Chapter One

Introduction

1.1 Background study

Plastics are a broad category of synthetic polymeric materials (including polypropylene, polycarbonate, nylon, polyvinyl chloride, polyethylene, and polystyrene) that can be used to make a wide range of final products (Leal et al., 2019). They are lightweight, durable, long-lasting, and inexpensive synthetic or semi-synthetic organic polymers (Van Eygen et al., 2017). As a result, single-use items like straws, food, packaging, disposable cups, cutlery plates, and cigarette filters are frequently made of plastic. As plastics are made to last, improperly dumped plastic products accumulate in nature for a long time, making it evident that plastic pollution could pose a serious threat to the environment (Andrady, 2003).

Plastics production on a large scale started in the 1950s, with a worldwide production of 2 million metric tons (Geyer et al., 2017). Plastics production in the world was 368 million metric tonnes in 2019 (Plastics Europe, 2020). Geyer et al. (2017) predict that by 2025 it will have risen to 500 million tons. Freshwater systems are a major source of plastic garbage reaching the marine ecosystem (Crawford and Quinn, 2017). Based on previous studies of plastic particle abundance in inland water environments, subsequent modeling studies have projected that rivers globally discharge around 1.2-2.4 MT of floating plastic contaminants from inland areas to the seas each year (Lebreton et al., 2017). Plastics are not only conveyed to the oceans but also settle down in the river bed, where they either deposit or are remobilized at higher flow rates (Waldschlager and Schuttrumpf, 2019). In later physical abrasion, high temperatures, and UV-B exposure are all environmental stresses that can assist plastic waste to disintegrate into a smaller form of plastic (Song et al., 2017). These smaller forms of plastic that are less than 5 mm are called microplastic (Egessa et al., 2020).

Microplastics were first reported in North American coastal waters in the 1970 (Carpenter et al., 1972). But nowadays, microplastic contamination has been a prominent topic of global concern as an emerging pollutant (Halden, 2015). It has been identified in almost all types of aquatic environments such as oceans, lakes, reservoirs, rivers, estuaries, even in the inhospitable regions of Antarctica and the Arctic Ocean (Villegas et al., 2021). Microplastics are often classified into primary and secondary microplastics based on their source (Sharma and Chatterjee, 2017). Primary microplastics are those that are designed to be microscopic in size and are commonly found in personal care products (e.g. cosmetics, facial cleanser), medicine, and air blasting media (Duis and Coors, 2016). Secondary microplastics originate from the fragmentation of larger plastic materials by environmental stressors such as ultraviolet light exposure, physical distortion, and hydraulic processes (Eriksen et al., 2014).

1.2 Significance of the study

Microplastics and the Karnaphuli River are the two most important aspects of this research. As a result, the significance of these two segments together raises the importance of the research.

1.2.1 Significance of microplastics

Microplastic contamination in the aquatic environment is an increasing environmental problem, not only because of their durability and accumulation in the environment but also of their propensity to harm aquatic biota (Wardrop et al., 2016). The United Nations Environment Program (UNEP) produced a document in 2016 addressing emergency environmental challenges, with microplastics being one of the six categories mentioned, covering the features of this pollutant as well as the issues associated with its entrance into the food chain (UNEP, 2016). Due to their smaller size range, microplastics are inadvertently ingested by organisms due to their smaller size range, which is comparable to the organism's natural food (Wright et al., 2013). The color and shape of microplastics also influence the opportunity of ingestion by organisms. Microplastics with colors and shapes that resemble biological foods like plankton are easily ingested by aquatic species (Dris et al., 2015). They are consumed by both low and high trophic animals, including vertebrates like fish, turtles, marine mammals, and sea birds, as well as invertebrates like zooplankton. Mussels have been documented with harmful health effects (Ivar do Sul Costa, 2014). Swallowed microplastics can cause physical problems such as gut obstruction, reduced capacity to evade predators, altered feeding behavior, and low energy levels, all of which have an impact on the exposed individuals' survival, growth, and reproduction (Wright et al., 2013). Many studies have shown that microplastics bioaccumulate and are transferred through the food chain, eventually reaching humans, posing a major health risk (Wagner et al., 2014). In addition, microplastics not only affect individual organisms but also modify an organism's habitat and have an impact on the population (Wright et al., 2013). According to the findings of Carson et al. (2011), the presence of more microplastics in the sediment enhanced its permeability and changed its maximum temperature. Moreover, microplastics may absorb other environmental contaminants such as polychlorobiphenyls (PCBs), heavy metals like dichlorodiphenyltrichloroethane (DDT), and polycyclic aromatic hydrocarbons(PAHs) and act as vectors for accumulating and transferring toxins into organisms (Teuten et al., 2009; Ziccardi et al., 2016). Considering all of these important effects of microplastics, it is imperative to do research on the identification and quantification of microplastics in aquatic environments, which will aid in the development of policies to limit plastic contamination.

1.2.2 Significance of Karnaphuli River

The Karnaphuli River, which originates in the Lushai Hills of India's Mizoram state, is the largest and most significant river in Chattogram and the Chattogram Hill Tracts. This river is significant for a variety of reasons, including navigation, fishing, docking yards, transportation, and the industrial use of river water (Siddique and Akter, 2012). The river is linked with many tributaries, minor rivers, and canals, all of which have had a significant impact on the Karnaphuli river's hydrobiology, contributing vast amounts of polluted water, solid waste, sewage, and other contaminants (Hossain et al., 2005). Almost 800 industries are located adjacent to the banks of the Karnaphuli in different areas, including tannery factories, fish processing plants, textile mills, oil refineries, chemical industries, Triple Super Phosphate (TSP) plant, Karnaphuli Paper Mills (KPM), paint and dye manufacturing units, Chattogram Urea Fertilizer Ltd (CUFL), and the Karnaphuli Fertilizer Company Ltd (KAFCO) (ADB, 2004; Hossain et al., 2005). All of these industries are polluting the Karnaphuli river in an indiscriminate manner that is exceeding the safer limit, and these pollutants contribute to the process of microplastics formation.

Microplastics have been examined for over 45 years, particularly in the marine environment (Carpenter et al., 1972; Bergmann et al., 2015), but only a few studies have been conducted in the freshwater environments (Blettler et al., 2018). Research regarding microplastics in Bangladesh is in a very early stage. Moreover, microplastic existence in freshwater environments is an unexplored issue in the microplastic research field of Bangladesh. So, there is an urgent need to develop a deep understanding of the identification and quantification of microplastics in the freshwater environment, which will aid in the development of guidelines for minimizing plastic pollution. In this thesis, it has been attempted to determine the amount of microplastics at Avaymitro Ghat in the surface water of the Karnaphuli river, Chattogram.

1.3 Objectives of the Study

- To determine the present status of microplastics abundance in the surface water of Karnaphuli river.
- To identify the microplastic particles according to their type, shape, size and color.
- To assess the seasonal variation of microplastics abundance in Karnaphuli river.

Chapter Two

Review of Literature

2.1 Microplastics in marine environments

The oceans are widely regarded as the ultimate microplastic sinks. The majority of marine microplastics come from land-based sources, such as beach litter degradation; freshwater sources, such as rainwater runoff, wastewater discharge; and airborne microplastics deposition (Zhang et al., 2017). Microplastics are conveyed and disseminated throughout the world's oceans, where they accumulate at remote locations such as seabed sediments, beaches, shorelines, and surface waters from the Arctic to the Antarctic (IMO, 2015). The density of the particles, the location of the sources, and the transportation with ocean currents and waves all have an impact on their distribution throughout the marine environment (Magnusson et al., 2016).

Isobe et al. (2015) studied microplastic concentrations in the East Asian Seas near Japan, and found 1.72 million pieces per square kilometer. Another study discovered microplastic concentrations ranging from 15,000 to 2,000,000 particles per km^2 in the surface water of San Francisco Bay (Sutton et al., 2016). In August 2016, microplastic concentrations were assessed from 11 sites in the Bohai Sea using a 330 mm trawling net, and the average microplastic items was 0.33 0.34 particles/m^3 (Zhang et al., 2017).

Microplastic type identification has been a hot topic in the research community in order to accurately determine the possible sources of plastic particles (Lo et al., 2018). A recent investigation of the available work by Shim et al. (2018) found that secondary microplastics are more plentiful in marine environments than primary microplastics, as the maximum research expeditions report fibers and fragments as the most common types of microplastics, which are formed by the fragmentation of larger plastic particles. Men'endez-Pedriza and Jaumot (2020) also found that only 21% of the total microplastics produced in marine systems each year are primary microplastics, with the remaining 79% being secondary forms.

2.2 Microplastics in freshwater environments

Research on microplastic pollution in freshwater environments is still in its early stages when compared to marine environments, but studies are rising, particularly since 2014 (Wagner et al., 2014). Studies on microplastics in freshwater environments are growing rapidly due to the large amounts of plastic particles recorded from lakes and rivers and the possibility of harmful impacts in these environments. The majority of early freshwater studies appear to have focused on lentic systems such as the Laurentian Great Lakes (Eriksen et al., 2013), but rivers are increasingly receiving more attention as possible microplastics pathways to the environment.

Moore et al. (2011) conducted an early study of microplastic pollution in riverine systems in the Los Angeles and San Gabriel Rivers, and the average microplastic concentration was 13.7 particles per liter. Mani et al. (2015) conducted a study in the Rhine River's surface water using a manta net of 300 µm mesh and found an average microplastic concentration of 892,777 particles per km^2. Secondary source microplastics were identified at higher concentrations, with fragments and fibers distributed unevenly across all sampling sites. These variances are caused by local sources and hydrological circumstances.

Zhang et al. (2015) conducted another study in the surface water of the Yangtze River's main stream and the estuarine areas of four tributaries. Microplastics abundance were found in the Yangtze River's main stream, ranging from 3407.7×10^3 to $13{,}617.5 \times 10^3$ particles per km^2, and in the estuary sections of four tributaries, ranging from 192.5×10^3 to $11{,}889.7 \times 10^3$ particles per km^2. Microplastics may be elevated in the Yangtze River's main stream as a result of anthropogenic activities at the port.

Zhang et al. (2017) investigated microplastics abundances in surface water of the Xiangxi River by using a trawl net of 112 µm and abundances varied from 0.55×10^5 to 342×10^5 items per km^2. Based on the morphological characteristics, the types of microplastics found are fragment, foam, lines, and sheet, with fragment and sheet being the most abundant.

2.3 Microplastics in river environments: sources and factors affecting transportation

The pollution of riverine systems with various forms of microplastic particles is the result of a complicated chain of events. Point and non-point sources such as surface runoff, sewage effluents, degradation from dumpling sites, and household activities all contribute to plastics entering the riverine ecosystem (Horton and Dixon, 2018).

Generally, microplastics' density, composition, and forms govern whether they float, or sink in aquatic habitats (Anderson et al., 2016). Seasons and climatic circumstances, such as heavy rains or flood-related phenomena, have an immense impact on the transport of microplastics to riverine systems (Scircle et al., 2020). While hydrodynamic variables such as flow velocity, turbulence, and depth influence the distribution of microplastics in the water column of rivers (Wong et al., 2020). Moreover, increased flow velocities caused by phenomena such as tides, wind-driven currents, or pressure can cause microplastics to upwell from the sediment to surface water (Wang et al., 2017).

Several studies on microplastic transmission in riverine ecosystems, including the Seine River in Paris (Dris et al., 2018), the Ganga (Singh et al., 2022), and Swiss rivers (Faure et al., 2015), have been published. Lechner et al. (2014) conducted research on plastic quantities in the Danube River, Austria for two years. Over both years of observation, the average plastic load of the Danube river was 316.84664.6 items per 1000 m^3, with 79.4% coming from industrial sources and 20.6% coming from other sources.

2.4 Temporal change of microplastics in river environments

The abundance of microplastics varies on a temporal basis, which can be related to both hydroclimatic and hydrodynamic conditions. Seasonal conditions, particularly precipitation, have an impact on microplastic concentrations (Xia et al., 2020). Precipitation has been reported to transfer land-based microplastics into aquatic ecosystems, and large abundances of microplastics have been observed in surface waters after such rain actions (Wong et al., 2020; Xia et al., 2020). The majority of a river's yearly surface water microplastic load may be directly connected to the rainy season, which is likely as a result of increased runoff delivering microplastics items to waters, in addition to resuspension of microplastics content from sediments (Hurley et al., 2018; Xia et al., 2020). Thus, significant changes in microplastic loads between the wet and dry seasons are not unusual, with evidence that the rainy season has higher abundances of microplastics content in surface waters (Eo et al., 2019; Campanale et al., 2020).

Several studies, such as Eo et al. (2019), Campanale et al. (2020), Chen et al. (2020), He et al. (2020), have reported significant positive relations between microplastics abundance and seasonality. Campanale et al. (2020) conducted research in Southeast Italy's Ofanto River. Five sampling campaigns were carried out for this study, with microplastic concentrations ranging from 0.9 ± 0.4 particles/m^3 to 13 ± 5 particles/m^3

They discovered increased concentrations of microplastics during wet seasons, demonstrating a land-based source, most likely from garbage produced by nearby agricultural areas.

Research on the spatiotemporal distribution of microplastics in the Nakdong River was investigated by Eo et al. (2019) in South Korea. The mean (± standard deviation) microplastic abundance in the Nakdong River ranged from 293 ± 83 particles/m^3 in water (upstream, February 2017) to 4760 ± 5242 particles/m^3 in water (downstream, August 2017). The temporal distribution revealed that the number of microplastics was quite high in August, which corresponded to the rainy season.

Microplastics are affected by water velocity in the sense that lower flow rates and weaker hydrodynamics may favor their accumulation (De Carvalho et al., 2021). As a result, lower quantities of microplastics have been detected in the center of river channels, with a greater number of microplastics identified along river margins (Dris et al., 2018). Several studies, including Campanale et al. (2020), Mani and Burkhardt-Holm, (2020), and Wagner et al. (2019), have found a positive relationship between microplastics abundance and river flow velocity.

2.5 Rivers as a microplastics transfer pathway

Rivers serve as a major route for plastic waste to travel into the ocean. Microplastics enter marine environments primarily through two routes: benthic transport, which transports high-density microplastics in the river's lower reaches; and pelagic transport, which transports low-density microplastics through estuaries and eventually to the seas (Eerkes-Medrano et al., 2015). The bulk transfer of microplastic content to the oceans is heavily influenced by population density, sewerage systems, riverine hydrology such as water flow velocity, seasonal variation of water flow, water depth, bottom topography, solid waste management methods, and wastewater treatment plant technologies (Van Wijnen et al., 2019). According to Lebreton et al. (2017), between 1.15 and 2.41 million tons of plastic trash reaches the ocean each year through rivers, with the top 20 polluted rivers in Asia accounting for 67% of all plastic emissions. Besides this, other research on microplastics transmission from rivers to seas has been conducted by Moore et al. (2011), Lechner et al. (2014), Schmidt et al. (2017), Siegfried et al. (2017), and Atwood et al. (2019).

Moore et al. (2011) investigated the transmission of plastic waste from two urban rivers, Los Angeles and San Gabriel, to marine systems and discovered 30 metric tons of plastic particles released into the marine environment over just a three-day period.

Lechner et al. (2014) also investigated the transfer of plastic particles from river to sea in Europe’s second largest river, the Danube. They revealed that some plastic manufacturing sites and an undetermined number of processing plants are located near the Danube and are responsible for the Danube's massive plastic production, which contributes 4.2 tonnes of plastic particles into the Black sea.

Similarly, Atwood et al. (2019) demonstrated that 785 to 1402 tonnes of microplastic are estimated to be transferred from the River Delta in Italy to the Adriatic Sea.

2.6 Impacts of microplastics ingestion and associated ecological concern

Microplastics are becoming a widespread contaminant in oceans, seas, estuaries, shorelines, lakes, rivers, marine and freshwater sediments, deltas, soil, air, wastewater treatment facilities, aquatic and terrestrial individuals, food, and beverage items. The constant entry of microplastics into these aquatic and terrestrial habitats causes plenty of difficulties for these environments and their inhabitants, particularly the organisms that live there.

Several studies, such as Peters and Bratton (2016), Zhang et al. (2017), Horton et al. (2018), Hossain et al. (2020), and Parvin et al. (2021), have reported that organisms can uptake particular quantities of microplastics in their body tissues by ingestion or filter-feeding mechanisms via their gills. According to these studies, a large amount of fibers has been reported to be ingested when compared to other microplastics. An extensive study has been done to measure the impact of microplastics on aquatic species, and the findings are frightening, with every trophic level in the food web exposed to health concerns and associated mortalities (De Sá et al., 2018).

The impacts of microplastic ingestion on organisms include digestive disorders, reproductive abnormalities, bioaccumulation, abnormal swimming, lethargy, pathological stress, developmental inhibition, oxidative stress and other health effects (Ferreira et al., 2018). For example, Zheng et al. (2019) extracted microplastics from the gastrointestinal tracts of fish in the Pearl River, China, for the riverine environment. Fibers and filaments can clog a fish's gastrointestinal tract, making it difficult for them to eat (Derraik, 2002; Wright et al., 2013).

Microplastics can also serve as an intermediate to concentrate and transmit organic pollutants. Commonly found chemicals in the freshwater environment are polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), polycylic aromatic hydrocarbons (PAHs) and dichlorodiphenyltrichloroethane (DDT) (Anderson et al., 2016). These organic contaminants sorbed by the microplastics may be released when consumed by individuals where digestive juice is present (Teuten et al., 2009). Rochman et al. (2013) discovered that 2 months of continuous exposure to a meal mixed with chemical pollutants absorbed from the sea environment resulted in persistent bioaccumulative and hazardous chemicals bioaccumulating in medaka, causing hepatic stress in the fish. Moreover, plastics contain a variety of hazardous components such as plasticizers, fillers, cross-linking compounds, antioxidants, pigments, stabilizers, surfactants, all of which have been shown to degrade the quality and lifespan of the receiving ecosystem (Lambert and Wagner, 2018).

Besides these, microplastics also have some ecological impacts, such as Green et al. (2015) have shown that rejected plastic bags create anoxic situations within the sediment, decrease primary productivity and organic substances, and reduce the richness of in faunal invertebrates.

Through the ingestion of freshwater species, humans are also in danger of being exposed to microplastics and related pollutants. The presence of microplastics in a fish's digestive tract does not suggest direct human contact because the tract is not consumed commonly However, there is still the possibility of organic pollutants and toxins leaking and accumulating in the fish's edible tissues (Wright and Kelly, 2017). Unlike fish, humans consume shellfish whole (excluding hard shell) without removing the digestive tracts, exposing them to microplastics directly (Wong et al., 2020).

2.7 Studies associated with microplastics pollution in Bangladesh

Bangladesh is one of the fastest-growing developing countries, with more people using plastic than ever before, posing a serious danger to the environment and biodiversity. According to statistics, plastic garbage has climbed from 1.74 percent in 1992 to 6.5 percent in 2014 in Dhaka's overall landfills (Bangladesh waste database, 2014). All of this plastic debris, as well as the uncontrolled usage of microbeads in skin care products, is responsible for the presence of so-called microbeads or microplastics in Bangladesh's aquatic and terrestrial habitats.

The Environment and Social Development Organization of Bangladesh, a pioneering organization, conducted a primary investigation into the level of microplastic pollution in Bangladesh's three major urban centers (Dhaka, Chattogram, and Sylhet). According to the survey, this is the first study about microplastic pollution in Bangladesh and they found that sixty of the most common and widely used personal care products, including face wash, tooth paste, body wash, detergent, body scrub, and so on, contain microbeads (ESDO, 2016).

Hossain et al. (2019) studied three marine fish from the Bay of Bengal and found 443 microplastics in their intestines, with fiber type microplastic dominating. Another study was done by Hossain et al. (2020) on microplastics abundance in the gastrointestinal tracts of brown shrimp (*Metapenaeus monocerous*) and tiger shrimp (*Penaeus monodon*) and they documented an average abundance of 3.40–3.87 items/g GI tract of shrimp. In this investigation, black fibers and fragments were the dominating types. According to the study, the microplastics may be transmitted to humans if the shrimp are eaten without having the intestines removed. On the other hand, Parvin et al. (2021) investigated microplastics in freshwater fishes and found 73.3% of freshwater fishes in Bangladesh contain microplastics in their gastrointestinal tract.

Microplastics were also identified from the salt pan of Bangladesh’s Maheshkhali Channel by Rakib et al. (2021), with average concentrations ranging from 78 ± 9.33 to 137 ± 21.70 particles per kg in the sediment of the salt pan.

Hossain et al. (2021) first investigated the abundance of microplastics in the intertidal soil of the world’s longest beach, Cox’s Bazar, with a mean abundance of 368.68 ± 10.65 plastics per kg, with fiber being the most abundant type of microplastic. Another investigation into the abundance of microplastics was done by Tajwar et al., (2022) in sediment samples from the Cox's Bazar coastal region and discovered that fibrous microplastics made up 70% of total microplastics.

Chapter Three

Materials and Methods

3.1 Study Area

This study was conducted in the surface water of the Karnaphuli River near Avaymitro Ghat, Chattogram. The Karnaphuli River which originates in the Lushai Hills in Mizoram, is one of the most important rivers in Chattogram and the hill tracts. It traverses over 180 kilometers of hilly wilderness before forming a tiny circle at Rangamati and then zigzagging its way to the Dhuliachhari and Kaptai loops. It follows a zigzag path through the area, eventually falling into the Bay of Bengal around 16 kilometers southwest of Chattogram town after a 170 km journey. Karnaphuli is slowly dying from uncontrolled dumping of domestic and industrial trash, unmanaged pollution, and unabated encroachment. Rajakhali Khal, Chaktai Khal, Kalurghat bridge, Avaymitro Ghat, and others are some of the Karnaphuli river's polluted areas. Avaymitro Ghat was chosen from among these sites for this study's investigation. This is a highly industrial area where fertilizer, oil refineries, shipbreaking, and fish processing industries are located, all of which contribute to the pollution of the Karnaphuli river. The GPS coordinates (Table 1) was recorded in the study area. Map of the study area is given in the figure 1.

Table 1. GPS coordinates

Point name	Latitude	Longitude
1a	22° 19' 33'' N	91° 50' 14'' E
1b	22° 19' 32'' N	91° 50' 31'' E
2a	22° 19' 20'' N	91° 50' 15'' E
2b	22° 19' 24'' N	91° 50' 30'' E
3a	22° 19' 11'' N	91° 50' 13'' E
3b	22° 19' 13'' N	91° 50' 32'' E

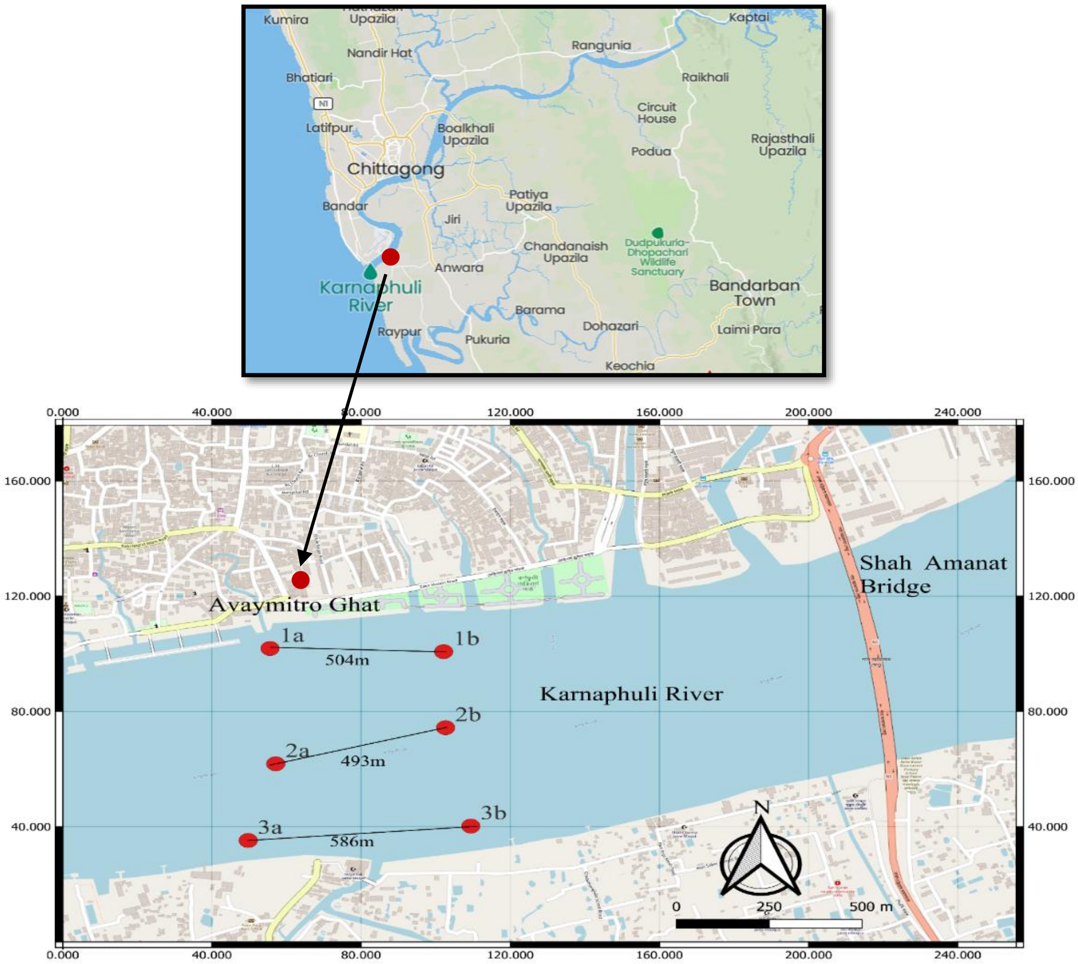

Figure 1. Map of Chattogram region and study site

3.2 Sampling Technique

From July 2021 to February 2022, sampling was conducted on a monthly basis to collect floating microplastics from the surface water of the Karnaphuli Rver near Avaymitro Ghat, Chattogram. During the sampling period, the rainy season was in July, August, September, and October, whereas the dry season was in November, December, January, and February. Three transects covering an area of about 500 m of the sampling site were selected for sample collection. Sampling area was measured by using GPS Speedometer-Odometer app. Two transects were chosen from the river's two sides, while a third was chosen from the river's middle. Each transect yielded two samples, bringing the total number of samples collected to six. Samples were collected using a manta net with a rectangular opening 20 cm high by 30 cm wide, and an 80 cm long, 200 µm net with a 20×10 cm^2 collecting pot. The net was towed from the side of the boat and floated on the surface water. Finally, the net was rinsed and flushed onsite with distilled water, ensuring that all samples reached the sample collector at the bottom of the net. Larger plastics and other waste were removed from the net. The samples in the collector were transferred to a 500 ml jar and immediately closed with the lid to avoid airborne plastic contamination.

3.3 Laboratory Analysis

The National Oceanic and Atmospheric Administration's (NOAA) laboratory method was followed in this experiment with some modifications. Laboratory work was conducted immediately after completing the sampling. Laboratory analysis includes the following steps:

- Wet sieving and drying
- Wet per oxidation
- Density separation
- Filtration
- Identification

3.3.1 Wet sieving and drying

- Stacks of 5.6 mm and 0.3 mm stainless steel mesh sieves were used to pour the samples through. All the leftover solids in the samples were transferred to the sieves using a spray bottle filled with distilled water.
- The items retained on the 5 mm sieve were discarded, while those retained on the 0.3 mm sieve were collected in a 500 ml weighted beaker.
- All beakers were placed in a hot air oven (Binder GmbH: ED 115) at 90 °C for 24 hours, or longer if sample dryness was required.

3.3.2 Wet per oxidation

Wet peroxide oxidation process was conducted to remove the organic material mixed in the sample. Wet per oxidation was done in the following ways:

- 20 ml of aqueous 0.05 M Fe(II) solution (Table 2) was added to the beaker containing the 0.3 mm size of collected materials.
- Then 20 ml of 30% hydrogen peroxide (H_2O_2) was added.
- The mixture was allowed to sit on the lab bench for five minutes at room temperature before moving on to the next stage.

- A magnetic stir bar was added to the beaker and covered with foil paper.
- On a hotplate (HSD 180), heat was applied at 75°C. When gas bubbles appear on the surface, the beaker was taken off the hotplate and placed in the fume hood until the boiling stops.
- The beaker was heated to 75°C for an additional 30 minutes.
- If any natural organic material remained, another 20 ml of 30% H_2O_2 was added to digest it, and the process was repeated until no natural organic material remained.
- To enhance the density of the aqueous solution (5 M NaCl), 6 g of salt (NaCl) per 20 ml of sample was added.
- The mixture was heated to 75 °C once more, until the salt had dissolved.

3.3.3 Density separation

The process of density separation was followed by Coppock et al. (2017) with some modifications. Density separation was conducted in the following ways:

- A density separator, which is made of a 1-foot-long PVC pipe with a 5 cm diameter, was used for this purpose.
- A ball bulb was used in the middle of the pipe to separate the low-density solution from the high-density solution.
- Zinc chloride ($ZnCl_2$) solution (table 2) was used as flotation media.
- 150 ml of filtered $ZnCl_2$ solution was added to each sample and then poured into the density separator.
- The unit was allowed to settle until all debris in the supernatant had been removed.
- A layer of microplastics floated upwards, while undissolved organic residues and inorganic materials settled at the bottom of the density separator, allowing less dense particles to be separated.
- The valve was then carefully closed, and the headspace supernatant was collected in beakers.
- To remove any leftover particles, the headspace was thoroughly cleaned with distilled water.
- Left-over materials on the bottom of the density separator were removed.

- Before and after performing each sample, all components of the density separator were cleaned with distilled water.

Table 2. Solution preparation

Solution name	Procedure
Iron (II) solution (0.05 M)	7.5 g of $FeSO_4.7H_2O$ was added to 500 ml of water, and then 3 ml of concentrated sulfuric acid was added to prepare this solution.
Zinc chloride ($ZnCl_2$) solution	500 g of $ZnCl_2$ was mixed with 1 L of distilled water to make the 1.5 g/cm^3 density solution.

3.3.4 Filtration

The supernatant collected from the density separator was filtered through a Vacuum pump filter machine (Rocker 300) with a cellulose nitrite filter paper with a pore size of 0.45 µm and a 47 mm diameter. The filter paper was then placed in a clean petri dish and examined under a microscope after it had been filtered.

3.3.5 Microplastics type, shape, color, identification

- Microplastics with a length of more than 2 mm were identified and quantified visually.
- Microplastics with a length of less than 2 mm were identified and quantified with an electron microscope (OPTIKA, B-192, Italy) at 40× magnification according to Masura et al. (2015).
- The images of microplastics were taken using a digital camera (OPTIKA-CB3) attached to the microscope.
- To separate microplastic particles from naturally occurring particles, microplastic criteria from Hidalgo-Ruz et al. (2012) was followed.
- Morphological characteristics such as color, shape, and detailed criteria described in a previous study Viršek et al. (2016) was followed for the identification of microplastics.

- According to Viršek et al. (2016) microplastics are divided into six types: fragments (rigid, thick, sharp curved edges and an uneven shape with variety of colors), filament (long or short, in various thicknesses and colors), film (thin, flexible and mostly transparent), foam (soft, irregular shape and the color ranges from white to yellow), pellets (irregular, round forms, and generally larger in size) and granules (compared to pellets, it has a regular round form, is often smaller in size, and comes in natural colors). The shapes and colors of the microplastics were also recorded.

3.3.6 Microplastics size measurement

The longest side of the microplastic particles was called the length of the microplastic (Isobe et al., 2014). Microplastics with a length of more than 2 mm were measured directly using a measuring scale, while the rest (smaller than 2 mm) were measured with the help of Proview digital camera software under an electron microscope by calibrating the stage micrometer scale.

3.4 Determination of microplastic abundance

Microplastic abundance in a sample was calculated by following the protocol of Viršek et al. (2016).

Here, net width = 30 cm = 0.3 m

Sampling length = 500 m

So, total area = (0.3 x 500) m^2 = 150 m^2 = 0.00015 km^2

Microplastic abundance in per km^2 = Number of microplastic counted / Total area

3.5 Statistical analysis

Microsoft Excel was used to analyze percentage data on the type, shape, size, and color of microplastics. Microplastic abundance in different months and microplastic type variation throughout the sampling seasons were analyzed by one-way ANOVA. A Tukey multiple comparison test was performed to draw a significant difference at a 95% confidence interval. Together with this, microplastic type variation and total microplastic abundance variation in the rainy and dry seasons were analyzed by performing an independent sample-t-test. Analyzed values are means ± SE. Statistical analyses were performed by using IBM SPSS statistics 26 software.

Chapter Four

Results

4.1 Abundance of Microplastics

4.1.1 Monthly variations of total microplastics abundance

Microplastics were identified in all months, and one-way ANOVA analysis revealed a significant difference ($P<0.05$) in microplastic abundance across all months. This study showed that the highest amount of microplastics abundance was found in the month of July with a mean abundance of 140370 ± 19586 particles per km^2 and the lowest amount of microplastics was found in the month of January with a mean abundance of 54815 ± 9220 particles per km^2 (Figure 2).

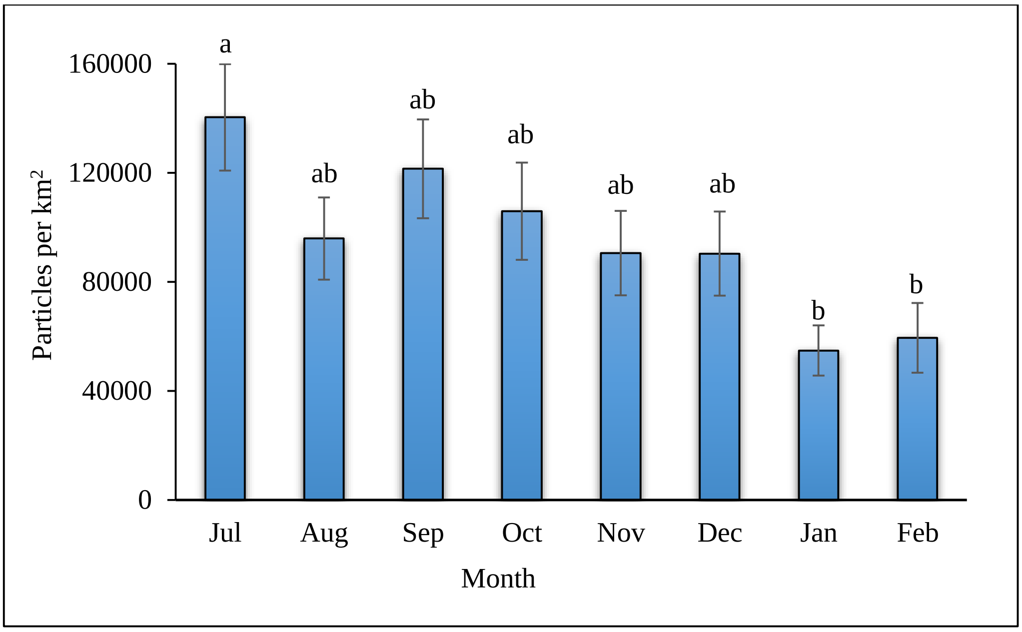

Figure 2. Monthly variations of total microplastics abundance

4.1.2 Seasonal variation of total microplastics abundance

An independent-sample-t test was performed to assess the significance difference in microplastic abundance between the rainy and dry seasons, and there was a significant difference found between these two seasons. The data illustration in Figure 3 showed that microplastic abundance was significantly higher in the rainy season (114639 ± 8845 particles per km^2) than in the dry season (73796 ± 6817 particles per km^2); $t(286)= 3.764$, $P<0.001$.

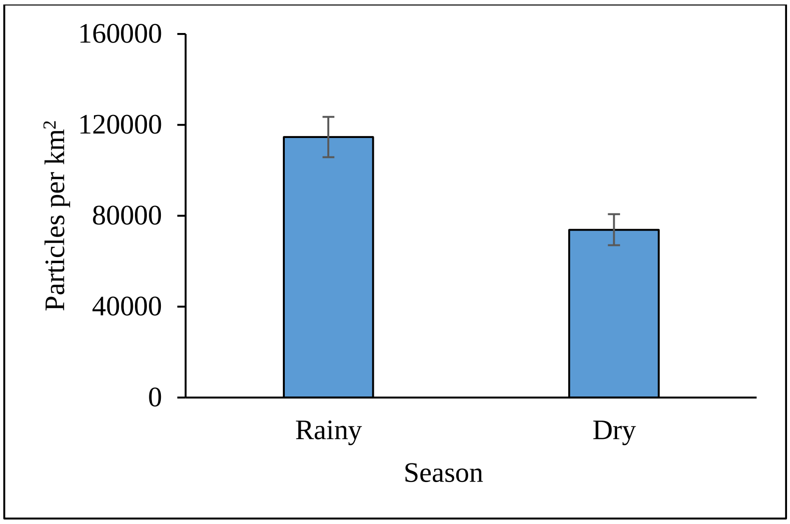

Figure 3. Seasonal variation of total microplastics abundance

4.1.3 Microplastic abundance variations by type

Six different types of microplastics were identified, including fragments, filaments, film, foam, granules, and pellets, which showed significant differences ($P<0.05$), with fragment and filament type microplastic abundance significantly higher, but there was no significant difference found between these two groups (Table 3). The results also revealed that granules and pellets were significantly lower and there were no significant differences between these two groups (Table 3).

Table 3. Abundances of six types (fragment filament, film, foam, pellet and granule). Values are means ± SE. Values with the different letters within each series indicate significant differences ($p < 0.05$) among the types.

Microplastic Type	Mean abundance (particles per km^2)
Fragment	205972 ± 11171^a
Filament	197083 ± 11530^a
Film	91389 ± 9728^b
Foam	46944 ± 5425^c
Granule	17222 ± 1796^d
Pellet	10556 ± 1221^d

4.1.4 Seasonal variations of microplastic abundance by type

The Independent-sample-t test revealed the significant difference of microplastic type between the rainy and dry seasons, and in this study, more fragment type microplastic was recorded in the rainy season (230000 ± 16082 particles per km^2) than in the dry season (181944 ± 14185 particles per km^2); t (46) = 2.241, P<0.01. The rainy season also had a higher amount of filament type microplastic than the dry season, accounting for 236667 ± 14950 particles per km^2 and 157500 ± 13548 particles per km^2 respectively; t (46) = 3.924, P<0.01. Pellet type microplastic was lowest in both seasons, but higher in the rainy season than in the dry season. Film, foam, and granule-type microplastics were also more prevalent from rainy to dry seasons (Figure 4).

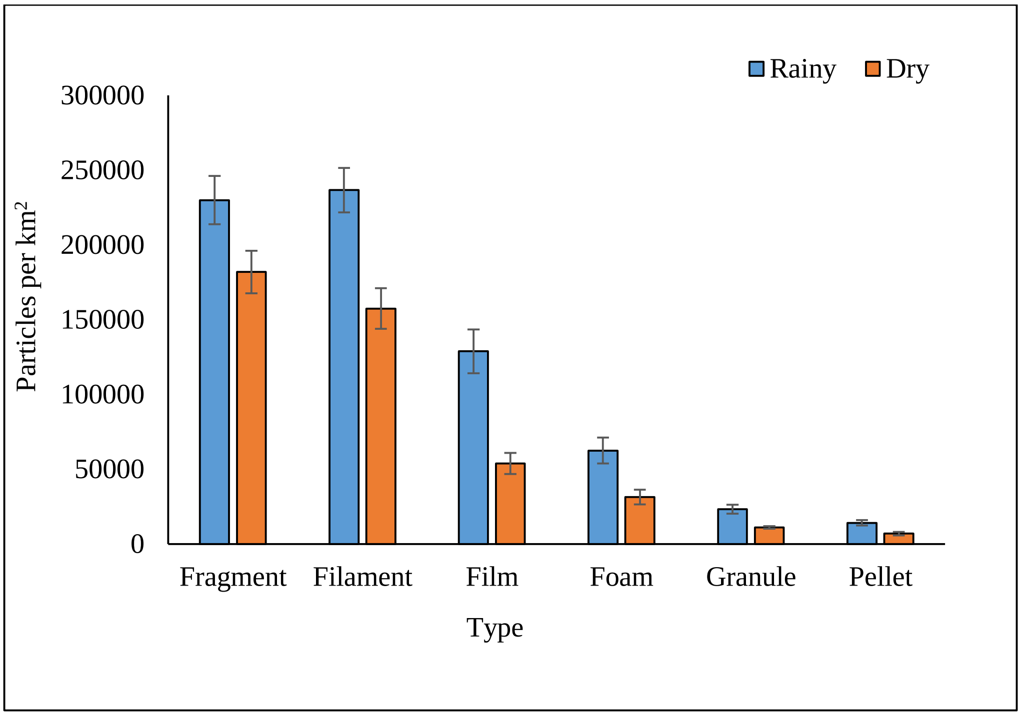

Figure 4. Seasonal variations of microplastic abundance by type

4.2 Characteristics of Microplastics

4.2.1 Types of microplastics

Fragment, filament, film, foam, pellet and granule all these types of microplastics were recorded in this study. The results showed that fragments (39.30%) and filaments (33.40%) predominated over the other microplastics like films (14.24%), foams (7.80%) and a low proportion of granules (3.15%) and pellets (2.10%) (Figure 5). The proportion of fragments and filament microplastics was comparatively high in both the rainy (37.43% and 33.19%, respectively) and dry (42.61% and 33.37%, respectively) seasons. Both the rainy and dry seasons had the lowest proportion of pellets, contributing 2.14% and 2.04%, respectively (figure 6, 7).

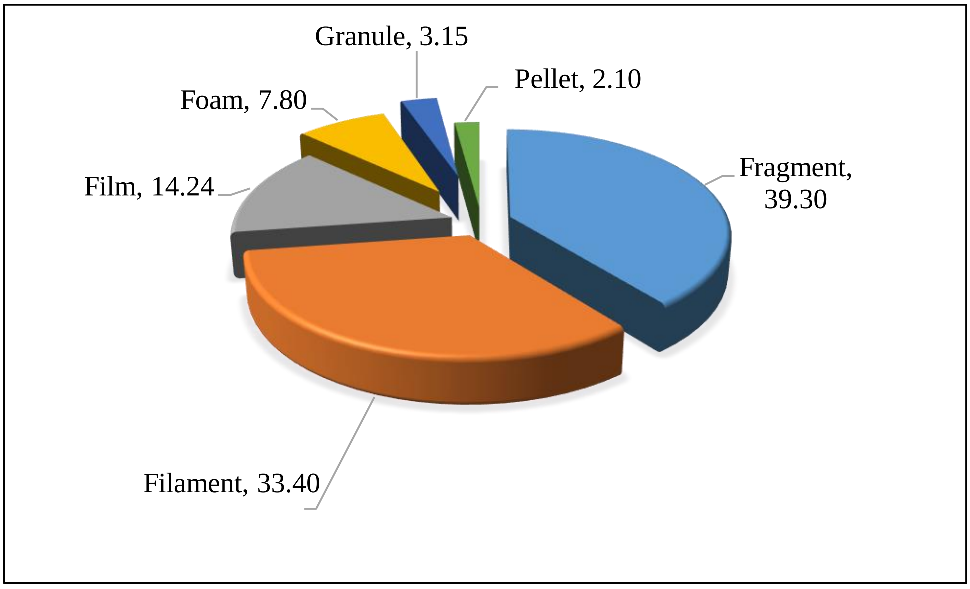

Figure 5. Percentage of different types of microplastics

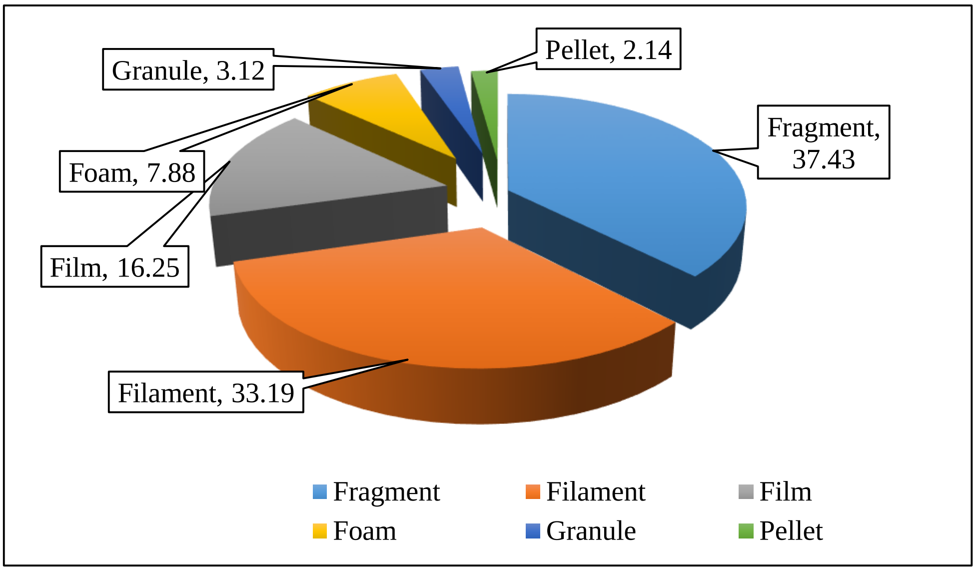

Figure 6. Percentage of different types of microplastics (rainy season)

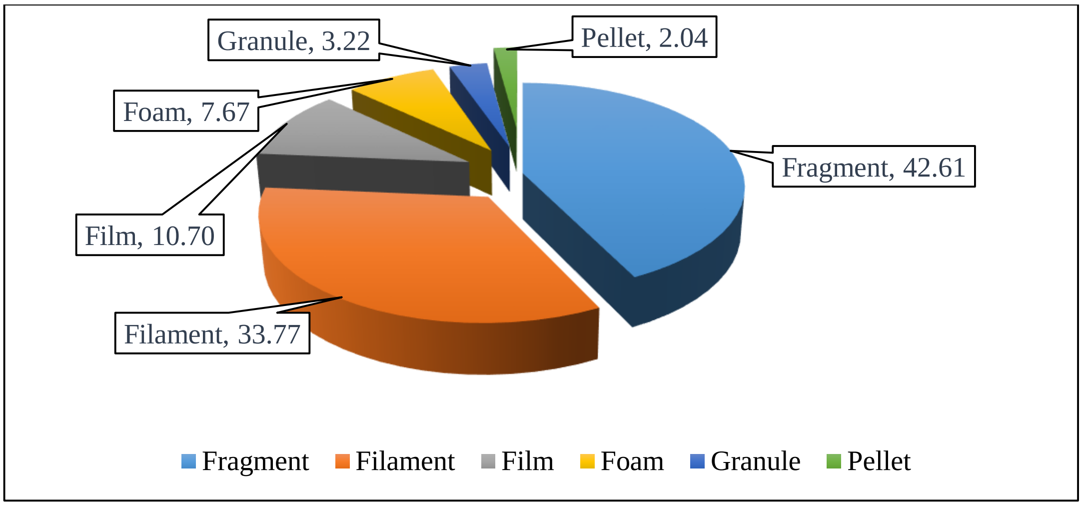

Figure 7. Percentage of different types of microplastics (dry season)

4.2.2 Colors of microplastics

The observed microplastics were classified into 10 different groups, with red (27.64%) and green (21.63%) being the most dominant colors, followed by transparent (12.42%), black (10.15%), white (8.28%), brown (5.47%), yellow (4.81%), blue (4.14%), pink (2.80%), and orange (2.67%) (figure 8). Red-colored microplastics were the most abundant in both the rainy and dry seasons, accounting for 25.37% and 31.62%, respectively. Orange-colored (2.31%) microplastics were the least abundant during the rainy season, whereas pink-colored (2.94%) microplastics were the least abundant during the dry season (figure 9, 10).

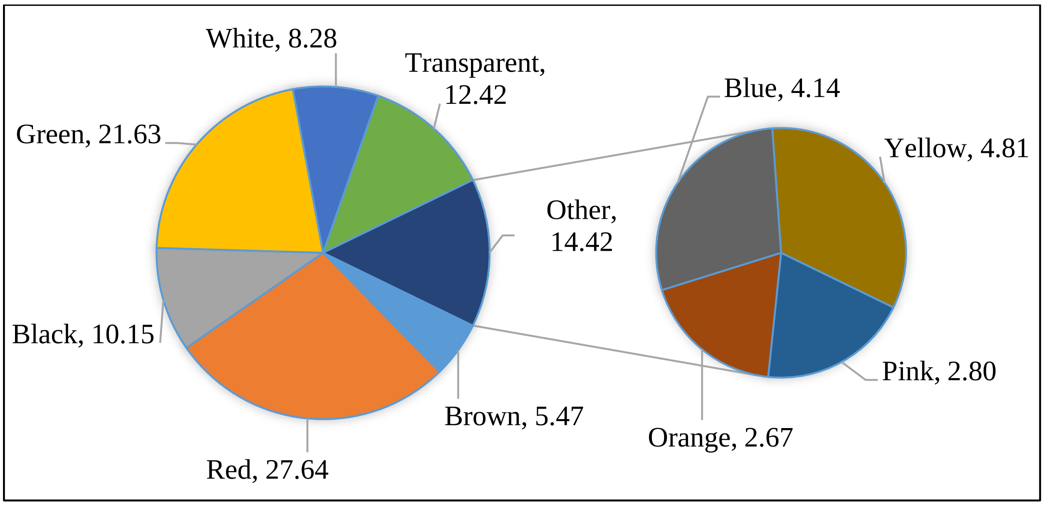

Figure 8. Percentage of different colors of microplastics

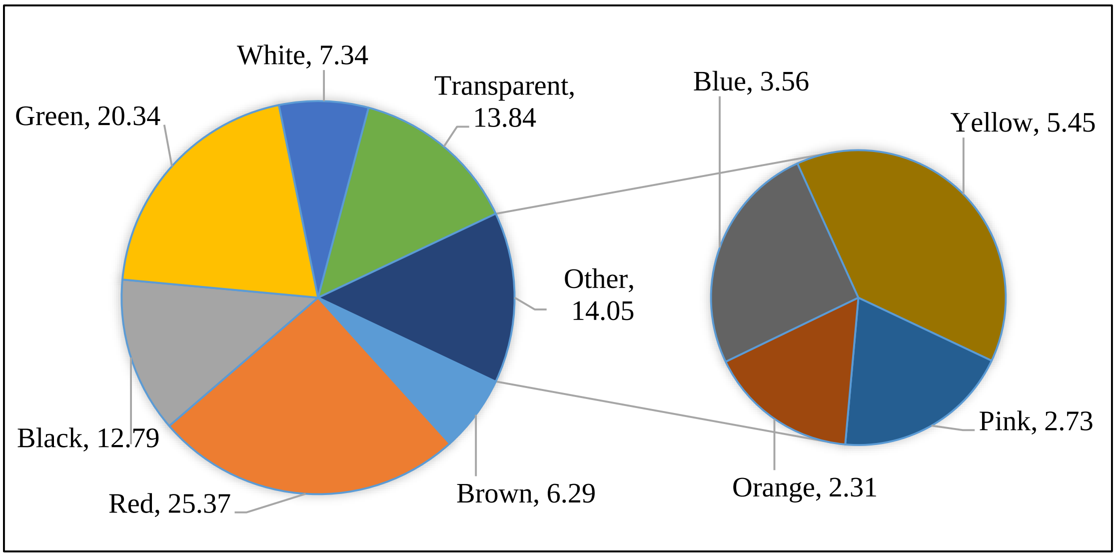

Figure 9. Percentage of different colors of microplastics (rainy season)

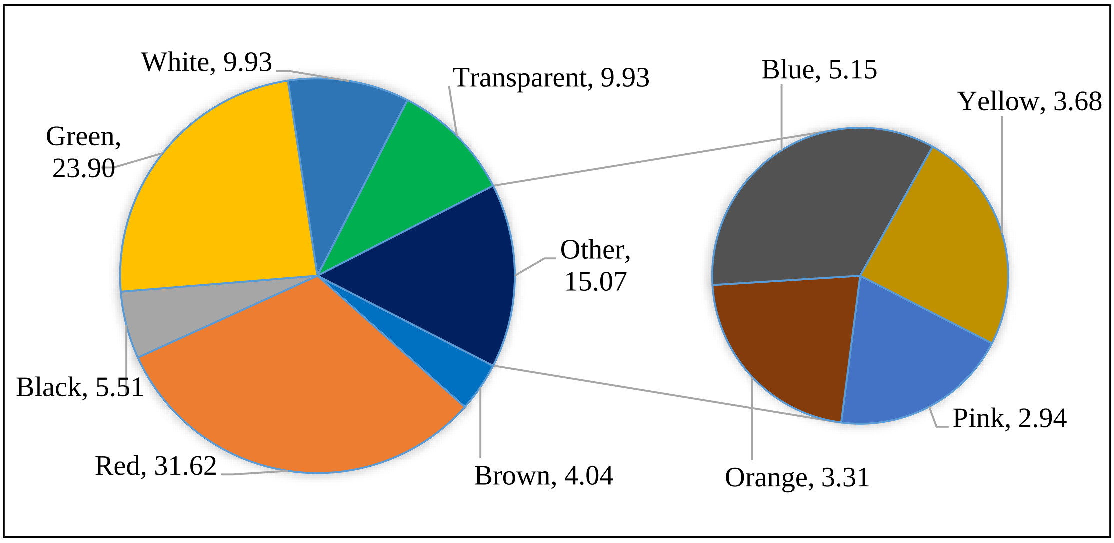

Figure 10. Percentage of different colors of microplastics (dry season)

4.2.3 Shapes of microplastics

The six shapes of microplastics observed were irregular, round, elongated, rectangular, cylindrical, and angular, with irregular (39.15%) and elongated (37.21%) being the most dominant (figure 11). Figure 12 showed the percentage of shapes of microplastics in the rainy season, where irregular-shaped microplastics dominated with 40.09% of the total. On the other hand, Figure 13 showed the percentage of shapes of microplastics in the dry season, where elongated-shaped microplastics dominated with 38.18% of the total. Cylindrical-shaped microplastics were the least prevalent during the rainy and dry seasons, respectively, accounting for 1.09% and 1.72%

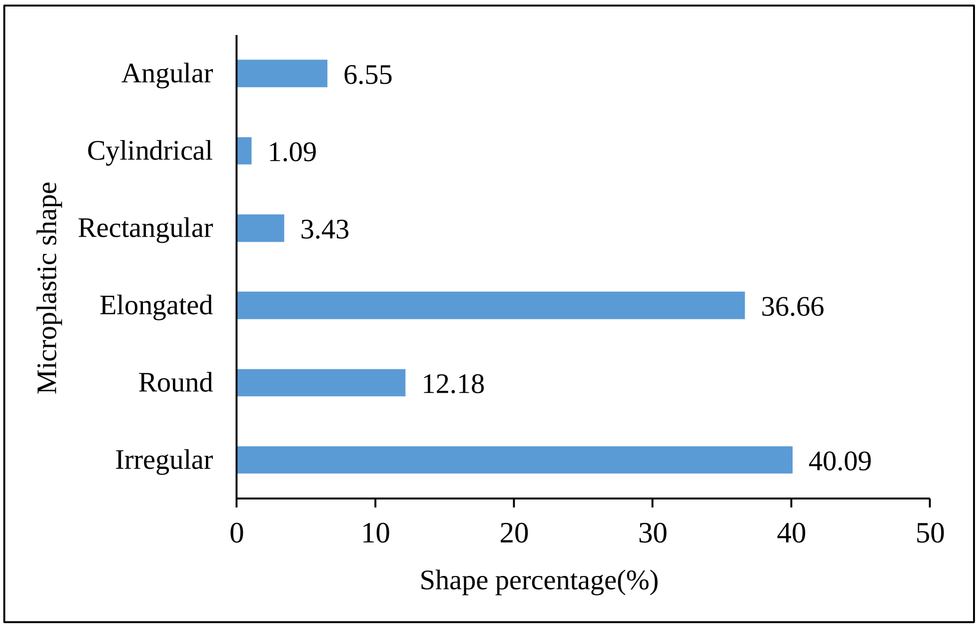

Figure 11. Percentage of different shapes of microplastics

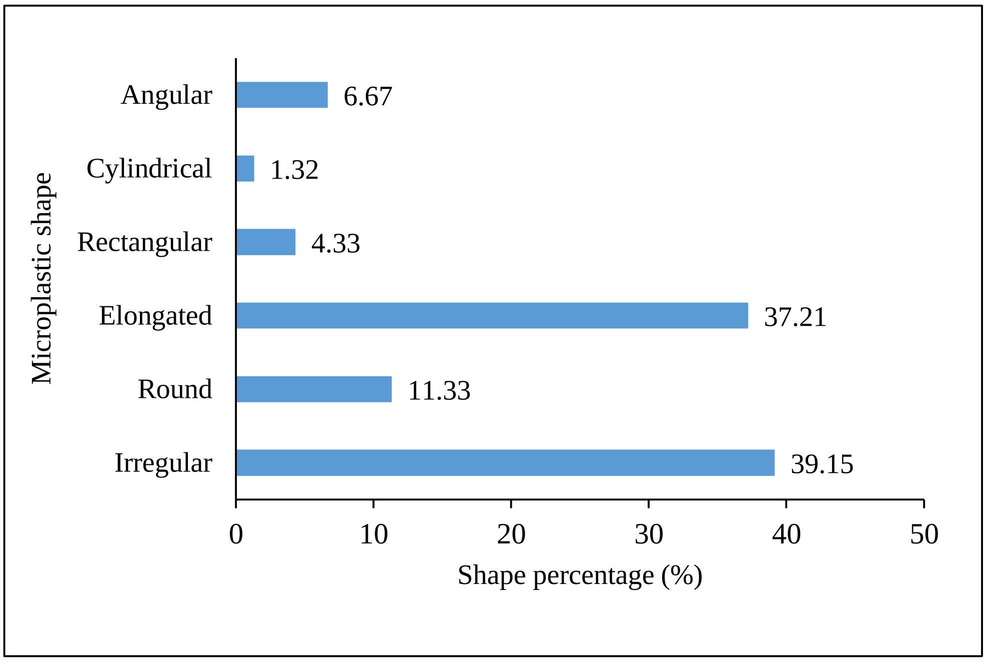

Figure 12. Percentage of different shapes of microplastics (rainy season)

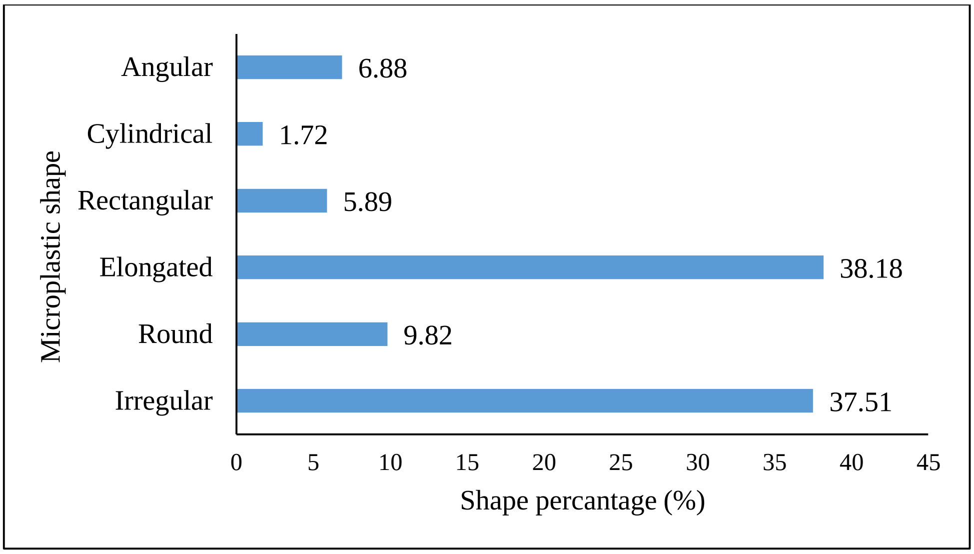

Figure 13. Percentage of different shapes of microplastics (dry season)

4.2.4 Sizes of microplastics

The identified microplastics were classified into 5 distinct size classes: 300 µm to <500 µm, 500 µm to < 1mm, 1mm to <2mm, 2mm to <3mm, 3mm to <5mm with a large proportion of microplastics were found in the class 1mm to <2mm, accounting for 32.97%. The second highest proportion of microplastics was observed in the 500 µm to < 1mm class, at 23.45%. The class 2mm to <3mm included the least quantity of microplastics, contributing 12.65% of the total (figure 14). In both the rainy and dry seasons, a large proportion of microplastics in the size class 1mm to <2mm were observed, contributing 30.53% and 37.34%, respectively (figure 15, 16).

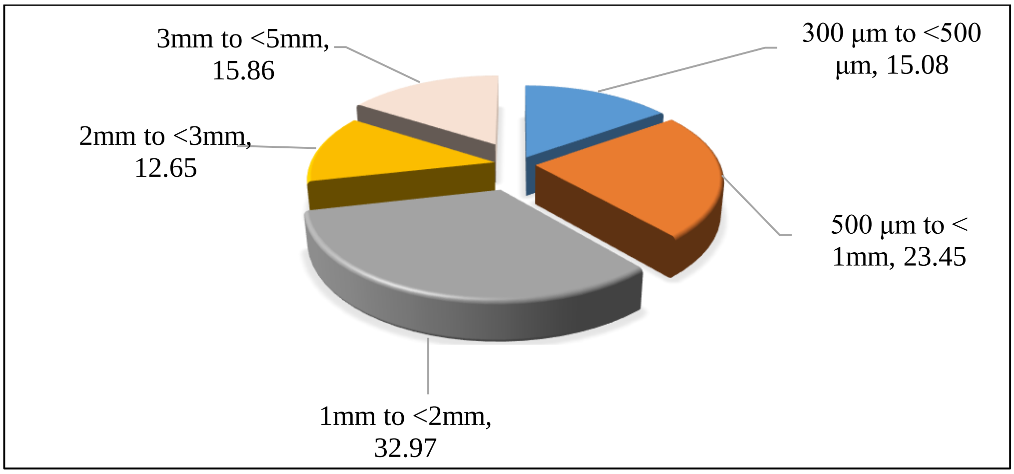

Figure 14. Percentage of different sizes of microplastics

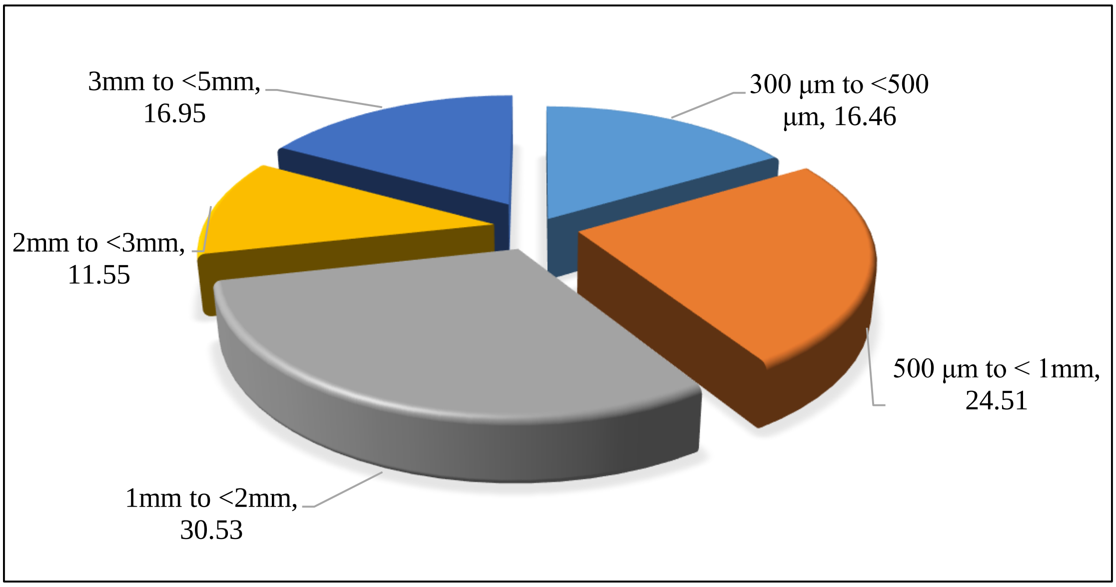

Figure 15. Percentage of different sizes of microplastics (rainy season)

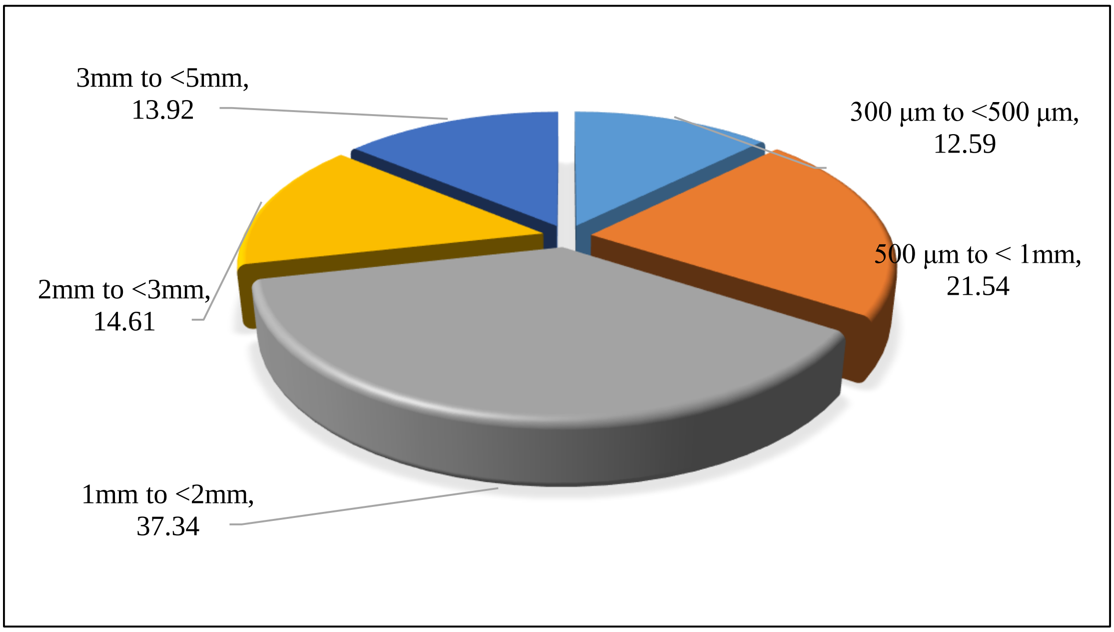

Figure 16. Percentage of different sizes of microplastics (dry season)

Chapter Five

Discussion

5.1 Monthly variations of total microplastics abundance

In the present study, July 2021 had the highest microplastic abundance (140370 ± 19586 particles per km^2) among the eight months of the investigation period. Almost 800 industries are located adjacent to the banks of the river Karnaphuli in different areas, and the river is linked with many canals, minor rivers, and tributaries, all of which contribute to the accumulation of plastic pollution (Hossain et al., 2005). According to BMD, (2021-2022) statistics, the study area received the highest rainfall (720mm) in July. As a result, increased runoff during periods of heavy precipitation may be linked to the higher levels of microplastics seen in July, which may have carried plastic trash from the watershed into the river.

On the other hand, the lowest microplastic abundances (54815 ± 9220 particles per km^2) were reported in January 2022. January is the driest month in Bangladesh, and according to BMD, (2021-2022) statistics, the average rainfall in Chattogram in January was 7 mm, which is associated with less runoff, resulting in less trash transfer from the watershed into the river.

Microplastics were identified in eight months where lower and higher abundance were 54815 ± 9220, 140370 ± 19586 particles per km^2 respectively. The current study found a lower abundance of microplastics than the Xiangxi River, with amounts ranging from 0.55×10^5 to 342×10^5 particles per km^2 (or, 55000 to 342000 particles per km^2) (Zhang et al., 2017). The mesh size of Zhang et al. (2017)'s manta net was 112 µm, whereas it was 200 µm in this study, which potentially generated lower abundance in present study. Microplastic concentrations in the Rhine River have also been found to be higher than in the current study, ranging from 1.45×10^5 to 30.7×10^5 particles per km^2 (or, 145000 to 3070000 particles per km^2) (Mani et al., 2015). The Rhine River is bordered by six different nations, and its basin is characterized by high population concentrations, which, combined with other factors, causes the Rhine River to have a higher quantity of microplastics (Mani et al., 2015). The Italian Subalpine Lakes had a relatively lower concentration of microplastics than the study being conducted now; these concentrations ranged from 4000 particles per km^2 to 57000 particles per km^2 (Sighicelli et el., 2018). These variations are caused by a variety of factors, including environmental differences, human activities, sample methodologies, net mesh size, and meteorological and hydrological circumstances.

5.2 Seasonal variation of total microplastic abundance

The abundance of microplastics was higher in the rainy season than in the dry season, while the mean abundance (rainy: 114639 $\pm$ 8845 particles per km^2; dry: 73796 $\pm$ 6817 particles per km^2) was higher in both seasons. Similar results were obtained in the Nakdong River, South Korea (Eo et al., 2019), the Pearl River Estuary, China (Li et al., 2021) and the Southern Indian Lake (Warrier et al., 2022), with these studies reporting a comparatively higher abundance of microplastics in rainy seasons compared to dry seasons. Heavy rain causes runoff in surrounding areas, which allows microplastics from the land to enter the river (Lima et al., 2014). Large river water outflow and rapid water flow, which occur during the wet period, re-suspend and convey microplastics that have previously been dumped on the river basin (Hurley et al., 2018). Microplastic concentrations in water are substantially increased by this process as a result of resuspension during the rainy season. Average precipitation during the dry season is low, and surface runoff input is predicted to be similarly low (Eo et al., 2019). As a result, microplastic concentrations are lower during the dry season.

5.3 Types of microplastics

In this investigation, six types of microplastics were identified: fragments, filaments, films, foam, granules, and pellets, with fragments, filaments, and film being the most common. This outcome was consistent with earlier investigations by Lin et al. (2018), Dikareva and Simon. (2019), Radhakrishnan et al. (2021), Saha et al. (2021). The current study also found that fragments were the most prevalent type of microplastic in both rainy and dry seasons, followed by filament and film, which is comparable to the results of Gupta et al. (2021). As the fragment (breakage of large size plastic) type microplastics were more abundant in this investigation, it can be inferred that secondary microplastics are more prevalent in the studied area.

Microplastics of the fragment type can originate from both land and sea-based sources (Gupta et al., 2021). Land-based sources of microplastics include packaging tools, industrial inputs, tire wear, fisheries, and aquaculture (Lusher and Pettersen, 2021). Contrarily, sea-based sources of microplastics include degradation of fishing gear, fishing, paintings from ships and other vessels, maintenance carried out in port regions, water sports events, etc. (Gupta et al., 2021). Filaments or fiber-type microplastics can originate from a variety of sources, including laundry, abandoned ropes, textiles, sewage treatment plants, and fishing operations (Browne et al., 2011). The main source of films is the degradation of products like plastic bags, single-use plastics, and trash discarded during tourist activities (Robin et al., 2020). The study found that the abundance of granule and pellet types of microplastics was lowest. The study by Wicaksono et al. (2021) reported a similar result, with pellets making up the lowest amount. The lower abundance of pellets in this study indicates that microplastics in the studied area do not originate largely from primary microplastics.

Granule-type plastics are frequently present in many cosmetic and cleaning products or are created when bigger degradable plastics break down (Cole et al., 2011). Pellets are commonly employed as feedstock for the manufacturing of plastics or in air blasting (Eerkes-Medrano et al., 2015). Foams are frequently used in the packaging and fishing industries (Wang et al., 2019).

5.4 Colors of Microplastics

In this study, ten different colors of microplastics were identified; the majority of these colors were red, green, transparent, blue, and white. Similar findings were obtained in the study by Castro et al. (2020). The color of microplastics can help determine their origin and level of weathering (Wicaksono et al., 2021). Colored MPs are typically produced from textiles, packaging, and a variety of other commercial uses (Xu et al., 2018). The predominance of colored microplastics in this study is therefore compatible with the idea that the microplastics observed here may have originated from consumer goods (e.g. clothes, plastic caps and cosmetics, plastic bags and plastic containers). In this investigation, 12.42% of the microplastics were observed to be transparent, which could be attributed to the widespread use of transparent plastic in packaging, containers, and fishing nets and lines.

Microplastic color may also impact a fish's preference to consume small plastic items. MPs with the same color as their food are preferred by fish (Wicaksono et al., 2021). Some fish and their young, which eat plankton, may misinterpret microplastics (e.g. brown, white and yellow plastics) with resemblances to their food (Boerger et al., 2010). Sea turtles frequently consume transparent and light-colored plastics, according to the study of (Boerger et al., 2010). However, the relationship between plastic color and the ingestion of microplastics by organisms has not been conclusively established (Zhang et al., 2017). Furthermore, the color of a microplastic is typically derived from a synthetic colorant, which can leach into the environment and pose a risk to aquatic organisms (Wicaksono et al., 2021).

5.5 Shapes of Microplastics

The identified microplastics were categorized into six distinct shapes: irregular, round, elongated, rectangular, cylindrical, and angular, with irregular (39.15%) and elongated (37.21%) being the most dominant in this study. Similar results were reported in studies of Bay of Bengal fish and sediment from the beach (Hossain et al., 2019; Hossain et al., 2021), where filamentous or elongated and irregular-shaped microplastics predominated. All of the fibers in this study had an elongated shape and came primarily from laundry and fishing net waste. Additionally, the majority of the fragments had irregular shapes and rough edges, which suggests that they may have been generated by the deterioration of larger plastic items.

In this investigation, microplastics with cylindrical shapes were the least common. These changes in microplastic morphologies may be caused by a number of factors, including waste source, macroplastic breakdown, debris quality, UV-B radiation, the way plastics are suspended in water, wind drift, and the rate at which plastics sink (Karthik et al., 2018).

5.6 Sizes of Microplastics

The microplastics observed in this study were classified into five different size classes, with a large proportion of microplastics found in the 1 mm to <2 mm class during both the rainy and dry seasons. A similar result was also found in the study of Zhang et al. (2019), where the majority of microplastic particles were <2 mm. The size of the microplastics did not vary with season in this study, and Wang et al. (2021) also observed that the size of the microplastics did not vary with season. These findings suggest that microplastics persist in the water for a prolonged period and also have a long-term effect on the environment.

The fragmentation of larger plastics could be aided by the high water flow in river systems and the abrasion caused by the tide (Browne et al., 2007), which likely contributes to the higher number of microplastics of small sizes. The size of microplastic particles influences whether or not they are easily consumed by organisms and reach the food chain. As a result, analyzing the size of microplastics aids in determining the biological threat of MP contamination.

Because of the size similarity of the microplastics with the lower trophic level organisms, smaller microplastics have a larger chance of being consumed by a variety of organisms (such as micro or nanoplankton) (Cole et al., 2011). Moreover, smaller size microplastics can also more easily enter the soft tissue of an organism, which puts the organism at risk. In this study, the large proportion of microparticles in the class of 1mm to <2mm was 32.97%, suggesting that aquatic biota was more likely to misinterpret microplastics as food in the studied areas.

Chapter Six

Conclusion

In the subject of environmental science, microplastic is a recent problem that is raising concerns all around the world. Bangladesh is one of the nations that could be at risk from this factor. This study has shown that there are extremely alarming amounts of microplastics in the surface water of the Karnaphuli River at Avaymitro Ghat. The month of July, when the average rainfall was highest, had the highest concentration of microplastics. In comparison to the dry season, the rainy season's microplastic concentration was substantially higher. Both during the rainy and dry seasons, fragments made up the majority of the microplastics, which were then followed by filaments, films, granules, and pellets. Since most of the microplastics are colored and mimic prey for living organisms, they are regarded as hazardous. Most of the identified microplastics were elongated and irregular in shape, with a range of sizes. Five different size classes of microplastics were identified where 1mm to <2mm microplastics were the most dominant. Overall, the varying sizes, shapes, and colors indicate that microplastics in this study site may have undergone distinct physical processes, resulting in a variety of sources of microplastic contamination. In this study area, the largest potential sources of microplastics are thought to be land-based sources, including road runoff, untreated wastewater discharges, tourism, and other anthropogenic wastes, with a minimal contribution from sea-based sources like fishing and port operations. These findings lay the groundwork for future research into the point and nonpoint causes of microplastic pollution. Furthermore, this study could serve as a baseline for managing the river's pollution. In addition to the current study, more research is required to ensure that annual changes in the quantity of microplastics and their toxicological effects on aquatic organisms and the environment.

Chapter Seven

Recommendations

The following recommendations may be made based on the findings of the current study:

- It is necessary to work on fixing microplastic pollution right now since it is a serious issue around the world. Controlling microplastic pollution at its source is the most efficient way to reduce it in the aquatic environment.
- Policymakers will play a critical role in developing the necessary regulatory framework to encourage mitigation efforts that contribute to reducing plastic waste at the source as well as encouraging cleanup of plastic pollution before it causes the most substantial damage.
- It is necessary to monitor pollution at the source and conduct public awareness programs to reduce consumption patterns and littering.
- Increasing demand for microplastic pollution monitoring at national and global levels requires the improvement of existing methods and the development of novel methodologies to reduce the identification time and effort.
- Future research should be done to determine the concentration of microplastics and how they impact aquatic organisms, humans and the environment.