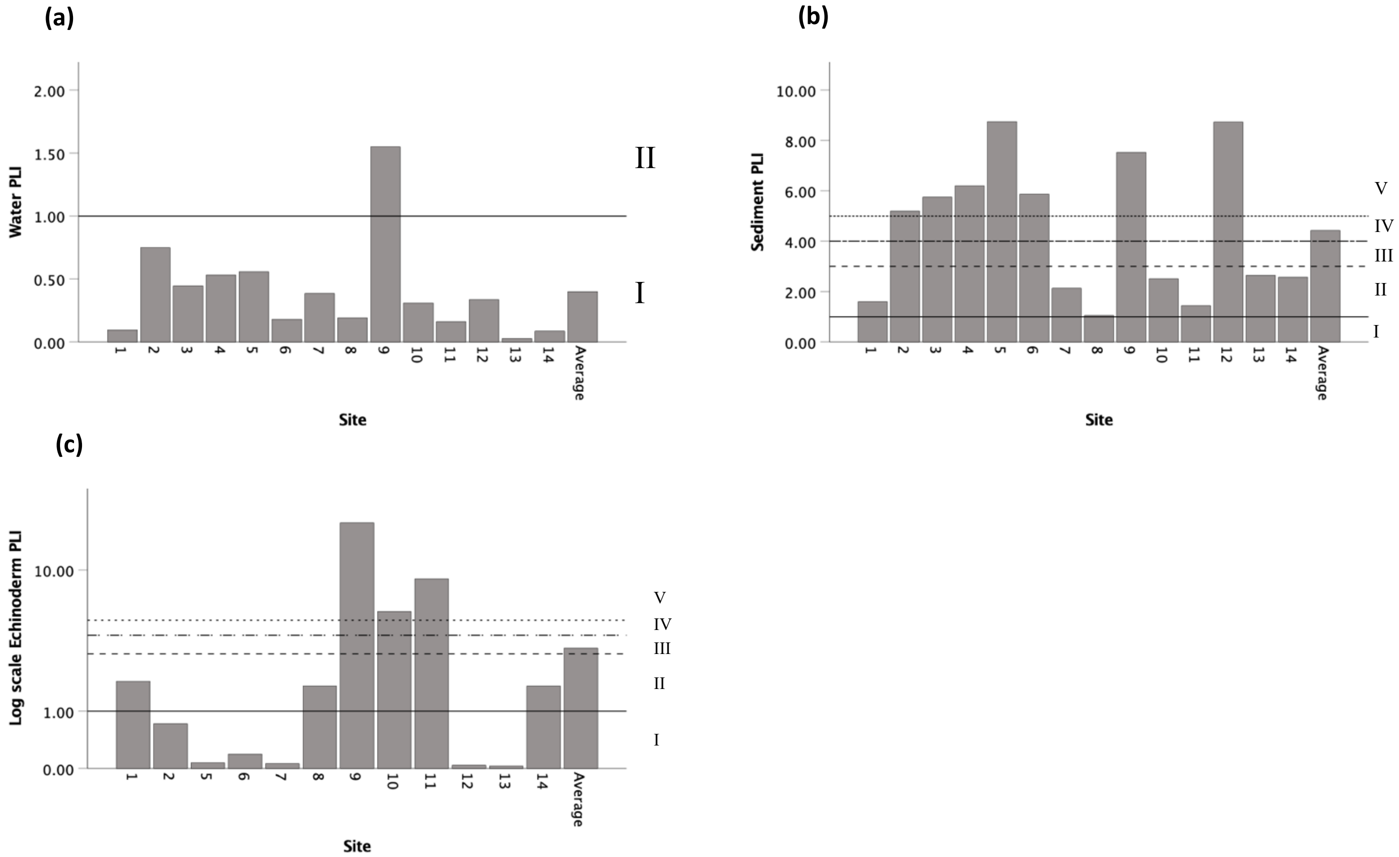

Figure 4.33: Microplastic Pollution Load Index (PLI) in a) water, b) sediment and c) log scale echinoderm samples collected at each site along the Western Cape coastline, South Africa. See Table 0.1 for categories of indices. Note the log scale for PLI in echinoderm samples.

The Polymer Risk Index (H) generally displayed high to very high levels in overall samples combined (Figure 4.34a) with noticeably very high levels of toxic chemicals associated with polymers recorded at Mouille Point (site 6) (1 399). The Pollution Risk Index (PRI) generally displayed a low risk for all sample types combined (Figure 4.34b), however Kalk Bay (site 9) and Mouille Point (site 6) displayed dangerous levels (5 579.91 and 2 909.73 respectively).

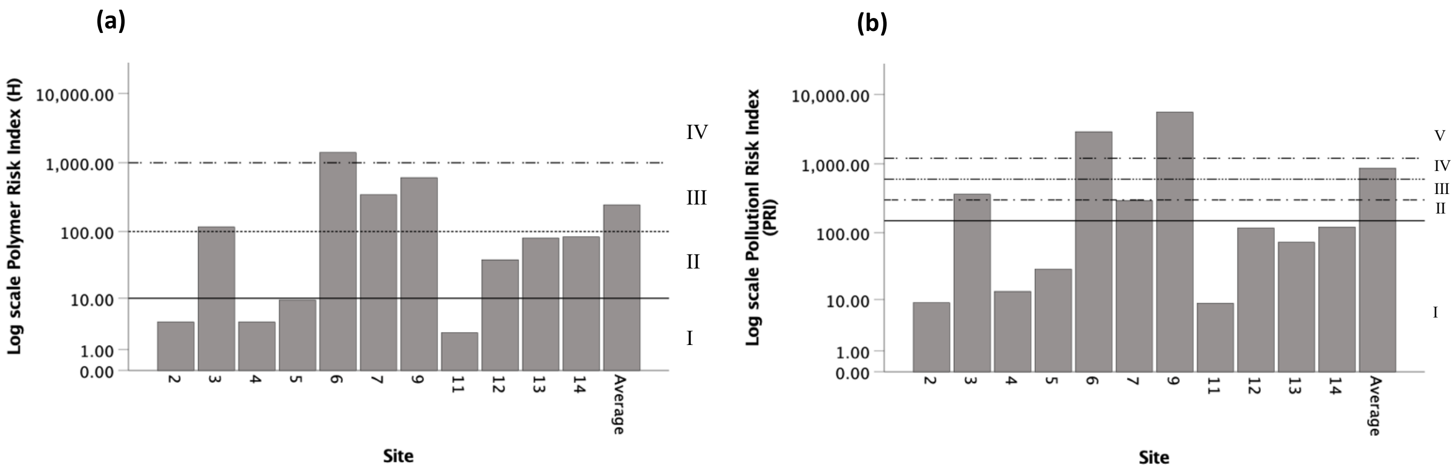

Figure 4.34: a) Log polymer risk index (H) and b) Log pollution risk index (PRI) of microplastics in all samples types combined at each site along the Western Cape coastline, South Africa. See Table 0.1 for categories of indices. Note the log scale for both polymer risk index (H) and pollution risk index (PRI).

Chapter 5

Discussion

5.1 Water samples

5.1.1 Concentration, abundance and MP classification in water samples

Of the 70 water samples, 69 (98.57%) contained microplastic (MP) particles. A total of 1840 MP particles was recorded , with a mean concentration of 1.33 particles per litre (MPs/L) (± 0.15 SE). MP concentrations was highest in water samples collected from urban areas (1.52 ± 0.20 MPs/L) ($p = 0.038$) (Figure 4.2b). This is directly linked to the high population density in urban areas (de Villiers, 2018) (Table 0.1) as well as the amount of outfall and storm water pipes (de Villiers, 2018; Naidoo et al., 2015), WWTW discharge points(de Villiers, 2018; Murphy et al., 2016; Nel et al., 2017; Sparks, 2020) harbours and their related (Nel et al., 2017; Paradas & Amado-Filho, 2007; Sparks & Awe, 2022). MP analyses of water samples collected at 14 sites along the Western Cape coastline indicated MP concentrations were highest at Kalk Bay (site 9) (4.97 ± 0.18 MPs/L) (Figure 4.2c). Kalk Bay is situated within 10 m of a stormwater pipe in a sheltered area experiencing weak water circulation, which most likely could account for the high MPs recorded. MP classification results indicated filaments and film were the most dominant MP type for all water samples combined (Figure 4.3a), with the highest percentage recorded in Simon's Town (site 8) and Saldanha Bay (site 4) respectively (Figure 4.3d). Filamentous MPs are commonly found in coastal waters all over the world (Nel & Froneman, 2015; Rodrigues et al., 2020). Black/grey and white MP particles were the most dominant MP colour for all water samples combined (Figure 4.4a) with the highest percentage recorded at Simon's Town (site 8) and Blouberg (site 5) respectively (Figure 4.4d). The most dominant MP size for all samples combined was between 1000 – 2000 µm followed by 2000 – 5000 µm (Figure 4.5a) with the highest percentage recorded at Lambert's Bay (site 1) and Eland's Bay (site 2) (Figure 4.5d). In addition, Kalk Bay had the highest percentage of MP particles > 5000 µm (Figure 4.5d). Kalk Bay experiences high pedestrian traffic and has a weak water circulation, which could account for the large MP sizes recorded during sampling event (Sparks, 2020). These results are comparable with results recorded in various studies around the world.

5.1.2 Microplastic polymer identification in water samples

10% of identified MPs was processed for FTIR analyses and 93.75% of MPs analysed were polymer based plastics with the remaining 6.25% being natural based plastics (cotton). For all the sites combined, the most dominant polymer types recorded in water samples was Polyethylene terephthalate (PET) and Polyethylene (PE) (Figure 4.6a). Filaments were predominantly identified as PET and film predominantly identified as PE (Figure 4.6b). PET applications include bottles which are susceptible to oxygen and UV degradation (Andrady & Neal, 2009) and could explain its abundance in the marine environment. PET has a high density and settling rate, however these particles have the ability to be resuspended in the water column through bioturbation by organisms, upwelling events, change in physical characteristics, such as size, shape and density, ageing and weathering (Carbery et al., 2018). PE is used to produce plastic film and applications include carrier bags, cling wrap and freezer bags (Andrady & Neal, 2009). False Bay displayed the highest variability in polymer type followed by Table Bay (Figure 4.7). Both regions experiences large scale winds (Fennel, 1999; Garzoli & Gordon, 1996; Lamprecht et al., 2013), allowing plastics with various densities from different areas to be blown into the ocean. In addition there are various products produced linked to sources that could potentially explain the variation in polymer types ranging from industrial areas, WWTW, river runoff, to harbour and fishing related activities.

5.2 Sediment samples

5.2.1 Concentration, abundance and MP classification in sediment samples

Of the 70 sediment samples, 69 (98.57%) contained MP particles. A total of 1281 particles were recorded, with a mean concentration of 185 particles per kilogram (MPs/kg) (± 15.25 SE). MP concentrations was highest in sediment samples collected from Table Bay (234.67 ± 31.42 MPs/kg) (Figure 4.9b). Table Bay is a shallow bay and surface currents are generally weak, thus allowing MP particles to settle in sediment. Table Bay forms part of Cape Town harbour where reports of spills and discharge from harbour activity have been linked to marine pollution. Other potential sources of MP contamination could possibly be linked to river systems (The Salt River, The Liesbeek River and The Black River) directly entering Table Bay. Black River has been identified as a source of marine pollution due to effluent from industrial and residential areas. Another notable feature about this region is the large amount of WWTW discharging effluent directly into near shore environments samples, which in studies have been identified as sources of MP contamination (de Villiers, 2018; Sparks, 2020). MP analyses of sediment samples collect from 14 sites along the Western Cape coastline indicated MP concentrations were highest at Gordan's Bay (site 12) (360.00 ± 36.74 MPs/kg) (Figure 4.9c). Gordan's Bay samples was collected in a semi-enclosed area 100 m outside of a harbour. Research has shown MP concentrations being linked to harbour and marine related (Paradas & Amado-Filho, 2007; Preston-Whyte et al., 2021; Sparks & Awe, 2022). In addition, semi-enclosed, shallow areas have weak water circulation, allowing MPs present in the water to settle in sediment. Other factors contributing to the high MP concentration in Gordan's Bay could be caused by the Sir Lowry's River receiving waste from the WWTW and entering the ocean at Gordan's Beach (City of Cape Town, 2019).

The results also displayed a weak relationship, the correlation and significant difference between sediment grain size and MP concentration was higher in fine sand ($r = -0.391$; $p = 0.001$) (Figure 4.11d).This suggests that MP concentrations are affected by sediment properties (Wang et al., 2020). The hydraulic equivalence of plastic particles of a particular shape, size and density behave similar to naturally occurring sediment particles of similar shape, size and density in the environment (Enders et al., 2019; P. T. Harris, 2020). However, the correlation between other grain sizes are not clear and had no significant. This could be linked to environmental conditions of the location (Wang et al., 2020) along the Western Cape coastline, potential sources and particle size, shape and density. The Western Cape coastline has a relatively narrow band. Hydrodynamics along the coastline is affected by the direct interaction between land and sea and associated air masses. Studies have found that the shape, width and circulation along coastlines affect hydrodynamic forces, which factors that influence deposition of MPs along shorelines (Wang et al., 2020).

Based on the classification of MPs analysed, filament and film was the most abundant MP type for all samples combined (66.96% and 28.13% respectively) (Figure 4.12a), with the highest percentage recorded in Kalk Bay (site 9) (97.56%) and Saldanha Bay (site 4) (57.21%) respectively (Figure 4.12d). Various studies have reported filament as the most dominant MP type (de Villiers, 2018; Sparks & Awe, 2022; Sun et al., 2021) followed by film. This is because filament and film behave differently in the ocean due to their surface area to mass ratio being greater than other MP types (Bergmann et al., 2015). In addition, biofouling rates are much higher on these MP types than on other MP types, causing MPs to sink sooner over a shorter period of time. Once on the beach, filaments are incorporated into the sediment matrix through pore-migration (P. T. Harris, 2020) . Filaments are more susceptible to being trapped within in sediment and this could explain the relatively high abundance of this MP type in the sediment (Cózar et al., 2014). Transparent, white and black/grey MP particles was the most dominant MP colour for all sediment samples combined (30.89%, 29.64% and 18.32% respectively) (Figure 4.13a). Kalk Bay (site 9) had the highest percentage of black/grey (35.35%), where Saldanha Bay (site 4) and Velddrif (site 3) had the highest percentage of white (57.17%) and transparent (57.41%) MP particles (Figure 4.13d). The colour variability of MPs could be a result of various sources. MP size varies across the regions (Figure 4.14b) and sites (Figure 4.14d), with the most abundant MP size for all sediment samples combined was between 2000 – 5000 μm (45.28%) (Figure 4.14a). Velddrif (site 3) and Strand (site 11) had the highest percentage of MP particles between 1000 – 2000 μm (57.51%) and 2000 – 5000 μm (39.24%) respectively. The size variability could be a result of secondary MPs being formed a combination of photo-degradation, mechanical transformation from wave action and biological degradation by organisms (Andrady, 2011; Browne, 2007; Cole et al., 2011). MP size is important for understanding how MPs are transported and spatially distributed in the marine environment via currents and waves relative to their hydraulic equivalence to natural sediment particles (P. T. Harris, 2020). Sand particles have the hydraulic equivalence with larger-sized plastic particles despite the differences in density (Enders et al., 2019) and studies have found a correlation between plastic particle size and wave/current energy (Enders et al., 2019; Ling et al., 2017). This could explain why MP particles in this study ranged between 1000 – 2000 μm, 2000 – 5000 μm and > 5000 μm.

5.2.2 Microplastic polymer identification in sediment samples

10% of identified MPs was processed for FTIR analyses and 68% of MPs analysed were polymer based plastics with the remaining 32% being natural based plastics (cotton). For all sediment samples combined (Figure 4.15a), the most dominant polymer types recorded was natural (cotton) (32%) followed by Polystyrene (24%) and Polyethylene terephthalate (PET) (16%). Filaments were either identified as natural (50%) or PET (50%), whereas film was predominantly identified as PE (66.67%) (Figure 4.15b). The West Coast region displayed the most variability in polymer types, with Table Bay having the highest percentage of PS (40%). The West Coast region is a mixed-used area with potential sources of MPs from activities such as fishing, stormwater outfalls, river input, agriculture to harbour and aquaculture. Products emanating from sources could explain the various in polymer type. The Table Bay region forms part of Cape Town harbour and has weak water circulation. As a result low density PS based plastics are able to experience biofouling, making plastic more dense causing it to sink and settle in sediment. Even though PS is generally water resistant, it can absorb water once it comes into direct contact, increasing its density and facilitating in it sinking.

5.3 Echinoderm samples

5.3.1 Concentration, abundance and MP classification in echinoderms samples

Of the 539 echinoderm samples, 523 (97.03%) contained MP particles. A total of 5988.22 MP particles was recorded, with a mean MP concentration of 1.44 MP particles per gram (MPs/g) (± 0.12 SE) and abundance of 11.7 MP particles per individual echinoderm (MPs/I) (± 0.76 SE). The concentration and abundance of MPs is directly linked to echinoderm type sampled at the site (Table 0.2), with Kalk Bay (site 9) having the highest concentration (2.90 ± 0.38 MPs/g) and abundance (59.95 ± 4.68 MPs/I) (Figure 4.19c). These results are reflective of the site conditions of Kalk Bay, being located 10 m from a stormwater pipe in a sheltered area experiencing weak water circulation. The MP abundance differed according to echinoderm type (Figure 4.21d) with the highest abundance recorded in non-selective filter-feeding sea cucumbers (17.57 ± 2.34 MPs/I). Studies have found that non-selective invertebrates ingest more MP particles that other feeding strategies (Iwalaye et al., 2020a; Setälä et al., 2016; Sparks, 2020; Taylor et al., 2016). Sea cucumbers acquire organic nutrients through filtering large amounts of water and sediment (Browne, 2007). Sea cucumbers indirectly ingest MPs by spreading their tentacles and feeding on MPs suspended in the water column and sediment (Iwalaye et al., 2020a). Whereas, the concentration of MPs was highest in predator echinoderms (2.47 ± 0.23 MPs/g) (Figure 4.21b). This suggests that predators are consuming prey that are already contaminated with MPs allowing MPs to not only be transferred from one trophic to the next, but has the potential to bioaccumulate and bio-magnify (Farrell & Nelson, 2013; Teuten et al., 2007).

Based on the classification of MPs analysed, the most abundant MP type ingested all by echinoderm samples combined was filament (94.48%) followed by film (4.75%) (Figure 4.22a). MP type ingested by echinoderms varied across all the sites, with Eland's Bay (site 2) having 100% filamentous particles and Gordan's Bay (site 12) having 10.29% of MP particles as film (Figure 4.23d). Filaments are mistakenly identified as food and accidentally consumed by echinoderms with various feeding strategies (de Sá et al., 2018; Nelms et al., 2018). These results are reflective of other studies conducted (Table *0.2*). MP colour varied with black/grey, transparent, blue/green and white particles being the most dominant MP colour (45.48%, 19.99%, 11.92% and 11.75% respectively) for all samples combined (Figure 4.24a). MP colours varied across the sites with Mouille Point (site 6) and Blouberg (site 5) having the highest percentage of white and transparent particles (21.09% and 35.77% respectively). Pringle Bay (site 14) and Eland's Bay (site 2) having the highest blue/green (20.01%) and black/grey (64.01%) respectively. The colour ingested is a result of various sources, with preference to darker colours. It is proposed that the ingestion of darker MPs is selectively preyed upon by filter-feeding organisms, suggesting that they ingest based on their prey preference (de Witte et al., 2014). However, there is not enough research to support this assumption as there is no evidence of filter-feeding organisms actively consuming MPs based on colour (A. L. Lusher et al., 2013). MP size varied across the regions (Figure 4.26b) and sites (Figure 4.26d), with the most abundant MP size ingested by echinoderms for all samples combined was between 1000 – 2000 µm (31.86%), 2000 – 5000 µm (25.86%) and >5000 µm (20.24%) (Figure 4.26a). Filter-feeding sea cucumbers in Maiden's Cove (site 7) ingested the biggest (>5000 µm) MP particles (55.46%) followed by sea cucumbers sampled in Kalk Bay (site 9) ingesting particles between 1000 – 2000 µm (45.14%). This suggests that in addition to feeding strategies smaller MPs facilitate the ingestion of plastic particles as opposed to larger sizes (Fang et al., 2018; A. L. Lusher et al., 2013). Other factors influencing the variation in MP sizes ingested could be a result of sources of contamination and bioavailability thereof.

5.3.2 Microplastic polymer identification in echinoderm samples

10% of identified MPs was processed for FTIR analyses and 98.18% of MPs analysed were polymer based plastics with the remaining 1.82% being natural based plastics (cotton). For all echinoderm samples combined (Figure 4.28a), the most dominant polymer type recorded was PET (49.48%). Filaments were predominantly PET (57.89%) (Figure 4.28b). False Bay displayed the most variability in polymer type, with PET being the most dominant type ingested by echinoderm species (Figure 4.29). These results are reflective of polymers recorded in water (Figure 4.7) and sediment (Figure 4.16) samples from this region, suggesting echinoderms acquire MPs directly from the environment. False Bay is a semi-enclosed region with weak water circulation aiding behaviour of this polymer type in the environment. PET has a high density and settling rate (Carbery et al., 2018), causing it to sink making it bioavailable to organisms, particularly filter-feeders inhabiting sedimentary environments. Various products (Crompton, 2007; Enders et al., 2019) used could be linked to potential sources explaining the variation in polymer types ranging from industrial areas, WWTW, river runoff, to harbour and fishing related activities.

5.4 Risk assessment of microplastics in water, sediment and echinoderm samples

The Pollution Load Index (PLI) was categorised as generally low (Lithner et al., 2011) for water (Figure 4.33a) and echinoderm samples (Figure 4.33c) however dangerous levels were observed in echinoderms collected from Kalk Bay (site 9) and Strandfontein (site 10). This dangerously high level is of concern, particularly filamentous MP polymers, as it is considered a high-risk for marine organisms (Qiao et al., 2019). Filaments are mistakenly identified as food and accidentally consumed by echinoderms with various feeding strategies (de Sá et al., 2018; Nelms et al., 2018). The PLI was categorised as generally very high (Lithner et al., 2011) in sediment samples across all the sites, however Blouberg (site 5) and Gordan's Bay (site 12) displayed dangerous levels (Figure 4.33b). These high concentration levels have the potential to threaten marine species inhabiting the sediment. In addition, it has potential to be resuspended through wave action and bioturbulance, which are factors that reintroduce MPs into the water column and could be ingested by marine organisms. The Pollution Risk Index (H) (Figure 4.34a) and the Polymer Risk Index (PRI) (Figure 4.34b) for all samples combined was noticeably very high and dangerously high respectively at Mouille Point (site 6). It is important to note that Mouille Point did not necessarily display the highest MP concentration, but had polymers with high assigned hazard scores (PUR and ABS) (Lithner et al., 2011). This indicates that the risk MPs poses on the environment is associated with the polymer type and not necessarily the MP concentration. This is important to note as the effects associated with polymer types could potentially pose a threat to marine organisms, particularly echinoderms, ingesting MPs.

5.5 Correlation between MP concentrations and sample type (water, sediment and echinoderms)

The result displays the manner in which MPs interact and reside within the marine environment. There is a strong correlation between MP concentrations in water (MPs/L) and sediment (MPs/kg) (samples (Figure 4.18a). This implies that MPs present in the water column eventually settle in marine sediment (Alomar et al., 2016; Pham et al., 2014). However, it is important to note that where MP concentrations were higher in water samples in relation to sediment samples, it could indicate MPs are being resuspended through bioturbation by organisms, wave action, upwelling events, change in physical characteristics, such as size, shape and density, ageing and weathering (Carbery et al., 2018). There is no relationship between the total number of MPs (n) and echinoderm weight (g) ((Figure 4.31), however sea cucumbers and sea urchins displayed a higher MP count in smaller organisms than bigger organisms (Figure 4.31). Suggesting that MP ingestion is dependent on the organism's feeding strategy, age, habitat, diet, size, age (Ryan, 1987; Ryan et al., 2020) and their interaction with water and sediment (Pinheiro et al., 2020). Smaller sea cucumbers swallow higher amounts of coarser sediment than larger sea cucumbers (Sabilu et al., 2021). These results are reflected in the correlation between MP concentrations in echinoderms and sediment ($r = 0.408$) between Lambert's Bay (site 1) and Kalk Bay (site 9) (Figure 4.32b) having a higher percentage of medium sediment (Figure 4.10b). Although the results show a weak, inversely proportional relation between MP concentrations in the overall water (MPs/L) and echinoderm (MPs/g) samples ($r = -0.131$) (Figure 4.32a), the results display when concentrations are high in water samples it is low in echinoderms samples collected from Saldanha Bay (site 4), Blouberg (site 5), Maidens Cove (site 6) and Eland's Bay (site 2). This could imply MPs are being resuspended into the water column via bioturbulance by sea cucumbers.

A summary of MP concentration in coastal water and sediment environments around the world (Table 0.1). Study areas included harbours and marinas, surface water, rivers, shorelines and beaches, intertidal zones and rocky shores.

Table 0.1: Microplastic abundance in different water and sediment samples from previous studies

Sample type	Location	Site description	Microplastic particles	Unit	Reference
Water	Western Cape, South Africa	Rocky shores	1.33	L	This study
	Durban, South Africa	Harbour	1.20	L	(Nel et al., 2017)
	Richards Bay, South Africa	Harbour	0.41	L	
	Sweden	Harbour	0.15 – 2.40		(Norén, 2007)
	Australia	Harbour	0.06 – 2.50	L	(Su *et al*., 2020)
	South-eastern coastline, South Africa	Surface coastal waters	0.26 – 1.22	L	(Nel & Froneman, 2015)
Sediment	Western Cape, South Africa	Rocky shores	185.07	kg	This study
	Simon's Town	Marina		kg	(Sparks & Awe, 2022)
	South Africa	beaches			(de Villiers, 2018)
	Sweden	Harbour	20 – 50	kg	(Norén, 2007)
	Belgium	Harbour	166.70	kg	Claessens *et al*., 2011
		Intertidal	92	kg	
	Slovenia	Shoreline	177.8	kg	(Laglbauer *et al*., 2014)
	Eastern Cape, South Africa	River	160.10	kg	(Nel et al., 2017)
	Germany	Beach (North 1)	106.39	kg	(Hengstmann *et al*., 2018)
		Beach (West)	76.27	kg	
		Beach (East)	94.41	kg	
		Beach (North 2)	63.11	kg	
	Bohai Sea	Beach	39.90	kg	(Yu *et al*., 2016)
	Hong Kong	Coastal beaches	49 - 279	kg	(Tsang *et al*., 2017)
	Hangzhou Bay		167	kg	(Teng *et al.*, 2020)
	Ireland	Intertidal	0 - 553	kg	(Mendes et al., 2021)

A summary of MP concentration and abundance in marine invertebrates ranging in feeding strategy around the world (Table 0.2). The table also displays classification of MPs, with filamentous particles being the most dominant MP type, black/grey being the most dominant colour and 500 – 1000 μm the dominant size.

Table 0.2: Microplastic abundance in biota samples from previous studies

Location	**Organism**	**Scientific name**	**Feeding type**	**MP particles per gram (MPs/g)**	**MP per individual (MPs/I)**	**Common MP Type**	**Common MP colour**	**Common MP size (µm)**	**Reference**
Western Cape, South Africa	Sea cucumber	*Roweia frauenfeldii* and *Roweia stephenson*	Suspension/deposit	1.14	17.75	Filaments	Black/grey	>5000	This study
	Sea urchin	*Parenchinus angulosus*	Grazer	0.42	15.67	Filaments	Transparent	1000 - 2000	
	Sea star	*Marthasterias africana*	Predator	0.14	7.75	Filaments	Black/grey	< 100	
	Cushion star	*Parvulastra exigua*	Predator	3.13	3.22	Filaments	Black/grey	500 - 1000	
KwaZulu-Natal	Sea cucumber	*Holothuria cinerascens*	Sususpension/deposit	8.13 – 10.23	-	Filaments	-	-	(Iwalaye et al., 2020a)
	Crab	*Dotilla fenestrata*	Predators/scavengers	8.13 – 12.07	-	Filaments	-	-	
	Redbait	*Pyura stolonifera*	Sususpension/deposit	3.57 – 4.60	-	Filaments	-	-	
Thailand	Striped barnacle	*Balanus amphitrite*	Filter	0.23 – 0.43	-	-	-	-	(Thushari et al., 2017b)
	Rocky oyster	*Saccostrea forskalii*	Filter	0.37 - 0.57	-	-	-	-	
	Periwinkle	*Littoraria sp.*	Grazer	0.17 – 0.23	-	-	-	-	
Germany	Blue mussel	*Mytilus edulis*	Filter	0.36	-	-	-	-	(van Cauwenberghe et al., 2015)
France	Oyster	*Crassostrea gigas*	Filter	0.47	-	-	-	-	
South Africa	Mussel	*Aulyacoma ater*	Filter	2.80	2.90	Filaments	Black/grey	500 - 1000	(Sparks, 2020)
	Mussel	*Choromytilus meridionalis*	Filter	1.80	5.60	Filaments	Blue/green	500 - 1000	
	Mussel	*Mytilus galloprovincialis*	Filter	2.80	3.40	Filaments	Black/grey	500 – 1000	
China	Mussel	*Mytilus edulis*	Filter	0.9 – 4.6	1.50 – 7.60	Filaments	-	< 250	(J. Li et al., 2016)
France, Belgium, Netherlands	Mussel	*Mytilus edulis*	Filter	0.20	-	Fragments	-	20 - 90	(Van Cauwenberghe *et al.,* 2015

Northeast Atlantic Ocean	Sea star	*Hymenaster pellucidus*	Predator	0.48 – 9.10	-	Filaments	-	200 - 5000	(Courtene-Jones et al., 2019)
	Brittle Star	*Ophiomusium lymani*	Deposit	1.96 – 3.43		Filaments	-		
Arctic and sub-Arctic region	Sea star	*Asterias rubens*	Predator	0.46	1.70	-	-	-	(Fang et al., 2018)
	Sea star	*Ctenodiscus crispatus*	Predator	0.25	0.30	-	-	-	
Southwest Atlantic ocean	Sea star	*Henricia obesa*	Filter	3.34	1.00	Filaments	Blue	500 - 1000	(Cossi et al., 2021)
	Sea star	*Odontaster penicillatus*	Predator	1.94	2.70	Filaments	Blue	100 - 500	

Chapter 6

Conclusion and recommendations

The results suggest that MP concentration and abundance could be linked to various sources, environmental factors, its interaction in the environment and MP characteristics. MP concentrations have been directly linked to population density (de Villiers, 2018; Nel et al., 2017), water circulation, WWTW, storm water pipes, industrial and harbour related activities (Preston-Whyte et al., 2021; Sparks et al., 2022; Sparks & Awe, 2022), with harbours and fishing areas being MP "hotspots" (Xu et al., 2018). MP characteristics, such as shape, size and density, determine where MPs reside within the marine environment, with sediment being classified as a MP sink (Alomar et al., 2016; Pham et al., 2014). Filamentous particles were the most dominant MP type across all sites which is reflective of other reports conducted around the world (A summary of MP concentration in coastal water and sediment environments around the world (Table *0.1*). Study areas included harbours and marinas, surface water, rivers, shorelines and beaches, intertidal zones and rocky shores.

Table *0.1*). Filaments were highest in filter-feeding echinoderms (94.38%), followed by water (73.29%) and sediment (66.96%) samples, suggesting that filaments present in the water column and sediment are consumed through non-selective feeding strategies. Bioturbation and excretion caused by organisms are able to resuspend MPs in the water column, which then eventually settle in sediment. Another potential reasons for MP consumption by organisms could be due to biofouling, however further research needs to investigate this. The colour of the MPs particles are linked to sources, with black/grey being the most dominant (41.12%) MP colour in all sample types combined, followed by transparent (20.84%) and white (15.17%). These results are reflective of other studies conducted around the world (Table 0.2). Possible reasons could be linked to sources but also UV exposure causing MPs to become lighter in colour over time. MP size varied across all samples types and sites. This could be due to a combination of photo-degradation, mechanical transformation from wave and wind action and biological degradation by organisms (Andrady & Neal, 2009). These processes compromise and reduce the structural integrity of larger plastics, resulting in MPs (Cole et al., 2011). PET were the most common MP type, followed by natural MPs, PE and PP. Common products produced with these polymers include clothes, cling wrap, bottle caps, packaging material, construction material, insulating material for cables and cosmetic products. The risk assessment indicates threats posed by MPs present in the environment are based on polymer type and not concentration. This is due to the high hazard score assigned to each polymer (Lithner et al., 2011).

Seasonal sampling is recommended to gain knowledge on the temporal distribution of MPs. Investigating environmental conditions such as upwelling, wind, current and water circulation could give insight on spatial distribution. The high MP concentrations reported provide a baseline for future studies, and it is evident that there is a need for investigations to focus on the effects of MPs on echinoderms in rocky shores environments along the Western Cape coastline, South Africa.

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Appendices

Appendices A: Airborne contamination

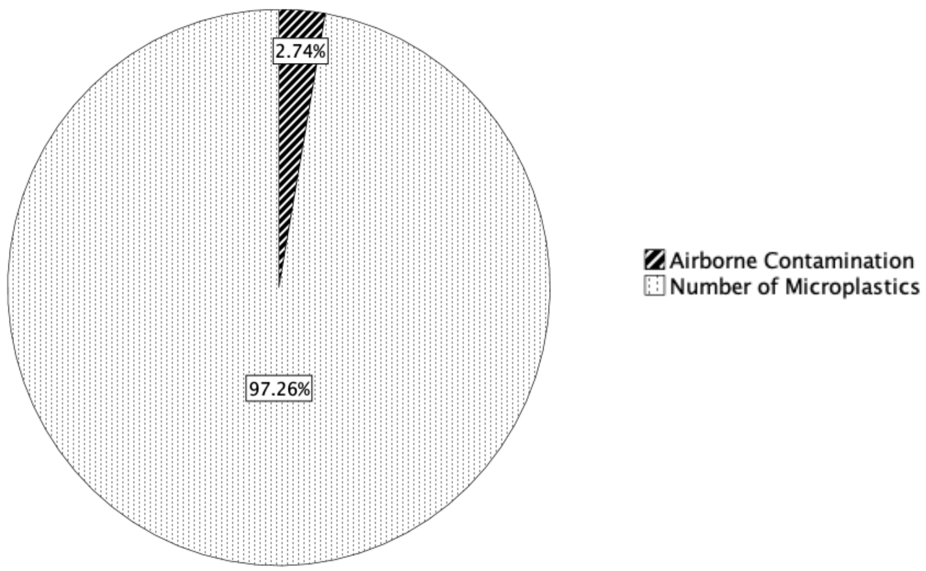


Figure 1: Percentage of airborne contamination.

Appendices B: Selected example of visual identification of MPs using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) scan.

(a)

(b)

(c)

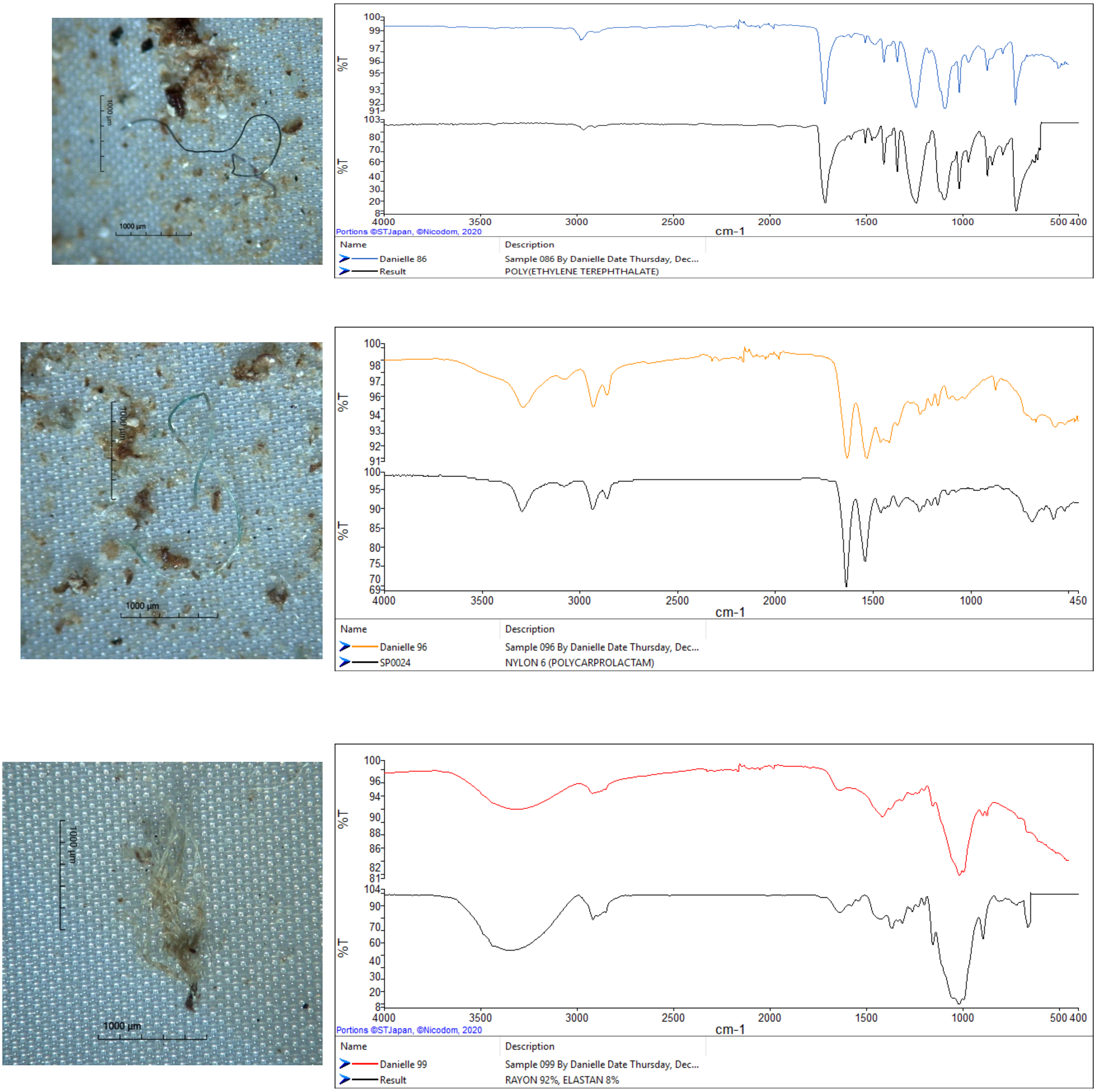
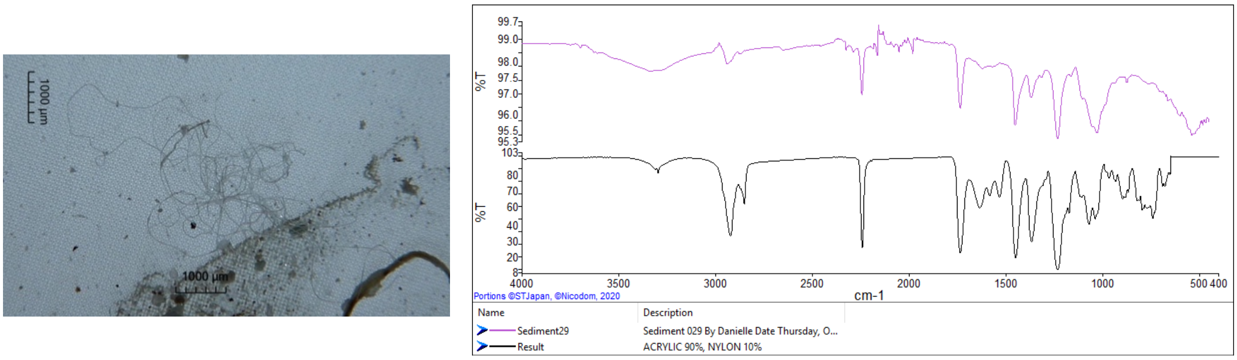


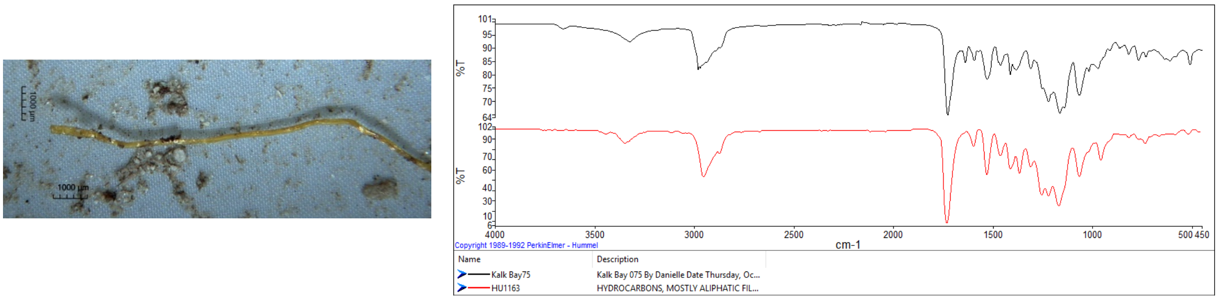
Figure 2: Selected example of visual identification of MPs using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) analysis a) black/grey PET filament, b) blue/green PA6 filament and c) white natural filament

Appendices C: Selected example of visual identification of MPs using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) scan.

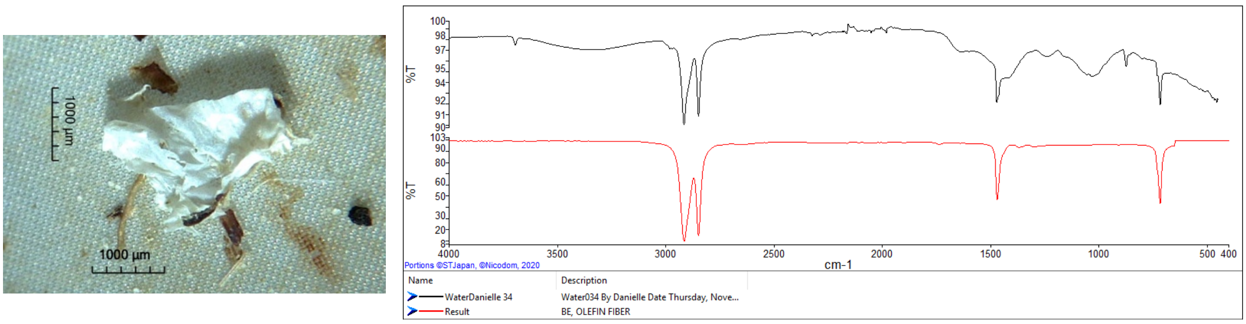
(a)



(b)



(c)



(d)

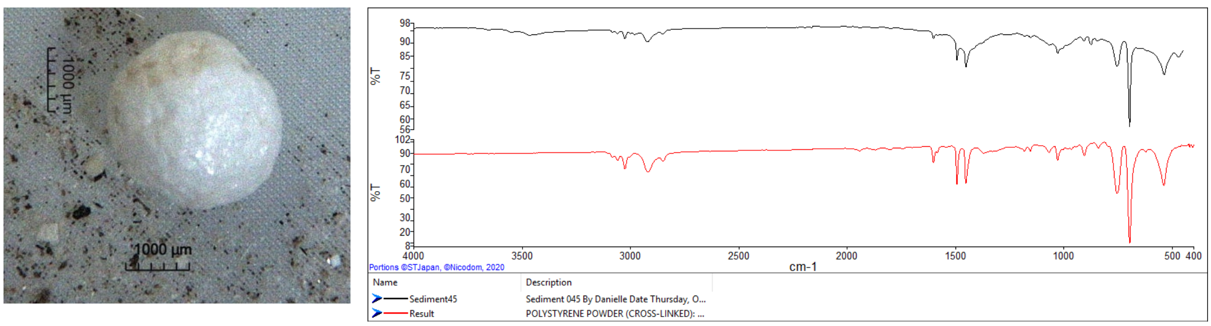


Figure 3: Selected example of visual identification of MPs using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) analysis a) white PAA filament, b) yellow PUR filament, c) white PE film and d) white PS foam

Appendices D: Selected example of visual identification of MPs using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) scan.

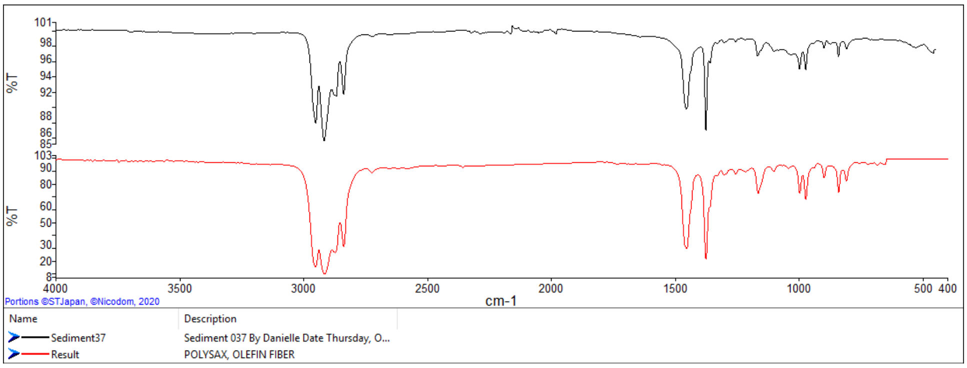


Figure 4: Selected example of visual identification of a red PP fragment using a microscope and Fourier Transform Infrared Spectroscopy (FTIR) analysis.