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ชื่อโครงการ Contamination and characterization of microplastics in different sediments of the inner Gulf of Thailand

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Title	**Contamination and characterization of microplastics in different sediments of the inner Gulf of Thailand**

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หัวข้อ	**การปนเปื้อนและลักษณะสมบัติของไมโครพลาสติกในตะกอนดินที่แตกต่างกันของอ่าวไทยตอนใน**
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บทคัดย่อ

ปัจจุบัน ไมโครพลาสติกเป็นประเด็นทางสิ่งแวดล้อมที่ทั่วทั้งโลกให้ความสนใจ มีงานวิจัยมากมายแสดงให้เห็นว่าสิ่งแวดล้อมทางน้ำมีการปนเปื้อนไมโครพลาสติก อย่างไรก็ตาม สถานะการปนเปื้อนและลักษณะสมบัติของไมโครพลาสติกในตะกอนดินชั้นผิวของอ่าวไทยตอนในยังมีการศึกษาไม่มากนัก การศึกษานี้จึงได้สำรวจไมโครพลาสติกเพื่อวิเคราะห์การปนเปื้อนและลักษณะสมบัติของไมโครพลาสติกในตะกอนดินที่แตกต่างกันของอ่าวไทยตอนใน ผลการศึกษาที่ได้แสดงให้เห็นว่าตะกอนดินชั้นผิวถูกปนเปื้อนไปด้วยไมโครพลาสติกทั่วทั้งอ่าวไทยตอนใน ปริมาณไมโครพลาสติกโดยเฉลี่ยในตะกอนดินชั้นผิวของอ่าวไทยตอนใน มีค่า 2534±1907 ชิ้นต่อกิโลกรัมตะกอนดินแห้ง และน้ำหนักไมโครพลาสติกโดยเฉลี่ย มีค่า 58±60 มิลลิกรัมต่อกิโลกรัมตะกอนดินแห้ง ไมโครพลาสติกขนาดเล็ก (300-100 ไมโครเมตร) ถูกพบอยู่ทั่วไปในตะกอนดินชั้นผิว และรูปร่างของไมโครพลาสติกที่พบบ่อยที่สุดคือ ชิ้นส่วนที่แตกหัก (56%) ขณะที่สีดำ สีน้ำตาล และสีขาวเป็นสีหลักที่พบท่ามกลางสีที่แตกต่างกันของไมโครพลาสติก จากผลการวิเคราะห์แสดงให้เห็นว่าตะกอนดินชั้นผิวของอ่าวไทยตอนในมีการปนเปื้อนที่ระดับความเข้มข้นค่อนข้างสูง และองค์ประกอบส่วนใหญ่เป็นขนาดเล็ก ซึ่งบ่งชี้ว่าการปนเปื้อนไมโครพลาสติกในตะกอนดินชั้นผิวอาจก่อให้เกิดผลกระทบและมีความเสี่ยงต่อชุมชนสิ่งมีชีวิตสัตว์หน้าดิน และอาจเพิ่มความรุนแรงไปสู่สิ่งมีชีวิตในน้ำได้ ยิ่งไปกว่านั้น ผลการศึกษาครั้งนี้เป็นข้อมูลทางวิทยาศาสตร์เกี่ยวกับมลพิษทางไมโครพลาสติกซึ่งสามารถนำไปใช้ในการพัฒนาการระบบการจัดการขยะพลาสติกและนโยบายสิ่งแวดล้อมในประเทศไทยได้

คำสำคัญ: ไมโครพลาสติก
ตะกอนดินชั้นผิว
สถานะการปนเปื้อน
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Project Title **Contamination and characterization of microplastics in different sediments of the inner Gulf of Thailand**

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Abstract

Nowadays, microplastics are an environmental issue in the world. There are many researches show that the aquatic environment is contaminated by microplastics. However, the contamination status and characterization of microplastics in surface sediment of the inner Gulf of Thailand have not many studies. The present study was investigated microplastics in order to analyze the contamination and characterization of microplastics in different sediments of the inner Gulf of Thailand. Result showed that surface sediment was contaminated by microplastics throughout the inner Gulf of Thailand. The average number of total microplastic concentration in surface sediments of the inner Gulf of Thailand was 2534±1907 pieces/kg SDW and the average mass of total microplastic concentration was 58±60 mg/kg SDW. The small size of microplastic (300-100 μm) was widely found in surface sediment and the most frequent microplastic shape observed was a fragment (56%). Black, brown and white were dominant among the different colors of microplastics. As a result, the microplastic concentrations in surface sediment of the inner Gulf of Thailand were relative high contaminations and high small size composition, which were suggested that the impact of the microplastics currently in the sediments might be potentially risk to the benthic community and potentially increase the magnitude into the pelagic community. Moreover, the result of this study provided scientific information on microplastic pollution to develop a plastic waste management and environmental policies in Thailand.

Keywords: Microplastics
Surface sediment
Contamination status
The inner Gulf of Thailand

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Rungrawin Anurakpradorn

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CHAPTER 1

INTRODUCTION

1.1 Significance of the Research

Microplastics are a global issue of attention. Research has found that microplastics appear to be virtually anywhere around the world such as seawater, beach sediments, atmospheric fallout and glaciers in high mountain areas (Ambrosini et al., 2019; Cai et al., 2017; Isobe et al., 2017; Stolte et al., 2015). As a result, everyone should be aware of microplastics problem. National Oceanic and Atmospheric Administration says, marine debris is defined as any persistent solid material that is manufactured or processed and directly or indirectly, intentionally or unintentionally, disposed of or abandoned into the marine environment or the Great Lakes. Marine debris tends to increase continuously (Jambeck et al., 2015). For this reason, microplastic problem is unlikely to be gone.

Microplastic is any piece of plastic with size ranging from 1 μm to 5 mm and can be divided into 2 types, including primary and secondary microplastics (Crawford and Quinn, 2017). Primary microplastics are small plastic that is produced by humans such as small spherical microbeads for use in personal care products, cosmetics and synthetic fibers for clothing. Secondary microplastic is macroplastic that degraded by biotic degradation (deterioration of plastic materials by biological organisms) or abiotic degradation (weathering of plastic materials from environmental factors, such as mechanical force, light and temperature etc.).

The small size of microplastic result in a lot of surface area. Chemicals or toxins can be attached to a microplastics. When contaminated microplastics were eaten by organisms, highly toxic chemical pollutants are getting into the food web (Crawford and Quinn, 2017). The toxins that are absorbed by microplastic can accumulate in tissues and organ causing damaging effects to the organism (Deng et al., 2017). The toxic substances that spread to the organism are also transmitted or accumulated from one creature to another. In addition, the creatures on top of the food chain are human. Accordingly, it is possible to tell where microplastics are in the environment, organisms in the food web can be all affected.

A study of microplastics is widespread throughout the world but areas that still lack of education about microplastics is Thailand. On the other hand, it was found that China, Indonesia, Thailand, Vietnam and the Philippines, a total of 5 countries, produce more than half of total plastic waste in the world (Leung, 2018). An important point is that Thailand is produce and reach plastic into the ocean in tremendous quantities, but Thailand still has insufficient information about microplastic for use in solving the problem. Four main estuaries in Thailand including Mae Klong, Tha Chin, Chao Phraya and Bangpakong rivers are play the important role in source of microplastics, which are accumulated in the inner Gulf of Thailand. When microplastics was swept into the Gulf of Thailand, which is the area that supported by 4 major rivers of Thailand. Some of these microplastics and toxic substances are accumulated in the sediment which is the final cumulative source. Moreover, the sediments can be a potential sink and source of microplastics in the inner Gulf of Thailand. Therefore, there is a risk of microplastic contamination in the inner Gulf of Thailand. The inner Gulf of Thailand is an important area because of natural resource, biodiversity, and economic zone. Therefore, it is necessary to find the quantity and quality of microplastic in sediment in the inner Gulf of Thailand. Status of microplastics contamination in the inner Gulf of Thailand can be used as information for waste management and legislation to reduce the effects of microplastic.

1.2 Research Objectives

To characterize and compare the contamination of microplastics including abundance (number and mass concentration), sizes, shapes and colors in different sediments of the inner Gulf of Thailand.

1.3 Scope of the Research

1) **Study areas:** the inner Gulf of Thailand is chosen to study on contamination and characterization of microplastics. Because, this specific area is the important marine resources of Thailand, which is additionally received microplastic pollutions from river runoff. Subsequently, microplastics can be distributed and contaminated entire the inner Gulf of Thailand.

2) **Sampling Points:** total 9 sampling points were established in the inner Gulf of Thailand, including 3 transect lines in different areas, which are various activities in the areas.

3) **Sampling:** A surface layer (top 5 cm layer of sediment) was taken from the previous study (Faculty of Fisheries, 2019), which was collected using the Smith McIntyre grab and Ekman grab samplers in December 2017.

4) **Quantitative analysis:** different sizes, shapes, colors and concentrations of microplastic in all sites were analyzed in order to assess the contamination status of microplastics in surface sediment.

5) **Laboratory analysis:** laboratory analysis was carried out during August 2019 to June 2020 at the Department of Environmental Science, Faculty of Science, Chulalongkorn University.

6) **Data analysis:** mean comparisons of different size and shape concentrations of microplastic between the transect lines were analyzed using one-way ANOVA and post-Hoc test.

1.4 Outcomes of the Research

1) Improved understanding of contamination status and characterization of microplastics in surface sediment of the inner Gulf of Thailand.

2) Finding data are used in order to develop plastic waste management and environmental policies in Thailand.

CHAPTER 2

THEORY AND LITERATURE REVIEWS

2.1 Plastics

2.1.1 Definition of plastic

Plastic is one of five synthetic organic polymers. The synthetic organic polymer is a man-made and long repeating chain of monomer that has carbon and hydrogen as the main compositions. Plastics can be divided base on sort of polymerization into two main types which are thermoplastic and thermosetting. Thermoplastic is producing by addition polymerization that is the same type of monomers join the polymer chain by adding on to the end of the last monomer such as polyethylene, polystyrene, and acrylic. Besides, thermoplastic is easily recycled. Thermosetting is producing by condensation polymerization that is the different monomers join by eliminated some parts of each monomer such as nylons, some polyesters, and urethanes. Thermoset polymer cannot be melted and reformed (Science history institute, 2020). The properties of plastics depending on the chemical composition and the arrangement of monomer. Plastics are commonly solids, poor conductors of electricity and heat, slow rate of degradation, and the deformation of mostly plastics are irreversible (Helmenstine, 2020). Plastic have various type depend on the species of monomer such as polyethylene (PE), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC) and polystyrene (PS) (Figure 2.1).

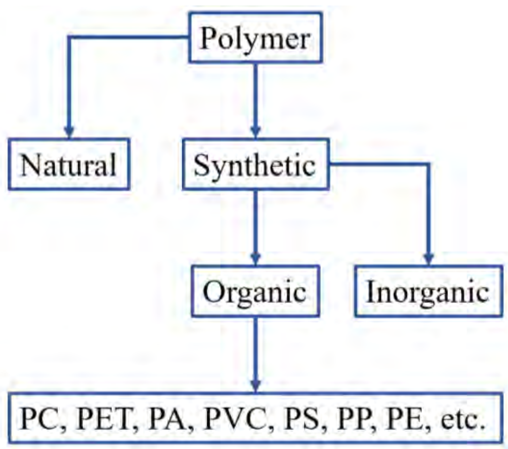

Figure 2.1 Schematic diagram of plastic definitions

2.1.2 Plastic productions

Because plastic is consumerism, convenient, and low-price so the production of plastic is increasing every year (Gourmelon, 2015). In the 1950s, plastics are produced 2 million tonnes per year but in 2015 the world produced plastics for 381 million tonnes that it is 200 fold of annual production (Ritchie and Roser, 2018). China, Europe, and North America are the most global plastics producer in 2014 (26%, 20%, and 19% respectively). Polyethylene and polypropylene are the most demand types of plastics. Besides, the biggest consumer of these materials is the packaging industry. The production of plastic has no trend to decrease. By 2050 plastic will be extra produced for 33 billion tonnes because of the global population (Crawford and Quinn, 2017).

In 2017, Thailand has produced, exported, and imported plastic bead more than 8.5, 5.2, and 2 million tonnes respectively. Also, 0.2 million tonnes of plastic scrap recycling is imported into the country. Thailand consumes 5.3 million tonnes of plastic in the country, with plastics being used in almost every industry. For instance, plastics are used for packaging, electronics, construction, auto part, and housewares. Most composition of using plastics is used for the packaging which is single-use plastics that is about 2.3 million tonnes or 41% of the total (Pollution control department of Thailand, 2019).

2.1.3 Types and uses of plastic

Plastics can be classified into many types using the chemical structure of the polymer's backbone and side chains. The seven most used plastic types are polyethylene terephthalate, high-density polyethylene, polyvinyl chloride, low-density polyethylene, polypropylene, polystyrene, and polycarbonate (Table 2.1). Polyethylene Terephthalate (PET or PETE) is used to produce water bottles, polyester film, containers for food, fibers for clothing, and carpets. High-Density Polyethylene (HDPE) is used to produce juice containers, grocery and trash bags, motor oil containers, detergent containers, and toys. Polyvinyl Chloride (PVC) is used to produce plumbing and sewage pipes, window frames, non-food packaging, cards, electrical cable insulation, and flooring. Low-Density Polyethylene (LDPE) is used in plastic bags, computer components, trays, and Ziploc frozen food bags. Polypropylene (PP) is used to produce flip-top bottles, plastic diapers, Tupperware containers, bottle caps, and even chairs. Polystyrene (PS) can be used to produce CD and DVD cases, single-use disposable cutlery, disposable razors, and smoke detector housings. Polycarbonate (PC) is used to produce baby feeding bottles, car parts, and water cooler bottles.

Table 2.1 Applications of plastics

Plastic	Abbreviation	Typical application
Polyethylene terephthalate	PET	Beverage bottles, Food containers, Film and sheeting, Fibres, Microwavable packaging
High-density polyethylene	HDPE	Chemical containers, Beverage bottles, Food containers, Tubing, Pipes
Polyvinyl chloride	PVC	Containers, Electrical conduit, Pipes, Window frames, Cable insulation, Footwear, Flooring
Low-density polyethylene	LDPE	Bags, Squeezable bottles, Food packaging, Carton linings, Waste bins, Outdoor furniture, Shower curtains and clamshell packaging
Polypropylene	PP	Bottle caps and container lids, Packaging tape, Pipes, Rope, Automotive Applications, Outdoor furniture, Drinking straws
Polystyrene	PS	Disposable cutlery and tableware, Rigid disposable food containers, Disposable cups
Polycarbonate	PC	Information storage discs, Traffic light lenses, Security windows, Eyeglasses

Source: Crawford and Quinn (2017)

2.1.4 Plastic wastes

Nowadays, single-use plastic become a serious problem in consumer behavior because of the enormous production and the low-price of plastic. Therefore, plastic waste is increased dramatically and most of that is end up in the environment. An assessment by the United Nations Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) concluded that 80% and 20% of waste in the marine environment originates from land and activities at sea respectively. Land-based sources of plastics are from urban discharge, industries discharge, dumping or unintentional spillage. Ocean-based sources of plastics are from shipping, fishery, recreation, offshore industries, and even accidental loss of shipping containers during storms (GESAMP, 2015). In Thailand, plastic wastes have approximately 12% of the total wastes generated in the country each year or about 2 million tonnes per year. Single-use plastics have 41.4% of the total plastic wastes such as plastic bags, plastic straws, etc. In terms of plastic wastes management in Thailand, 20.21% of the plastic wastes were reused, 78.24% are mismanaged plastic wastes and causing environmental problems and 1.55% are swept into the environment (Pollution control department of Thailand, 2018).

2.1.5 Marine debris and their impacts

Plastics are lightweight, durable, strong, corrosion-resistant, and designed to be disposable. Because of its property, plastic remains in the environment for a long time. Therefore, plastic debris has a high opportunity to give critical negative impacts on the environment and organisms. When plastics swept into the marine environment because of various reasons, it will be called marine debris. Especially, many reports show that marine debris has a significant impact on the marine environment. For example, fishing gear is the main cause of California's coastal marine wildlife injury (Dau et al., 2009) and a lot of plastic fragments are ingested by mesopelagic fishes in the North Pacific Subtropical Gyre (Davison and Asch, 2011). In addition, marine debris is affecting marine habitats and biodiversity of many organism species (Gall and Thompson, 2015).

2.2 Microplastics

2.2.1 Definition of microplastic

Microplastics are solid synthetic materials with high polymer content that have smaller than 5 mm. and insoluble in water. In addition, microplastics are not degradable or use a long time to degrade (Verschoor, 2015). In addition, Crawford and Quinn (2017) reported that “microplastics are any piece of plastic smaller than 5 mm to 1 μm in size along its longest dimension”.

2.2.2 Types of microplastic

Microplastics can be divided into 2 types include primary microplastics and secondary microplastics. The primary microplastics are microplastics that intentionally manufactured for use in many purposes such as small spherical microbeads for use in cosmetics or personal care products, small colored pellets for melted down and molded to form larger plastic artefacts, etc. The secondary microplastics are microplastics that unintentionally produced from degradation of larger pieces of plastic such as plastic bags, bottles, and nets (Crawford and Quinn, 2017).

2.2.3 Fate and transportation of microplastic

Macroplastics and primary microplastics are manufactured for a wide range of applications so, there are in all shapes and sizes. Used plastics are mismanaged then, transported into the environment. Freshwater environments can contaminate these plastics by industrial effluent, urban runoff, and wastewater treatment effluent. In addition, macroplastics and primary microplastics are swept into the marine environment via river runoff, illegal dumping, urban discharge etc. (Horton and Dixon, 2018). Macroplastics in the marine environment can break down to microplastics by different degradation processes. For instance, photo-oxidative degradation by ultraviolet light (UV) and Thermal-oxidative degradation by temperature changing. Microplastics that denser than seawater will settle down and accumulate in the deep-sea sediment. On the other hand, low-density microplastics can be swept to other areas by the ocean current and aggregate with organic matter or small particles such as persistent organic pollutions (POPs), heavy metals, etc. Causing microplastic to be denser than seawater then, sink and accumulate in deep-sea sediment (Figure 2) (Crawford and Quinn, 2017).

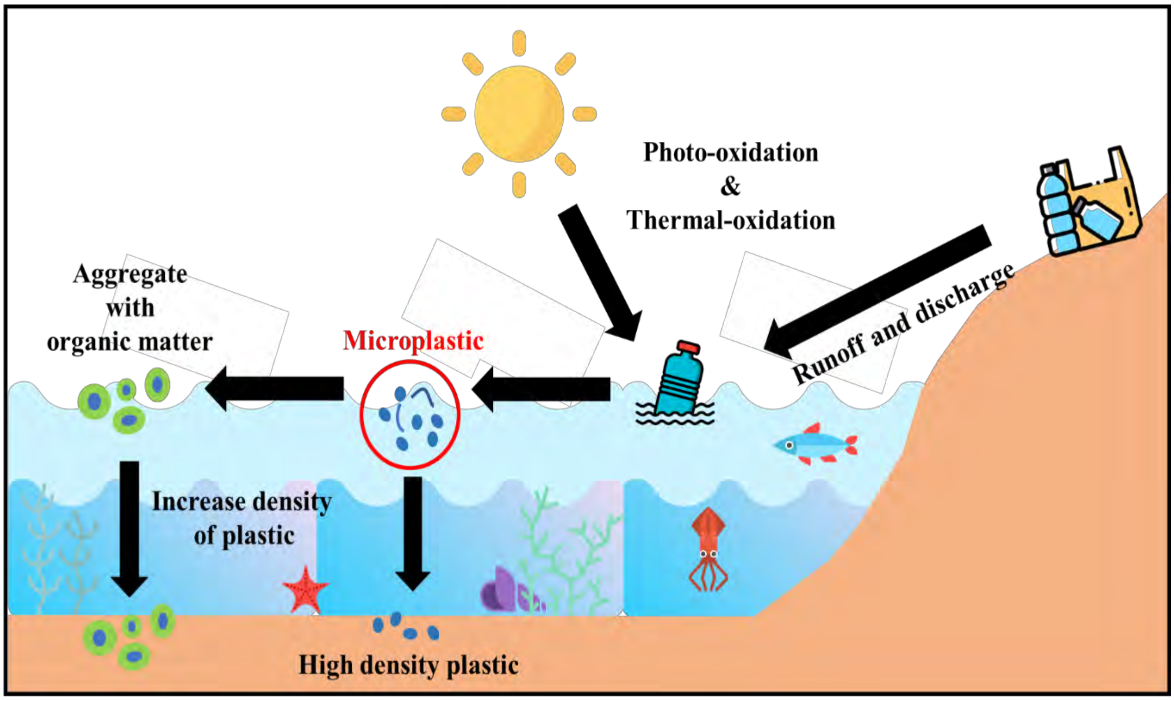

Figure 2.2 Fate and transportation of microplastics in the marine environment

2.2.4 Impacts of microplastic

Aquatic organisms can expose microplastic into their body through the gills or ingestion. The important factors that affect the microplastic concentration in an organism are particle size, shape, and character of the organism. Microplastic can transfer from the digestive system to the circulatory system and accumulate in cells, tissue, and organs or excrete depend on their size, shape, and composition of the particle. Microplastic can desorption, absorption, and adsorption of the wide range of chemical pollutants or metal from water because of the large surface area, polymer type, hydrophobicity of the substance. The most chemicals were absorbed by microplastic are persistent organic pollutants (POPs), persistent, bioaccumulative and toxic substances (PBTs). Therefore, the microplastic is a media of chemicals transport into the aquatic organisms. Microplastic and chemical pollutants are getting into the food web by predator-prey relationships and trophic levels. Microplastic have two types of effects including physical and chemical effect. Physical effect is relating to the result of the physical properties of microplastic to organisms, for example, the obstruction of the digestive tract that leads to death. Chemical effect is about the result of their large surface area, the toxicity of the polymer and absorbed contaminants to organisms such as polyvinyl chloride (PVC) can effect in genetic changes (GESAMP, 2015).

2.3 The inner Gulf of Thailand

The inner Gulf of Thailand has an area of approximately 100 x 100 square kilometers and the water level is at a depth of -1 to -54 meters from the mean sea level. The east area is deeper than the west and north. Coastal areas have very low slopes. The seafloor in the middle and east areas of the inner Gulf of Thailand are characterized by alternating hills, puddles positioned north-south. Also, characteristics of surface sediment of the inner Gulf of Thailand in approximately 60 percent, consisting of mud sediment mixed with shell debris which the sediment is grayish-green, gray, black, brown and dark brown. Followed by 20 percent of mud sediment mixed sandy silt, sediment is grayish-green. The climate in the Gulf of Thailand is under the influence of the monsoon include the southwest monsoon which will cover between mid-May to mid-October and northeast monsoon which will cover around mid-October until mid-February. The tide is flowing clockwise in the southwest monsoon and counterclockwise in the northeast monsoon. The inner Gulf of Thailand is a large estuary receiving freshwater from 4 main rivers, including Mae Klong, Tha Chin, Chao Phraya, and Bangpakong River. The upper coast of the inner Gulf of Thailand is a lane beach in the tide area that is covered by mangrove forest with a width of 1 to 10 kilometers. The coast of the inner Gulf of Thailand is a habitat, food sources, and shelter for marine animals that are economically valuable (Department of mineral resources of Thailand, 2012).

2.4 Literature Reviews

2.4.1 International microplastic contaminations

Nowadays, microplastics are a hot issue in the world. There are many researches show that the aquatic environment is contaminated by microplastics. The abundance of microplastics in the Changjiang estuary in China was 121 particles per kg. of dry weight and the most shape, color and size were found are fiber (93%), transparent (43%) and smaller than 1 mm. microplastics (58%) respectively. They indicate that source of microplastics was from washing clothes because the most abundant types are rayon, polyester, and acrylic (Peng et al., 2017). Reed et al. (2018) has the same source of microplastic because of the characteristics of detected microplastic. Guanabara Bay in southeast Brazil is one of the most polluted environments on the Brazilian coastline. There are 12 to 1300 microplastic particles per m^2 on the beaches sediment (de Carvalho and Baptista Neto, 2016). Claessens et al. (2011) found that Belgian marine sediments from coastal harbors have the highest concentration of microplastics which up to 390 particles per kg of dry sediment. Similarly, to the microplastics concentration in the sediment from Deep Bay, Tolo harbor, Tsing Yi, Victoria harbor in Hong Kong that are 49 to 279 particles per kg. (Tsang et al., 2017). Klein et al. (2015) show that there are 4000 particles of microplastics per kg. of river shore sediments of the Rhine-Main area in Germany and large abundant types are polyethylene, polypropylene, and polystyrene. Same as Wessel et al. (2016) that reported polypropylene and polyethylene were most abundant in beach sediments in the northern Gulf of Mexico estuary. The vast majority of microplastics in coastal sediments from southern Portuguese shelf water are rayon fibers and polypropylene fragments (Frias et al., 2016). Yu et al. (2016) and Martin et al. (2017) found that microplastic accumulate in surface sediment more than deep sediment because of the abundance of microplastic in different sediment depth. Van Cauwenberghe et al. (2013) show that there are microplastics in deep-sea sediment ranging in depth 1100-5000 m. and Cordova and Wahyudi (2016) found that microplastics present in the western Sumatera sediments at more than 2000 m. deep. Therefore, microplastics pollution is widespread throughout the world's aquatic environment.

2.4.2 Microplastic contaminations in Thailand

Matsuguma et al. (2017) found that the microplastics concentration in sediment cores collected in the Gulf of Thailand is 100 pieces per kg. of dry sediment and polyethylene is the most abundant type in four sampling points. They suggest that Thailand has large plastic consumption in the 1960s because it cannot detect microplastic in the 44-46 cm. of sediment layer (1950s). Also, the abundance of microplastics is increase in the shallower layer of sediment. Therefore, the global microplastic pollution is increasing over time. Microplastics have been found in two species bivalve (*Donex* sp. and *Paphia* sp.) at Chaolao and Kungwiman beach, Chanthaburi province. Microplastic contamination in *Donax* sp. in Chaolao beach and Kungwiman beach are similarly which were 3.13±2.75 particle/individual and 2.98±3.12 particle/individual respectively. The vast majority is fiber and the most microplastics colors are black in Chaolao beach blue in Kungwiman beach (Tharamon et al., 2016). The filter-feeding organisms can use as an indicator of microplastic contamination. Therefore, Thushari et al. (2017) is studied in 3 most abundant sessile and intertidal invertebrates (Rock Oyster: *Saccostrea forskalii*, Striped Barnacle: *Balanus amphitrite*, Periwinkle: *Littoraria* sp.) in 3 beaches of the eastern coasts of Thailand for indicate the microplastics contamination. The results showed that microplastics accumulated in the invertebrates at rates of 0.2–0.6 counts/g. So, it represents the potential health risk of seafood consumers.

CHAPTER 3

MATERIALS AND METHODS

The present study is partially research of the research program of Development of Socio-Ecological Based on Effective Fishery Management Policy for Good Governance in Sustainable Fishery of the inner Gulf of Thailand, under research project of assessments of impacts from climate changes and specific hydro-ecological characteristics of surrounded estuaries on the water quality and pollution status of the inner Gulf of Thailand, which was carried out during 2017-2018 (Faculty of Fisheries, 2019). Although, the surface sediment samples of this study were already collected, the remaining analysis is mainly part of this research.

3.1 Study Areas and Sampling Points

This study chooses the inner Gulf of Thailand to be study area, which were received pollution loadings from 4 main river estuaries including the Mae Klong, Tha Chin, Chao Phraya, and Bangpakong rivers. As well as in each area have many human activities such as agriculture, fisheries, industrial, aquaculture, water transportation route and are a tourist attraction. So, a lot of plastic wastes with mismanagement are reached the river estuaries. Similarly, when river estuaries are runoff into the inner Gulf of Thailand therefore, many plastic wastes are transferred and become marine debris. Plastic waste was fragmented into the smaller piece called microplastic by physical, chemical or biological processes. Besides, microplastic settling down to the bottom sediment. Especially, the 4 main river estuaries and the inner Gulf of Thailand are habitat, food sources and shelter for marine animals that are economically and ecologically valuable. For this reason, microplastic contamination is a direct effect on the marine environment in Thailand. Human activities around the inner Gulf of Thailand vary according to population density and land use management. Therefore, microplastics contamination may differ depending on the area of the inner Gulf of Thailand.

Sediment samples were collected at 9 points in total, divided into 3 transect lines across the inner Gulf of Thailand. First line was located in the western coastal line including station GT-W3 that is located away from Hua Hin Sub-district, Hua Hin District, Prachuap Khiri Khan for about 1.3 km, GT-W2 that is located away from Hat Chao

Samran Sub-district, Mueang Phetchaburi District, Phetchaburi for about 1.5 km, and GT-W1 that is located away from Ban Laem Sub-district, Ban Laem District, Phetchaburi for about 7.4 km (western zone). Second line was located in middle of the inner Gulf of Thailand including station GT-M1 that is located away from Laem Chabang Sub-district, Sriracha District and Bang Lamung District, Chon Buri for about 37.0 km, GT-M3 that is approximately 43.0 km away from Hua Hin Sub-district, Hua Hin District, Prachuap Khiri Khan, and GT-M2 that is located 42.0 km away from Bang Kao Sub-district, Cha-Am District, Phetchaburi. Third line was located in eastern areas including station GT-E1 that is located away from Sriracha Sub-district, Sriracha District, Chonburi for about 1.5 km, GT-E2 that is located away from Na Chom Thien Sub-district, Sattahip District, Chon Buri for about 3.5 km, and GT-E3 that is located away from Sattahip Sub-district, Sattahip District, Chon Buri for about 4.5 km. (Figure 3.1).

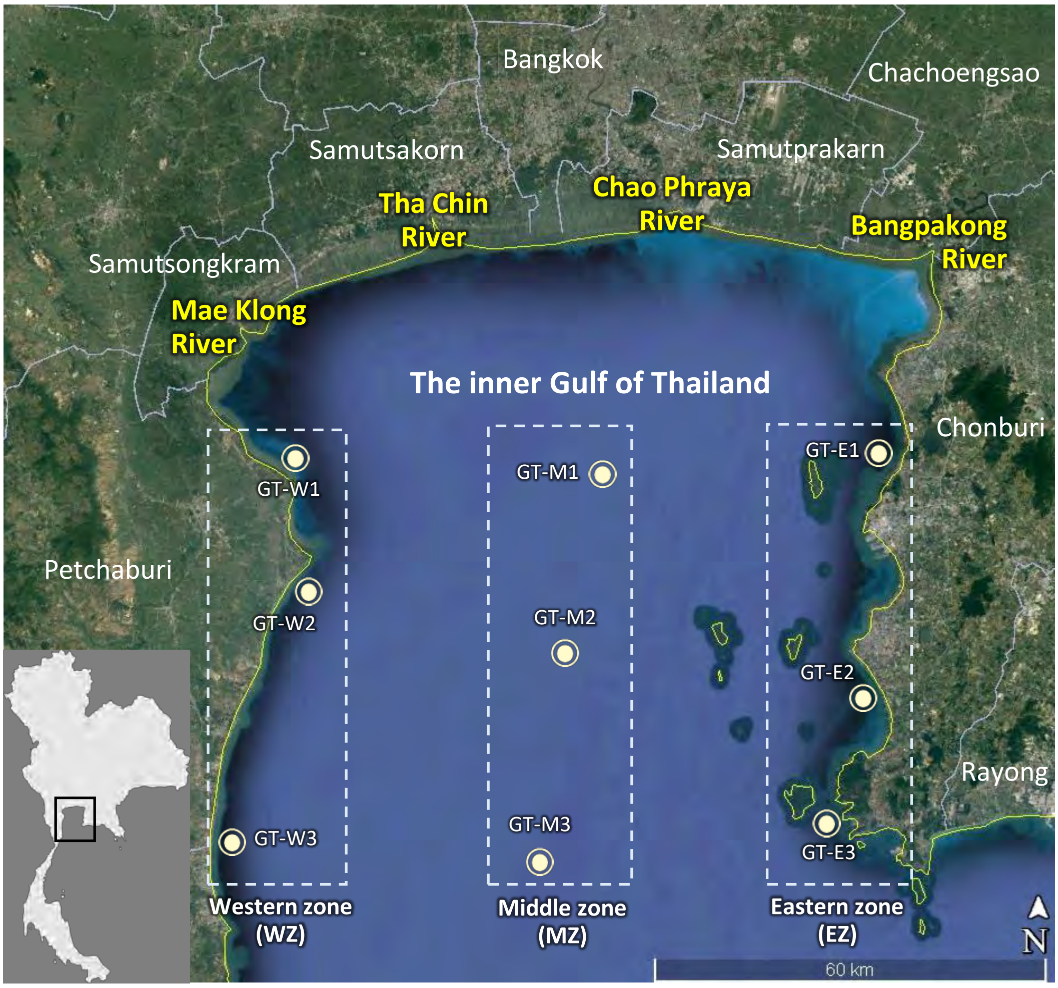

Figure 3.1 Study areas and sampling points, where were established in different areas of the inner Gulf of Thailand, where were western long line (GT-W1−3), middle long line (GT-M1−3) and eastern long line (GT-E1−3)

3.2 Research Materials

3.2.1 Laboratory instruments

1) Digital balance 2 digits (Mettler Toledo, ML1602/01, Switzerland)
2) Digital balance 5 digits (Mettler Toledo, MS204S, Switzerland)
3) Drying oven (Memmert, 600, Germany)
4) Stereo microscope 30X magnification (Shodensha, Trinocular Stereomicroscope NSZ405J3, Japan)
5) Laboratory mixer (Humboldt, H-4260.5F, United State)

3.2.2 Laboratory equipment

1) Filter paper (Whatman, No.42, UK)
2) Glass beakers
3) ASTM test sieves
4) Standard Metal Forceps
5) Distilled water bottle
6) Metal spatula
7) Watch glass
8) Laboratory hot plate
9) Separation unit
10) Retort stand
11) Spring clamp
12) Aluminum foil
13) 4-mL glass vials

3.2.3 Chemical substances

1) Iron(II) sulfate
2) 30% Hydrogen peroxide
3) Sodium chloride
4) Calgon 5%

3.3 Sampling Methods

Sediment samples were collected along the margin of the inner Gulf of Thailand by using the 15x15 cm Ekman grab sampler and the middle of the inner Gulf of Thailand by using the 20x20 cm smith McIntyre grab sampler. When the sediments were collected, the excess sediments were sliced off. Then put the sediment samples into a Ziplock plastic bag (precisely identified) and collect it in a cooling box at 4 °C for laboratory analysis.

3.4 Laboratory Analysis

3.4.1 Quantitative analysis

1) Weight the foil and put 1 gram of wet sediment sample into the foil.
2) Dried in an oven at 105 °C for one day or until sample dries.
3) Weight the dried sample and calculate the water content by use the following formula:

$$Water\ content\ (\%) = \frac{Wet\ sediment\ (g) - Dry\ sediment\ (g)}{Wet\ sediment\ (g)} \times 100$$

4) Weight the 100 grams of wet sediment in the beaker and put the beaker in an oven at 60 °C until sample dries.
5) Add 100-200 ml of Calgon 5% into the beaker and mixed by laboratory mixer for 30 minutes.
6) Sieved the sediment sample using ASTM test sieves into three sizes including 4000 to 1000 µm, 1000 to 300 µm and 300 to 100 µm.
7) Dried the sample in an oven at 60 °C until sample dryness. For high volume of sediment samples, added the NaCl solution with a density 1.17 g/cm^3 into the beaker and pour the sample into the density separator for density separation.
8) Leave it overnight. Microplastics and some small particles in the sample were floated and other sediments were settled down.
9) Drain settled sediments out of density separator for quality control test. Collected microplastics and other floated particles were transferred into the beaker. Then, dried the sample in an oven at 60 °C until sample dryness.
10) Added HCl to remove contaminated $CaCO_3$. Next, heated the beaker at 75 °C about 15 minutes using hotplate to remove excess chloride.
11) Added 20 ml of $FeSO_4$ and 20 ml of H_2O_2 into the beaker to remove organic materials from microplastics. Mixed by stir bar (150 rpm) at room temperature.

12) Heated the beaker at 75 °C for 30 minutes. Then, 12 grams of NaCl were added to increase the density of aqueous solution to 5 M NaCl and stop heating. Next, mixed the solution by stir bar (150 rpm) for 10 minutes.
13) Transfer the sample to the separation unit and leave it until there are not settled sediments. After that, microplastics are filtered from the solution by using the 12 µm polycarbonate Whatman nucleopore track-etch membrane.
14) Used a stereomicroscope to identify color, shape and the amount of microplastic (NOAA, 2015).

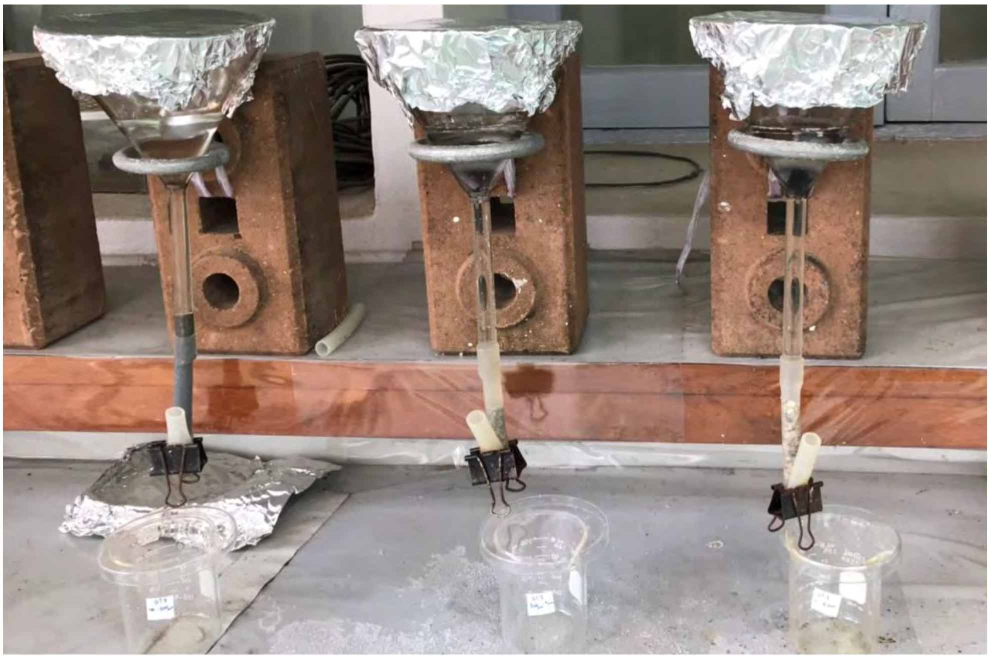

Figure 3.2 Density separation for high-volume of sediment samples

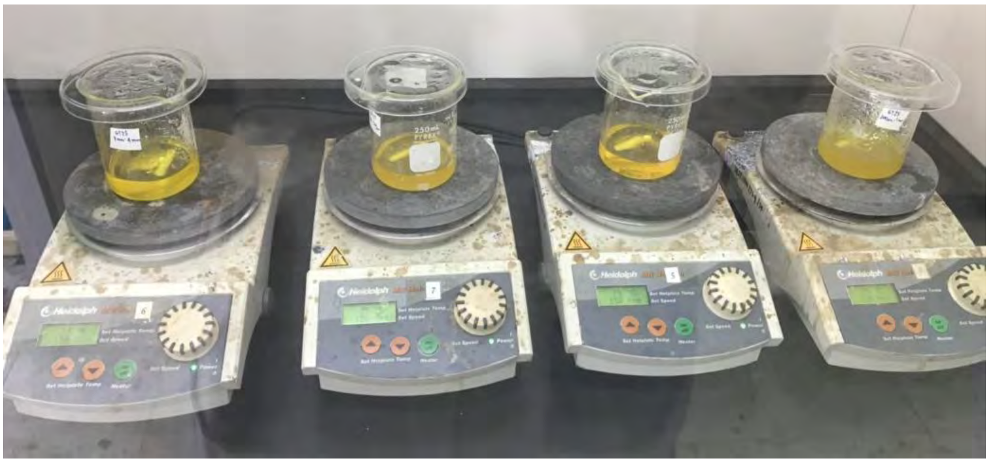

Figure 3.3 Wet Peroxide Oxidation (WPO)

Figure 3.4 Density separation using separation unit

3.4.2 The identification of shapes and colors

For plastics smaller than 5 mm in size, further sorting is undertaken based on their appearance. Microplastics should be sorted into their appropriate type, based on their size, shape and basic composition, by referring to Table 3.1. The next step requires that all pieces of microplastic are given an individual color by visual decision.

Table 3.1 Categorization of microplastics based on morphology

Shape	Abbreviation	Definition
Pellet	PL	A small spherical piece of plastic less than 5 mm to 1 µm in diameter
Fragment	FR	An irregular shaped piece of plastic less than 5 mm to 1 µm in size along its longest dimension
Fiber	FB	A strand or filament of plastic less than 5 mm to 1 µm in size along its longest dimension
Film	FI	A thin sheet or membrane-like piece of plastic less than 5 mm to 1 µm in size along its longest dimension
Foam	FM	A piece of sponge, foam, or foam-like plastic material less than 5 mm to 1 µm in size along its longest dimension

Source: Crawford and Quinn (2017)

3.5 Data Analysis

3.5.1 Potential differences in microplastic abundance between 3 zones of the inner Gulf of Thailand were evaluated using one-way ANOVA and Tukey's HSD (Honestly Significant Difference).

3.5.2 Contamination status of microplastics in three zones of the inner Gulf of Thailand were analyzed with stereomicroscope examination and gravimetric method. The data are calculated using Microsoft Excel and SPSS. Reporting units are in the form of pieces/kg dry sediment and mg/kg dry sediment.

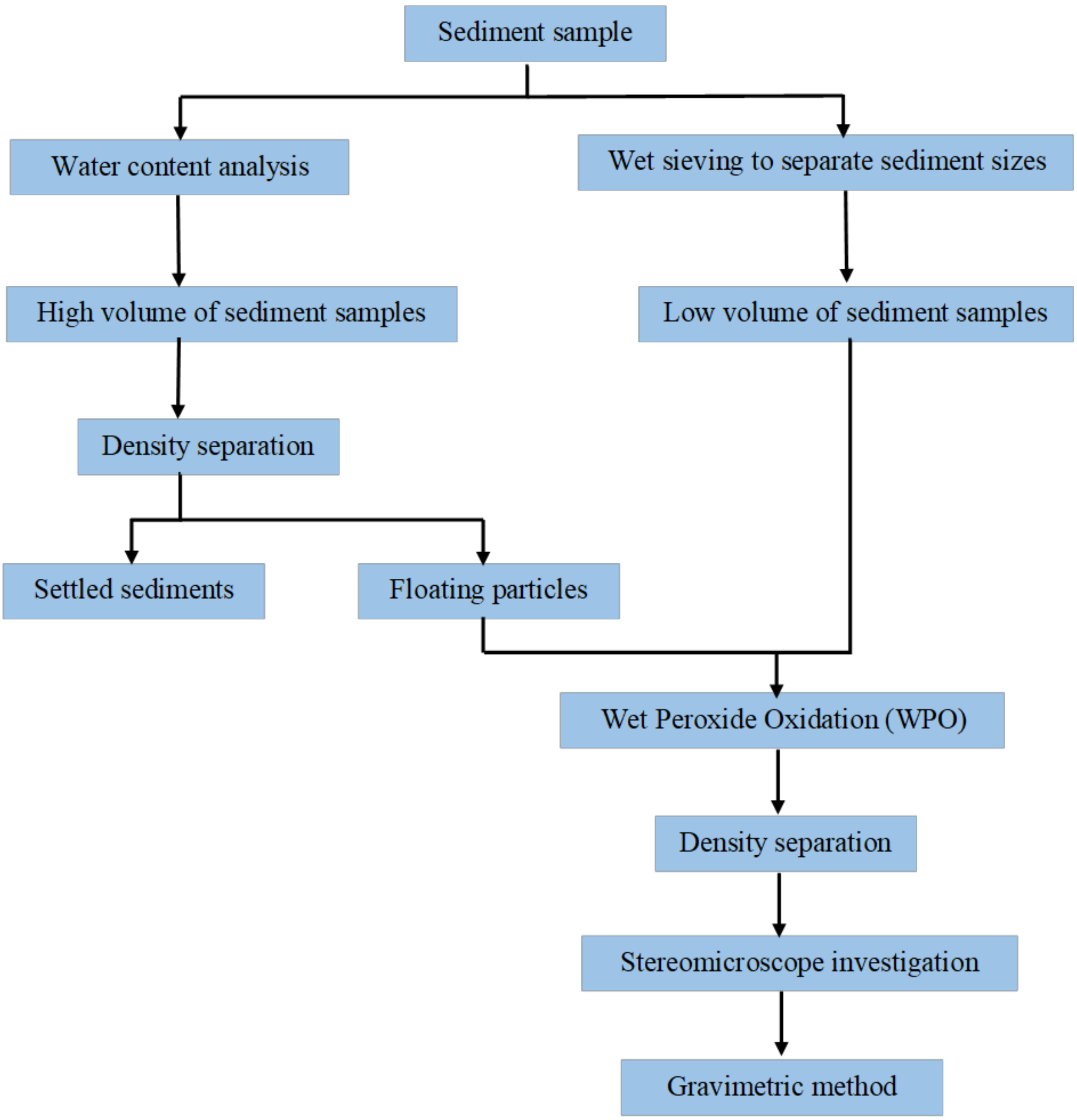

Figure 3.5 Diagram of samples preparation and surface sediment analysis including microplastic extraction and quantity identification

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overviews of Microplastic Analysis

Microplastic analysis of surface sediment at 9 sampling points in 3 zones in the inner Gulf of Thailand showed that microplastics were contaminated in all sediment samples (Table 4.1). As a result, microplastics in 3 different sizes of sediment samples (4000-1000, 1000-300 and 300-100 μm) were found all shapes (pellet, fragment, fiber, film, foam) in each sediment samples. Also, the middle zone is discovered microplastics with all colors including red, blue, green, brown, black, white, transparent, and others in the sediment samples. On the other hand, the western zone and the eastern zone is discovered microplastics with all colors accepted green and others. These results indicated that there are various sizes, shapes, and colors of microplastics, which were contaminated in different surface sediments of the inner Gulf of Thailand. As a result, it is possible to say that there are many sources of plastic waste, which were caused the contamination of marine debris and microplastics in the inner Gulf of Thailand.

Table 4.1 Overall detection of microplastics analysis in the present study

Abundance/ Characteristics	Categories	The inner Gulf of Thailand		
		Western zone	Middle zone	Eastern zone
Total concentration	mg/kg SDW	✓	✓	✓
	pieces/kg SDW	✓	✓	✓
Size composition	4000-1000 μm	✓	✓	✓
	1000-300 μm	✓	✓	✓
	300-100 μm	✓	✓	✓
Shape composition	Pellet	✓	✓	✓
	Fragment	✓	✓	✓
	Fiber	✓	✓	✓
	Film	✓	✓	✓
	Foam	✓	✓	✓
Color composition	Red	✓	✓	✓
	Blue	✓	✓	✓
	Green	×	✓	×
	Brown	✓	✓	✓
	Black	✓	✓	✓
	White	✓	✓	✓
	Transparent	✓	✓	✓
	Others	×	✓	×

4.2 Total Microplastic Concentration

The results from the microscopic analysis were showed in Figure 4.1. Total microplastics of the western zone is in range of 1201 to 5678 pieces/kg SDW with the average 3598±2255 pieces/kg SDW, the middle zone is in range of 670 to 1787 pieces/kg SDW with the average 1412±643 pieces/kg SDW, and the eastern zone is in range of 991 to 5272 pieces/kg SDW with the average 2592±2335 pieces/kg SDW. The highest number of microplastics (5678 pieces/kg SDW) was observed at GT-W2 in the western zone. The lowest number of microplastics (670 pieces/kg SDW) was observed at GT-M1 in the middle zone (Figure 4.1). One-way ANOVA indicated no statistically significant difference in total microplastic abundance in term of pieces/kg SDW between 3 zones in the inner Gulf of Thailand (p=0.427) (Figure 4.2).

The results from the gravimetric analysis showed that total microplastics of the western zone is in range of 20.24 to 62.85 mg/kg SDW with the average 47.40±23.59 mg/kg SDW, the middle zone is in range of 17.02 to 22.60 mg/kg SDW with the average 20.67±3.17 mg/kg SDW, and the eastern zone is in range of 10.48 to 193.71 mg/kg SDW with the average 105.76±91.84 mg/kg SDW. The highest weight of microplastics (193.71 mg/kg SDW) was observed at GT-E1 in the eastern zone. The lowest weight of microplastics (10.5 mg/kg SDW) was observed at GT-E3 in the eastern zone (Figure 4.1). One-way ANOVA indicated no statistically significant difference in total microplastic abundance in term of mg/kg SDW between 3 zones in the inner Gulf of Thailand (p=0.230) (Figure 4.2).

The total microplastic abundance between western, middle and eastern zones of the inner Gulf of Thailand was no statistically significant difference. This result indicated that the tides in the inner Gulf of Thailand were affected the distribution of microplastics by causing the microplastic to spread throughout the inner Gulf of Thailand. In addition, discarded plastic from areas with a lot of human activity were still need time to decompose into secondary microplastics, causing the currents to blow away these plastics from the source and spread throughout the inner Gulf of Thailand. Another reason was that the sampling points were not far enough apart to separate the source of microplastics. Therefore, microplastics from many sources were accumulated in the surface sediment around the inner Gulf of Thailand. However, when the consideration was made in individual site, different concentrations were found in western and eastern zones for unit of pieces/kg SWD and in eastern zone for unit of mg/kg SWD (Figure 4.1). Additionally, Pearson analysis between unit of pieces/kg SDW and mg/kg SDW was not significant correlated (*p*>0.05), which was indicated that number and weight of microplastics cannot use in single unit for total microplastics in the inner Gulf of Thailand (Figure 4.3).

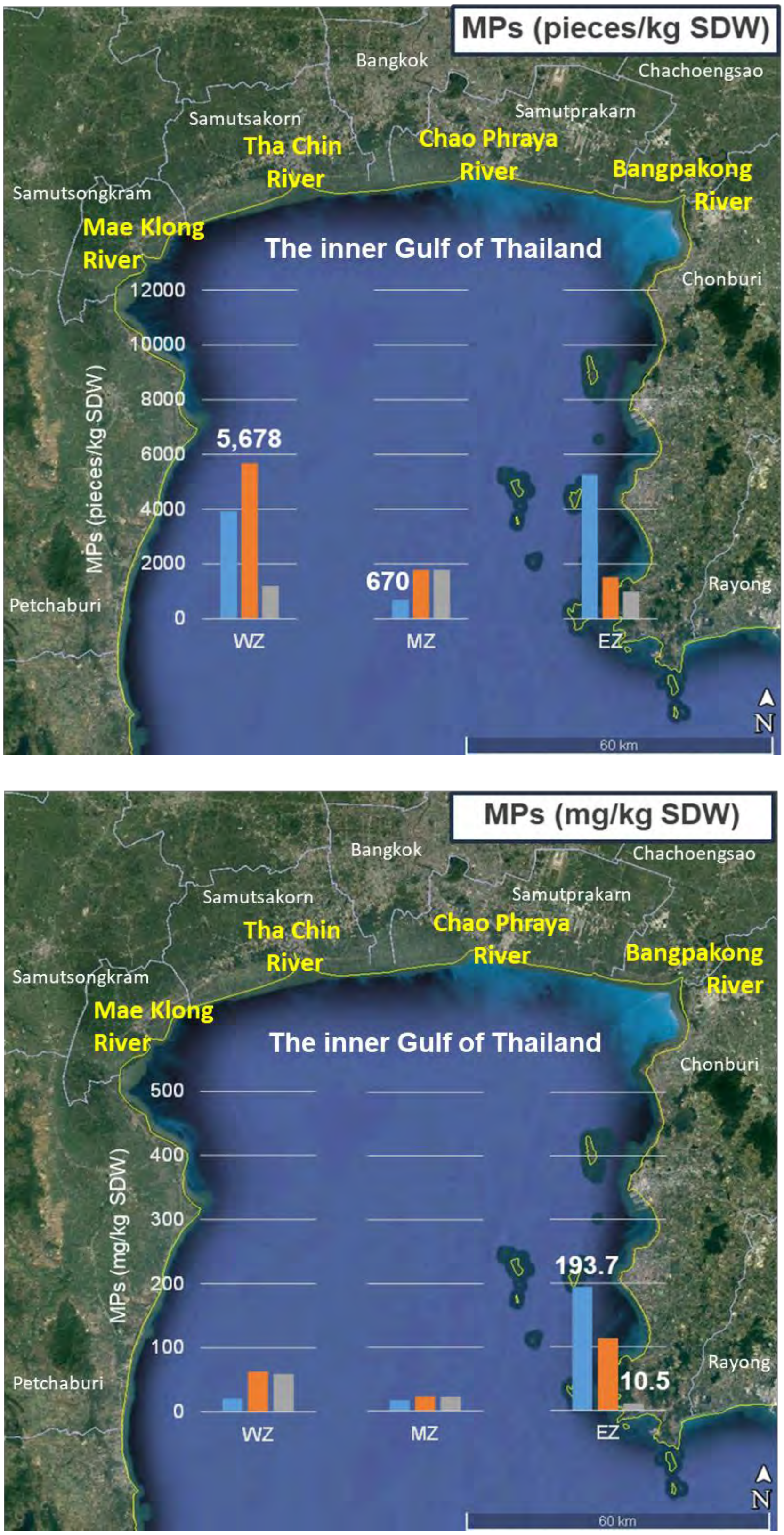

Figure 4.1 Total concentrations of microplastic contamination in different surface sediments of western zone (WZ), middle zone (MZ) and eastern zone (EZ) of the inner Gulf of Thailand

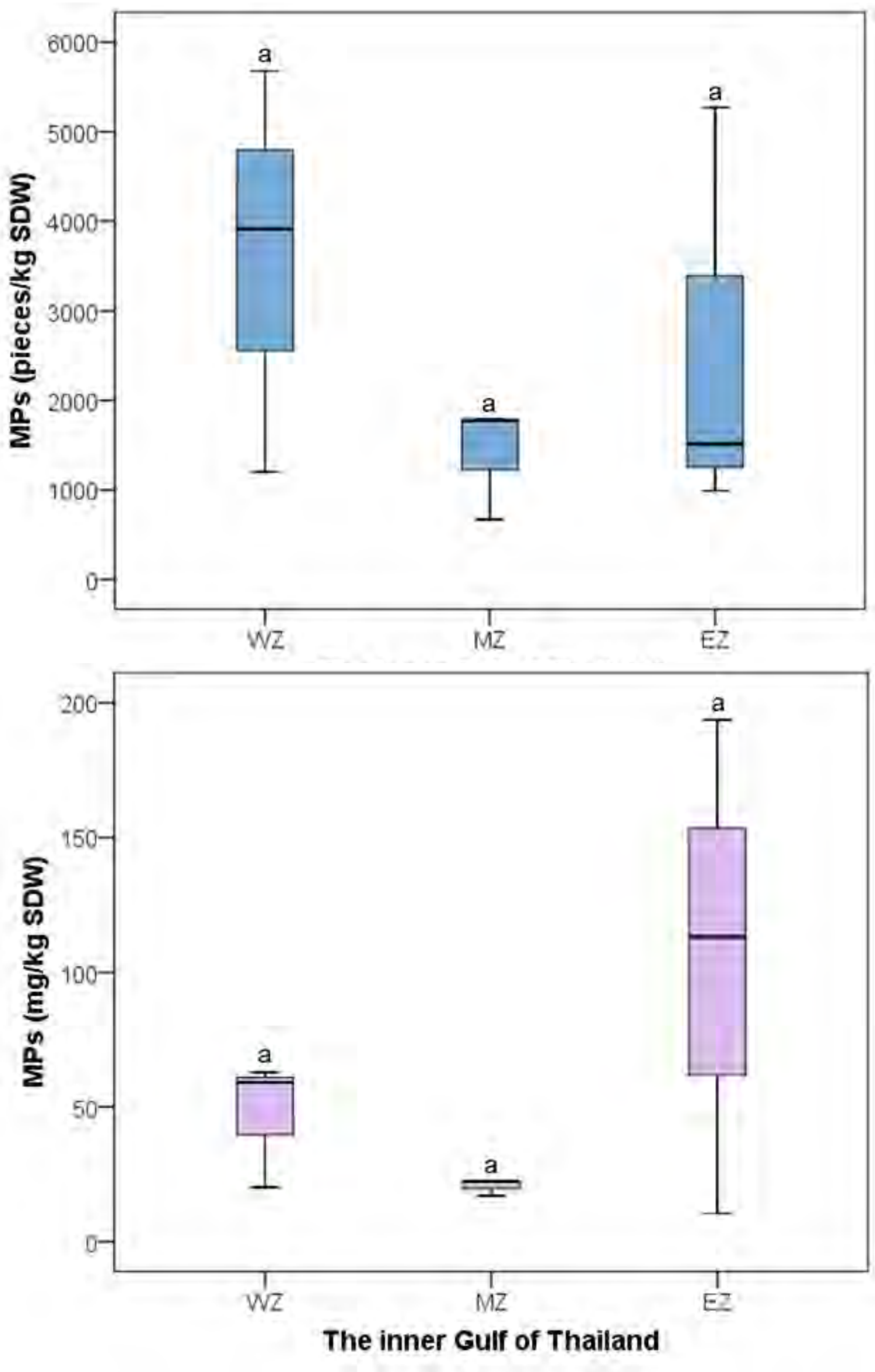

Figure 4.2 The statistical comparisons of microplastic contamination in different surface sediments of western zone (WZ), middle zone (MZ) and eastern zone (EZ) of the inner Gulf of Thailand

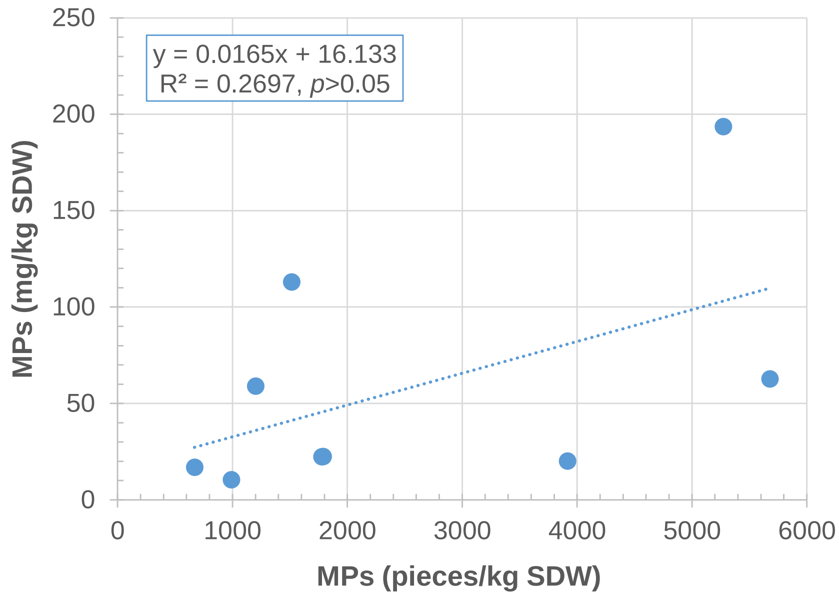

Figure 4.3 Pearson correlation analysis of total microplastics in pieces/kg SDW and mg/kg SDW of the inner Gulf of Thailand

4.3 Microplastic Sizes

Size composition analysis of microplastics was demonstrated in Figure 4.5. The result found that all zones in the inner Gulf of Thailand including western zone, middle zone, and eastern zone have various sizes of microplastic (4000-1000, 1000-300, 300-100 µm) contaminated in the surface sediment. The most microplastics size that discovered in the western zone and the eastern zone were 300-100 µm (48 and 51%), followed by 1000-300 µm (38 and 42%) and 4000-1000 µm (14 and 8%), respectively. While the most microplastics size that discovered in the middle zone was 1000-300 µm (34%), followed by 4000-1000 µm (34%) and 300-100 µm (32%), respectively. Most sizes of microplastic found in surface sediment are small size (300-100 µm). As a result, the small particles have a lot of surface area so, it can indicate that there is more toxicity and marine organisms can exposure through ingestion easier than larger particles (GESAMP, 2015). Comparison of microplastic size in global surface sediment shows that the surface sediments collected from Sishili Bay, China and coastal sediments of Jakarta Bay, Indonesia has the most abundance microplastic in size of <500 µm (Zhang et al., 2019) and 100-500 µm (Anggresia et al., 2017) respectively. In addition, sediment cores collected in Japan, Thailand, Malaysia, and South Africa have the most number of microplastics in size range of 315–1000 µm which in this study is measured microplastics between 315 µm and 5000 µm (Matsuguma et al., 2017). Therefore, most microplastic size in the inner Gulf of Thailand has the same trend as other areas. This indicated that the number of microplastics will increase when the size becomes smaller.

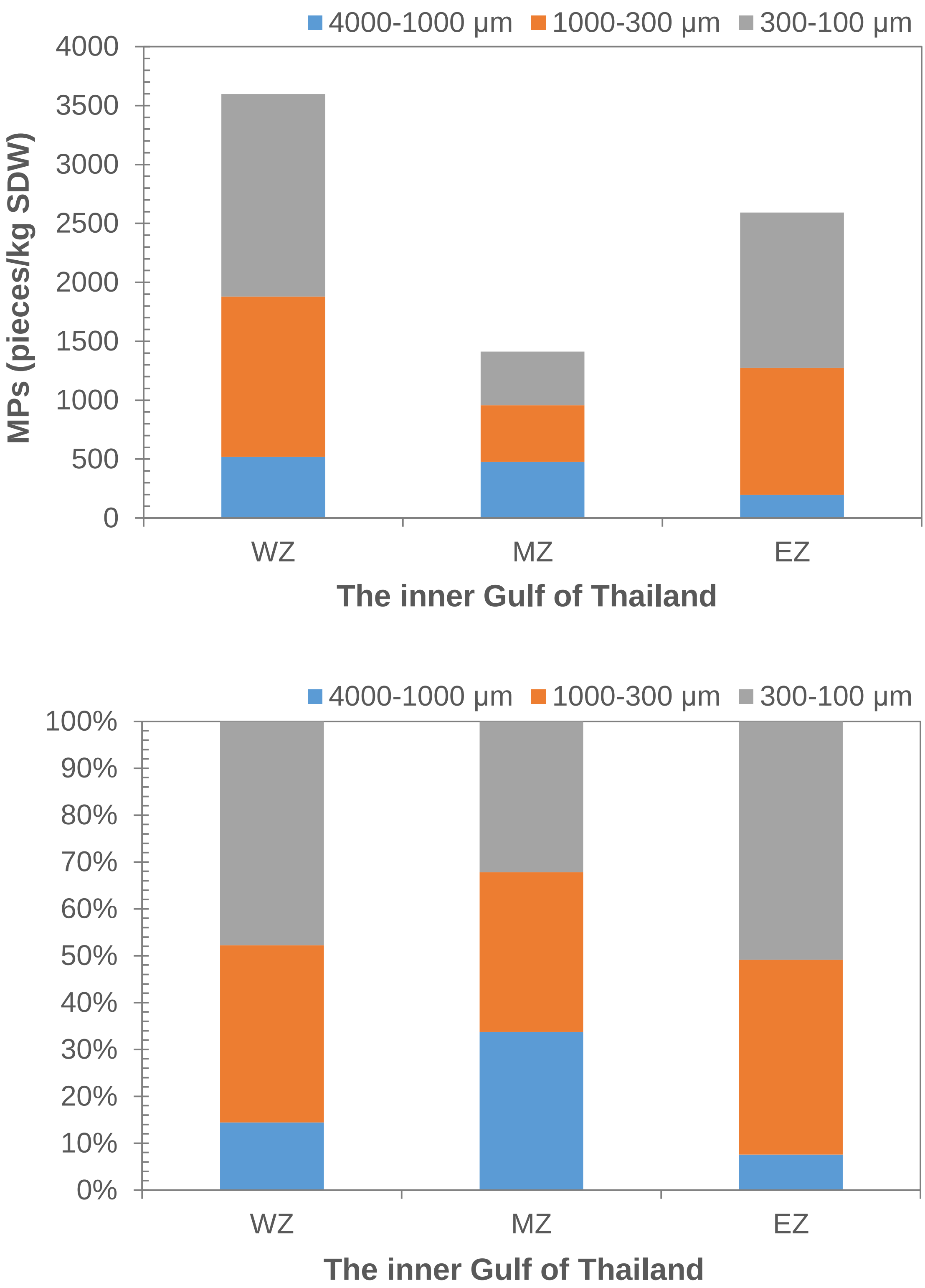

Figure 4.4 Size compositions of microplastics in different surface sediments of the inner Gulf of Thailand

4.4 Microplastic Shapes

The result showed that 3 zones of the inner Gulf of Thailand have all shapes of microplastic (pellet, fragment, fiber, film, foam) contaminated in the surface sediments (Figure 4.6). In terms of spatial distribution, microplastic shapes in the western zone of the inner Gulf of Thailand were found in arrangement of foam (1563 pieces, 43%), fragment (1541 pieces, 43%), pellet (199 pieces, 6%), fiber (182 pieces, 5%) and film (114 pieces, 3%). While, the middle zone were found in arrangement of fragment (825 pieces, 58%), foam (238 pieces, 17%), pellet (209 pieces, 15%), fiber (123 pieces, 9%) and film (17 pieces, 1%). Finally, the eastern zone were found in arrangement of fragment (1924 pieces, 74%), foam (342 pieces, 13%), pellet (193 pieces, 7%), fiber (73 pieces, 3%) and film (61 pieces, 2%). Which the same shape composition in 3 zones in the inner Gulf of Thailand. The most frequent microplastic shapes observed for all zones were fragments (56%), followed by foam (28%), pellet (8%), fiber (5%), and film (3%). Because fragment and foam are the most microplastic shapes we found so, nearly all of the microplastics identified were secondary microplastics (i.e. resulting from the breakdown of larger plastics). The possible primary microplastic discovered in this project is the pellet that may come from the manufacturing for used in cleansers and cosmetics. On the other hand, we cannot be excluded the possibility that fragments were polished to a form of pellet by physical forces. Fibers that can be detected may come from washing processes and sewage treatment which not completely removed. Then, it is emitted into the inner Gulf of Thailand through river runoff. Comparison of microplastic shape in global surface sediment shows that fiber was the majority shape of microplastics in Sishili Bay in China (Zhang et al., 2019), the Irish Continental Shelf (Martin et al., 2017), the northern Bering and Chukchi Seas of the Arctic ocean (Mu et al., 2019). However, coastal sediment in Jakarta Bay of Indonesia (Anggresia et al., 2017) and marine sediments of Hong Kong (Tsang et al., 2017) has fragment as the most common shape. This is indicated that the shape of microplastic was varied by the area.

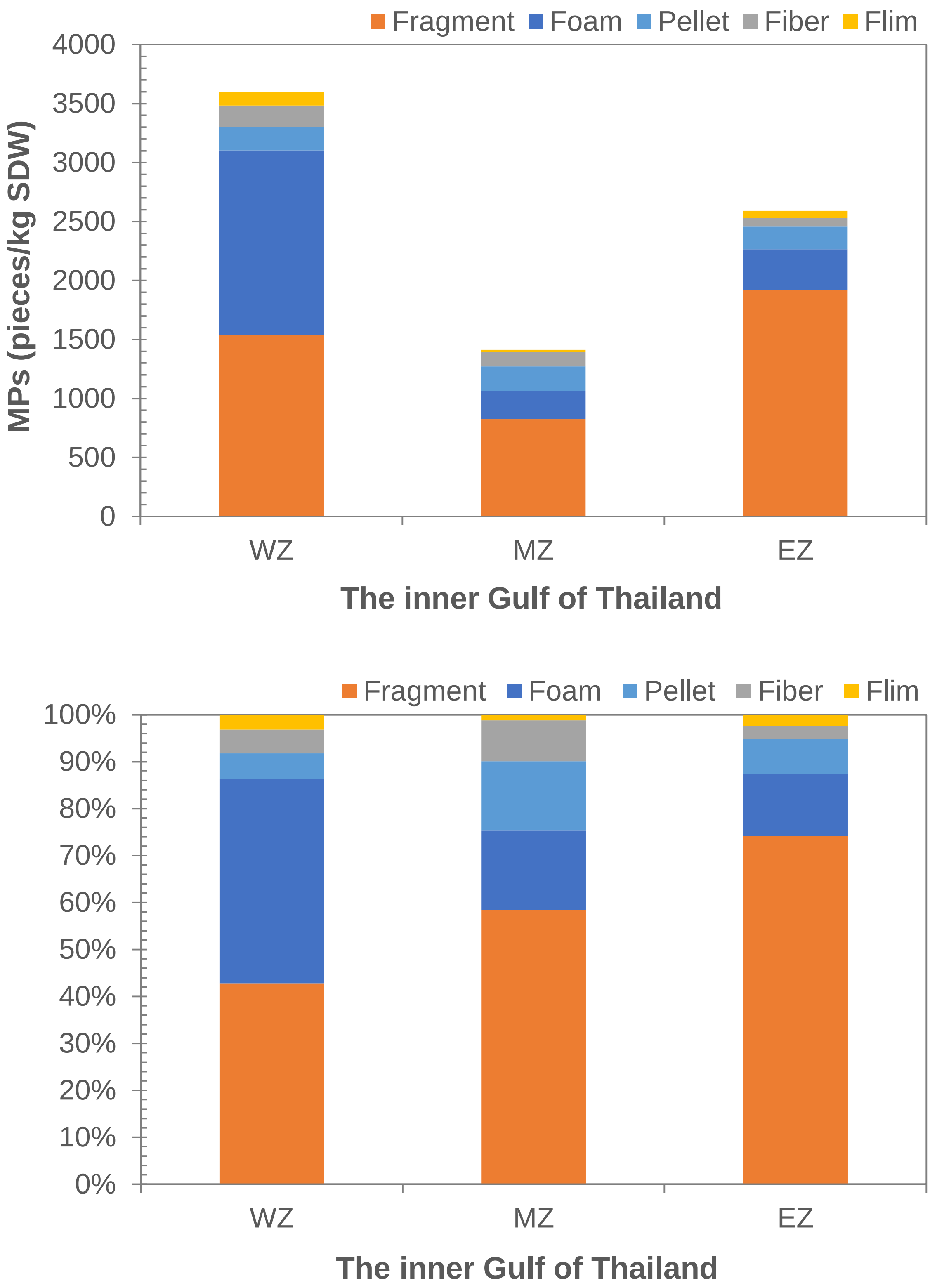

Figure 4.5 Shape compositions of microplastics in different surface sediments of the inner Gulf of Thailand

4.5 Microplastic Colors

Our finding shows that microplastics found in the middle zone have all colors (red, blue, green, brown, black, white, transparent, others). Only green microplastic was excluded in the western zone and eastern zone. In terms of spatial distribution, microplastic colors in the middle zone were found in arrangement of black (439 pieces, 31%), white (266 pieces, 19%), brown (250 pieces, 18%), transparent (229 pieces, 16%), blue (199 pieces, 14%), red (12 pieces, 0.85%), others (11 pieces, 0.81%) and green (5 pieces, 0.38%). While, the western zone were found in arrangement of brown (1199 pieces, 33%), white (1174 pieces, 32%), black (822 pieces, 23%), transparent (307 pieces, 8%), blue (106 pieces, 3%) and red (12 pieces, 0.33%). Finally, the eastern zone were found in arrangement of black (1457 pieces, 57%), brown (432 pieces, 17%), blue (381 pieces, 15%), white (186 pieces, 7%), transparent (84 pieces, 3%) and red (24 pieces, 0.95%). Color compositions of microplastics in surface sediments at 3 zones were black (36%), brown (25%), white (21%), blue (9%), transparent (8%), red, green, and others (1%). Brown microplastic is the most color found in the western zone. Black microplastic is also most commonly found in the middle zone and the eastern zone of the inner Gulf of Thailand. The microplastic color analysis shows that there were various colors of microplastic contamination in surface sediment. Which will result in a misunderstanding of the food intake of zooplankton or benthic organisms. A comparison of microplastic color in global surface sediment shows that transparent microplastics were the dominant color among all samples in Changsha, China (Wen et al., 2018). Red and blue were the most abundant colors in Bohai Bay, China (Wu et al., 2019). However, black and blue were the majority color of microplastics in bivalve at Chaolao beach and Kungwiman beach respectively (Tharamon et al., 2016), which are the same as the microplastics color found in the inner Gulf of Thailand.

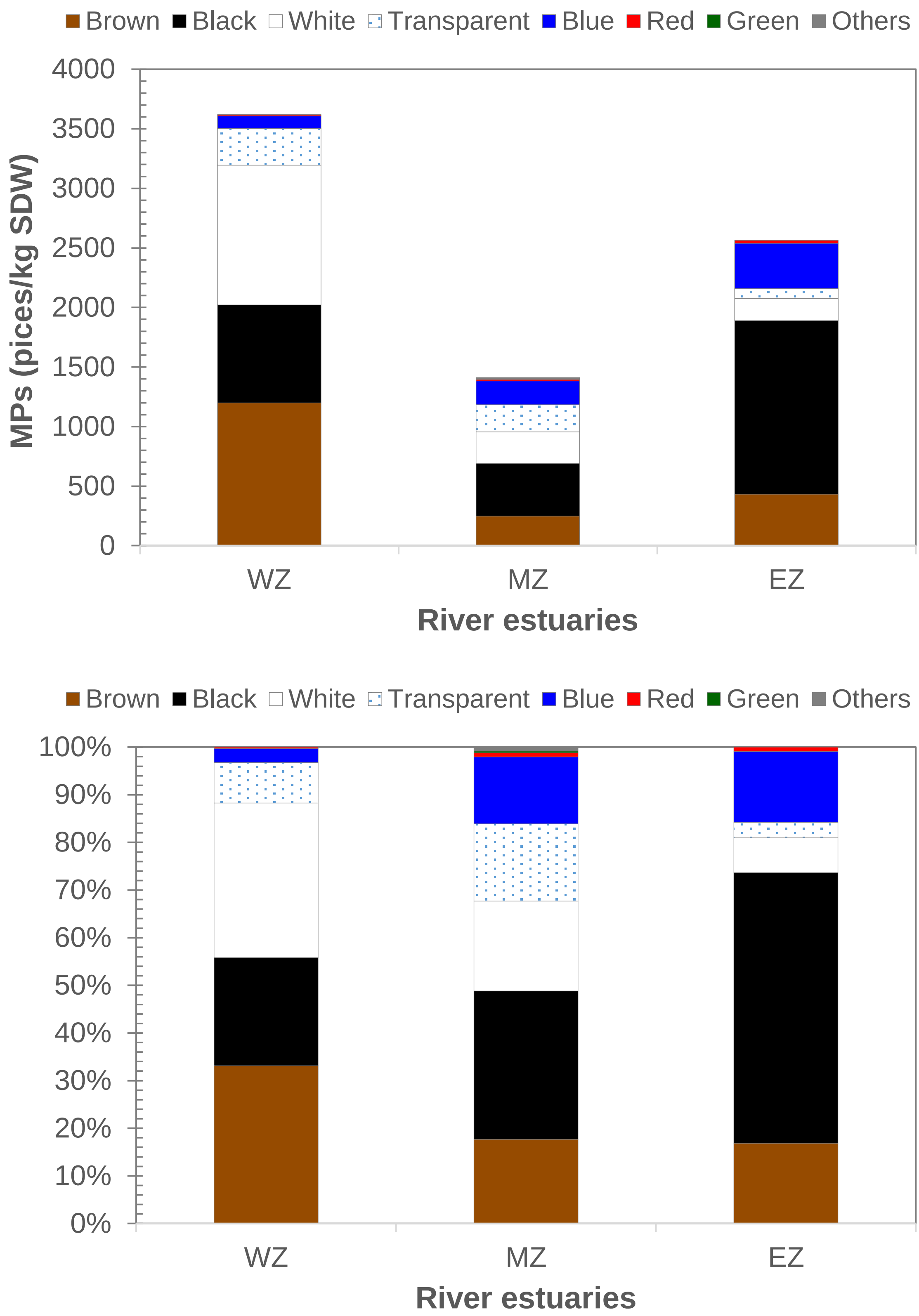

Figure 4.6 Color compositions of microplastics in different surface sediments of the inner Gulf of Thailand

4.6 Status of Microplastic Contamination in the inner Gulf of Thailand

Microplastic abundance in the inner Gulf of Thailand (2534±1907 pieces/kg) is similar to Tokyo Bay in Japan, the Straits of Johor in Malaysia, Durban Bay in South Africa (1845 to 5385 pieces/kg) and Xiangshan Bay in China (1739±2153 pieces/kg) than other areas. Xiangshan Bay is an advisable area for mariculture activities and Tokyo Bay is the largest industrialized area in Japan. Therefore, microplastics abundance in the inner Gulf of Thailand is about the same as Xiangshan Bay and Tokyo Bay because of level of human activities. Also, microplastic abundance in the downstream areas of Pluit and Ancol in Jakarta Bay in Indonesia (18,405 to 38,790 pieces/kg) is higher than the inner Gulf of Thailand because of surface sediment in the downstream areas is direct received microplastic from the source. In the inner Gulf of Thailand, microplastics concentration was 9, 10, and 6 times larger than Deep Bay, Tolo harbor, Tsing Yi, Victoria harbor in Hong Kong (49 to 279 pieces/kg), Hiroshima Bay in Japan (24 to 253 pieces/kg), and Sishili Bay, North Yellow Sea in China (499±370 pieces/kg) respectively. Microplastics in the Belgian coast, Maine and Florida in the USA, the Irish Continental Shelf, the northern Bering and Chukchi Seas were less than the inner Gulf of Thailand. This may be caused by a low level of human activities (Table 4.2). By comparing the data of this study with research around the world. Show that microplastic contamination in the surface sediment of the inner Gulf of Thailand was at a high level. It may cause impacts and risks to ecosystems in the inner Gulf of Thailand.

Table 4.2 Literature on the comparison of microplastic abundance in global surface sediment of coastal bays

Study area	Size (mm)	Concentration (piece/kg)	Reference
Jakarta Bay, Indonesia	<5	18,405 to 38,790	Anggresia et al. (2017)
Deep Bay, Hong Kong	0.01-4.7	49 to 279	Tsang et al. (2017)
Tokyo Bay, Japan	<5	1845 to 5385	Matsuguma et al. (2017)
Hiroshima Bay, Japan	0.3-5	24 to 253	Sagawa et al. (2018)
Sishili Bay, China	<5	499±370	Zhang et al. (2019)
Xiangshan Bay, China	<5	1739±2153	Chen et al. (2018)
Belgian coast	0.038-1	390	Claessens et al. (2011)
Irish Continental Shelf	0.25-5	62	Martin et al. (2017)
Northern Bering and Chukchi Seas	0.10-4.86	0-68	Mu et al. (2019)
Maine and Florida, USA	0.25-4	105-214	Graham and Thompson (2009)
Western zone	**0.1–4**	**3598±2255**	**This study**
Middle zone	**0.1–4**	**1412±643**	**This study**
Eastern zone	**0.1–4**	**2592±2335**	**This study**
The inner Gulf of Thailand	**0.1–4**	**2534±1907**	**This study**

CHAPTER 5

RESEARCH CONCLUSIONS

5.1. Conclusions

In this study, the abundance, characterization and distribution of microplastics in different sediments of the inner Gulf of Thailand were investigated. Four main conclusions were drawn from the results as follow:

1. The surface sediments of the inner Gulf of Thailand were ubiquitously contaminated with microplastics. The average concentration of microplastic was 2534±1907 pieces/kg SDW and the average weight of microplastic was 58±60 mg/kg SDW.
2. The most frequent microplastic shape observed was a fragment. Black, brown and white were dominant among the different colors of microplastics. The most sizes of microplastic found in surface sediment are small size (300-100 μm).
3. The total microplastic abundance between western, middle and eastern zones of the inner Gulf of Thailand was no statistically significant difference (p=0.230). And Pearson analysis between the unit of pieces/kg SDW and mg/kg SDW was not significantly correlated (p>0.05).
4. The results of this study have revealed that surface sediment is a potential sink and source of microplastics. And our data shows that there was necessary to develop plastic waste management and environmental policies in Thailand.

5.2 Research Suggestions

Firstly, the result indicates that plastic waste management and environmental policies in Thailand have not well enough to protect the aquatic environment in the inner Gulf of Thailand from microplastics pollution. Therefore, it is seriously important to develop plastic waste management in Thailand to reduce the effects of microplastic on the aquatic ecosystem in the inner Gulf of Thailand. Secondly, future microplastic research, the sampling points should be increased to cover the area of the plastic source in order to more clearly analyze the origin of the microplastics. In addition, the toxicity and risk of microplastic contamination to the organisms and ecosystems should be studied.

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