Are Microplastics more Prevalent in Seabed Sediment at Shallower Depths in the Red Sea?

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'I confirm that this piece of work that I have submitted is all my own work and that all

references and quotations from both primary and secondary sources, including the internet,

have been fully identified and properly acknowledged'

Signed_____ Dated_____

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Abstract

Microplastic pollution is a growing problem threating the health of global marine ecosystems. Floating microplastics on surface water only account for 1% of the plastic debris estimated to be in the ocean. It has been suggested that much of the plastic that enters the marine environment sinks to the sea floor. This can occur due to the plastic being denser than sea water or due to chemical changes to the polymer over time. It is believed that sediment acts as a long-term sink for plastics.

As the majority of microplastics found within the ocean are secondary microplastics, it was predicted that there would be greater concentrations of plastic at shallower depths. Secondary microplastics occur because of the degradation and fragmentation of larger plastics. It was estimated that the factors which facilitate degradation such as UV exposure would be more prevalent at shallower depths.

Core sediment samples were taken from five depths and three transects, starting at 0m and decreasing at 10m increments until 40m (n=75). Extraction of microplastics from the sediment samples was completed following NOAA guidelines. The difference between depth and concentration of microplastic concentration was found to not be statically significantly different (F $(4, 68) = 1.692$, $p = 0.162$).

Introduction

The marine environment is facing a plethora of ever increasing pressures due to human activity and population growth. The most severe of these pressures are referred to as planetary boundary threats. These can influence global biogeochemical systems (Steffen *et al.*, 2015). Marine debris is one such issue (Galway and Lewis, 2016). Of all the litter that enters the ocean, 80 to 85% is comprised of plastic (Auta *et al.*, 2017). Jambeck *et al.*, (2015) predicted that of the 275 million metric tons (MT) of plastic waste generated in 192 coastal countries, in a single year, between 4.8 to 12.7 million MT entered the ocean. It has been shown that plastic debris enters the ocean due to insufficient treatment capacity or a lack of infrastructure allowing for the disposal of plastic waste. Other factors are accidental inputs, littering, or illegal dumping (Barnes *et al.*, 2009; Hopewell *et al.*, 2009).

Plastic is defined as a generic term by The International Union of Pure and Applied Chemistry (IUPAC) for "polymeric material that may contain other substances to improve performance and/or reduce costs" (Vert *et al.*, 2012). Plastics are versatile and can be used for a variety of purposes. It is lightweight, durable, corrosion-resistant, has insulation properties and is cheap to produce, ensuring it is a popular construction material. These same characteristics ensure that they can travel large distances once in the marine environment. Plastics may last for centuries if not removed as most plastics are highly resistant to aging and biological degradation. (Moore, 2008; Thompson *et al.*, 2009).

The first scientific reports of plastics being found on the surface of the oceans began to appear in the 1970's (Carpenter and Smith, 1972; Colton *et al*., 1974). Plastics also began to be found in the guts of fish during this time (Carpenter *et al*., 1972). Despite this, little was done to stem the influx of plastic into the marine environment. In 2006, Ng and Obbard drew attention to the high abundance of microscopic fragments of plastics in seawater. By 2009, it was shown that minute fragments of plastic were ubiquitous within the ocean (Barnes *et al*., 2009).

Plastic debris is often categorised based on the it's size. Macroplastics are larger than 25mm, mesoplastics are between 5 and 25mm and microplastics are smaller than 5mm (Lee *et al*., 2013). In recent years, researchers have shown that plastic can break down into particles smaller than 100 nm, these are referred to as nanoplastics (Koelmans *et al*., 2015). The size limit for nanoplastics is currently not agreed upon. Different studies have used both 1μm or 100 nm as the upper size limit (Gigault *et al*., 2018).

Microplastics are either classified as primary or secondary. Primary microplastics are manufactured as such. They are micron-sized particles, which are frequently found in products such as exfoliants, and industrial abrasives. It can also be argued that microfibres from synthetic clothes can also be placed in this category, others argue that they are a secondary microplastic. (Gregory, 1996; Mathalon and Hill, 2014). The majority of microplastics in the marine environment are secondary plastics, these are plastics which have broken down from meso or macroplastics. Break down occurs due to mechanical, photolytic, and/or chemical degradation (Mathalon and Hill, 2014).

It has been shown that many organisms within marine ecosystems are impacted by plastics of all sizes. The most common problem caused by microplastics is ingestion. As the size of the particle decreases, it becomes available to an increasing number of organisms to ingest (Moore, 2008). Once mistaken for food, this plastic can be further transported through food webs (Eriksson and Burton, 2003). Eriksson and Burton showed that fur seals which had consumed pelagic fish excreted plastic in their faeces. They hypothesised that the fish had ingested plastic which had then been ingested along with the fish as they were consumed. It has also been shown that in some mollusc's uptake of microplastics can occur through ventilation (Watts *et al.*, 2014).

Egypt is estimated to be 1 of the top 20 countries responsible for producing marine debris (Jambeck *et al.*, 2017). Jambeck *et al.*, have so far complied the most data to demonstrate the scale of the plastic pollution problem in Egypt. They estimated that Egypt generated over 1,000 tonnes of plastic waste daily. They also estimated that Egypt mismanaged 60-80% of it's plastic debris.

A recent study in the Southern Baltic Sea showed that microplastic concentrations are lower in marine bottom sediments (0–27 particles kg^{-1} d.w) than beach sediments (25 particles kg^{-1} d.w. to 53 particles kg^{-1} d.w) (Graca *et al.*, 2017). This will likely be replicated in other seas.

The aim of this study is to establish whether sediment from the Egyptian coast of the Red Sea contains a greater concentration of microplastics at shallower depths. The investigation will concentrate on samples taken from between 0m and 40m depth. The mass of plastic from each sample will be determined and an Infrared spectrophotometer will be used to determine the type of plastics recovered. The hypothesis is that the concentration of microplastics found within samples will decrease as the depth they were taken from increases.

Literature Review
Plastic Pollution Globally

Plastics were first developed in the 1940s and then industrialised in the 1950s (Jambeck *et al.*, 2015). There are now nearly 5,000 grades of plastic material available (Leslie *et al.,* 2016). In 2017, the cumulative global production of plastic reached 8.3 million MT. Approximately 50% of this total has been produced within the last 13 years. This is only expected to increase. By 2015, only 9% of plastic ever produced had been recycled and 12% was incinerated. The remaining 79% was disposed of in landfill or made its way into the natural environment (Geyer *et al.*, 2017).

Of the plastic that makes its way into the natural environment every year, 4.8 to 12.7 million MT is predicted to have entered the ocean (Jambeck *et al.*, 2015). It is estimated that 80% of the plastic debris in the oceans has come from land-based sources. Densely populated urban areas, and industrialised areas are both a major source of plastic. The main contributors from these areas are littering, plastic bag usage, wastewater treatment, and solid waste disposal (Derraik, 2002). Lee *et al.,* (2013) showed that much of the floating and beached plastic debris they found had originated from land-based sources. Waste water effluent and refuse site leachate have also been shown to be a source of plastic pollution (Browne *et al.,* 2010). Plastics are now found within every marine ecosystem, including Arctic ice (Obbard *et al.,* 2014). These ecosystems now act as a final reservoir for plastic debris (Gregory, 2009; Thompson *et al.,* 2004).

Extreme weather has also been found to increase the transfer of plastic from land to the marine environment (Barnes *et al.*, 2009). One study in California showed that plastic particles per cubic metre (pp m^3) increased after such an event (10 pp m^3 to 60 pp m^3). It was also found that this weather event caused plastic to be deposited at greater distances from the source than normal (Moore *et al.,* 2002). With severe weather expected to increase in intensity with climate change, this may cause the land-based plastic entering the ocean to increase.

Although plastic pollution is found worldwide, studies have found higher plastics loads within areas of high anthropogenic activity (Romeo *et al.,* 2015). Nor and Obbard (2014) also reported lower levels of microplastic concentrations in areas with lower anthropogenic activity. Other studies refute this. Alomar *et al.,* (2016) found no difference in microplastic load between a Marine Protected Area and a populated coastal zone. Klein *et al.,* (2015) also demonstrated that there was no significant correlation between population density and density of microplastic loads. Reisser *et al.,* (2013) reported similar findings from Australia. This suggests that microplastics are dispersed through hydrodynamic processes (Claessens *et al.,* 2011). Moreira *et al.,* (2016) reported that tidal range and the oscillations of waterlines are likely to have a major impact on the distribution of microplastics in beach sediments.

Microplastics

When a plastic particle is smaller than 5mm, it is considered a microplastic. Microplastics can either be manufactured as microplastics (primary) or breakdown from larger plastic particles (secondary). Examples of primary microplastics include, nurdles, exfoliants and microparticles used for sandblasting (Andrady, 2011). It is currently believed that the majority of microplastics present within the ocean are of the secondary category (Hidalgo-Ruz *et al*., 2012). Macroplastics can be broken into smaller pieces through use or through degradation processes. Plastic has been shown to breakdown when exposed to UV radiation and physical abrasion (Barnes *et al*., 2009; Cole *et al*., 2011; Moore *et al*., 2011). The physical abrasion of a single syntenic garment during a wash cycle for example, can release up to 1,900 fibres (Browne *et al*., 2011).

Degradation of plastic is the reduction of average molecular weight of the polymer due to chemical changes. The polymers molecular integrity is also reduced (Singh and Sharma, 2008). This leads to embrittlement and fragmentation. Rates of degradation vary depending on both environmental factors and the type of polymer. Polymer topography and the presence of chemical additives also impact degradation rate. Environmental factors include temperature and availability of oxygen. Plastic degradation has been shown to occur more slowly in colder sea waters than on beaches, although UV radiation may also be a factor here (Andrady, 2011).

Impacts of Microplastics on Marine Organisms

Once in the marine environment, plastics can have many detrimental impacts on the ecosystem. Although some impacts such as entanglement and ingestion are well documented, other impacts are less well understood, but are still likely to be detrimental to the health of marine ecosystems (Dris *et al*., 2015).

The ingestion of plastic particles is not a new phenomenon, with the first reports of plastic within fish being published in the 1970s (Carpenter *et al*., 1972). Most research towards the ingestion of plastic focused on the damage caused by ingesting macroplastics. Stomach volume which is occupied by plastic may cause a false feeling of satiation. This will reduce drive to feed and therefore reduce food intake. Eventually organisms may starve to death as their stomachs fill with plastic. Organ failure is also a possible effect, as are abrasions and ulcers within the gastrointestinal tract (Day *et al.,* 1985).

Research is now being carried out into the potential negative ecotoxicological effects microplastic ingestion may have (Browne *et al.,* 2015). Potential impacts include; inability to predate effectively, reduced survival, reduced reproductive success, and uptake of persistent organic pollutants (POPs). All impacts of microplastic pollution have so far have been found to be more significant to organisms in early life stages (Oliveira *et al.*, 2013).

Microplastics can also enter organisms through inspiration across the gills, as was shown in the shore crab (*Carcinus maenas*). Fluorescently labelled polystyrene microspheres (8-10 µm) were used to determine how inspiration across the gill occurred. It was found that the uptake of the microspheres was significantly higher in the posterior gills. It was also found that the microspheres were retained in the body tissues for up to 21 days (Watts *et al.,* 2014). Filter feeding bivalves have also been found to uptake microplastics through gills due to ventilation mechanisms (Browne *et al.,* 2008).

Research has been carried out into these impacts through field work, however field work conditions mean it is difficult to distinguish impacts of microplastic exposure from other stressors (Avio *et al.,* 2017). Laboratory studies have also been carried out to predict potential risks. Bhattacharya *et al.*, (2010) demonstrated that microplastics can enter the base of the food web. Under laboratory conditions, they found that microplastic particles were adsorbed by the cellulose of two algal species (*Chlorella* and *Scenedesmus).* They also showed that this adsorption hindered photosynthesis and led to oxidative stress. If these findings are true for all algae, not just within laboratory conditions, then this may impact on the sustainability of the aquatic food chain. However, laboratory studies have often used concentrations of microplastics that greatly exceed current environmental concentrations. This could lead to overestimated effects being reported (Huvet *et al.,* 2016).

Plastics hydrophobic nature allows sorption of hydrophobic organic pollutants. One such pollutant is POPs. POPs can be adsorbed by plastic debris and then transported via ocean currents (Mato *et al,* 2001). POPs are highly persistent man-made chemicals, of which there are many different kinds. POPs have many different uses including; pesticides, flame retardants, and other household and industrial purposes (Clukey *et al.,* 2018).

Many POPs are lipophilic meaning they can enter foodwebs through being stored in lipids. Once there, POPs can biomagnify (Clukey *et al.,* 2018). A correlation between POP toxicity and the amount of plastic ingested has been found (Mato *et al.,* 2001). When ingested, digestive surfactants cause contaminants within the plastic to become more bioavailable. This also causes an acceleration in desorption rate of the chemicals adsorbed into the plastic (Voparil and Mayer, 2000; Teuten *et al.,* 2007). The desorption rate of POPs from plastic has been found to be higher in the presence of digestive surfactants than in sea water, potentially increasing the bioavailability to marine species (Bakir *et al*., 2014).

One such POP that plastic has been observed to adsorb is polychlorinated biphenyls (PCBs) (Zarfl and Matthies, 2010). Betts (2008) found that the concentration of PCBs in microplastics can be higher than the surrounding water by up to one million times. One study found that unpolluted plastic particles readily adsorbed hydrophobic contaminates over a period of 7 days (Mato *et al.,* 2001). A correlation has also been found between the amount of ingested plastic and the concentration of PCBs in fat tissue of great shearwater (Ryan, 1988). These results were mirrored by findings in North Pacific pelagic seabirds (Yamashita *et al*., 2011).

Another POP is polycyclic aromatic hydrocarbons (PAHs). These compounds are also hydrophobic and can be adsorbed by plastic. PAHs are toxic and have the potential to be carcinogenic, and mutagenic (Fisner *et al.,* 2013). One suspected effect of high POP concentrations is endocrine disruption. This has been documented in arctic fish following exposure to polychlorinated dibenzo-p-dioxins (PCDDs) and PCBs (Li *et al.,* 2016). The potential biomagnification and trophic transfer can be observed in the glaucous gull. These gulls fed on arctic fish contaminated with POPs which has led to changes in their endocrine system (Verboven *et al.,* 2008).

Ecological and Oceanographical Characteristics of the Red Sea

The Red Sea starts at the strait of Bab el Mandab to the south, where it meets the Indian Ocean (Marti *et al.,* 2017). It then travels in a North by North West direction for roughly 2,000km to the Gulf of Aqaba and Gulf of Suez (Fishelson, 1971). The Red Sea is now connected to the Mediterranean Sea via the Suez Canal (Marti *et al.,* 2017). The water within the Red Sea is sheltered due to the limited openings to other water bodies. There are no permanent rivers in its catchment area. As a result, the Red Sea has a stable high salinity. High rates of evaporation also contribute to this high salinity. Average salinity in the Red Sea ranges from 36% in the south to 41% in the north (Fishelson, 1971). Water from the Indian Ocean is imported into the Red Sea at the surface, whilst hypersaline water is exported at depth (inverse estuarine circulation) (Smeed, 2004). Tidal currents within the Red Sea are particularly weak, and mostly driven by wind (Churchill *et al.,* 2014). The prevailing southern winds move water masses towards the north (Fishelson, 1971).

The Benthic Community Within the Red Sea

It is generally accepted that there is a lack of understanding the ecology of the Red Sea. This is due to permitting regulations, which have made much of the Red Sea inaccessible for research, therefore making it a poorly studied area. Most published research available regarding the Red Sea comes from the Eastern Red Sea. Little is available for regions in Africa. Despite this, the Red Sea is recognised as a marine biodiversity hotspot with high levels of endemism (Ellis *et al,* 2017).

Fishelson (1971) reported that around 500 biotic benthic species can be found in the infratidal and shallow subtidal areas of the Red Sea. Most species found within the benthic community feed on the detritus and micro-organisms found within, or on the surface of the sediment. The benthos found within the infratidal and shallow subtidal areas change with the habitat type and depth. Ellis *et al.,* (2017) also found that community composition differed with location and depth. Many taxa found within the Red Sea benthos have the potential to be impacted by microplastic pollution.

Plastic Pollution in Egypt and the Red Sea

Egypt has an estimated population of 95.7 million, of which, 21.8 million live within 50km of the coast. In 2017, Jambeck *et al*., reported on the countries with the most mismanaged plastic waste, using data from 2010. Egypt was ranked 7th worst in the world for mass of mismanaged plastic waste. Per person, per day, 1.37kg of plastic waste was generated. It is estimated that 69% of this waste is mismanaged, totalling 0.97 million MT a year. Of this, 0.15-0.39 million MT is expected to become plastic marine debris annually.

Although no data is available for floating plastic in the Red Sea on the Egyptian coast, it is available for the Arabian coast. An average concentration of microplastic particles (excluding fibres) for the Eastern Red Sea is 1.08 g km^{-2}. This is the lowest abundance reported for any coastal sea (Marti *et al.,* 2017). In the Mediterranean Sea, concentrations of 423 g km^{-2} and 672 g km^{-2} have been reported (Cózar *et al*., 2015; Suaria *et al*., 2016).

Marti *et al.,* (2017) suggest that this low abundance may be due to low plastic waste inputs from land. A second possibility may be fast removal rates of the debris from the surface. The lack of rivers discharging into the Red Sea may also play a part in the lower than expected abundance. Rivers have been shown to be a major source of plastic pollution entering the ocean (Lebreton *et al*., 2017). It is estimated that 1.15-2.41 million tons of plastic that enters the oceans every year is from rivers and other bodies of water (Lebreton *et al*., 2017). These possible reasons are not mutually exclusive. Egypt mismanages more waste than Saudi Arabia, so the low abundance may not be true for Egypt.

It has also been suggested that the hydrodynamic pattern between the Red Sea and the Indian Ocean means that floating plastic debris may be imported from the Indian Ocean into the Red Sea. The Red Sea may be acting as a trap for said debris as there is limited capacity for the debris to be exported (Cózar *et al*., 2015).

Sediments as a Microplastic Sink

Van Sebille *et al.*, (2015) estimated that the global amount of microplastic particles floating on the oceans surface ranged from 15 to 51 trillion particles. This was estimated to weigh between 93 and 236 thousand MT. This accounts for approximately 1% of the global plastic waste which Jambeck *et al.,* (2015) predicted enters the ocean. It is therefore likely that much of the plastic which enters the ocean sinks into seafloor sediment.

Microplastics in sediment were first reported by Thomson *et al.*, 2004. It has been suggested that sediment will be a long-term sink for microplastics (Law *et al.*, 2010; Cozar *et al.*, 2014). Alomar *et al.,* (2016) argue that marine coastal shallow waters are in fact a sink for microplastics. Plastics which are denser than sea water ($>1.02 \text{ g cm}^{-3}$) sink to the sea floor, where it accumulates in the sediment. Plastics which have a low-density float at the surface or in the water column (Van Cauwenberghe *et al.*, 2015). Beach sediments have also been demonstrated to be a repository for microplastics (Browne, 2007; Hidalgo-Ruz *et al,* 2012).

Density modification can occur to plastic once it is in the marine environment due to biofouling. This can increase the plastics density which can cause it to sink. Biofouling occurs when the surface is covered by a biofilm, an algal mat and finally invertebrates (Andrady, 2011). Studies by Lobelle and Cunliffe (2011) showed that polyethylene bags submerged in seawater for 3 weeks would develop a biofilm. Cozar *et al.*, (2014) suggested that a sediment sink may partially explain why the global plastic load within the ocean is lower than expected when compared to expectancy models. However, they also stated that other sinks such as ingestion by microorganisms may be responsible, as the biofilm may be lost as the plastic begins to sink.

Graca *et al.,* (2017) determined that higher concentrations of microplastics were found in sediment samples closer to the shore when compared to samples taken from 70–106 m deep sites. Carson *et al.,* (2011) found that the presence of microplastics in sediment increased the permeability of the sediment. It has also been shown that there is not a clear relationship between sediment grain size and microplastics abundance (Alomar *et al.,* 2016). Overall, it is still not fully understood what happens to microplastics once they have settled in sediment (Zalasiewicz *et al*., 2016).

Plastics with high molecular weight are considered biodegradtion resistant. These plastics will fragment but remain within the environment. However, if the polymer chain reaches a low enough molecular weight, microorganisms can decompose them (Zheng *et al.,* 2005). A few microbal strains have been found that can biodegrade some plastics such as non-oxidized PE (Shah *et al*., 2008).

Materials and Methods
Study Site

The sediment samples were collected from the house reef of Roots Luxury Camp (RLC) (26°12'18.8"N 34°13'09.4"E). RLC is located at Abu Sauatir on the main Safaga Road. The closest urban area is Hamrawein, which is a small fishing village 6km north of RLC. The nearest city is El Quseir, a small city located 13km south of RLC and 138 km south of Hurghada.

The house reef can be described as a gentle, sandy slope located in a channel between the north and south reefs. The channel gradually widens before opening into a lagoon. The north and south reefs then turn to follow the shoreline, whilst the lagoon continues deepen (figure 1).

Figure 1. Aerial view of the study site, RLC house reef. Taken from Google Earth (2018).

Collecting Sediment Samples

A pole is present on the shoreline at the entrance to the channel, which is roughly central. This point was selected as the location for transect line 2. Transect line 1 was located 5m north of this centre point. Transect line 3 was 5m south of the centre point. The transects followed a bearing of 79 degrees, from 0m down to 40m. A Mares Puck dive computer was used to determine the depth.

In total, 75 samples were collected, 5 samples were taken from 0m, 10m, 20m, 30m and

40m on each transect. To determine where the samples should be taken from, a $1m^2$

quadrat was divided into 100 squares. Microsoft Excel was then used to generate 5 random

numbers for each depth location. This was used to determine which 5 random squares the

samples would be taken from at each quadrat location. Due to the potential confusion

effects of nitrogen narcosis when diving, cable ties were tied on the top left and bottom

right of the squares that were required for sample taking (figure 2).
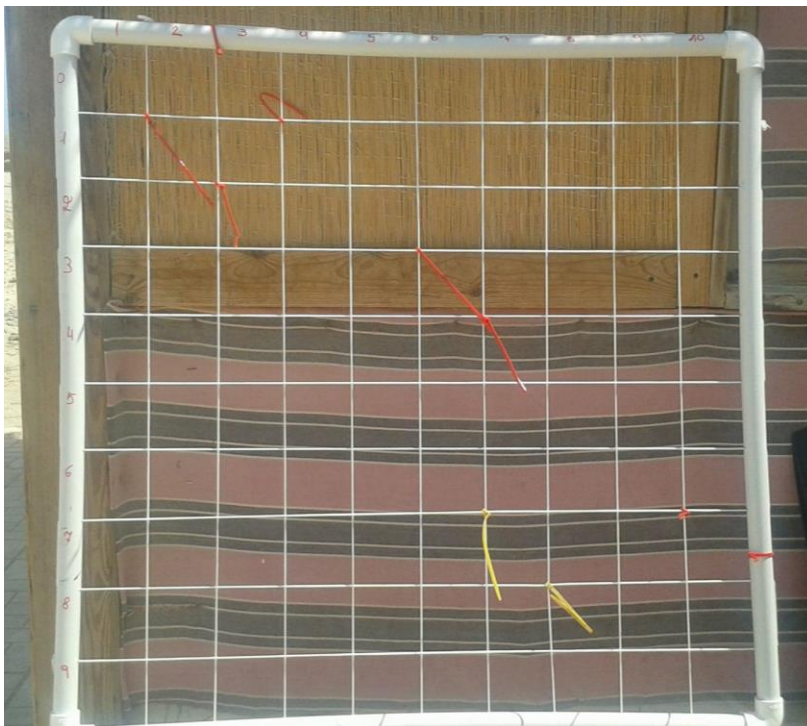
Figure 2. A $1m^2$ quadrat divided into 100 squares. Cable ties where attached to the top left

and bottom right corner of squares which were required to have samples taken from them.

This was done to minimise time taken to find the squares and to combat potential nitrogen

narcosis.

Samples were collected using a corer constructed out of PVC piping (3cm x 12cm). The corer was slipped over the neck of a 100ml plastic bottle. Once assembled, the corer was inserted into the sediment found beneath the correct square of the quadrat. Sediment was then scoped into the bottle from that square until full (Figure 3). Once full, the lid was placed on tightly to prevent contamination from other potential sources of plastic. Bottle lids were labelled prior to diving. Paper labels were added to bottles after sediment collection.
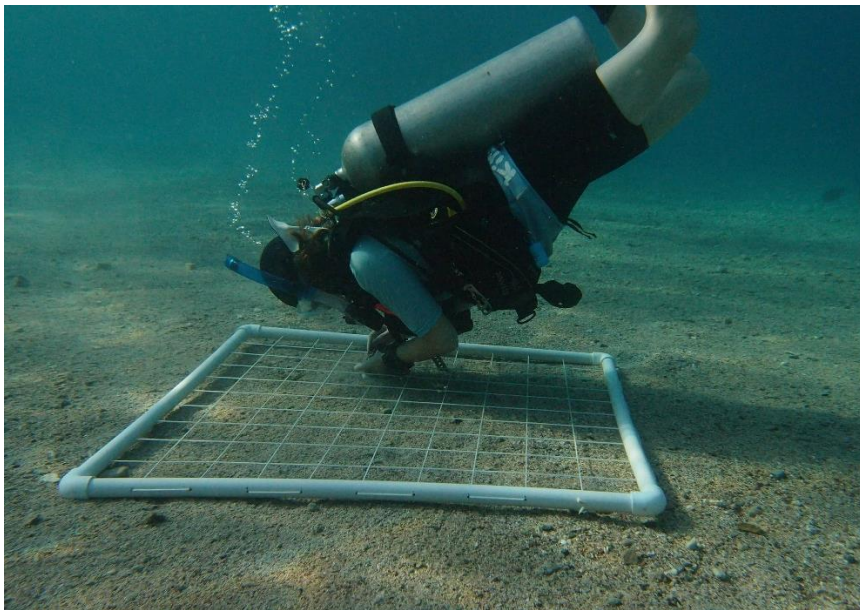
Figure 3. A practice collection sample, hence the lack of cable ties. The corer is being placed onto the plastic bottle, prior to sample collection.

All samples were collected between the 20/08/2016 and the 29/08/2016. When planning which sample would be collected when, decompression limits had to be considered. For that reason, a 40m sample was always collected on the first dive on any transect. The second dive started at 30m on a transect and followed the back bearing (259 degrees) to 20m and then 10m. The third dive was then available to make any corrections should they be required. This was repeated for the other 2 transects on following days.

Extracting Microplastics from Sediment

The methodology for extracting any microplastics from the sediment samples was taken from the 'Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments.' This was developed by the National Oceanic and Atmospheric Administration in 2015 (Masura *et al.,* 2015).

This methodology did call for 400g of sediment for each sample. In some cases, there was not enough sediment, so the 400g requirement was halved to 200g for all samples. Any required substances for chemical analysis also had their weights or volume halved as a result.

To ensure that the methodology was effective, and carried out correctly, 5 control samples were also prepared. This was done through adding a known weight of grated plastic to a known weight of builder's sand.

Samples were dried in a drying oven at 90°C for 24 hours or until sample dryness. Following this, 200 ml of potassium metaphosphate (5.5 g per litre of water) was added to each solution. The NOAA methodology called for a stir bar to be used at high revolutions per minute. This was unsuccessful, so a shaker was used to disaggregate the samples instead. The samples were then sieved through the 5mm sieve and the 0.3mm sieve. Anything larger than 5mm was discarded. Anything smaller than 5mm was kept and dried in the oven until dry.

Next a density separation was carried out. It was not possible to obtain 5.4 M lithium metatungstate (d=1.62 g/mL) solution, so 5 M Sodium chloride solution (d=1.15g/mL) was used instead, as suggested by NOAA. Sodium chloride solution (150ml) was added to each sample before being stirred vigorously for several minutes. Floating solids were then transferred to the 0.3mm sieve, placed into a new container and dried in the drying oven.

A wet peroxide oxidation was then carried out by adding 10 ml of aqueous 0.05 M Fe(II) solution (Prepared by adding 7.5 g of $FeSO_4°7H_2O$ (= 278.02 g/mol) to 500 mL of water and 3 mL of concentrated sulfuric acid) and then adding 10 ml of 30% hydrogen peroxide. This was then left to sit for 5 minutes. The sample was then placed on a heat plate at 75°C with a stir bar. A watch glass was placed on top. As soon as the sample showed signs of boiling, it was removed from the heat. If the sample seemed like it was going to boil over, distilled water was added to slow the reaction. Once signs of boiling had subsided, the sample was heated to 75°C for a further 30 minutes. If all organic matter had been removed, 6g of salt (NaCl) per 20 mL of sample was added. This was heated until all the salt had dissolved.

For the next stage of density separation, the mixture was transferred to a density separator (figure 4) and left to settle for 24 hours. Any floating plastics were the removed from the surface via a 0.3mm sieve. Finally, these remaining solids were checked under a microscope to ensure that it was not organic. These microplastics were then counted.

Figure 4. A density separator constructed to NOAA's guidelines.

Infrared Spectrophotometry

There was only one microplastic large enough to be tested by using an infrared spectrometer. OMNIC software was used to test the available microplastic sample. The appropriate library databases were input before a background scan was completed. The sample was then tested, and the results compared to the OMNIC database.

Statistical Analysis

SPSS was used to test for normality. Differences in concentration of microplastic concentration by depth was evaluated by a one-way ANOVA followed by a Tukey's HSD.

Results
Overview
Pilot Study

A known weight of microplastic particles were added to 5 samples of builder's sand. After extraction, the plastic recovered was weighed again. Of the 1.514g of microplastic added to the builder's sand, 1.115g was recovered using the NOAA extraction methodology. A percentage of plastic recovered for each sample was then determined (table 1).

Table 1. Table to show the weight of plastic added (g), the weight of plastic recovered (g) and the resulting percentage of microplastics recovered from the pilot study samples.

Sample	Plastic Added (g)	Plastic Recovered (g)	Percentage Recovered
1	0.100	0.100	100.000
2	0.204	0.145	71.078
3	0.305	0.245	80.328
4	0.404	0.258	63.861
5	0.501	0.367	73.253

Microplastic Recovered from Samples

One sample from transect 3, 10m (T3 10m) was removed due to its high value (132). This value was not in line with any observable pattern so was excluded as an outlier. Using 73 samples, the total weight of sediment tested was 14.6kg. This gives an estimated microplastic concentration of 96.3 particles per kg of sediment.

Originally 75 samples were collected. One sample was lost in transit. For this reason and the removal of the T3 10m data point, an average of the number of microplastic particles found per depth and transect was taken (figure 5).
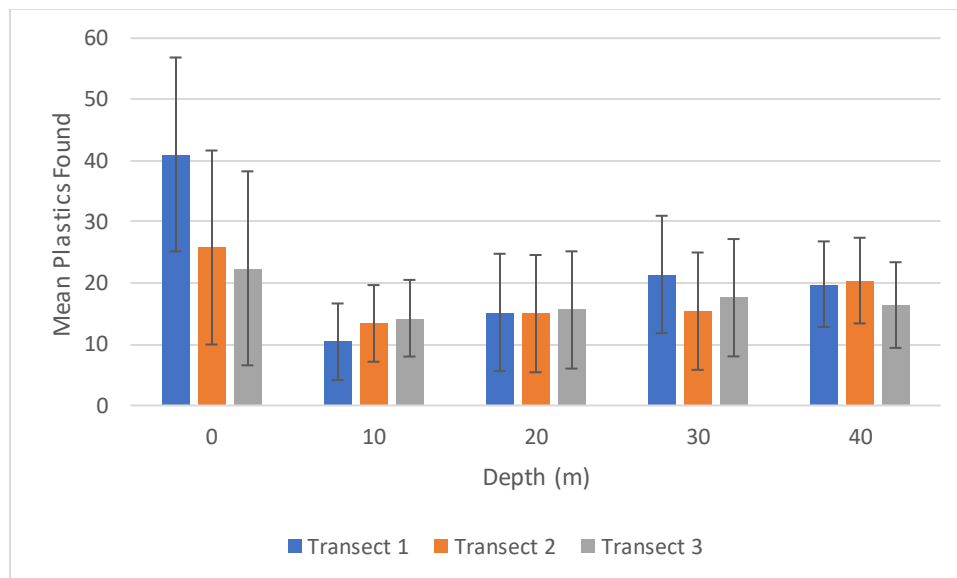
Figure 5. Graph to show the average pieces of microplastic recovered from the samples (n = 5, except T3 10m and T3 30m where n=4) at each depth per transect. Error bars represent standard deviation.

Test of Difference
Pilot Study

A Sharpio-Wilk's test showed that the plastic added to the samples and the plastic recovered from the samples followed a normal distribution ($p = 0.809$) (Appendix 1). The extraction methodology had a mean recovery percentage of 77.704% from the pilot samples (n=5).

A paired t test was carried out to determine whether there was a difference between the plastic added to the samples and the plastic recovered from the samples. T The results show that there is a statistically significant difference between the plastic added and the plastic recovered ($p = 0.041$) (Appendix 2).

Microplastic Recovered from Samples

After the exclusion of a T3 10m, 15 (14 for T3 10m and T3 30m) samples were normality tested by depth. Shapiro-Wilk normality testing showed that the data for all depths followed a normal distribution ($p = < 0.05$), except for 0m ($p = 0.008$, s total = 10.479) (Appendix 3).

Despite 0m not following a normal distribution, a one-way ANOVA test was used to determine whether there was a significant difference between the average amounts of microplastics found at each depth. One-way ANOVA was determined to be robust enough to use on a minor deviation from normal distribution (0m) (Blanca *et al.,* 2017). This test showed that there was no statistically significant difference. ($F_{(4, 68)} = 1.692$, $p = 0.162$), therefore the null hypothesis could not be rejected (Appendix 4). To determine whether there was a statistically significant difference between any of the depths, a post-hoc Tukey test was also carried out (Appendix 5). A one-way ANOVA test was also used to determine

that there was no significant difference between mean plastic found over each transect across all depths (p = 0.482) (Appendix 6).

Infrared Spectrophotometry
Pilot Study

Infrared spectrophotometry analysis of the plastic pieces used for the pilot study returned a 79.22% match to atactic polypropylene. The first 8 results are all variations of polypropylene (appendix 7). This gives a high level of confidence for the samples being Atactic Polypropylene. For full IR spectrophotometry profile see appendix 8.

Microplastics Recovered from Samples

Only one microplastic sample was large enough to test with the IR spectrophotometer. This was from one of the samples at T 1, 0m. IR spectrophotometry analysis of this microplastic gave a 49.01% match to cellophane (appendix 9). The first two results were cellophane; however, the rest are not plastics. As a result of the low percentage match, and the number of different potential compounds suggested, it is not certain that the sample is cellophane. For full IR spectrophotometry profile see appendix 10.

Discussion

Density Separation

Density separation (DS) is the most effective means of extracting plastic from sediment. Karlsson *et al.,* (2017) tested DS on 5 different household polymers and found that recovery rates varied from 64% to 81%. This pilot study suggested that NOAA's extraction technique recovered 77.704% of plastic within the samples. The t test suggests that this was a significant recovery rate. However, the pilot study only used one polymer type (atactic polypropylene). Polypropylene has a density of 0.895 and 0.92 g/cm^3, making it less dense than the NaCl solution used (d=1.15g/mL). Karlsson *et al.,* (2017) identified problem areas within the glassware used during testing as a source of loss of microplastics. They found that plastic pieces would get stuck in these areas. They suggest the addition of a drop of olive oil to the sample. Plastic gathered in the oil and reduced the loss of plastic recovered.

Most polymers are less dense than NaCl solution, so will float, however some heavier polymers such as polyvinyl chloride (PVC) may not (Karlsson *et al*., 2017). Imhof *et al.,* (2012) created an improved DS with a validated recovery rate of 100% for microplastics ranging from 1-5mm, and 95.5% for microplastics smaller than 1mm. They achieved this through the construction of an apparatus, the so-called Munich Plastic Sediment Separator (Appendix 11). They also recommended they use of $ZnCl_2$ (1.6–1.7 kg/L) as the separation fluid. The higher density allows for the recovery of a greater number of polymers. Raman microspectroscopy is recommended for the identification and quantification of microplastics. Particles down to 1 μm can be recovered following this methodology.

Further data analysis was available for the pilot study. A calibration of the recovery rate could have been completed. Using regression, a formula could be generated to predict the total weight of microplastics within the sediment, considering the recovery percentage (Karlsson *et al.,* 2017). This would have proved useful to determine the total amount of microplastics within the sediment, rather than just those recovered. However, the pilot study results were measured by weight, whilst the main study was measured by number of microplastic particles. Originally, the intent was for the main studies results to be weighed and counted, but there was not the capacity to measure the weights of the microplastics, as it was so small. As such, the formula generated could not be used to determine the total weight. As this study was designed to test whether there is a difference between depth and concentration of plastic, rather than just amounts of plastic, the calibration was rejected.

Microplastic Abundance in the Red Sea

The average microplastic concentration found within all samples (n=74) is 96.3 particles per kg of sediment. Comparing this to similar studies from around the world, we can see that this is similar to results found on the beaches and continental shelf of Belgium (table 2). Claessens *et al.,* 2011 state that their results were linked to the geometry of the compartments in which the microplastics were found. This may be the case for the RLC reef. They also suggest freshwater rivers being an important source of microplastics, this has no bearing on the results of this study due to the absence of freshwater rivers in the Red Sea catchment area.

Table 2. Abundance of microplastics in sediment worldwide. Only studies which have

measured abundance by items per kg have been listed for comparison.

Country	Location Specification	Measured Abundance (items per kg)	Reference
Germany	Beach	1.3 – 2.3	Dekiff *et al.,* 2014
Belgium	High Tide Mark	9.2	Van Cauwenberghe *et al.,* 2015
Belgium	Low Tide Mark	17.7	Van Cauwenberghe *et al.,* 2015
Singapore	Mangrove	36.8	Nor and Obbard, 2014
Belgium	Beach	92.8	Claessens *et al.,* 2011
Egypt	Infralittoral/Subtidal	96.3	This study
Belgium	Continental Shelf	97.2	Claessens *et al.,* 2011
Slovenia	Infralittoral	170.4	Laglbauer *et al.,* 2014
Slovenia	Beach	177.8	Laglbauer *et al.,* 2014
Italy	Subtidal	672-2175	Vianello *et al.,* 2013

Difference between Microplastics and Depth

The most microplastic particles were found at 0m as expected, however the least were

found at 20m rather than 40m. The one-way ANOVA test found no statistically significant

difference between depth and concentration of microplastics within the sediment ($F_{(4, 68)}$

= 1.692, p = 0.162. This contradicts the findings of Graca *et al.,* (2017), who found a

statistically significant difference between microplastic concentrations in beach sediment

and bottom sediment (up to 53 particles kg^{-1} d.w and 0–27 particles kg^{-1} d.w respectively).

The authors suggest that this may mean that most of the plastic debris found within the

study sites come from land-based sources rather than from the sea.

Microplastic particles being found in greatest abundance at 0m may suggest that land-based plastics are the greatest contributor to the microplastic pollution. A low abundance of floating microplastics have been found on the Arabian coast of the Red Sea (Marti *et al.,* 2017). It is possible that this is true for the Egyptian coast. This too would support the idea that 0m had the greatest concentration of microplastic due to land-based sources.

An often-cited source of microplastic particles in shallow depths are waste water outlets (Browne *et al.,* 2010). Although there is not such an outlet within the immediate facility of the reef, there may be such outlets further along the coast. Once in the sea, microplastics from such sources can travel away from the entry point (Cole *et al.,* 2011). It is possible that the higher than expected concentrations of microplastics found at 30m and 40m, may be due to this.

The layout of the reef means that the 10m and 20m depths are more sheltered than the 30m and 40m sample locations. The channel between the north and south reef houses the 10m depth, the channel continues to widen for 20m. By the time 30m and 40m has been reached, the sampling sites are out in the open and not protected by the reefs. This may also be why less plastic was found at 10m and 20m. When the tide changes, a strong rip current travels through the channel. This rip current could potentially transport any microplastics on the surface of the sediment to deeper depths.

Of all the microplastics found, the majority were microfibres. Microfibres are the result of the shedding from synthetic clothing (Verschoor *et al.* 2014). The beach at RLC and the reef itself are a draw to the local population and tourists. It is not known how many people visit the site, but many swim or dive there annually. Microfibres are constantly shed from synthetic clothing, however physical abrasion increases the rate of shedding (Verschoor *et al.* 2014). The high percentage of microfibres found may be a result of the shed from synthetic clothing used in the pursuit of recreation at the site, such as swim wear and SCUBA diving equipment. Another potential source of the microfibres is the ropes that have been placed within the channel to help guide divers. These ropes are synthetic and under constant motion due to the channels current. It is highly likely that these ropes are shedding microfibres.

The site is also a popular location for fisherman. Local fisherman will walk on top of the reefs to fish with both net and monofilament line. These items are often caught in the coral and discarded. Even though the reef is regularly cleaned by guests and staff from RLC, not all line and netting can be easily removed. Netting and monofilament fishing line is also fibrous and can therefore breakdown into microfibres (Moore, 2008). It is possible that some of the microfibres found were from these sources. The fishermen tend to use line for fishing deeper sites. This may also contribute to the greater microplastic concentrations found at 30m and 40m.

Human error during testing may mean that the results are not accurate. The microplastics within the sediments were counted by eye under a microscope. It is possible that some were missed or counted twice. However, this is unlikely to cause a major impact when testing for a difference between depth and concentration of microplastic particles.

Improvements and Further Research

Although great lengths were taken to avoid contamination of samples, some compromises had to be made. Ideally, samples should be kept in glass rather than plastic bottles (Rocha-Santos and Duarte, 2015). Plastic bottles are a potential source of extra microplastics should abrasion from sediments cause minor fragmentation. Due to the need to transport 75 samples from Egypt to the UK, it was deemed more appropriate to use plastic bottles. The need to bring the samples to the UK also limited the size of the samples that could be collected. NOAA's methodology suggests using 400g of sediment for testing (Masura *et al.,* 2015). This was not possible due to transit constraints.

There was the potential for further contamination during the extraction process within the laboratory. It has been found that microfibres can become air born (Gasperi *et al.,* 2018). Microplastics have also been found it tap water (Kosuth *et al.,* 2018). Should this procedure be replicated in the future, controls should be put in place to test whether airborne or tap water born microplastics had an impact on the microplastics found within the sediment samples.

Further improvements would be the testing the procedure on multiple types of polymers during the pilot study and having the facilities to measure the weight of the microplastics (weights of total microplastic concentrations found per sample were < 0.000g). The construction and implementation of a Munich Plastic Sediment Separator and the use of $ZnCl_2$ (1.6–1.7 kg/L) as a separation solution may increase the percentage of microplastics extracted from samples. This would give a more accurate representation of microplastic concentrations. Finally, the microplastic samples found were too small to test with the IR spectrophotometer available. Future studies could use Raman microspectroscopy (Imhof *et al.,* 2012).

To better understand the distribution of microplastics and their fate once in sediment to progress, a standardised testing procedure should be agreed upon. It is currently difficult to compare microplastic studies due to the many different methodologies and reporting methods. Some concentrations are presented as particles per kg, others by particles per km^2 or m^2, whilst others are reported by weight (Rocha-Santos and Duarte, 2015).

Most studies either concentrate on microplastics found on a beach or in bottom sediment. Graca *et al.,* (2017), have been one of the few studies to examine both. Further studies such as this need to be carried out to determine whether there is a difference between concentrations of microplastics found at shallower depths and deeper depths.

For a greater understanding of the microplastic load in sediment within the Egyptian Red Sea, studies to analysis this should be conducted across multiple sites. Findings from these studies would show where the greatest concentrations of microplastics were found. This could potentially be used to help determine microplastic hotspots and sources. However, as Ellis *et al.,* (2017) state, information about the Red Sea is not readily available to researches. The political climate within the region has not been conductive to carrying out research. As such, this may need to stabilise before meaningful research can be carried out.

Conclusion

This is one of the first studies to look at the concentration of microplastics in sediment on the Egyptian coast of the Red Sea. Microplastics were found within all samples. An average of 96.3 particles per kg of sediment was determined when all microplastics found was compared to weight of sediment tested. As there are no comparable studies available for the coast of Egypt, it cannot be determined whether this is a high or low abundance.

The aim of this study was to determine whether concentrations of microplastic were greater at shallower depths than at greater depths. It was determined that there was no statistically significant difference between depth and concentration of microplastic particles found, and the null hypothesis could not be rejected. This contradicts findings from other studies. There are many potential reasons for why there is no difference. Human error and environmental factors at the study site and within the laboratory may account for the results.

This study also used IR spectrophotometry to determine the polymer type found. This was mainly unsuccessful, with only one sample being tested. This was because the microplastics found were too small to test. This study highlights the need for a standardised methodology for testing microplastic concentrations. It also demonstrates that further studies testing the relationship between depth and microplastic particle concentrations are required.

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Appendices

Appendix 1. Sharpio-Wilk test of the percentage of microplastic recovered from the pilot samples.

A: Table to show the results of the Sharpio-Wilk test on the amount of plastic added to the samples and the amount of plastic recovered. The data follows a normal distribution ($p=$ 0.809). Created in SPSS (IBM, U.S.A).

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Recovered	.183	5	.200[*]	.960	5	.809

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix 2. Paired- Sample t test on the difference between plastic added to the pilot

samples and plastic recovered from the pilot samples.

A: Table to show the results of the paired- sample t test on the difference between plastic

added to the pilot samples and plastic recovered from the pilot samples. The results show

that there is a statistically significant difference between the plastic added and the plastic

recovered (p = 0.041). Created in SPSS (IBM, U.S.A).

Paired Samples Test

| | | Paired Differences | | | | | | | |
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	Plast_Add - Plast_Recov	.079800	.060235	.026938	.005009	.154591	2.962	4	.041

Appendix 3. Sharpio-Wilk test of the average microplastics found at each depth.

A: Table to show the results of the Sharpio-Wilk test on the average found at each depth across all transects. The data follows a normal distribution ($p= >0.05$) except for 0m ($p = 0.008$). Created in SPSS (IBM, U.S.A).

Tests of Normality

	Depth	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Pieces	0	.251	15	.012	.825	15	.008
	10	.196	14	.150	.904	14	.127
	20	.139	15	.200*	.956	15	.630
	30	.166	14	.200*	.896	14	.100
	40	.150	15	.200*	.883	15	.052

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix 4. One-way ANOVA on the average amounts of microplastics found at each depth.

A: Table to show the results of the One-way ANOVA test to determine whether there was a significant difference between the average amounts of microplastics found at each depth. The data shows that there is no statistically significant difference between depths ($p = 0.162$). Created in SPSS (IBM, U.S.A).

ANOVA

Pieces

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	715.486	4	178.871	1.692	.162
Within Groups	7190.186	68	105.738		
Total	7905.671	72			

Appendix 5. Tukey test on the average amounts of microplastics found at each depth.

A: Table to show the results of the Tukey test to determine whether there was a significant difference between the average amounts of microplastics found at each depth. The data shows that there is no statistically significant difference between any of the depths (p <0.05). Created in SPSS (IBM, U.S.A).

Multiple Comparisons

Dependent Variable: Pieces

Tukey HSD

(I) Depth	(J) Depth	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0	10	8.562	3.821	.177	-2.15	19.27
	20	5.867	3.755	.526	-4.66	16.39
	30	1.919	3.821	.987	-8.79	12.63
	40	1.600	3.755	.993	-8.92	12.12
10	0	-8.562	3.821	.177	-19.27	2.15
	20	-2.695	3.821	.955	-13.40	8.01
	30	-6.643	3.887	.435	-17.53	4.25

	40	-6.962	3.821	.370	-17.67	3.75
20	0	-5.867	3.755	.526	-16.39	4.66
	10	2.695	3.821	.955	-8.01	13.40
	30	-3.948	3.821	.839	-14.66	6.76
	40	-4.267	3.755	.787	-14.79	6.26
30	0	-1.919	3.821	.987	-12.63	8.79
	10	6.643	3.887	.435	-4.25	17.53
	20	3.948	3.821	.839	-6.76	14.66
	40	-.319	3.821	1.000	-11.03	10.39
40	0	-1.600	3.755	.993	-12.12	8.92
	10	6.962	3.821	.370	-3.75	17.67
	20	4.267	3.755	.787	-6.26	14.79
	30	.319	3.821	1.000	-10.39	11.03

Appendix 6. One-way ANOVA on the average amounts of microplastics found per transect across all depths.

A: Table to show the results of the One-way ANOVA test to determine whether there was a significant difference between the average amounts of microplastics found per transect across all depths. The data shows that there is no statistically significant difference between transects across all depths ($p = 0.482$). Created in SPSS (IBM, U.S.A).
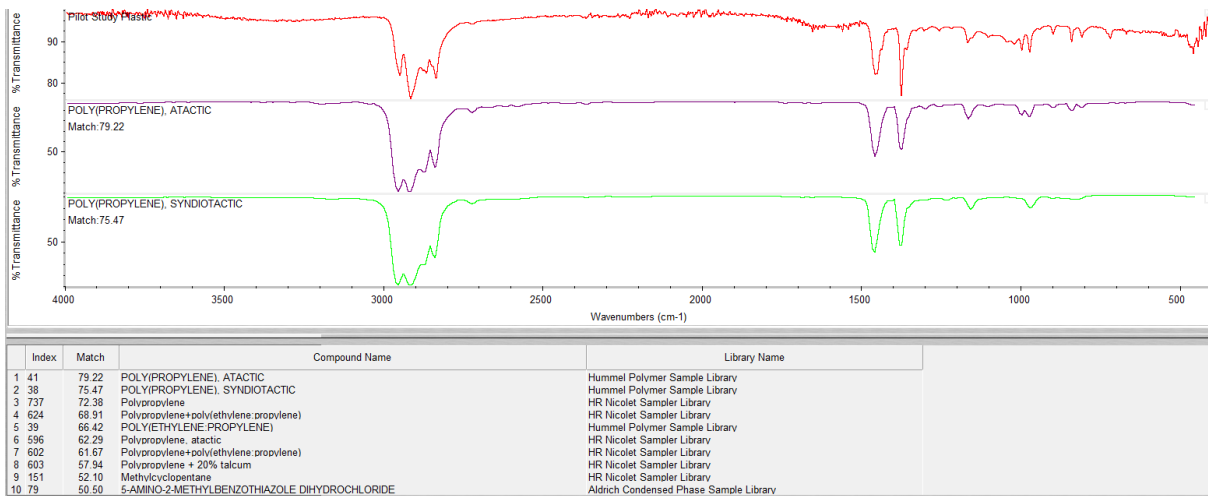
ANOVA

Pieces

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	163.076	2	81.538	.737	.482
Within Groups	7742.595	70	110.608		
Total	7905.671	72			

Appendix 7. Results of the infrared spectrophotometry analysis of the plastic pieces used for the pilot study.

A: Results of the infrared spectrophotometry analysis of the plastic pieces used for the pilot study. A 79.22% match to atactic polypropylene was found. The first 8 results are all variations of polypropylene. This gives a high level of confidence for the samples being Atactic Polypropylene.

	Index	Match	Compound Name	Library Name
1	41	79.22	POLY(PROPYLENE), ATACTIC	Hummel Polymer Sample Library
2	38	75.47	POLY(PROPYLENE), SYNDIOTACTIC	Hummel Polymer Sample Library
3	737	72.38	Polypropylene	HR Nicolet Sampler Library
4	624	68.91	Polypropylene+poly(ethylene:propylene)	HR Nicolet Sampler Library
5	39	66.42	POLY(ETHYLENE:PROPYLENE)	Hummel Polymer Sample Library
6	596	62.29	Polypropylene, atactic	HR Nicolet Sampler Library
7	602	61.67	Polypropylene+poly(ethylene:propylene)	HR Nicolet Sampler Library
8	603	57.94	Polypropylene + 20% talcum	HR Nicolet Sampler Library
9	151	52.10	Methylcyclopentane	HR Nicolet Sampler Library
10	79	50.50	5-AMINO-2-METHYLBENZOTHIAZOLE DIHYDROCHLORIDE	Aldrich Condensed Phase Sample Library

Appendix 8. Full IR spectrophotometry profile of the plastic used for the pilot study.

A: Full IR spectrophotometry profile of the plastic used for the pilot study, with peaks added.
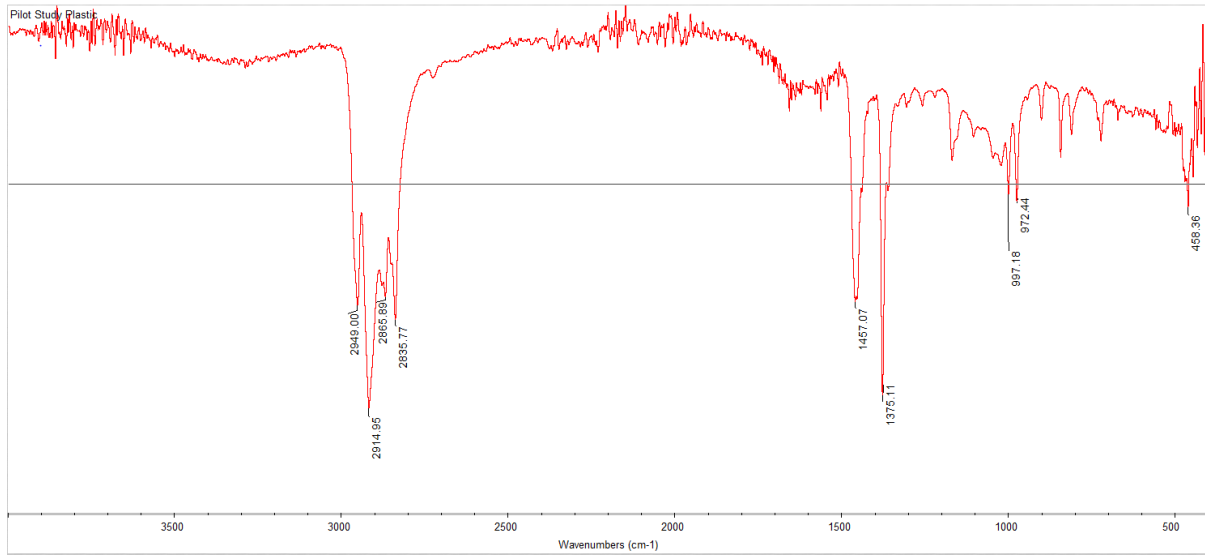
Appendix 9. Results of the infrared spectrophotometry analysis of the microplastic large

enough to be tested.

A: Full IR spectrophotometry profile of the microplastic found at T 1, 0m. The analysis of the

profile returned a 49.01% match to cellophane. The first 2 results were both cellophane, the

rest where a mix of substances that were not plastics. This gives a low level of confidence

for the samples being cellophane.

	Index	Match	Compound Name	Library Name
1	40	49.01	CELLOPHANE	Hummel Polymer Sample Library
2	8	42.96	CELLOPHANE	Hummel Polymer Sample Library
3	121	42.46	DEXTROSE ANHYDROUS POWDER IN KBR	Georgia State Crime Lab Sample Library
4	109	38.75	SUCROSE IN KBR	Georgia State Crime Lab Sample Library
5	14	37.25	METHYL ALCOHOL, 99.9%, SPECTROPHOTOMETRIC GRADE	Aldrich Vapor Phase Sample Library
6	122	37.20	DEXTROSE MONOHYDRATE POWDER	Georgia State Crime Lab Sample Library
7	119	36.88	INOSITOL POWDER IN KBR	Georgia State Crime Lab Sample Library
8	7	35.99	(+)-BETA-D-LACTOSE	Aldrich Condensed Phase Sample Library
9	12	34.64	NONANOIC ACID, 98%	Aldrich Condensed Phase Sample Library
10	290	34.30	Formaldehyde - aqueous solution	HR Nicolet Sampler Library

Appendix 10. Full IR spectrophotometry profile of the microplastic found at T 1, 0m.

A: Full IR spectrophotometry profile of the microplastic found at T 1, 0m. with peaks added.
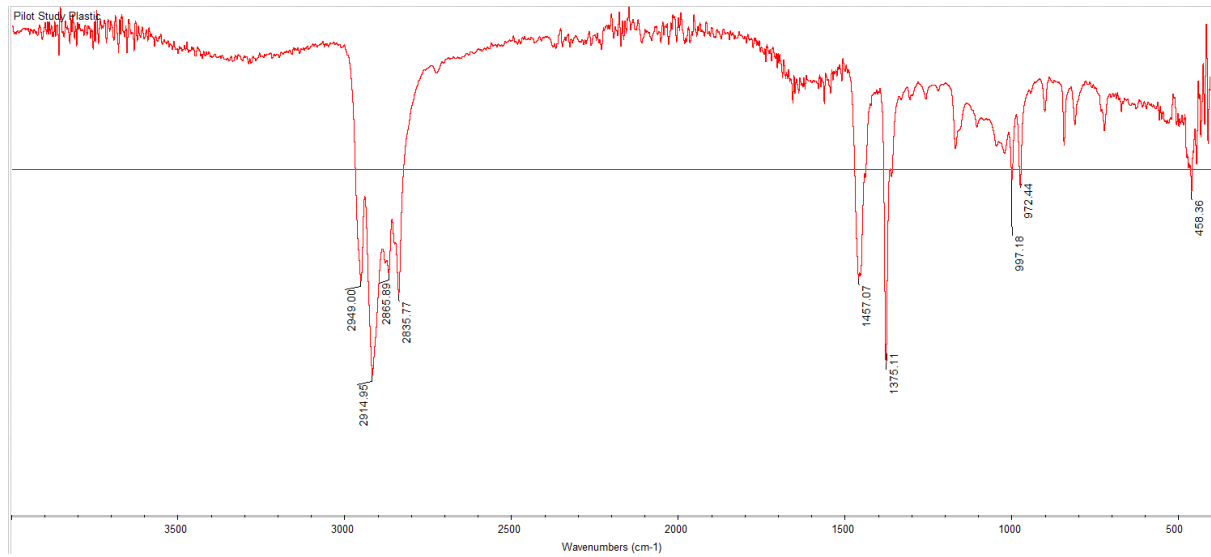
Appendix 11. Sectional drawing of the Munich Plastic Sediment Separator (Imhof, 2012).