A STUDY ON THE PRESENCE OF MICROPLASTIC IN COMMERCIAL SALT AND RAW SEA SALT FROM BANSKHALI AND KUTUBDIA REGION, BANGLADESH

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Roll No: 0120/21

Registration No: 903

Session: January-June, 2020

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Public Health.

One Health Institute

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

30 JUNE, 2022

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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30 JUNE, 2022

Acknowledgements

All praise is dedicated to the Almighty for his graces, courage, aptitude, patience, and for allowing me to pursue higher education and finish my thesis for the Master of Science (MS) in Public Health degree. First and foremost, I would like to thank the Ministry of Science and Technology (MOST) of Bangladesh for providing me with the opportunity to conduct research through the National Science and Technology (NST) fellowship 2020-2021.

I would like to express my heartfelt gratitude to Professor **Dr. Goutam Buddha Das**, Vice-Chancellor of Chattogram Veterinary and Animal Sciences University (CVASU) in Bangladesh, for providing this unique opportunity and research facilities.

I would like to express my deepest appreciation and gratitude to **Ms. Sharmin Chowdhury**, Professor and Head, One Health Institute, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, and chairperson of the thesis evaluation committee, for her precious advice, instruction and gracious approval of my thesis.

I am grateful to my supervisor, **Ms. Shireen Akhter**, Associate Professor, Department of Food processing and Engineering, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, who relentlessly led and encouraged me and kept me motivated throughout my study effort. It has been an honor and a privilege to have navigated this difficult endeavor under her intellectual direction, compassionate supervision, insightful ideas, and constructive and continual inspiration throughout the whole study period.

I would like to thank **Mr Avijit Talukder**, Assistant Professor, Department of Marine Bioresource Science, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, who selflessly helped and guided me with his precious suggestions instructions and motivated me throughout the whole study.

I would like to express my gratitude to **Mr. Dr. Subrata Kumar Shil**, Associate Professor, Department of Anatomy and Histology, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh, for his selfless guidance and motivation throughout the entire study.

I would like to take this opportunity to thank all of my all technical officers and supporting staffs at the Department of Food Processing and Engineering, the Department of Marine Bioresource Science, and the Department of Applied Chemistry and Chemical Technology, Chattogram Veterinary and Animal Sciences University (CVASU) in Bangladesh for their invaluable contributions to my research and thesis.

In addition, I would want to convey my profound and sincere appreciation to both of my parents as well as the rest of my family for their roles in shaping who I am today. Without their encouragement, moral support, kindness, and unending love, I never would have been able to make it this far.

Last but not least, I am grateful to my supportive friends, lab mates, and well-wishers for their insightful talks, cheerfulness, and inspiration throughout this research. Their motivation and company kept me motivated and focused throughout the course of this research.

The Author,

June, 2022.

Table of content

List of abbreviations

Words	Abbreviations
MP	Microplastic
%	Percent
Kg	Kilogram
G	Gram
Cm	Centimeter
Mm	Millimeter
Nm	Nanometer
Mm	Micrometer
Ml	Milliliter
Rpm	Rotation per minute
◦c	Degree Celsius
CN	Cellulose Nitrate
PE	Polyethylene
PP	Polypropylene
UV	Ultraviolet
PS	Polystyrene
PET	Polyethylene terephthalate
PVC	Poly vinyl chloride
PUR	Polyurethane
HDPE	High density polyethylene
LDPE	Low density polyethylene
SEM	Scanning electron microscope
FTIR	Fourier-transform infrared spectroscopy
ATR-FTIR	Attenuated total reflection Fourier-transform infrared spectroscopy
FT-MIR-NIR	Fourier Transform Mid-IR and Near-IR

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Abstract

Microplastic (MP) is an environmental issue due to its pervasiveness and bioaccumulative capacity. Marine salts have been found to contain microplastics (MPs) that indicate plastic pollution in marine environment. This study has been done to investigate the presence and abundance of microplastics in commercial salt and coarse salt from salt pans in the Banshkhali and Kutubdia region, Bangladesh. Sample collection was executed by obtaining nine raw sea salt samples from Banskhali and Kutubdia regions each, along with nine individual samples from nine commercial salt brands. The samples were prepared by digestion of the sample with 30% H_2O_2 followed by filtration, and the MPs were observed under microscopes and identified using Raman spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR). The average number of MPs in per kg of raw salt samples from Banskhali and Kutubdia region were 5072.2 ±740.40 and 5700.0 ± 578.79 respectively. In contrast, nine commercial salt brands had an average of 3283.33±640.80 MPs per kg of salt. Statistical analysis showed a significant difference ($p=0.00$) in the abundance of MPs between raw sea salt and commercially processed salt. Microscopic analysis (stereomicroscope and scanning electron microscope) revealed that fiber (85.39%) was the dominant shape of MPs followed by fragment (13.99%) and foam (0.62%). The most prevalent color of MPs was purple (28.26%), followed by blue (23.16%), red (23.12%), and colorless (14.03 %). The most dominant polymers identified in the salt samples were polyethylene (PE), high density polyethylene (HDPE), polyethylene terephthalate (PET), polypropylene (PP) and nylon. Results of the present study indicate that people are consuming salt contaminated with microplastics on a regular basis hence posing a serious threat to public health.

Key words: Raw sea salt, Commercial salt, Microplastics, Public health.

Chapter-I: Introduction

1.1 Background

Over the last few decades, the amount of anthropogenic litter in aquatic and terrestrial habitats has expanded considerably, with plastic accounting for 60-80 percent of that litter (Derraik, 2002). Plastics are synthetic polymers that can be easily molded (flexible) and modified into a variety of shapes. Plastics are made up of a long chain of polymers containing carbon, oxygen, hydrogen, silicon, and chloride, which are derived from natural gas, oil, and coal. Plastic waste is difficult to break due to the basic element of polymer. Plastic garbage from domestic and industrial activity is tossed on the ground, and some are thrown into the river, which eventually reaches the sea, contaminating the ecosystem (Warsito and Triadi Putranto, 2020). Plastics production began in the 1950s and now surpasses 280 million tons worldwide (Akdogan and Guven, 2019). Each year, 4.8 to 12.7 million metric tons of mismanaged plastic garbage is predicted to enter the oceans from coastal countries (Jambeck et al., 2015). As a result, experts have already warned that if current trends continue, the number of plastics in the ocean would exceed fish by 2050 (Anik et al., 2021).

Based on their size, plastics present in the environment are classified into five categories such as- megaplastics (>50 cm), macroplastics (>5 to 50 cm), mesoplastics (>5 mm to 5cm), microplastics (≥1 μm to < 5 mm), nenoplastics (<5 mm) (Anik et al., 2021). Microplastics (MPs) are particles smaller than 5.0 mm in size that are repeatedly shredded and fragmented from plastics by weathering agents such as mechanical forces exerted by wave action, oxidative properties of the atmosphere, and hydrolytic properties of seawater, among others, and are converted to smaller particles (Zafar et al., 2020).

Nowadays people are exploring diverse sea beaches due to globalization, and more hotels and restaurants are being established near the shore. These tourist, beachside hotels and restaurants produce a considerable amount of plastic debris, which are frequently discarded on the beach and ocean. MPs have been found on beaches, in surface waters, in the water column, and on the deep bottom in both open and enclosed seas are pernicious to the marine environment because they can enter the food chain through bioaccumulation and bio magnification in aquatic organisms as MPs are mistaken as food by marine biota (Parvin *et al.*, 2022). Moreover MPs are capable to adsorbing various chemicals, including heavy metals, due to their comparatively large surface area and hydrophobic nature. Hazardous pollutants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) may be transferred to aquatic life through MPs (Akdogan and Guven, 2019). In humans and other mammals, MPs have the ability to translocate between living cells to the lymphatic and circulatory systems, potentially via the intestine (Zafar et al., 2020). So research on micro plastic pollution and its effect on aquatic life and environment is important. But it's difficult to estimate the amount of MPs in seawater. As a result, researches have focused on monitoring sea salt as a source of MPs pollution (Parvin et al., 2022).

1.2 Rationale and significance of the study

Table salt is made from mineral rock mining or the evaporation of seawater sources. Saltwater is subjected to a variety of physical processes during the manufacturing process. It is pumped into evaporation ponds, then concentrated and crystallized by the sun and wind, before being cut and packaged for sale (Rakib et al., 2021a). If MPs are present in sea water, there is a high risk of MP contamination in sea salt, as it is produced by evaporating sea water. People consume foods that contain significant amounts of salt. According to the World Health Organization adults should consume 6 grams of salt per day (WHO, 2012). Moreover, daily salt intakes as high as 15 g/day and 21 g/day have been reported in Bangladesh based on industrial output and hospital patient data reports (Zaman et al., 2017). A recent research evaluated the MP availability in table salts obtained from various countries, utilizing sea salt as a saltwater MP pollution indicator, and discovered that Asia had much more MP prevalence than the other continents (Kim et al., 2018a). Though salt consumption rate is high, there is a scarcity of information on the presence of MP in Bangladeshi table salt. Therefore, the current study aims to investigate the abundance, properties, and polymer composition of MP pollution in commercial salts and salts from salt pans of Banskhali and Kutubdia region along the Chattogram coast of Bangladesh.

1.3 Objectives:

1. To evaluate the abundance and morphological characteristics of microplastics in coarse sea salt and commercial salt of Chattogram region.
2. To identify the polymer composition of microplastics in raw sea salt and commercial salt.

Chapter-II: Literature Review

2.1 Definition and global distribution of plastic

In the present world, reliance on plastic polymers for the production of customer goods is inevitable, especially in lessening the production costs for lightweight but durable items. During and after World War II, when these 'pliable and easily moulded' polymers gained wide acceptance in substituting scarce natural items such as ivory, wood, metal, stone, bone, tusk, and horn, plastic production and the manufacturing of plastic goods boomed (Hamid et al., 2018). Plastics are long, chain-like molecules with a high average molecular weight and synthetic polymers with other chemicals added to improve performance. They are typically manufactured from petrochemical sources and have a wide range of molecular mass and flexibility. Many common types of plastics are made up of hydrocarbons produced mostly from fossil fuel and biomasses of different origin (Law, 2017). Plastics have become widely used in recent years due to its convenience of production, inertness (chemical, temperature, and light resistance), low cost, high strength/weight ratio, and water resistance (Sharma et al., 2017). As a result, plastic production has increased dramatically during the previous few decades.

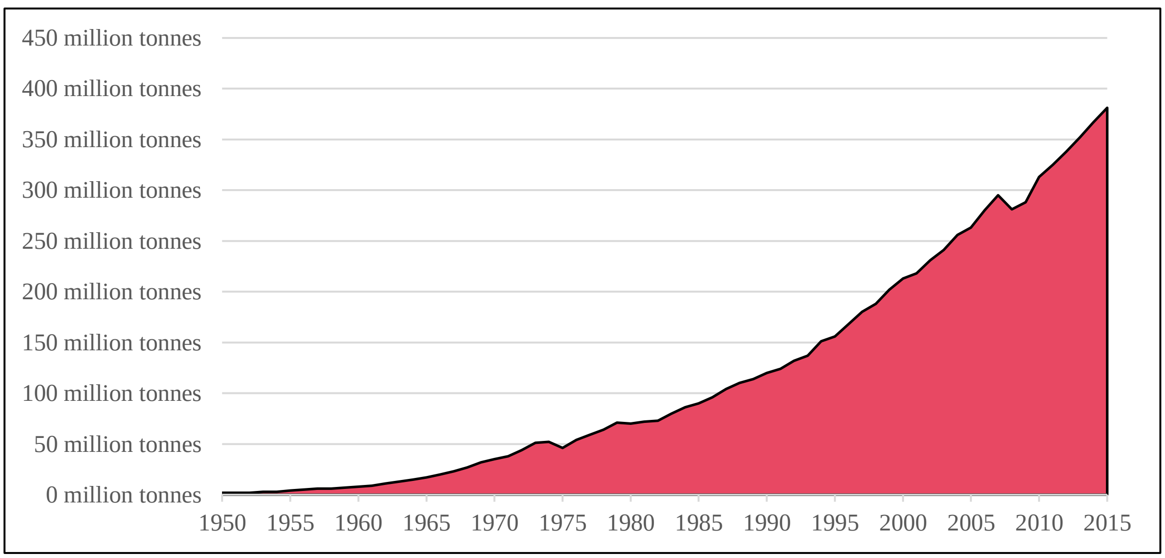

Figure 1: Global plastic production from 1950 through to 2015 (Geyer et al., 2017).

According to a study, only 2 million tonnes of garbage were produced annually in 1950, but that number has increased more than 200 fold since then, exceeding 381 million tonnes during 2015 (Geyer et al., 2017).

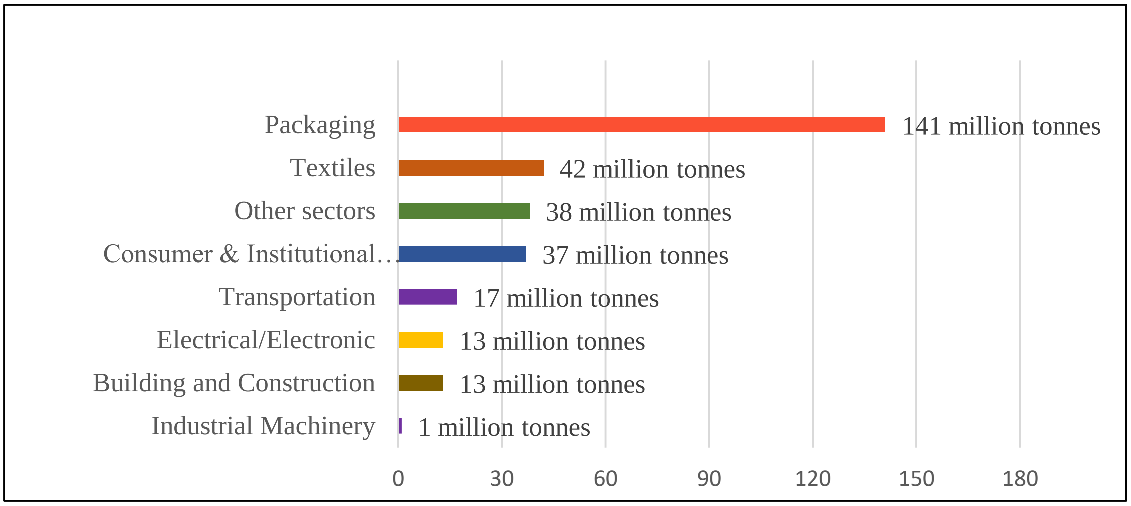

Figure 2: Global plastic waste generation by industrial sector, 2015 (Geyer et al., 2017).

In 2015, a study was conducted to observe global plastic garbage production by different industrial sector. According to that study packaging industry was the largest plastic generating sector. Packaging industries used the most primary plastics, accounting for 42 percent (146 million tonnes) of all plastics in 2015 (Jambeck et al., 2015).

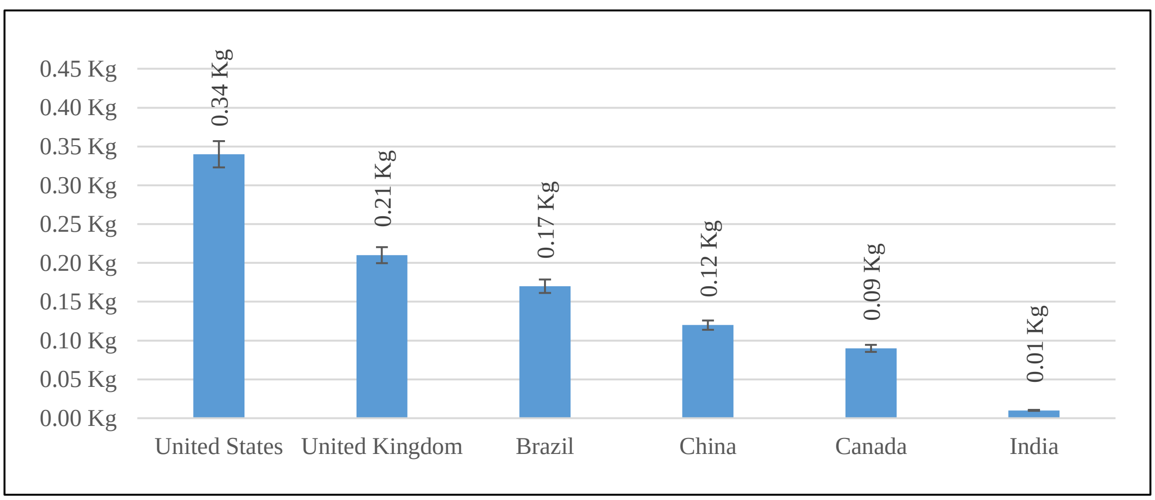

Figure 3: Worldwide plastic waste generation per person, 2010.

According to Jambeck et al. (2015) high income countries like the United States and members of the European Union (EU-28) had considerable plastic waste discharged to the ocean in 2010. The study showed the per capita rate of garbage production, measured in Kg per person per day by different countries. The United States generated the highest amount of plastic waste (0.34 Kg) per person followed by the United Kingdom, Brazil, China, Canada and India (Jambeck et al., 2015).

2.2 Types of plastics:

The thermoplastics, theromosets, and elastomers are the three types of plastic polymers. Thermoplastics are made up of one-dimensional carbon-carbon chains that are long, linear, and saturated. As thermoplastic molecules are not cross-linked, they can move independently. Due to melting and solidifying repeatedly by heating and cooling, thermoplastics can be reused (Robertson, 2013). Thermosets, unlike thermoplastics, form irreversible crosslinks between chains during processing and cannot be re-melted or reprocessed. Elastomers are rubber like elastic polymers and are made up of long chain molecules. After being stretched to extremes, these plastic polymers are able to retrieve their original shape. Elastomers have numerous crosslinks between particles that allow molecular chains to move, resulting in soft and flexible properties (Plank et al., 2018). Thermoplastics are the most extensively used plastics due to their mechanical qualities, responsible for more than two-thirds of all polymers used globally (Robertson, 2013).

Table 1: Classification of plastics (Kershaw, 2015)

Plastic		
Thermoplastic	Thermoset	Elastomer
Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyvinyl chloride (PVC)	Polyurethane, Epoxy, Alkyd	Styrene-butadiene rubber (SBR), Polyisoprene (IR), Polybutadiene (BR), Chloroprene rubber (CR)

Polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC) are the most popular thermoplastics, while thermosets include polyurethane, epoxy, and alkyd. Many different varieties of plastic are manufactured around the world, but six types of plastic dominate the market: polyvinyl chloride (PVC), polyethylene (PE, high and low density), polypropylene (PP), polyurethane (PUR), polystyrene (PS, including expanded EPS) and polyethylene terephthalate (PET) (Kershaw, 2015).

2.3 Microplastics:

Microplastics is a term used to describe a totally heterogeneous combination of particles with sizes ranging from a few microns to several millimeters in diameter, and shapes ranging from perfectly spherical to elongated fibers (Thompson, 2015). Since 2004, when Thompson et al.(2004) used the term to illustrate and describe the accumulation of truly microscopic pieces of plastic in marine sediments and in the water column in European waters, the term microplastics has become widely used in relation to anthropogenic debris (Thompson et al., 2004). The US National Oceanographic and Atmospheric Administration (NOAA) convened the first International Microplastics Workshop in Washington in 2008, and as part of that meeting, a broader working definition was created to cover all particles less than 5 mm in diameter (Arthur et al., 2009). Following the United States, the European Union has approved a 5-mm upper bound for microplastic classification in the Marine Strategy Framework Directive (MSFD) (Galgani et al., 2010).

Microplastics come from a variety of places, but they can be divided into two major categories: primary and secondary. Primary MPs are the direct discharge of tiny particles, such as when pellets or powders are released, and secondary MPs are such as when bigger items are fragmented (Thompson, 2015). Polyethylene (PE), polypropylene (PP), and polystyrene (PS) particles are primary sources of microplastics in cosmetic and medical items (Horton et al., 2017). Manufacturers and other chemical agencies purposefully synthesize primary MPs for use in cosmetics, personal care products, skin exfoliators, and other applications (Vaid et al., 2021). Again, physical, chemical, and biological processes that break plastic trash, produce secondary microplastics. A secondary fraction of MPs is formed by the fragmentation of bigger plastic objects such as food packaging, fishing gear, synthetic textiles, plastic bottles, paints, vehicle tyres and cosmetics (Gabriel et al., 2016). Again based on their size, plastics present in the environment are classified into five categories such as- megaplastics (>50 cm), macroplastics (>5 to 50 cm), mesoplastics (>5 mm to 5cm), microplastics (≥1 µm to < 5 mm), nenoplastics (<5 mm) (Anik et al., 2021).

Table 2: Classification of plastics based on size (Anik et al., 2021)

Plastics				
Megaplastics	Macroplastics	Mesoplastics	Microplastics	Nenoplastics
(>50 cm)	(>5 - 50 cm)	(>5 mm -5cm)	(≥1 µm - < 5 mm)	(<5 mm)

2.4 Sources of MPs:

Marine plastic contamination is an anthropogenic substance that derives from either terrestrial sources (which account for 80% of maritime garbage) or coastal practices such as unintentional loss or illegal dumping by fishermen while fishing or other operations (Coyle et al., 2020). Microplastics can be found in a variety of forms, including synthetic fibers. Fibrous microplastics are thought to enter the aquatic environment through a variety of mechanisms and thought to be derived from synthetic garments or macroplastics (Dris et al., 2016). Laundry washing machines have been found to discharge substantial volumes of microplastics into effluents (up to 1900 fibers in one wash) (Browne et al., 2011). Facial cleansers, tooth paste, resin pellets, and cosmetics such as shower/bath gels, peelings eye shadow, scrubs, blush powders, deodorant, make-up foundation, shaving cream, mascara, baby products, hair coloring, bubble bath lotions, insect repellents, nail polish, and sunscreen are all sources of primary plastics (Auta et al., 2017). Tourism and fishing operations, the outflow of effluent from industries and urban run-off, and marine transport activities are all anthropogenic sources of microplastic pollution (Shahul Hamid et al., 2018). Polyethylene (25%), polyethylene terephthalate (16.5%), polyamide (12%), polypropylene (14%), and polystyrene (8.5%) are most common microplastics found in the maritime environment. Many research have been carried out to quantify their abundance in marine environment (Koelmans et al., 2019).

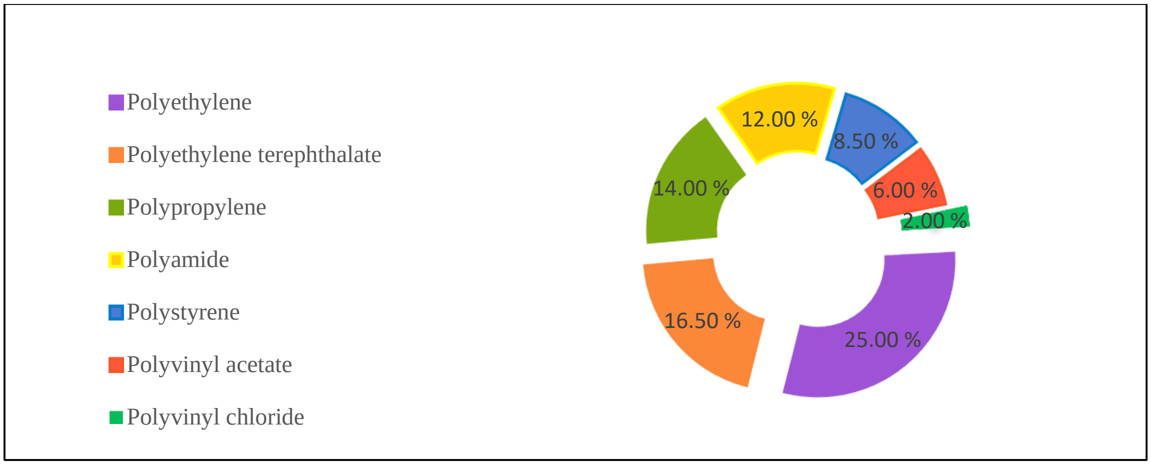

Figure 4: Abundance of MPs in marine environment (Koelmans et al., 2019)

2.5 Factors affecting abundance of MPs

MPs can arise from a wide range of land- and sea-based sources in both freshwater and marine habitats. Despite this, 80 percent of marine trash comes from land-based sources, with the rest coming from marine-based activity (Jang et al., 2020). The key factors influencing the quantity of MPs in the ecosystem have been identified as density of population and proximity to urban centers. Furthermore, higher MP concentrations have been associated with increased rainfall, and the stronger the rainfall, the greater the erosion effect of surface discharge on land, and the greater the volume of plastic waste transported. Industrial and domestic sewage leakage could be a major source of MP pollution in marine and freshwater systems (Elizalde-Velázquez and Gómez-Oliván, 2021). Environmental and anthropogenic forces are the key determinants of microplastic abundance and dispersion. Environmental factors include tides, cyclones, wind directions (Browne et al., 2011; Kukulka et al., 2012; Thiel et al., 2013; Sadri and Thompson, 2014) wave currents (Kim et al., 2018a), that define the distribution of microplastic. Anthropogenic causes, on the other hand, are human actions that cause the aggregation of plastic garbage in the environment (Shahul Hamid et al., 2018).

2.6 Generation of microplastics:

Primary MPs are generated directly during manufacturing of commercial commodities. But secondary MPs are generated from large plastic particles by photo-oxidation process.

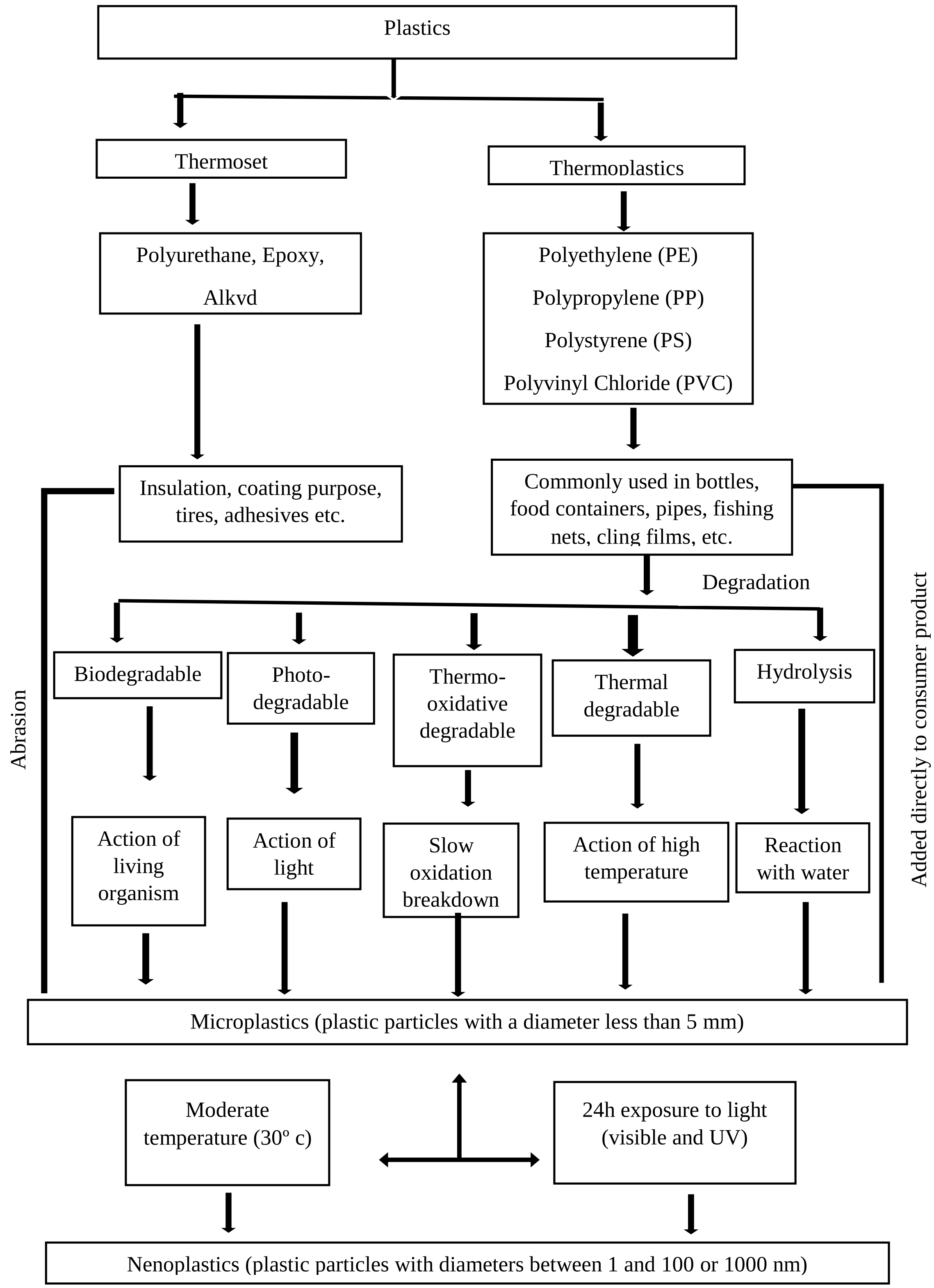

Figure 5: A summary of possible environmental degradation pathway of plastics. This schematic is drawn based on (Jiang et al., 2020)

The photo-oxidation of plastic is catalyzed by ultraviolet (UV) radiation, causing it to become fragile and fragment into microplastics. Plastic marine trash on beaches and floating in water, loses its mechanical integrity due to weathering degradation as it is exposed to UV radiation from the sun (Kershaw, 2015). Two environmental factors influences the production of microplatics in the ocean such as 1) UV radiation from the sun promotes oxidative breakdown of polymers, causing them to lose mechanical strength 2) Mechanical degradation, such as wave, wind, ocean current, human action and animal biting, can further break down the polymer into tiny fragments (Ogunola and Palanisami, 2016). Heat and sunlight, as well as well-aerated environments, are favorable for producing microplastics via repetitive fragmentation processes but cold and anoxic conditions in marine ecosystem and deposits can lead plastic particles to degrade very slowly over decades (Akdogan and Guven, 2019)

2.7 Previous study on microplastics

Numerous research have been conducted to identify, characterize, and analyze microplastics in the environment. MPs have been detected in many marine sources such as marine environment (Lusher, 2015), beach sediments (Bridson et al., 2020), both in fresh and marine water fish (Jabeen et al., 2017), fresh water (Li et al., 2018), salt (Iñiguez et al., 2017) and fish meal (Castelvetro et al., 2020). Since 2020, there has been an increase in research on MPs in Bangladesh. In 1972, in the southern region of England, the very first study on microplastics in fish was conducted. The study became a watershed moment for many other nations in the coming years. Polystyrene feldspars were discovered as trash in the marine waters of the area throughout the study. The particle was discovered in both transparent and white addle forms, having diene rubber coloring (Carpenter et al., 1972).

MPs were described as "marine debris" in another study conducted in Spain in 2017 using commercial salt as samples. The researchers observed that the most common MP in the salt was polyethylene terephthalate (PET), with abundance of 50-280 MPs/kg of salt, followed by polypropylene and polyethylene. The study also found MPs in well-packaged salts ranging from 120 -185 MPs/kg. But the study found no correlation between packaging and MPs in salt (Iñiguez et al., 2017).

In a study conducted in Taiwan in 2019, researchers found 43 microplastics in 4.4 kg of salt, with an average of 9.77 MPs/kg of salt. Polypropylene, polyester, polystyrene, polyethylene, polyethylene terephthalate, polyetherimide and other MPs were identified in that study. Polyethylene terephthalate, polypropylene, and polyethylene were found to be the most abundant microplastics in salt, according to the study (Lee et al., 2019).

The first study on Turkish table salt used microscope and Raman spectroscopic inspection to evaluate the microplastic content of 16 different brands of table salt on the market. Microplastic content was determined in sea salt at 16-84 items/kg, lake salt at 8-102 item/kg and rock alt at 9-16 item/kg. Polyethylene and polypropylene were the most abundant plastic polymers (22.9 % and 19%) (Gündoğdu, 2018). A similar research was carried out in 8 South African countries with 23 brands of table salts. According to that study (0-1.33 ± 0.32 particles/kg) was the highest concentration of MPs found in Nigeria, Cameroun, and Ghana in South Africa. Polyvinyl acetate, polypropylene, and polyethylene were the most found MPs in that study. However, the researchers found no MPs in salt samples from other countries like Zimbabwe, Malawi, Kenya, and Uganda (Fadare et al., 2021).On the presence and quantification of salts, another comprehensive investigation was conducted in the Atlantic Ocean, Indian Ocean, Mediterranean Sea, Pacific Ocean, and Continental Ocean. Seventeen salt samples were gathered from eight different nations, and -Raman Spectroscopy revealed that 88 % of the salt samples contained MPs. MPs were not found in the French salt sample, but were found in the other samples in amounts ranging from 1 - 10 kg^{-1}. Plastic polymers made up 42 % of the MPs, whereas 29 % of the MPs were undefined. The most frequent plastic polymer discovered in the study was polypropylene (Karami et al., 2017).

In 2022, a revolutionary investigation was carried out in Sri Lanka to evaluate and quantify the abundance of MPs in salt products. MPs were found in all salt samples and varied from 11 to 193 items per kg in commercial salts, 64 items per kg in rock salts, and 253±8.9 items per kg in lab grade NaCl. Fibers were the most common type of MP, followed by fragments. Low-density polyethylene (LDPE; 17%), resin dispersion (15%), and high-density polyethylene (HDPE; 12%) were the most abundant of the 23 varieties of MPs found (Kapukotuwa et al., 2022).

In the year 2021, a study was conducted in Parangipettai and Marakkanam, Tamil Nadu, India. The goal of the study was to determine the amount of MPs in commercial table salts and table salts from salt pans. According to that study, the amount of MPs in salts from salt pans (3.67 ± 1.54 to 21.33 ± 1.53 nos./10 g) was higher than that of commercial salts (4.67 ± 1.15 to 16.33 ± 1.53 nos./10 g). According to that research, the most abundant MPs were colorless, red, black, green, blue, brown, and white fibers. The results of the FT-IR analysis revealed that the samples contained four different polymers: polypropylene (PP), nylon, polyethylene terephthalate (PET), low density polyethylene (LDPE) (Nithin et al., 2021).

A baseline study was carried out in South India in 2020. From salt pans in the Tuticorin coastal region, 25 different varieties of sea salt samples were collected. The diameter of 60 % of all MPs identified in salt samples was smaller than 100 μm. Polypropylene (PP) was the most abundant MP in the sea salts, followed by polyethylene (PE), nylon, and cellulose (Selvam et al., 2020).

Another global study was administered in 2018 with 39 salt samples from six different continents. The study recommended salt as an index of MPs pollution in marine localities. Three types of salts were investigated in the research with the finding of MPs concentration as following: sea salts (0-1674MP/kg), rock salts (0-148 MPs/kg) and lake salts (28-462 MPs/kg). The study also specified Asia as an epicenter for plastic pollution (Kim et al., 2018b). A similar global investigation was conducted with samples of commercial sea salts from 12 well-known brands. Fibers were discovered to be the most common anthropogenic pollutant in salt, with 47-806 particles per kg (Kosuth et al., 2018).

In the recent year, the presence and concentration of MPs in Bangladesh have been a topic of concern. In the year 2021, a study in Cox's Bazar revealed the scenario of MP contamination in salt. The research examined 10 branded salt samples and 3 unbranded salt samples. The analysis found 2676 MPs/kg of salt with a diameter of 0.5 mm. With the exception of Croatia and Indonesia, the concentration of MPs in the salt was substantially greater (Parvin et al., 2022a).

Another research conducted on the Maheshkhali channel highlighted concern about MPs in salt in the same context. MPs were found in all eight samples taken from the typical salt pans, according to the researchers. The average amount of MPs accumulated in salt ranged from 78 ± 9.33 per kg to 137 ± 21.7 per kg maximum. Fragmented plastics made up 48% of the MPs identified in the salt sample. The presence of polyethylene terephthalate (PET) in the highest quantity was determined using the Fourier Transform Mid-IR and Near-IR (FT-MIR-NIR) (48%). Other MPs included polypropylene, polyethylene and polystyrene (Rakib et al., 2021b).

As a result of the mismanagement of plastics debris, MP have been found in the marine environment and on beaches. Many studies have been carried out to investigate the scenario of microplastic contamination in beaches sediments. In 2014, a similar investigation on beach sediments was conducted in the German Baltic region. Microplastic particles with a concentration of 0-7 particles per kg and 7-11 fibers per kg were found in the study. The most common sources of high microplastic contamination, according to the study, were municipal outflows, commercial fishing, industrial output sites, and tourism (Stolte et al., 2015).Almost all plastic waste in developed countries is recycled, incinerated or dumped in well-managed dumping sites. If plastic is not properly managed it will end up in rivers and the ocean. In 2021 a research was conducted to investigate the global mismanaged plastic garbage, where Asia was in top position (Meijer et al., 2021).

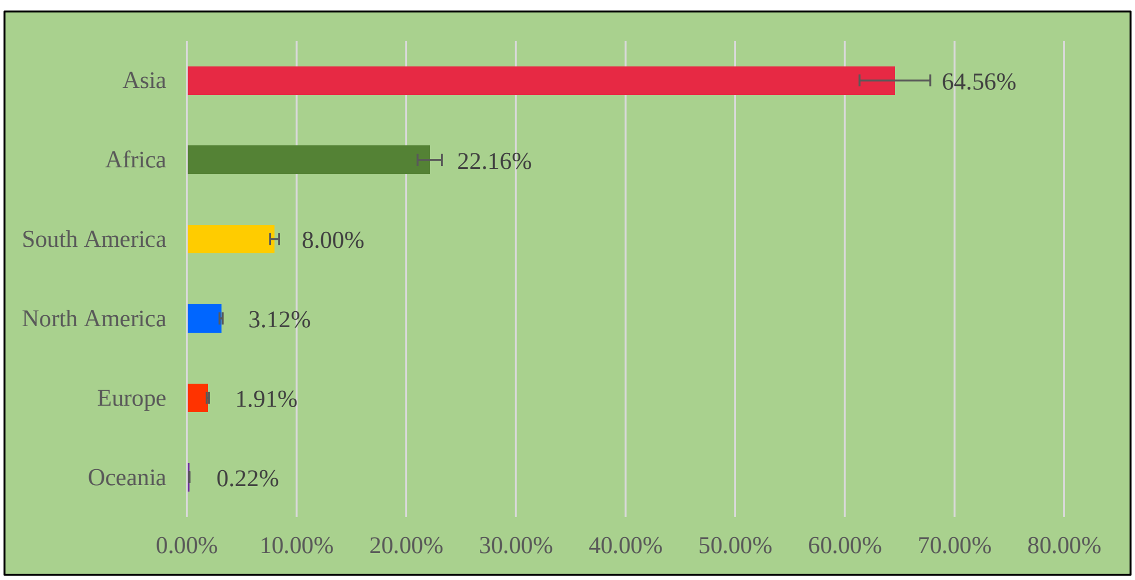

Figure 6: Share of global mismanaged plastic waste, 2019

A macro scale study was carried out to investigate MPS pollution in beach sediments throughout Europe. The study examined samples from 13 different countries. The researchers discovered a remarkable variation in the amount of MPs in the beach sediments, which ranged from 72 ± 24 to 1512 kg-1 of dry beach sediments. The persistent pollutants found in the samples were colorful fibrous MPs. Raman spectroscopy identified the MPs found in the samples as polystyrene, polyethylene, and polypropylene (Lots et al., 2017).

Another study was conducted to investigate the relationship between the tide line and microplastic pollution on marine beaches. Pyrolysis Gas Chromatography/Mass Spectroscopy was used to identify the polymer types, with polyethylene and polypropylene emerging as the most important MPs. However, no significant relationship was discovered between the tide line and MPs pollution (Doyen et al., 2019). Since the last decade, several studies in the Indian region have been regulated. A study was conducted in Karnataka in 2021 to examine and characterize MPs in beach sediments. The results revealed an abundance of microplastic in dry sediments ranging from 264 to 1100 per kg, with polyethylene and polypropylene were contributing the most microplastic in the Karnataka samples. The illustrative imaging of the samples by Field Emission Scanning Electron Microscope (FESEM) signified their constant interaction with the marine environment (Yaranal et al., 2021).Another study was conducted in Puducherry to determine the prevalence of MPs in beach sands. An abundance of 72.03 ± 19.16 MPs/100 gram of sand samples was identified after six beaches were analyzed for the purpose. The researchers also found a significant association between MP amplitude and fishing (high correlation, p=0.0103) and tourism (weak correlation, p=0.932). Polypropylene, high density polyethylene, low density polyethylene, and polyurethane were the most common MPs found in Puducherry sediments (Dowarah and Devipriya, 2019).

Another baseline study was carried out in Kanyakumari, India's southernmost coast. Along the Indian Ocean, samples were taken from fishing villages/residential beaches, tourist beaches, harbors, and undisturbed coastal areas. After analyzing 50 g of dry sediments (d.s.) from each location, the researchers discovered 343 particles (67 % fiber and 33 % fragment) from eight different locations. The highest concentration was found on tourist beaches (150 particles/50 g d.s.), followed by harbors (99 particles/50 g d.s.). Microplastics were found in the lowest amount on the undisturbed beach, accounting for only 4.3 % of the total retrieved items (Sundar et al., 2021). Another study of MPs was conducted in Bangladesh in 2020. MPs in beach sediments in Cox's Bazar were investigated by the researchers. The result was 8.1± 2.9 MPs/kg sample, posing a major hazard to the marine environment. Polypropylene was disinterred in the highest percentage across various types of polymers (47%). According to the researchers, the beach face and swash zone had less MPs than the wrack line. The researchers also discovered a link between the buildup of MPs in beach sediments and tourist activity (Rahman et al., 2020).

A similar study was conducted in the Kuakata region to describe the massive inflation of MPs in 2022 (Banik et al., 2022). The authors claimed to have discovered more MPs in beach sands than everywhere else on the planet, with 232 ± 52 MPs per kilogram of dry weight measured. PET was found in the highest concentration, followed by polyethylene and polypropylene. The researchers found a link between grain size and the accumulation of MPs in beach sediments. The study region of Kuakata fell into risk category I, which presented a risk and concern for the country, according to the pollution load index analyzed by the researchers. Marine water has also been extensively studied around the world to evaluate MP pollution.

In Hong Kong, a study to determine MP pollution in marine water was conducted from 2015 to 2016 (Tsang et al., 2017). The researchers found 51 to 27,909 MP particles per 100 m^3 of water sample. The polymer types were identified using ATR-FTIR, which revealed the presence of Polypropylene, Styrene acrylonitrile. LDP, HDP, Polypropylene and ethylene propylene. Another study was carried out in Qatar to investigate the prevalence of microplastic in coastal water. For sampling, the Exclusive Economic Zone (EEZ) was used. MPs were found to be 0.71/m3 in water. The samples were analyzed using ATR-FTIR to determine the polymer types. Polyamide, Polypropylene, low density polyethylene, cellophane, polystyrene and polyethylene were all found in abundance (Castillo et al., 2016).

In Bangladesh, a study on abundance, characteristics, and types of MPs in various freshwater fish species was conducted in 2021. To investigate the differences in MPs ingestion rates among different feeding zones, 48 fishes from eighteen species were collected from different feeding territories. According to Fourier Transform Infrared analysis, 73.3 % of the fish studied had MPs in their gastrointestinal tract, including high density polyethylene, polypropylene-polyethylene copolymer, and ethylene vinyl acetate. Among all the fish species studied, *Mystus vittatus* had the highest concentration of MPs. Microscopic investigations (polarized light, SEM) revealed that the majority of the MPs were fiber-shaped and transparent in color (Parvin et al., 2021).

Again, in 2022 researchers were investigated MPs in two commercially imported dried fish-Bombay duck (*Harpadon nehereus*) and ribbon fish (*Trichiurus lepturus*), collected from two sites on the Bay of Bengal (Cox's Bazar and Kuakata) of Bangladesh. Dried Bombay duck and ribbon fish from Kuakata had significantly higher MP levels (41.33 g^{-1} and 46.00 g^{-1}, respectively) than in samples from Cox's Bazar (28.54 g^{-1} and 34.17 g^{-1}, respectively). Fibers (41–64 %) were the most predominant type of MP found in all samples, followed by fragments (22–34 %), microbeads (9–16 %), films (3–4%), foams (1–4%), and pellets (0–2%). In the dried fish samples, ATR-FTIR analysis indicated three different forms of MP polymer: polyethylene (35–45%), polyamide (30–45%) and polystyrene (20–30%) (Hasan et al., 2022).

A similar study was administrated in 2021 in Bay of Bangle with 100 commercial marine fish from 10 different species. There were 215 microplastics retrieved in total, with an average abundance of 2.2 ± 0.89 microplastics per individual. Fibers, films, fragments, foams, and granules contributed 53.4%, 40%, 3.3%, 1.9%, and 1.4% of the total microplastics found. Green (39%) was the most found color, while most found size was 500 μm (85%). Polyethylene (55%) was the most common polymer, followed by polypropylene (33%), polyester (6%), polyurethane (2%), ethylene propylene diene monomer (2%), and styrene butadiene rubber (2%) among all the MPs (Ghosh et al., 2021).A global study was conducted in China, in 2019. The study was aimed at identifying the presence of MPs in both digestive and non-digestive tissues of fish from major fish farms and mariculture areas. Six major wild fish species (including *Thryssa kammalensis, Odontamblyopus rubicundus, Amblychaeturichthys hexanema, Chaeturichthys stigmatias, Cynoglossus semilaevis* and *Collichthys lucidus*) was collected from major fish farms and mariculture areas, Haizhou Bay, China. *T. kammalensis,* a filter-feeding species that lives in estuaries, had the highest MP abundance of 22.21 ± 1.70 items/individual or 11.19 ± 1.28 items/g whereas *C. stigmatias* ((1.61 ± 0.56 items/g) and *C. semilaevis* (13.54 ± 2.09 items/individual) had the lowest MP abundance. Fibrous, black or grey colored, and cellophane made up MPs had dominated the majority of the MPs in fish. In total the number of MPs on skin (800) and gills (746) was higher than in the gut (514) (Feng et al., 2019).

In 2019, another investigation was carried out in Chile to identify and characterize MPs presented in commercially important six fish species from different trophic levels of the oceanic and coastal habitats. The gastrointestinal content of the fish was collected, extracted, examined, and characterized using a microscopy linked with Fourier-transform infrared spectroscopy (FT-IR) to analyze MPs. Red microfibers (70%-100%) dominated the MPs found in fish samples with diameters ranging from 176 - 2842 μm. The most frequent polymers found were polyethylene (PE), polyester and polyethylene terephthalate (PET). Microfibers of a larger size and abundance were found in coastal species (71%) compared to oceanic species (29%) implying a higher risk of exposure (Pozo et al., 2019).

Microplastic ingestion was compared between detritus-feeding mullet and herbivorous rabbitfish, as well as between marine fishes and freshwater, in a study conducted in Philippine in 2021. MPs in the guts of fish collected from different feeding guilds, sizes, and body weights as well as MPs in the gut of fishes and their surrounding waters were compared in the study. The researchers found that herbivores consumed more MPs (58.57%) than their detritivore counterparts (30.0 %). For both species, a Pearson correlation of 0.06 revealed a weak association between fish weight and MPs level. The study also revealed that ingestion of MPs in marine fishes (66%) is higher than that of fresh water fishes (45%) (Cabansag et al., 2021).

Another study was conducted in the Agulhas Bank, south of South Africa in 2020. The researchers found MPs in 86.67% of fish sampled with abundances ranging from 2.8 to 4.6 items/fish. Fibrous (95.14%), black colored (38.11%) and size ranging from 1000 -500 μm

(35.55%) were the most dominant MPs in marine fish according to the study (Sparks and Immelman, 2020).

A global study was administered to identify microplastics in the sediment and mudskipper fish (*Periophthalmus waltoni*) in mangrove forests in southern Iran. The researchers yielded a total of 2,657 plastic particles of various sizes, colors, shapes, and taxa from sediment samples and 15 microplastics were identified from mudskippers. The highest and lowest abundances of extracted microplastics from sediments were found in the mangrove forests of Bidkhoun (urban area) and Bordkhon, respectively, but no microplastics in fish tissue were found at those areas. 60% of the MPs found in beach and fish were black in color and 26% of total MPs were polystyrene. Again according to the study polycarbonate was the lowest polymer found in sample (3%) (Maghsodian et al., 2021).

Another study was administrated with six species of inhabiting fish, namely carp (*C. carpio*), bluegill (*L. macrochirus*), crucian carp (*C. cuvieri*), bass *(M. salmoides*), snakehead (*C. argus*) and catfish (*S. asotus*) in South Korea in 2020. The researchers found the concentration of microplastics in surface waters (0 m) was 0–42.9 particles/m3 (mean: 7.0 ± 12.9 particles/m3), whereas at a depth of 2 m, the concentration was 20.0–180.0 particles/m3 20.0–180.0 particles/m3 (mean: 102.0 ± 50.3 particles/m3). Polystyrene, polyethylene (PE) and silicone had dominated plastic in river, while polyethylene, polytetrafluoroethylene (PTFE) and polyester were the most common in the tributaries. Fragments were the most abundant MP found in this study (73%). MPs concentration in fish intestine and gut were ranging from 4 - 48 particles/fish (mean: 22.0 ±16.0 particles/fish) and 1-16 particles/fish (mean: 8.3 ± 6.0 particles/fish) respectively (Park et al., 2020). A similar study was conducted in central Europe. A total 187 roach and 202 gudgeon samples were collected from Lowland River (Widawa R., SW Poland). MPs were presented in 53.9% of roach and 54.5% of gudgeon sample. However the study found no relation between MPs and type of feeding, collection site and sex (Kuśmierek and Popiołek, 2020).

In 2021, another study was carried out to detect MPs in GI tract and gills of commercial marine fish from Malaysia. 158 fish samples were collected from 16 different species from two different locations. MPs were found approximately 92% in the gills and 86% in the

GIT of tested fish sample. MPs were higher in fish near urban areas (9.88 MPs per individual) than in fish near less urbanized areas (5.17 MPs per individual). Fiber (80.2%) was the dominant MPs followed by fragment (17.7%) and filament (3.1%). Raman spectroscopy and Infrared analysis ascertained the presence of polypropylene (PP), polyethene (PE), acrylonitrile butadiene styrene (ABS), polyethylene terephthalates (PET) and polystyrene (PS). Heavy metal like iron (Fe) and chromium (Cr) were detected on the surface of green colored PE fragments by EDX (Jaafar et al., 2021).Another research was administrated with eight commercially available fish species common to the Arabian (Persian) Gulf, which were collected locally from the State of Kuwait. Various microparticles were found in the intestines of *Eleutheronemaa tetradactylum*, *Acanthopagrus latus* and *Lutjanus quinquelineatus* including three fragmented MP particles (Al-Salem et al., 2020).

2.8 Possible impacts of MPs to human health:

Ingestion of contaminated food is one of the most common nano and microplastic entry routes into the human body. Nano and microplastics were discovered in sugar, salt, alcohol, and bottled water with concentrations of 0.44 MPs/g, 0.11 MPs/g, 0.03 MPs/g, and 0.09 MPs/g, respectively, in a study (Cox et al., 2019). Plants (fruits and vegetables) that accumulate MPs through uptake from polluted soil could also provide an estimated 80 g of microplastics per day to humans (Ebere et al., 2019). As a result of dietary exposure, absorption in humans is possible, as proven by synthetic particles smaller than 150 μm has ability to pass the gastrointestinal epithelium in mammalian bodies, resulting in systemic exposure. Only 0.3 percent of these particles are likely to be absorbed, according to scientists, while a smaller percentage (0.1%) containing particles larger than 10 m should be able to reach both organs and cellular membranes, as well as pass through the blood–brain barrier and placenta (Barboza et al., 2018). Particles smaller than 2.5 μm can enter the gastrointestinal system via endocytosis by M cells (Specialized epithelial cells of the mucosa- associated lymphoid tissues). Particles are transported from the intestinal lumen to the mucosal lymphoid tissues by M cells or via paracellular persorption. Mechanical kneading of solid particles into a circulatory system occurs through gaps in the single-layer epithelium at the villus tips of the gastrointestinal tract. Because of the persistent character of MPs, as well as their specific features such as hydrophobicity and chemical properties, the ensuing toxicity is thought to be inflammatory, with an accumulative action that is dose sensitive (Wright and Kelly, 2017).

Inhalation is another route for microplastics to enter the human body. Microplastics can be carried by wind or deposited in the atmosphere and they can also come from agricultural lands being eroded, synthetic clothing fabric, dried sludges and wastewater treatment products, roar dust, industrial emission and marine aerosol. Men may experience respiratory distress, cytotoxic and inflammatory consequences and autoimmune illness as a result of the spread. Furthermore, human lung has a large alveolation surface of around 150 m^2 and a relatively weak tissue barrier of less than 1 m, allowing nanoparticals to infiltrate the blood and the entire body. Polystyrene particles with a diameter of 50 nm have been found to have cytotoxic and genotoxic effects on macrophages and pulmonary epithelial cells (Campanale et al., 2020). In general, the response to inhaled particles can be expressed as diffuse interstitial fibrosis and granulomas with fiber inclusions (extrinsic allergic alveolities, chronic pneumonia), immediate bronchial reactions, interalveolar septa lesions, inflammatory and fibrotic changes in the bronchial and peribronchial tissue (chronic bronchitis) depending on individual metabolism and susceptibility (Prata, 2018). The final route for microplastics to enter the human body is through skin contact with during washing or using scrubs and cosmetics containing micro and nanoplastics.

Additionally, these plastic wastes adsorb and concentrate high levels of hydrophobic organic contaminants (HOCs) (e.g., PAHs, organochlorine insecticides, polychlorinated biphenyls), metals (e.g., lead, chromium, cadmium, selenium), non-metals, and additives/monomers (e.g., bisphenol-A, polybrominated diphenyl ethers, octylphenol and nonylphenols) and consequently, be transported to the lymphatic system together (Peixoto et al., 2019). Even at low concentrations, direct exposure to persistent organic pollutants (POPs) and other related compounds absorbed by MPs can disrupt biological systems and represent unique hazards to people and animals (Barboza et al., 2018; Smith et al., 2018). MPs are bio persistent and may cause unfavorable biological responses in humans such as inflammation, genotoxicity, oxidative stress, cell apoptosis, and tissue necrosis, resulting in localized cell and tissue damage, fibrosis, and possibly carcinogenesis (Peixoto et al., 2019). Microbes can colonize the surface of consumed MPs and act as a vector for pathogenic bacteria within the body, causing severe direct physiological impacts (Smith, M. et al., 2018). Ingestion of MPs may cause genetic alterations in humans, resulting in infertility, obesity, and cancer (Sharma and Chatterjee, 2017).

Microplastics as contaminants in the environment pose a threat to human health because they have been proven to be consumed by a variety of aquatic animals, both ocean and freshwater, and therefore have the ability to accumulate through the food web (Galloway, 2015).

2.9 Microplastic effects in fish:

The combination of microplastics and pyrene have a toxic effect on *Pomatoschistus* sp. causing oxidative damage and a reduction in isocitrate dehydrogenase activity. This mixture also inhibited IDH activity, indicating a toxicological association between microplastics and pyrene, while increasing the quantity of biliary pyrene byproducts, implying that microplastics could affect both pyrene bioavailability and biotransformation (Oliveira et al., 2013). A loss in ability of juvenile gobies to catch prey and avoid predators could be caused by decrease of predatory activity due to simultaneous exposure to heavy metal (Cr) and microplastics. As a result, there's a chance that individual fitness, and thus population fitness, will suffer, with implications for juvenile development rates and species survival (Guzzetti et al., 2018). The accumulation of microplastics in gills, liver and gut of *Daino rerio* caused a number of deleterious consequences including inflammation, abnormal metabolic pathways and increased enzymatic activity (catalase and superoxide dismutase) (Lu et al., 2016). Moreover microplastics can change the genetic expression of aquatic fish due to their toxicity (Gola et al., 2021).

2.10 Microplastic effects in marine biota:

Microplastics cannot be digested or absorbed by aquatic animals due to lack of necessary metabolic pathways to break synthetic polymers. As a result they are classified as bio-inert substances (Andrady, 2011). Ingestion of MPs and entanglement in plastic matter can cause chronic and acute injuries as well as an increase in pollutant loads, causing pathogenic disease and mortality in cetaceans (Baulch and Perry, 2014). High level of microplastics in

GI tracts of baleen whales may impede digestion process and block the intestinal tract (Guzzetti et al., 2018). Moreover MP consumption by marine turtles can cause severe digestive system damage and can clog the intestinal tract as well as diminish the feeding impulse and stomach capacity which can lead to food dilution and starvation, and eventually death (Nelms et al., 2015). The presence of microplastics in aquatic bodies can have an impact on the population of algae, which serve as a food source for many other aquatic creatures. Microplastics have adverse effects on algae, including a decrease in photosynthetic activity and oxidative stress. Microplastics can limit photosynthetic activity of algae, which has a detrimental impact on algae biomass resulting reduction in the overall food production for other marine creatures that rely on algae (Gola et al., 2021).

Chapter-III: Materials and Methods

3.1 Study area:

Salt industry has a vital role in the output, employment, and industrialization of Bangladesh. In the coastal regions of Bangladesh, particularly in Chattogram and Cox's bazar, there are lots of salt industries which produce salt from the salty seawater. To conduct the study salt samples were collected from Banskhali region located at 22.0485°N 91.9416°E and Kutubdia region located at 21.8167°S 91.8583°E.

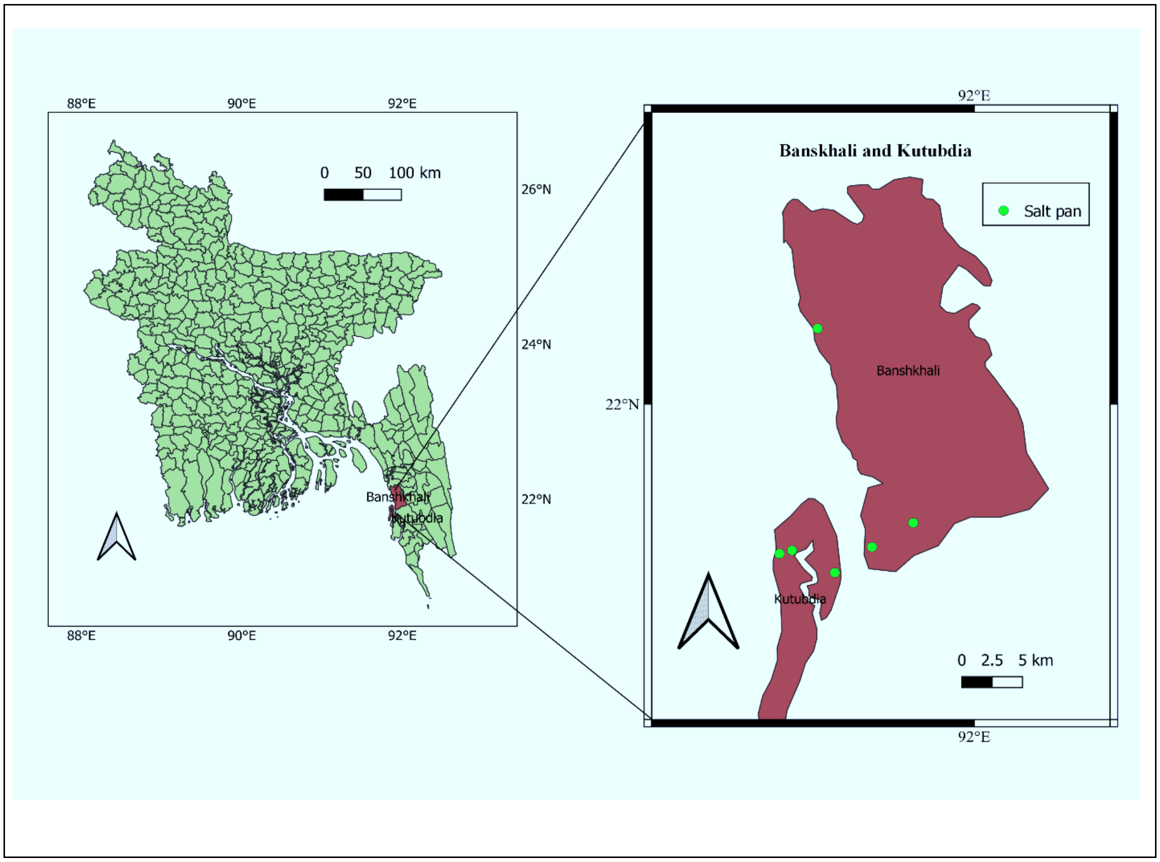

Figure 7: Geographical representation of sampling area

3.2 Study period:

Present study was conducted between the months of January 2022 and May 2022 at a variety of laboratories including Department of Food Processing and Engineering, Department of Marine Bioresource Science, Department of Anatomy and Histology located in Chattogram Veterinary and Animal Sciences University (CVASU) and Bangladesh Council of Scientific and Industrial Research (BCSIR).

3.3 Sample collection:

Nine samples were collected from Baharchada, Puichadi and Chanua at Banskhali and nine salt samples were collected from Bakkhali, Chander ghona and Hazipara at Kutubdia region. Samples from nine commercial salt brands were also collected to investigate microplastics in commercial salt.

3.4 Sample preparation and segregation of microplastic from salt:

The methodology that was used to prepare the samples, which include some minor adjustments and optimizations, was comparable with that of previous research (Rakib et al., 2021a). Briefly, in order to digest the organic matter, a sample of salt weighing roughly 100 grams was combined with 100 ml of a solution containing 30 percent H_2O_2 and then placed in an incubator at a temperature of 65 degree Celsius for 24 hours. After that, the solution was stirred using a magnetic stirrer at a speed of 80 revolutions per minute (rpm) for a period of four hours. To complete the process of dissolving the remaining salt samples into the solution, approximately 800 milliliters (ml) of deionized water was added to the solution. The procedure required the use of deionized water because of its resilience to the impacts of plastic materials (Parvin et al., 2022b). The solution was moved in the process and put into a vacuum pump in order to filter the solution. During the filtration procedure, Cellulose Nitrate (CN) filter paper was used. This particular paper was made by Sartorius in Germany and featured the following specifications: 0.22 micron pore size and 47 millimeter diameter. When it comes to removing particles, CN filter sheets are an excellent choice due to their low cost and the biobased alternative (Selvam et al., 2020). The rate of flow was 1 milliliter per minute. After the filtration procedure was finished, the filter papers were collected, allowed to dry at ambient temperature for one hour, and then placed in an incubator at a temperature of 25 degrees Celsius until further examination. It was observed that the filter papers contained the particles that were resistant to digestion or insoluble. Each sample was given three identical copies, or replicates. In addition, a blank sample with no salt solution was passed through the filter paper and examined to eliminate the probable chances of operational contamination.

3.5 Microscopic observation of microplastic:

The microplastics were initially visualized using a stereo microscope with a magnification of 10 times, which allowed the researchers to recognize the microplastics on cellulose nitrate filter paper. After that the filter papers were moved to a different stereo microscope (Leica® EZ4 HD) that included a built-in camera and magnification of 35 times so that MPs could be observed and captured images. The camera was used to collect images in order to differentiate the microplastics according to their morphology, which include factors such as color, size and shape. Further classification of microplastics into fiber, foams, filaments and pieces was carried out (Parvin et al., 2022a)

3.6 Fourier Transformed Infrared Spectroscopy (FTIR) analysis:

The microplastics that appeared to be the same were first separated from the filter papers and placed in a small glass vial. The polymer type was then determined by analyzing the glass vial. The tests were carried out using a Perkin Elmer Fourier Transformed Infrared Spectroscopy (IR spectrum ES version 10.6.2). The samples were measured using the Attenuated Total Reflection (ATR) method. The absence or presence of functional groups and bonds in the fiber polymers can be determined by using FTIR-ATR (Anderson and Voskerician, 2009). With a modest modification of the approach outlined in a similar research, the wavelengths were adjusted from 4000 – 400 cm^{-1} (Hossain et al., 2019). After that, the spectra were additionally matched to the absorption spectrum of each plastic polymer that were revealed in previously published literature in order to prevent the possibility of false negative results.

3.7 Scanning Electron Microscope (SEM) imaging:

The filter papers were then sent to the laboratory, where they were analyzed for the pattering on the surface of the microplastics and photographs were taken using a scanning electron microscope with a high resolution (SEM).

3.8 Raman Spectroscopy:

Raman spectroscopy is a direct and simple method that has proven to be more effective in the identification of microplastics in a variety of environmental samples and have a high degree of dependability. The samples were treated with a monochromatic laser light source for Raman spectroscopic evaluation. The typical range for laser wavelengths is between 500 to 800 nm (Löder and Gerdts, 2015). For this study, samples were exposed to a wavelength of 785 nm, with a minor modification of the protocol outlined in a previous study (Anger et al., 2018). There was an interaction between the radiation and the sample, a very small fraction of the scattered photons had an energy change and this change provided information on the motions of molecules in the sample (Käppler et al., 2016).

3.9 Procedural contamination control:

Precaution was taken at every step to avoid procedural contamination. The vacuum pump, petri dishes, conical flasks, and beakers were all cleaned with distilled water. Plastic bags were not used in the process to avoid plastic contamination. Gloves were worn while handling the chemicals (Devriese et al., 2015). All of the glass and metal items were washed with distilled water three times. To avoid misleading negative results, a blank sample with no salt was processed through a filter paper. Covers and foil papers were used to keep all glass and metal objects safe. The experimental methods were carried out as promptly as possible. Cotton aprons were worn as protective clothing during research as recommended by a procedure published by a Portuguese study (Neves et al., 2015). Both aerial contaminants and synthetic fibers can cause cross contamination in the process (Banik et al., 2022).

3.10 Statistical analysis:

Differences in the abundance of microplastics between raw sea salt from salt pan and commercial salt were examined by statistical analysis. The amount and morphologic characteristics of MPs found in salt samples were enrolled into a sheet of MS Excel-2007 programs. Then the data was exported to STATA® version 13.

Chapter-IV: Results

4.1 The abundance and morphologic characteristics of MPs in salt:

4.1.1 Abundance of microplastics in salt:

After examining the salt from the salt pans of Banskhali and Kutubdia it was observed that the abundance of microplastics per kg in Banskhali salt ranges from 4150 to 6300 MPs/kg and in Kutubdia salt ranged from 5200 to 6600 MPs/kg. Commercial salt had a lower amount of MP 2550 to 3700 MPs/kg compared with raw salt from Banskhali and Kutubdia salt pans.

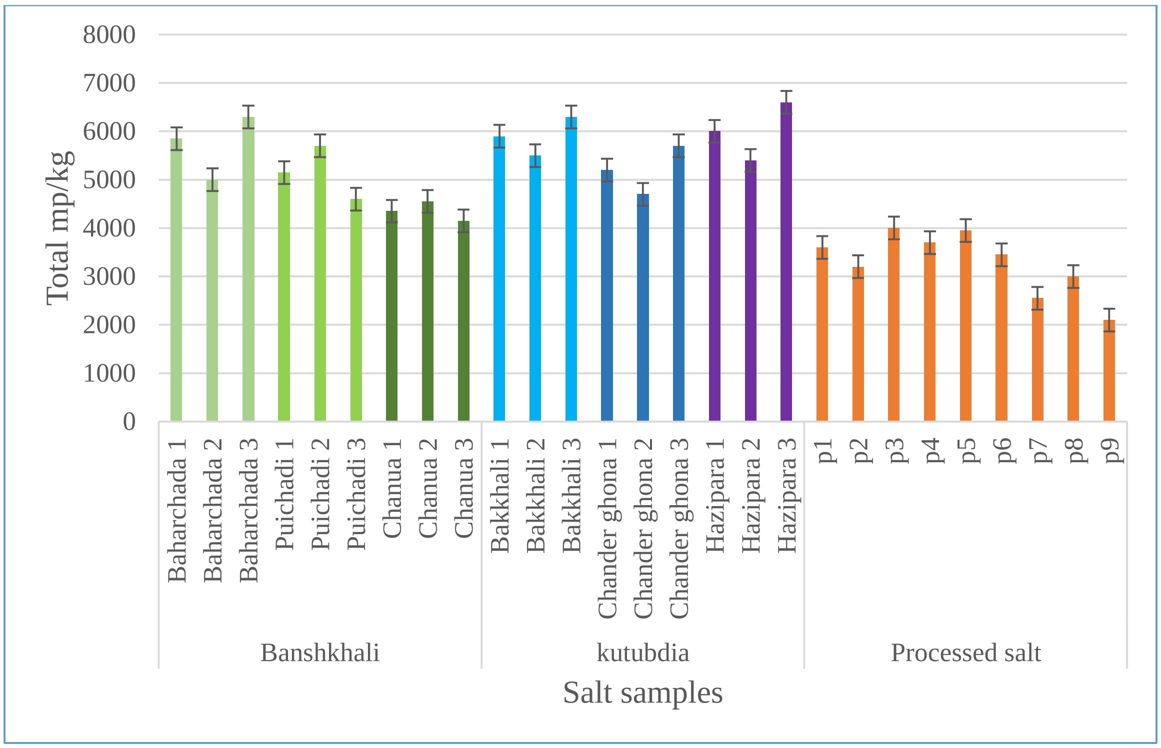

Figure 8: Amount of MPs in salt samples collected from both salt pans and commercial brands

4.1.2 Color composition of MPs:

Among all the samples investigated in the Banskhali region, purple was the most dominant color (27%), followed by red (22%), blue (20%), colorless (19%), brown (4%), yellow and green (3%) and white (2%). Similarly, purple was found to be the most significant color in the kutubdia region (30%). Other major colors found in abundance were blue and red (24%) and colorless (13%). The minor coloring MPs were brown and white (4%).Blue and purple colors were widely found in the commercial salt samples (27%), meanwhile red colored MPs were also found to be in the major quantity (24%).

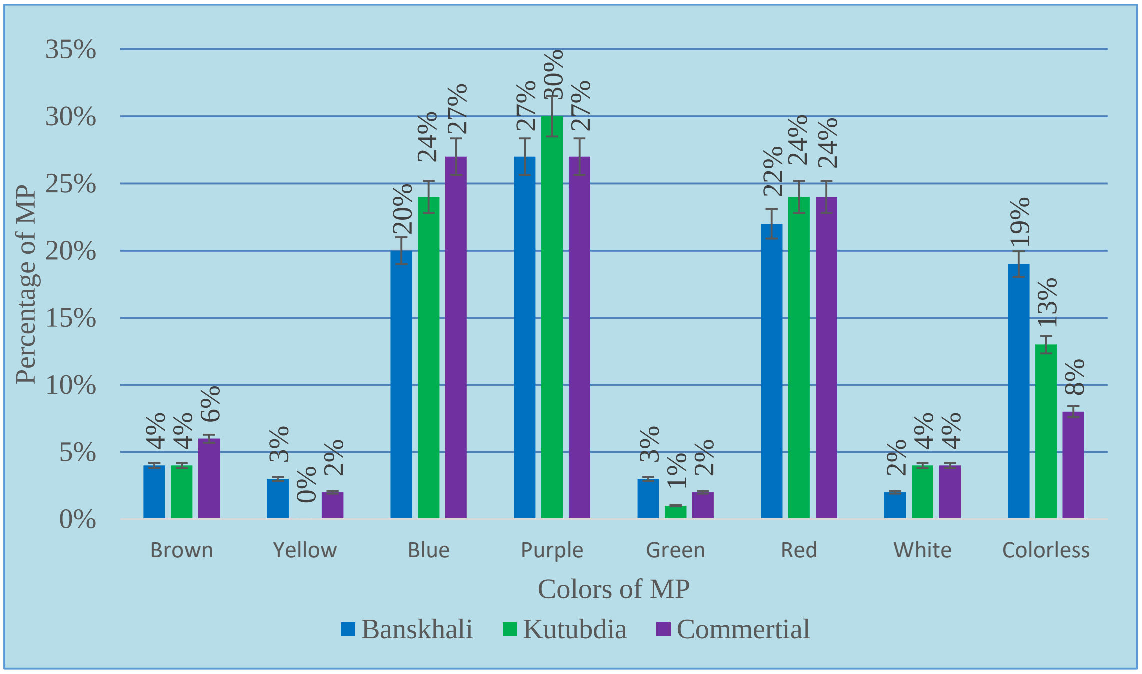

Figure 9: Color composition of MPs in salt samples collected from both salt pans and commercial brands

4.1.3 Morphotype of MPs:

Fiber was the most common type of shapes among all salt samples at Banskhali region followed by fragments and foam. In kutubdia region the most abundant shape of MPs was fiber. Other type of shapes found in salt samples were fragments and foam. Similarly among all commercial salts investigated in this study, fiber was the most common type of shapes followed by fragment and foam.

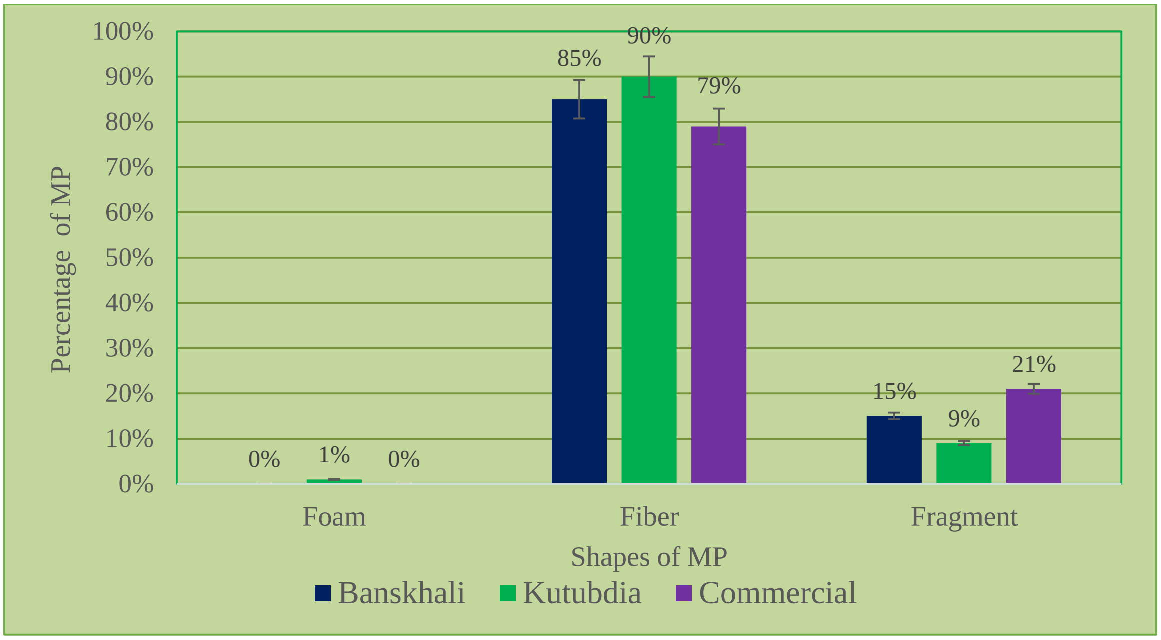

Figure 10: Shapes of MPs in salt samples found in both salt pans and commercial brands

4.2 Stereo microscopic image of microplastics:

Figure 11: Stereo microscopic image of MPs

4.3 Scanning Electron Microscopic (SEM) image:

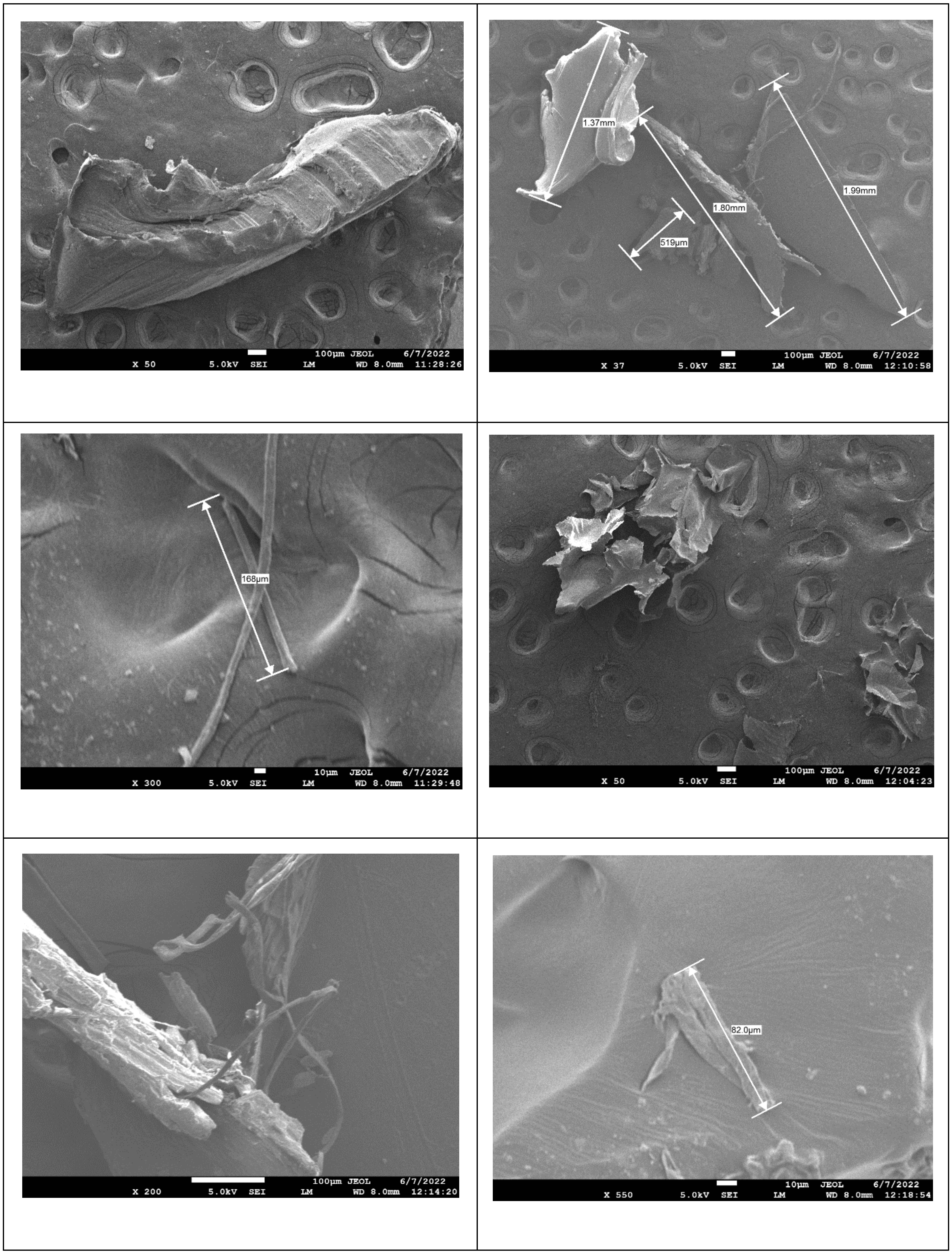

Figure 12: SEM image of different microplastics

4.4 Polymer composition of MPs:

4.4.1 FTIR result:

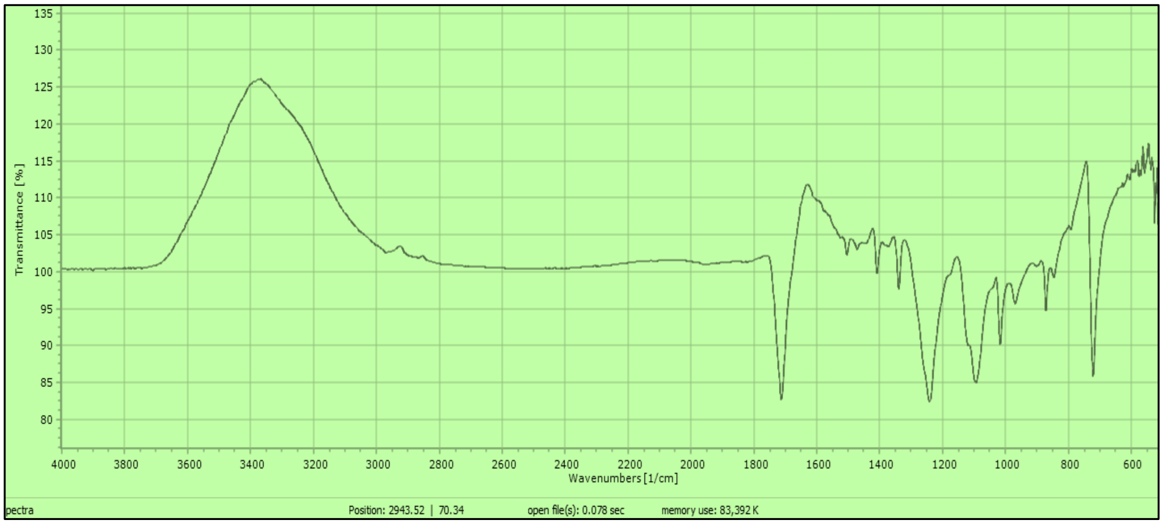

Figure 13: FTIR spectrum of sample 1

In fig 13, significant FTIR spectra of sample 1 were found at different wavenumbers including 1716 cm^{-1}, 1410cm^{-1} and 1340 cm^{-1}, 1240 cm^{-1} and 1094cm^{-1} and at 720 cm^{-1}.

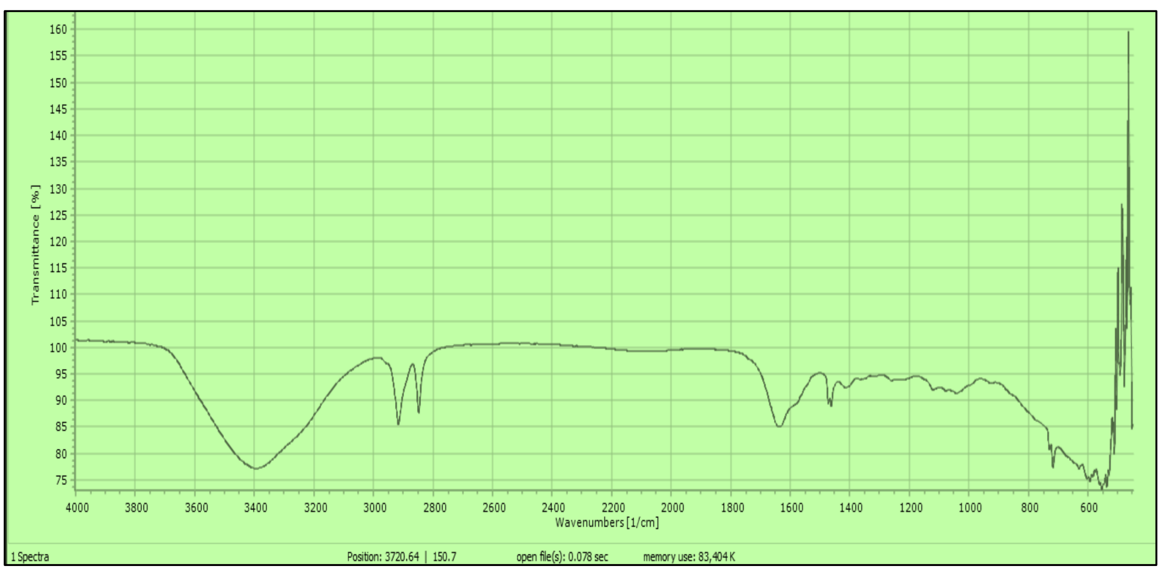

Figure 14: FTIR spectrum of sample 2

Again significant peak for sample 2 was observed at wavenumber 2922, 2852, 1663 and 720 cm^{-1}.

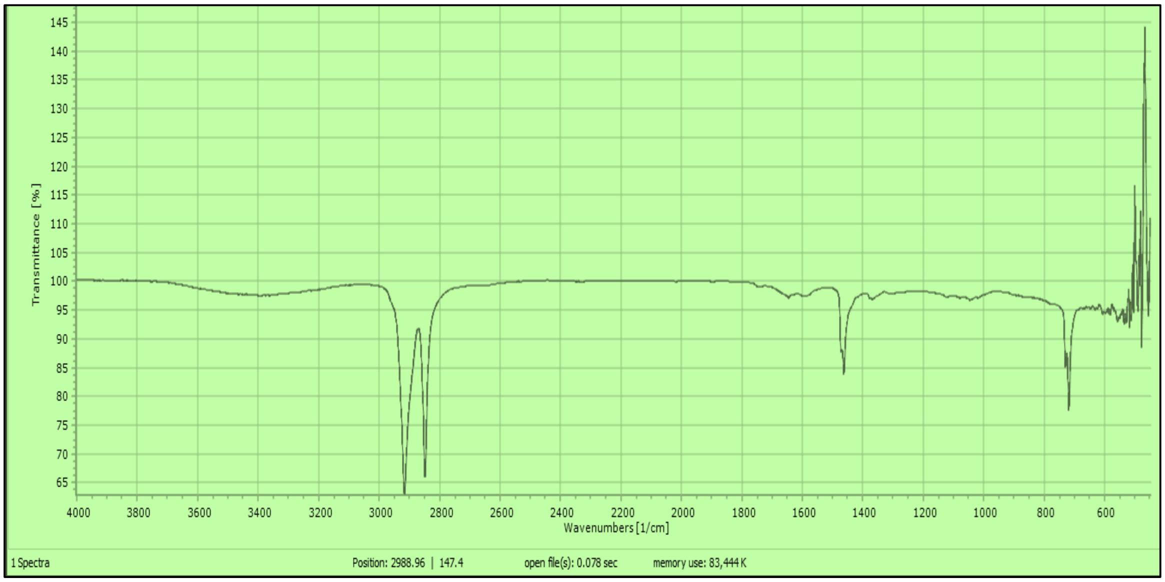

Figure 15: FTIR spectrum of sample 3

Observing the FTIR spectrum of sample 3 the distinctive peaks were found at wavenumber 2922, 2852, 1663 and 720 cm^{-1}.

4.4.2 Raman spectroscopy result:

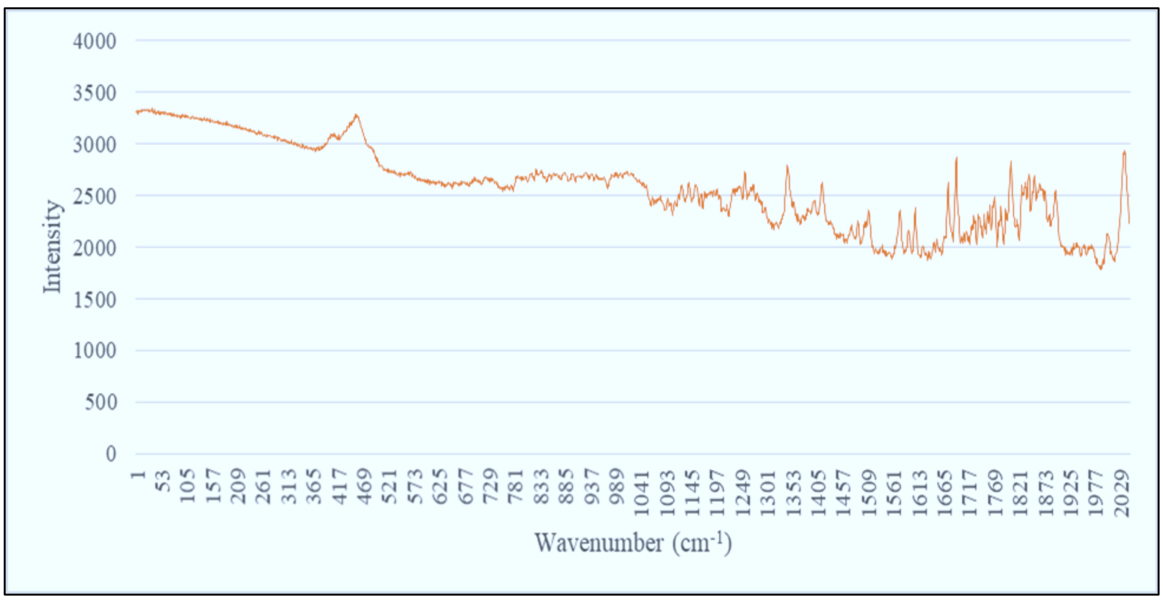

Figure 16: Raman spectrum of sample 1

The Raman spectrum analysis of sample 1 (fig 16) indicated the peaks at different wavelengths such as 399, 808, 841, 976, 1001, 1034, 1155, 1331, 1460, and 2887 cm-1.

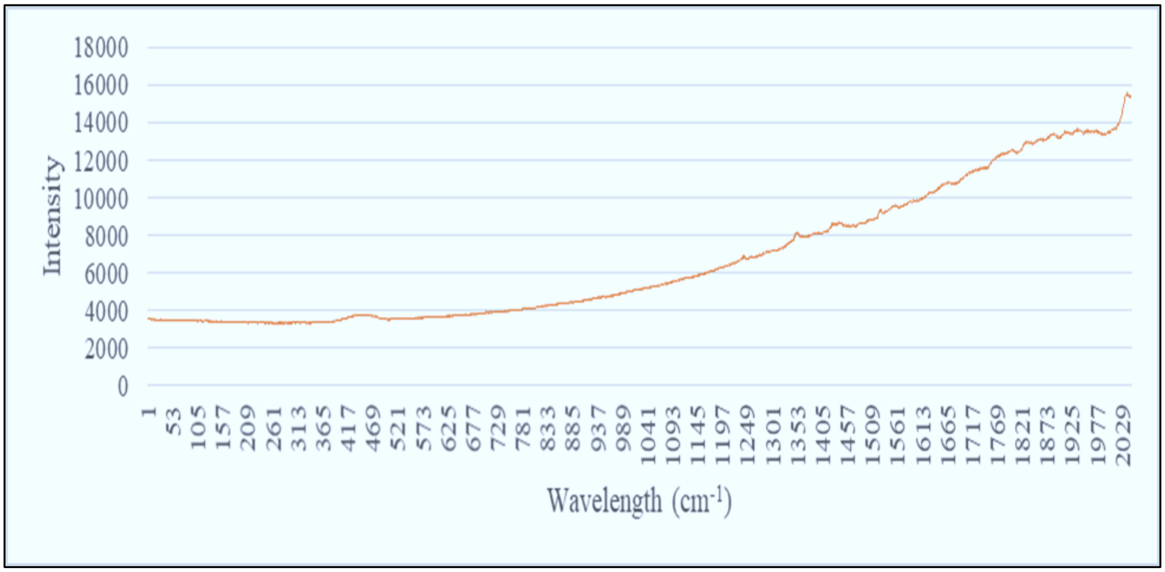

Figure 17: Raman spectrum of sample 2

Raman spectroscopy of Sample 2 (fig 17) exhibited a variety of peaks at numerous identifiable wavelengths including 1125 cm^{-1}, 1310 cm^{-1}, 1445 cm^{-1}, 1642 cm^{-1} and at 2920 cm^{-1}.

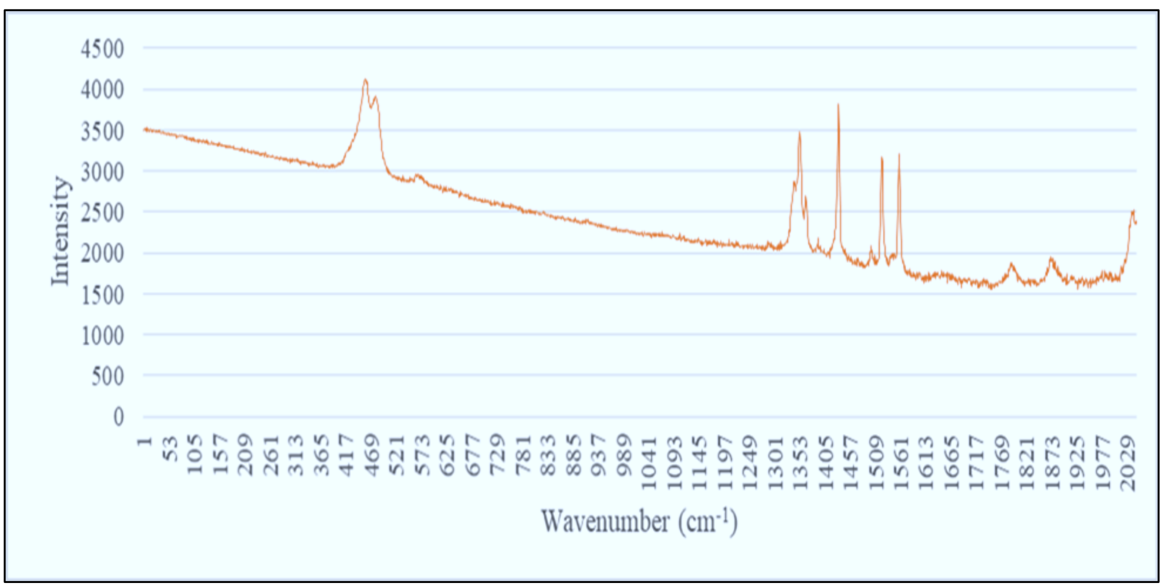

Figure 18: Raman spectrum result of sample 3

Raman spectroscopic peaks of sample 3 (fig 18) were found at 1061, 1130, 1295, 1441, 2850 and 2880 cm^{-1}.

4.3 Statistical analysis:

Table 3: Difference between the amount of MPs in commercial salt and raw sea salt.

Total MP(Area)	Contrast	Std. Err.	P> | t |	Tukey 95% CI	
2 vs 1	62.77778	30.95779	0.127	-14.53	140.09
3 vs 1	-178.8889	30.95779	0.000*	-256.20	-101.57
3 vs 2	-241.6667	30.95779	0.000*	-318.98	-164.36

*1= raw salt from Banskhali, 2= raw salt from Kutubdia and 3= commercial salts

According to statistical analysis it was observed that the amount of microplastics in raw sea pan salts and in commercial salts had a significant difference. But there was no difference in the amount of microplastics in raw sea salts from two regions.

Chapter-V: Discussion

5.1 The abundance and morphologic characteristics of MPs in salt:

5.1.1 Abundance of microplastics in salt:

Banskhali and Kutubdia salt pans had microplastic concentration of per kg sample between (4350 – 5717 MP/kg) and (5200 – 6000 MP/kg) while the abundance of MPs in commercial salt ranges from 2550 to 3700 MP/kg. Bangladeshi commercial packaged salts were found to contain microplastics ranged from 390-7400 MP/kg (average 2676 MP/kg) in a previous research (Parvin et al., 2022a). In another study, the microplastic content of Croatian sea salt was reported to range from 13500 to 19800 MPs/kg (Renzi and Blašković, 2018). Coarse salt from salt pans had higher MP concentration than commercial salt, according to this study. This may be due to manual removal of visible debris without filtration during raw salt production (Kapukotuwa et al., 2022). This high MP concentration in sea salt might be attributed to plastic pollution in beach or salt producing site. In recent years, Banskhali and Kutubdia beaches are popular tourist spots. Locals and tourists from across the country visit the beaches regularly. But there is no study on microplastics in these beaches. Though Banskhali and Kutubdia beaches have not been studied for microplastics, seashore of Cox's Bazar and Bay of Bengal are extensively contaminated with MPs due to human activity and tourism (Rahman et al., 2020; Hossain et al., 2021). Therefore, the high amount of MPs in coarse salt from Banskhali and Kutubdia salt pans might be associated with increased human activity or tourism.

5.1.1.1 Statistical analysis on the abundance of MPs in raw sea salts and commercial salts:

Pre Shapiro-Wilk test was conducted to check the normal distribution of samples and found samples were normally distributed. Then statistical investigation was carried out to ascertain the mean differences in MP content among different salt samples (n=27). Abundance of MPs between commercial salt and coarse salt from salt pans of Banskhali and kutubdia were significantly different ($p = 0.00$* and $p = 0.00$* respectively). But there was no significant difference in the abundance of MPs in salts among salt pans at Banskhali and Kutubdia ($p = 0.127$), according to tukey’s test. The seawater is not filtered to eliminate debris during the making of raw salt from it raw salt from seawater, whereas commercial salt is filtered (Kapukotuwa et al., 2022). This filtration procedure can help to minimize the high load of microplastics in commercial salt. Additionally, there are a few additional processing steps that commercial processed salt must go through, such as washing, purifying and refining. These processing stages may be the cause of lower MPs load in commercial processed salt. Though the load of microplastic in commercial salt is low compared to raw sea salt, still the load is too high to be consumed by people.

5.1.2 Color composition of MPs:

Among all the samples investigated in the Banskhali region, purple was the most dominant color (27%) followed by red (22%), blue (20%), colorless (19%), brown (4%), yellow and green (3%) and white (2%) (fig 9). Similarly, purple was found to be the most significant color in the kutubdia region (30%). Other major colors found in abundance were blue and red (24%) and colorless (13%). The minor coloring MPs were brown and white (4%) (fig 9). Again blue and purple colors were widely found in the commercial salt samples (27%), meanwhile red colored MPs were also found to be in major quantities (24%) (fig 9). In this investigation, the following was the order in which the color of MPs was observed: purple > blue > red > colorless > brown > yellow and green > white. Some previous studies also found purple, blue, red, colorless, white colored microplastic in marine environment and sea salt (Hossain et al., 2021; Parvin et al., 2022a). Bottles, plastic glasses and plastic glasses are transparent or white in color and are thrown by tourists after use, could be the source of microplastics with these colors. Most of the Bangladeshi fishing nets are made of nylon and blue in color. So, the rejected fishing nets might be a source of blue colored microplastics in salt.

5.1.3 Morphotype of MPs:

Fiber was the most common type of shapes among all salt samples at Banskhali region followed by fragment and foam (fig 10). In the kutubdia region the most abundant shape of MPs was fiber. Other types of shapes found in salt samples were fragments and foam (fig 10). Similarly among all commercial salts investigated in this study, fiber was the most common type of shapes followed by fragment and foam (fig 10). These findings are in line with those found in earlier research on microplastic in salt from all over the world (Iñiguez et al., 2017; Karami et al., 2017; Kim et al., 2018b; Sathish et al., 2020; Parvin et al., 2022a). There was relatively little foam in salt from salt pans and commercial salts in general, therefore fiber and fragments accounted for the majority of the MP.

5.2 Polymer composition of MPs:

5.2.1 FTIR results:

The FTIR spectra of sample 1 in fig 13 was identical to the FTIR spectra of PET described by other studies (Noda et al., 2007; Chércoles Asensio et al., 2009; Jung et al., 2018). In fig 13 spectra of sample 1 was found at different wavenumbers including 1716 cm^{-1}, 1410cm^{-1} and 1340 cm^{-1}, 1240 cm^{-1} and 1094cm^{-1} and at 720 cm^{-1} assigned to stretching of C=O, C-O stretching, C=O stretching and C-H aromatic plane bending which were familiar to the stretching and bending peaks of PET.

Again spectra of FTIR for sample 2 (fig 14) were found at 2920 cm^{-1} due to CH_2 asymmetric stretching, 2850 cm^{-1} due to CH_2 (C=H) stretching , 1472 cm^{-1} for CH_2 bending deformation and 720 cm^{-1} for CH_2 for rocking deformation. These spectrum in Fig 14 correspond to the FTIR spectra of HDPE described by other researchers (Noda et al., 2007; Jung et al., 2018; Parvin et al., 2021). The absorption bands of PE are well represented in the FTIR spectrum of the sample 3 illustrated in fig 15 (Jung et al., 2018). The distinctive peaks for PE were found at wavenumber 2922, 2852, 1663 and 720 cm^{-1}. These wavelengths demonstrate the existence of asymmetric and symmetric stretching in CH_2 groups at 2922 cm^{-1} and 2852 cm^{-1}; C=O amide I or stretching vibration of aromatic ring in C-C at 1663 cm^{-1} and plane bending in C-H at 720 cm^{-1}.

But in the FTIR wavenumber, some identical picks of those plastic polymers were missing while others were present. The most likely reason is that as plastic waste ages, it can get weathered due to some physical, chemical or photo degradation. The natural degradation activity might be altered by adding or deleting various functional groups such as adding hydroxyl group through a delayed hydrolysis process which might obscure distinctive picks of some secondary microplastics (Parvin et al., 2022a).

5.2.2 Raman spectroscopy result:

Particles as small as 20 microns can be detected using FTIR spectroscopy, but Raman spectroscopy can visualize very small microplastics that were previously undetected due to better resolution of up to 1 μm (Ivleva et al., 2017). FTIR spectroscopy was used to evaluate a total of 5 samples. The FTIR analysis recognized three samples as HDPE (fig 13), PET (fig 14), and PE (fig 15), but the remaining two samples were unidentifiable. All of those samples were then subjected to Raman spectroscopy to detect any samples that had been missed by FTIR and to confirm the validity of the FTIR results for HDPE, PET, and PE.

The Raman spectrum analysis of sample 1 (fig 16) indicated the peaks at different wavelengths such as 399, 808, 841, 976, 1001, 1034, 1155, 1331, 1460, and 2887 cm^{-1}. The peak that appears at a wavelength of 399 cm^{-1} is caused by the wagging of CH_2 and the bending of CH. Sharp peaks like 976, 1001, 1034, and 2887 are created by CH_3 rocking and C-C stretching at 976 cm^{-1}, CH_3 rocking, C-H and CH_2 bending at 1001 cm^{-1}, C-C stretching and CH_2 bending at 1034 cm^{-1}, and CH_3 symmetrical stretching at 2887 cm^{-1}. Other significant peaks were observed at 808 cm^{-1} due to the presence of CH_2 rocking and C-C stretching, 841 cm^{-1} for the occurrence of CH_2, CH_3 rocking, and C-C stretching, 1155 cm^{-1} for C-C stretching, CH_3 rocking, and CH bending, 1331 cm^{-1} due to CH_2 twisting and CH bending, and 1460 cm^{-1} because of CH_2 bending and CH_3 asymmetric bending. These aforementioned wavelengths in sample 1 correspond to the Raman wavelength of Polyethylene (Piyawardhana et al., 2022).

Raman spectroscopy of Sample 2 (fig 17) exhibited a variety of peaks at numerous identifiable wavelengths. Some sharp and identical Raman peak of wavelengths were found at 1125 cm^{-1}, 1310 cm^{-1}, 1445 cm^{-1}, 1642 cm^{-1} and at 2920 cm^{-1}. These mentioned wavelengths of sample 2 are representative of the Raman spectrum of Nylon 6 (Menchaca et al., 2003). The occurrence of characteristic peaks at 1125, 1310, 1445, 1642 and 2920 cm^{-1} reflects the presence of C-C stretching, CH_2 twisting, CH_2 bending, amida I and CH_2 asymmetric stretching. Raman spectroscopic peaks of sample 3 (fig 18) were found at 1061, 1130, 1295, 1441, 2850 and 2880 cm^{-1}. These peaks are caused by C-H banding, C-C stretching, CH_2 twist, CH_2 banding and CH_2 stretching respectively. The Raman spectrum of HDPE is the same as these wavelengths (Silva and Wiebeck, 2019).

In this study sea salt was discovered to have most common polymers including HDPE, PP, nylon and PET. In sea water and salt nylon is a prevalent polymer as it is a typical raw material for fishing lines and nets as well as fish crates or bags (Parvin et al., 2022a). Bottles for beverages, medicine packages, cloths and carpet fibers are all made from PET plastic. There are a variety of household products made from HDPE such as containers for oil and milk as shampoos, conditioners, soaps and detergents (Hossain et al., 2021).

Chapter-VI: Conclusion

In the modern world, microplastic contamination in marine environment has become a global issue. Growing plastic manufacturing and improper disposal have led to microplastic contamination which are the result of fragmentation and degradation of plastic material. A lack of treatment in waste water plants has allowed plastics to reach the marine environment which is damaging marine biota, fish and human health as well. Microplastic contamination in marine water is also caused by human activities such as fishing, tourism, maritime transport etc. Microplastic pollution in marine environment and water has been highlighted by the proliferation of MPs in sea products such as seafood, sea salt etc. According to the findings of this study salts from the salt pans of Banshkhali and Kutubdia region as well as commercial brands are substantially contaminated with high amounts of microplastics. Particles of all shapes, sizes and colors were identified in the salt samples especially the fibrous shaped, blue and purple colored MPs dominating the field. Nylon, HDPE, PET and PP made up the majority of the polymers detected by using FTIR and Raman spectroscopy. Microplastics have harmful effects and can transport hazardous substances into our body according to previous studies (Rios et al., 2007). The widespread use of these Bangladeshi sea salts could put a substantial proportion of population at risk for health related problems linked with microplastic consumption. An effective refining procedure should be developed and consumption of salts should be diminished to reduce MPs in body. The situation of microplastic proliferation in edible salt is poorly understood by the people of our country due to lack of available data. This study will help the people to understand the present scenario of microplastics in sea salts but more research is needed on this regard.

Chapter-VII: Recommendation

The amount of microplastics in terrestrial and marine habitants is rising at an alarming rate, and this is now a global issue. According to this study, there were precariously high amount of microplastics in raw sea salt and commercial salt. This chapter provides the recommendation and future perspectives of the present study on the basis of the prevalence of microplastics in coarse sea salt and commercial salt.

- In this particular investigation, microplastics were viewed through a stereo-microscope that had 10x magnification. A microscope with a higher magnification would be able to distinguish more microplastics of a smaller size.
- Samples were collected from two distinct regions of Bangladesh, in this study. Samples from more salt producing regions of Bangladesh would provide a more accurate scenario of microplastic contamination in edible salt.
- Despite taking precautions at every stage of the laboratory operations, there was still a possibility that the sample would be tainted or that it would be lost. Additionally, there was a possibility of making mistakes during visually identifying and classifying the different types of microplastics.
- The relationship between microplastics and tourism, as well as the association between microplastic and heavy metals were not investigated in this particular study. Additional research is needed in this regard.
- There has been no study on microplastics on the beaches of these two regions and the potential source of microplastic in salt is still unknown. More study is needed to identify the potential source of microplastics in marine environments and salt for this reasons.

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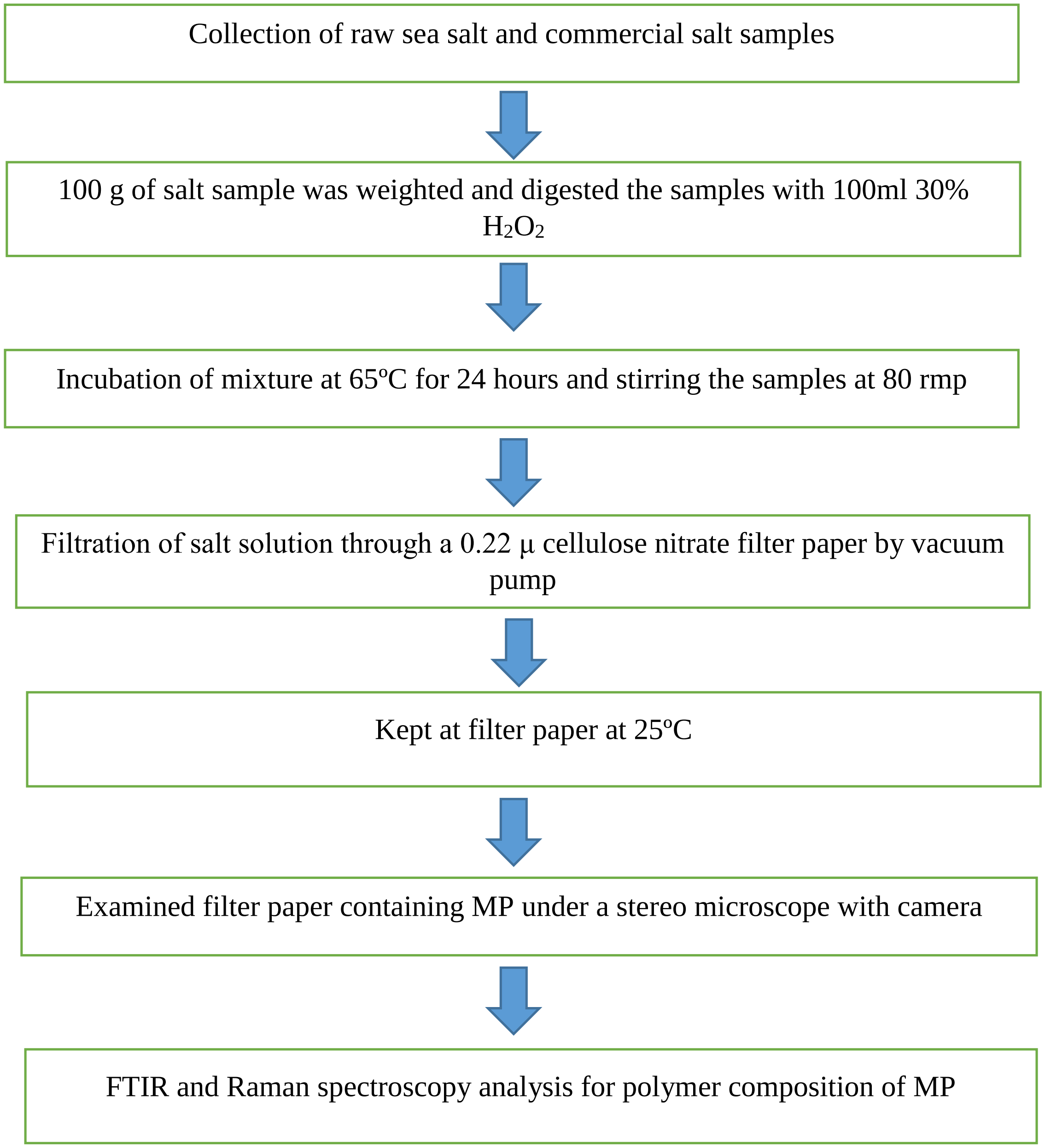
Appendix A: Overall study design of this research.



Appendix B: Salt sample collection point from Banskhali and kutubdia region in Chattogram, Bangladesh

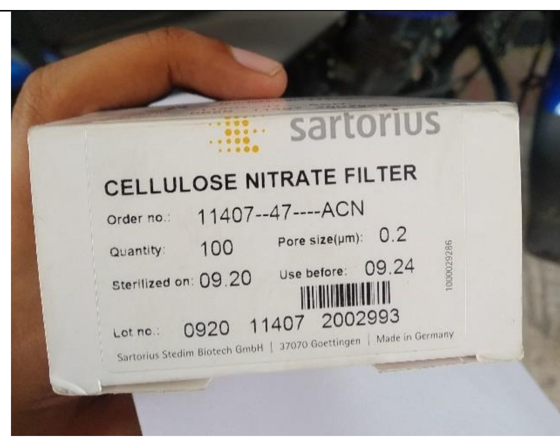
Appendix C: Salt samples digestion, filtration and identification of MP procedure

Measuring the weight of salt sample

Transfer weighted samples into conical flax

Digestion of samples with 30% 100 ml H_2O_2

Incubation of samples



Cellulose nitrate filter paper

Vacuum filtration of digested samples

Filter papers are kept in petri dish

SEM

Stereo microscopic analysis of filter paper

Brief Biography

Arpan Mitra Chowdhury has graduated with a Bachelor of Science (B.Sc. Honors) in Food Science and Technology from the faculty of Food Science and Technology (FST) at Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh with a 3.56 CGPA on a 4.00 scale. After completing graduation, he has enrolled himself as an applicant for the MS in Public Health at One Health Institute, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Previously he successfully completed an academic internship in Universiti Malaysia Terengganu (UMT), Malaysia. He also received training from Chattogram Food Testing Laboratory, Chattogram where he worked on a project association of food safety knowledge and practice with microbiological contamination in street vended fruit juice. In addition to that he also received training from a variety of well-known companies as well as from the Training Institute for Chemical Industries (TICI), Bangladesh. Through participation in these trainings and field work experiences he has gained the self-assurance and self-confidence necessary to take on challenges relating to the nutrition, public health, food safety and environmental issues. He has a deep interest in both training and scientific research. His main areas of research interest are food safety and public health, food microbiology, animal and human health and environmental problems. His skills and strengths include the investigation and identification of microbes in food, emerging pollutants (heavy metal, antibiotics and pesticides) screening and extraction, instrumental analysis of UV-Vis, AAS, GC-MS and statistical analysis (STATA). He has great interest and passion in sharing his experience and conducting research, and he would be excited to participate in furthermore opportunities.