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ชื่อโครงการ Contamination and characterization of microplastics in different sediments of the river estuaries (the inner Gulf of Thailand)

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Title	**Contamination and characterization of microplastics in different sediments of the river estuaries (the inner Gulf of Thailand)**		
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หัวข้อ **การปนเปื้อนและลักษณะสมบัติของไมโครพลาสติกในตะกอนดินที่แตกต่างกันของปากแม่น้ำในอ่าวไทยตอนใน**

โดย นางสาวภัคจิรา ทัพจันทร์ รหัสประจำตัวนิสิต 5933335023

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บทคัดย่อ

ไมโครพลาสติกเป็นปัญหาสิ่งแวดล้อมที่ทั่วโลกกำลังกังวลต่อผลกระทบเชิงลบโดยเฉพาะอย่างยิ่งกับระบบนิเวศทางทะเล และสุขภาพของมนุษย์ อย่างไรก็ตามปริมาณการปนเปื้อนไมโครพลาสติกที่เกิดขึ้นในสิ่งแวดล้อมทางทะเลนั้นยังไม่เป็นที่ทราบแน่ชัด โดยเฉพาะอย่างยิ่งในพลวัตของตะกอนปากแม่น้ำ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อวิเคราะห์การปนเปื้อนและลักษณะสมบัติของไมโครพลาสติกในตะกอนดินที่แตกต่างกันบริเวณปากแม่น้ำแม่กลอง ท่าจีน เจ้าพระยา และบางปะกง ซึ่งเป็นแม่น้ำสายสำคัญของอ่าวไทยตอนใน ผลการศึกษาพบว่าปริมาณไมโครพลาสติกทั้งหมดบริเวณปากแม่น้ำแม่กลอง ท่าจีน เจ้าพระยา และบางปะกง มีค่าตั้งแต่ 981 ถึง 11,920 ชิ้นต่อกิโลกรัมของตะกอนดินแห้ง หรือเท่ากับ 18.06 ถึง 78.83 มิลลิกรัมต่อกิโลกรัมของตะกอนดินแห้ง ขนาดของไมโครพลาสติกที่พบมากที่สุดในปากแม่น้ำท่าจีน เจ้าพระยา และบางปะกง คือ ขนาด 300-100 ไมโครเมตร ในขณะที่ปากแม่น้ำแม่กลองพบขนาด 1,000-300 ไมโครเมตร มากที่สุด รูปร่างและสีของไมโครพลาสติกที่พบมากที่สุดในบริเวณปากแม่น้ำ คือ รูปร่างแตกหักร้อยละ 66 และสีน้ำตาลร้อยละ 43 จากผลการศึกษาครั้งนี้สามารถใช้เป็นฐานข้อมูลในการพัฒนานโยบายการจัดการขยะพลาสติกและนโยบายสิ่งแวดล้อมของประเทศไทยได้ อีกทั้งผลการศึกษายังแสดงให้เห็นถึงปริมาณ รูปแบบการกระจายตัวเชิงพื้นที่ และลักษณะสมบัติของไมโครพลาสติกในตะกอนดินปากแม่น้ำ และเป็นแหล่งข้อมูลที่เป็นระบบสำหรับการวิจัยเพิ่มเติมเกี่ยวกับไมโครพลาสติกในสิ่งแวดล้อมปากแม่น้ำทั่วโลก

คำสำคัญ: ไมโครพลาสติก
ตะกอนดินชั้นผิว
สถานะการปนเปื้อน
ปากแม่น้ำ

Project Title **Contamination and characterization of microplastics in different sediments of the river estuaries (the inner Gulf of Thailand)**

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Abstract

Microplastic pollution is an environmental problem worldwide, which concerns over the negative impacts of microplastics particularly on marine ecosystem and human health. However, the occurrence of microplastics in marine environment remains largely unknown, especially in dynamics of river estuarine sediments. The present study was aimed to analyze contamination and characterization of microplastics in different sediments of the The Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries of the inner Gulf of Thailand. The results showed that Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries were found total microplastic concentration ranges from 981 to 11,920 pieces/kg dry SDW or 18.06 to 78.83 mg/kg dry SDW. The most dominant size of microplastics was 300-100 μm in the Tha Chin, Chao Phraya and Bangpakong river estuaries while the Mae Klong river estuaries were found with size 1000-300 μm. While, the most commonly microplastic shapes and colors in river estuaries was fragment (66%) and brown (43%). This study, to be used as a database in order to develop a plastic waste management and environmental policies in Thailand. Moreover, this study reveals the abundance, spatial distribution patterns, and characteristics of microplastic pollutants in river estuarine sediments, and provides systematic data for further research on microplastics in estuarine environments worldwide.

Keywords: Microplastics
Surface sediment
Contamination status
River estuary

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CHAPTER 1

INTRODUCTION

1.1 Significance of the Research

Microplastics are described as plasticle sizes less than 5 mm, which can be divided into two types, including primary and secondary microplastics. Primary microplastic is a plastic produced by the industry's intention to manufacture such as microbeads as a component in various products such as cosmetics, toothpaste, facial foam, and personal care products. Which primary microplastic can flow into the sewage and contaminate the aquatic environment. Secondary microplastic is a large plastic such as plastic bags, water bottles, foam box, and mesh, etc. That is breakdown into small pieces by photodegradation process or mechanical force such as wave and wind (GESAMP, 2015). Microplastics are become worldwide serious problems in the global environments. Particularly, the pollution caused by microplastics in the rivers (Cheung et al., 2018) the estuaries (Zhao et al., 2015), the oceans (Isobe et al., 2017), deep sea (Van Cauwenberghe et al., 2013) and even in ice of the Arctic (Peeken et al., 2018) and Antarctic (Waller et al., 2017) is now well established. Moreover, microplastics could be contaminated in surface water (Eriksen et al., 2013), beaches (Stolte et al., 2015), marine sediment (Claessens et al., 2011) as well as in the marine biota (Cole et al., 2013).

According to contamination of microplastics, various effects have been occurred due to the small size and the large surface area of microplastics, which were adsorbed persistent organic pollutants (POPs), heavy metal, and other toxins (Crawford and Quinn, 2017). Also, microplastics are like an intermediary in carrying toxic chemicals to contaminate and spread throughout the environment. Organisms in the aquatic environment can ingest microplastics by ingestion, they can transfer toxic chemicals into tissues of the organism resulting in the toxic chemical is accumulated in trophic level and food web (GESAMP, 2015).

In Thailand, 4 main river estuaries including the Mae Klong, Tha Chin, Chao Phraya and Bangpakong rivers are play the important role in source of microplastics, subsequently are accumulated in water, sediment and organism of the inner Gulf of Thailand. Moreover, the sediments can be a potential sink and source of microplastics in the inner Gulf of Thailand. Some of these microplastics and toxic substances are accumulated in the sediment which is the final cumulative source. The other was swept into the Gulf of Thailand, which is the area that supported by 4 major rivers of Thailand. Therefore, there is a risk of microplastic contamination in both 4 main estuaries of Thailand. Therefore, it is necessary to find the quantity of microplastic in sediment in four main river estuaries. Status of microplastics contamination in 4 main river estuaries 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 Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries.

1.3 Scope of the Research

1) **Study areas:** the river estuaries are a source of microplastics due to river runoff, subsequently microplastics can be distributed and contaminated entire the inner Gulf of Thailand. Therefore, the Mae Klong, Tha Chin, Chao Phraya and Bang Pakong river estuaries are chosen to study on contamination and characteristics of microplastics.

2) **Sampling Points:** total 3 sampling points are chosen from each the river estuaries. The first point is located in riverine area, second and third points are located in western and eastern areas of river mouth.

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

4) **Quantitative analysis:** concentrations, sizes, shapes and colors of microplastic 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:** the comparisons of different concentrations, sizes and shapes of microplastic between the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries was analyzed using one-way ANOVA and Post Hoc multiple comparison.

1.4 Outcomes of the research

1) Improved understanding of contamination and characteristics of microplastics in surface sediment of the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries.
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

Plastics are synthesized to meet the need of humans. The synthesis of plastics consists of polymers that have long chains. The polymer is divided into a homopolymer and copolymer. A homopolymer is an identical type of monomer in long-chain, a copolymer is a combination of a dissimilar type of monomer. Also, polymers can be softened when heated and can be molded (Crawford and Quinn, 2017).

Based on both industrial and chemical parameters, leads to classifying plastics relative to their polymeric structure and underlying monomers. Polymers consist of a repetition of the same element, a monomer, in the form of chains. Plastics are then manufactured using these monomer chains. Differences in the physicochemical characteristics of these chains create differences in plastic polymer properties and end uses.

2.1.2 Plastic productions

Plastic was first invented in 1860 and in the 1920's it was developed for use in the industry. Plastic production grew significantly in 1940, which has become one of the fastest-growing industries in the world. The global plastic production continues to increase, with 359 million metric tons of plastic produced in 2018, an increase of 3.16% from the output in 2017 (Statista, 2020), with relatively low-cost plastic materials and ease of use. The plastic demand of consumers is increasing due to the increase in the world population. Also, plastics are found in many manufacturing industries such as textiles, healthcare, food products, telecommunications, transportation, construction, and consumer products (Gourmelon, 2015).

2.1.3 Types and uses of plastic

Plastics can be classified according to the chemical structure and the properties, which can be divided into 7 classification (Crawford and Quinn, 2017) as follows:

1) Polyethylene terephthalate (PET) is a polyester that is chemically stable and chemical formula is $(C_{10}H_8O_4)_n$ which PET can be used in a variety of applications, such as food containers, fibers in clothing, beverage bottles, film, and sheeting.
2) High-density polyethylene (HDPE), the chemical formula is $(C_2H_4)_n$ which HDPE is highly safe, so it can be used as a chemical container and can also be used for other uses, such as food containers, crates, buckets, tubing, pipes, and beverage bottles.
3) Polyvinyl chloride (PVC), the chemical formula is $(C_2H_3Cl)_n$ and there are 2 types of products: rigid and flexible. Rigid type is an extremely durable hard material with fire resistance properties such as containers, conduits, pipes, and window frames. Flexible plastics are formed by the addition of phthalate to soften the polymer, such as insulation, cables, garden hose, and shoes.
4) Low-density polyethylene (LDPE), the chemical formula is $(C_2H_4)_n$ which LDPE is tough and used to make products such as bags, squeezable bottles, food packaging, and pallet sheets.
5) Polypropylene (PP) is a polymer that is lightweight, durable, flexible, heat resistant and has a chemical formula is $(C_3H_6)_n$ which polypropylene can be made into various products such as bottle caps, packaging tape, pipes, and rope.
6) Polystyrene (PS) has a chemical formula of $(C_8H_8)_n$ which is transparent, hard, and brittle. Polystyrene can be used to make products such as disposable cutlery and tableware and rigid disposable food containers.
7) Polycarbonate is strong, stable and transparent, the chemical formula is $(C_{16}H_{18}O_5)_n$. Polycarbonate can be used in a variety of applications such as information storage discs, traffic light lenses, and eye protection.

2.1.4 Plastic wastes

The plastic produced is used for the benefit and in the production of plastic each year, some types of plastic are designed to not be reused approximately 33% makes it only used once and has to be discarded, which causes the amount of waste has increased (Crawford and Quinn, 2017). A lot of waste in the marine environment comes from the land up to 80% such as urban discharge, industries discharge, wastewater treatment and another 20% is a result of recreational activities of tourists, fishery and marine transportation. In 2010, plastic waste is released to enter the ocean by about 4.8 to 12.7

million tons (Crawford and Quinn, 2017). Which in Thailand ranks 6th in the world for dumping large quantities of plastic into the ocean (Jambeck et al., 2015). In addition, it is predicted that each year, as many as 8 million tons of plastic will be emitted into the ocean and it is expected that by 2050, plastic waste will increase to 32 million tons per year (Crawford and Quinn, 2017).

2.1.5 Marine debris and their impacts

Currently, there is not enough waste disposal to meet the demand for plastic, so much plastic waste can enter the ocean through the flow of wastewater and wind or blows along the stream (Clark et al., 2016). Plastic waste has a slow rate of degradation and floats on the surface in the ocean, causing marine debris, which can persist for many years and can accumulate in sediment (Clark et al., 2016). Therefore, marine debris is marine pollution that causes environmental concerns that affect living organisms and the ecosystem including the economy as well. Marine debris is associated with many chemicals and living organisms that receive chemicals such as dye. Which marine debris can absorb insoluble organic substances such as DDT, marine debris can float or sink into the seafloor, may be transported over long distances, including crossing or even adjacent oceans (Bergmann et al., 2015). As a result, chemicals in contaminated marine debris are contaminated with sediment. If these chemicals are released from the degradation of materials, sediment may be a source of chemical contamination in marine organisms and benthic animals by ingesting substances that may cause endocrine disruption or cell toxicity (Clark et al., 2016).

2.2 Microplastics

2.2.1 Definition of microplastic

A small piece of plastic any particle size of less than 5 mm in diameter is term a microplastic (GESAMP, 2015). Microplastics are solid particles that are formed both from the synthesis of polymers or caused by large plastic breakage. Microplastics can be found in a variety of products such as cosmetics, synthetic clothing, plastic bags, and bottles (Crawford amd Quinn, 2017).

2.2.2 Types of microplastic

Microplastic can be divided on their origin into two types including primary and secondary microplastics. Primary microplastics are deliberately manufactured as small plastic particles for used in various fields such as microbead in facial cleansers and cosmetics, polyester microplastic scrubbers in air blasting technology. Secondary microplastic is the small pieces of plastic that are a result of the fragmentation process of larger plastic such as fibers from the washing of synthetic clothing. Microplastic has many different sizes and shapes because of their origin and type, for example, fragment, fiber, and film (Shim et al., 2018).

2.2.3 Fate and transportation of microplastic

Macroplastic is swept into the marine environment by river runoff, urban discharge, etc. Macroplastic floats in the marine water are breakdown into microplastic by various degradation process. For example, photo-oxidative degradation by ultraviolet light (UV) and Thermal-oxidative degradation by temperature changing. Microplastic can aggregate with 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, On the other hand, a low density of microplastic can be swept to other areas by the ocean current (Figure 2.1) (Crawford and Quinn, 2017).

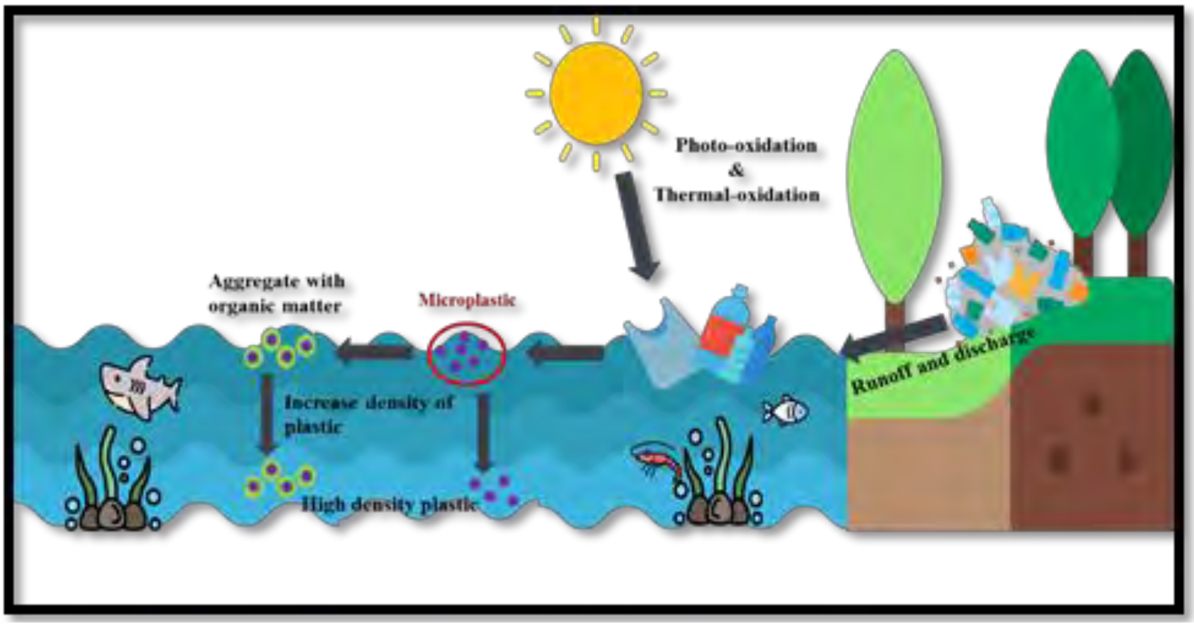


Figure 2.1 Fate and transportation of microplastic in the marine environment

2.2.4 Impacts of microplastic

Microplastics are widespread found in oceans around the world, and the accumulation of microplastics raises concerns about the impact of microplastics on marine organisms (Bergmann et al., 2015). Microplastics are exposed to chemicals by additive chemicals of polymers such as phthalates and alkylphenols. This additive is harmful to organisms that act as chemicals, interfere with the endocrine glands. In addition, microplastics able to adsorb organic chemicals such as PCBs, DDT which microplastics contain small particles that have a large surface area. Therefore, it is absorb a lot of chemical substance and become more toxic (GESAMP, 2015). Microplastics are ingested by marine organisms such as fish and invertebrates (lugworms, mussels, sea cucumbers, zooplankton) (GESAMP, 2015). Microplastics can transfer the toxic chemical to the food web; besides, they still impact on environmental values and economic for tourism and marine-related industries (Avio et al., 2017).

2.3 The Gulf of Thailand

The inner Gulf of Thailand is approximately 100 x 100 square kilometers, which is a large estuary that receives freshwater from 4 rivers, namely Mae Klong River, Tha Chin River, Chao Phraya River and the Bangpakong River. The top coast of the Gulf of Thailand is a mudflat in the tide-covered area that is covered by a 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 of marine animals that economically valuable.

2.3.1 The Mae Klong River

The Mae Klong river begins in Tenasserim Range and other mountains in Tak province. Then, flowing through Sangkhlaburi, Sisawat, Mueang district at Kanchanaburi province, which is called the Kwai Yai river. Next, combined with the Kwai Noi river at Mueang Kanchanaburi district and become the Mae Klong river. Finally, flows into the inner Gulf of Thailand at Mueang district, Samut Songkhram province. The total length of the river is approximately 140 kilometers. The Mae Klong river is an important source for agriculture, fisheries, communities, industrial, aquaculture, water transportation route and a tourist attraction.

2.3.2 The Tha Chin River

The Tha Chin river is separated from the Chao Phraya river at Mueang district, Uthai Thani province and Wat Sing district, Chainat province. Tha Chin river flows through Chainat, Suphanburi, Nakhon Pathom and Samut Sakhon province before flowing into the inner Gulf of Thailand at Mueang Samut Sakhon district, Samut Sakhon province. The total length of the river is approximately 325 kilometers. The Tha Chin

River is an important source for agriculture, communities, industrial, aquaculture, water transportation route and a tourist attraction.

2.3.3 The Chao Phraya River

The Chao Phraya river is a combination of Ping and Nan rivers at Nakhon Sawan province. Then, it flows down to Chainat province which passes the lower Chao Phraya Plain and divided into 2 main riverways. The river that flows to the west is the Tha Chin River. On the other hand, the river that flows to the east is also separated into 2 the riverway, the Noi River and the Lopburi river which is 135 kilometers and 87 kilometers long respectively. Finally, flow combination as the Chao Phraya river and flowing into the inner Gulf of Thailand at Samut Prakarn province. The total length of the river is approximately 380 kilometers. The Chao Phraya river flows through large urban communities and the river is used for agriculture, industry and fishery areas.

2.3.4 The Bangpakong River

The Bangpakong river originates from the Nakhon Nayok river flow combine with the Prachinburi river at Bang Nam Priao district, Chachoengsao province. Then, flowing south through the low plain in Bang Khla district and Mueang district, Chachoengsao province. Finally, the Bangpakong river flows into the inner Gulf of Thailand at Bang Pakong district, Chachoengsao province. The total length of the river is approximately 120 kilometers. Bang Pakong River is an essential economic driver in the central region that utilize both consumptions, agriculture, industry, and fishery.

2.4 Literature Reviews

2.4.1 International microplastic contaminations

Microplastics are pollution, which is now a problem that many countries around the world are interested in and concerned about the spread that may affect aquatic organisms., aquatic ecosystems, including the economy. Currently, the studies of the distribution and quantity of microplastics in many areas in which Di and Wang (2018) investigates microplastic in surface water and sediment from Three Gorges Reservoir, China. That found microplastic concentration ranges from 1,597 to 12,611 particles per m^3 and 25 to 300 particles per kg of wet weight respectively. The shape and color of the most common microplastics were fiber and transparent and the most common types of polymer were polystyrene (38.5%), followed by polypropylene (29.4%) and polyethylene (21%). Similarly, Microplastic concentration in the Yangtze Estuary with a range from 500 to 10,200 particles per m^3 (Zhao et al., 2014). Peng et al. (2017) investigated microplastic in the sediment of Changjiang Estuary which means concentration was 121±9 particles per kg of dry weight. The shape, color, and size of the most common microplastics are fiber (93%), transparent (42%) and small microplastics (<1 mm) (58%) which most common types of polymer were rayon, polyester, and acrylic. In addition, the main sources of microplastic in the Changjiang Estuary has been identified as washing clothes. Wu et al. (2019) reports the occurrence and distribution of microplastics of two typical estuaries in Bohai Bay, China. Haihe Estuary has the means concentration of microplastics in sediment was 216.1±92.1 particles per kg dry weight whereas Yondingxinhe Estuary has the means concentration of microplastics in sediment was 85.0±40.1 particles per kg dry weight. The shape of the most common is fiber and the color of microplastics was found transparent, white, green, blue, yellow, red and black. Also, the main sources of microplastic pollution in estuarine areas comes from human activity and river runoff. The occurrence of microplastics in the sediments of Vembanad Lake, India reported by Sruthy and Ramasamy (2017) found an abundance of microplastic with a range from 96 to 496 particles per m^2 and the most common types of polymer was Low density polyethylene. Gray et al. (2018) reports the occurrence of microplastic contamination in two South Carolina Estuaries consist of Charleston Harbor and Winyah Bay, found the average concentration in intertidal sediments of Charleston Harbor was 413.8±76.7 particles per m^2 and Winyah Bay was 221.0±25.6 particles per m^2. The most common microplastic shapes and colors was a fragment and black and were believed to come from tire wear particles. The abundance of microplastics in five different rivers of the Tibet Plateau found microplastics concentration ranged from 50 to 195 particles per kg in the sediment, fiber and transparent microplastics were found the high concentration. The types of polymers found were as follows: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (PA) (Jiang et al., 2019).

2.4.2 Microplastic contaminations in Thailand

The investigated the occurrence and distribution of microplastics in surface sediments of the Gulf of Thailand reported by Wang et al. (2020) found an abundance of microplastics ranged from 25.0 pieces per kg to 362.5 pieces per kg and the average was 150.4±86.2 pieces per kg dry weight and found small microplastics (0.5–1 mm) >70% of total microplastic. Also, the shape of the most common microplastics was fiber and the most common types of polymer were rayon (37%) and polyester (PES: 16%) which assumed that municipal sewage discharge was the main source of microplastics. Microplastics in the sediment core collected from the Gulf of Thailand found that abundance was 100 pieces per kg of dry sediment and layers of sediment depth 2-4 cm has a high abundance of microplastics was detected (Matsuguma et al., 2017). Kasamesiri and Thaimuangphol (2020) investigate microplastics ingested by eight fish species in the Chi River found microplastics at the mean concentration of 1.76±0.97 particles per fish in which omnivorous fish *Puntioplites proctozysron* was found the highest percentage of microplastic concentration about 86.7%. The most common size, color and shape of microplastics ingested by fish were over 0.5 mm (47.5%), blue (56.9%) and fiber (86.9%) respectively, they indicate major sources of microplastic contaminants in the Chi River was from fishing nets and fish cages. Thushari et al. (2017) investigates microplastic contamination in sessile and invertebrates: rock oyster (*Saccostrea forskalii*), a striped barnacle (*Balanus amphitrite*), and periwinkle (*Littoraria* sp.) from the intertidal zone on the 3 beaches including Angsila, Bangsaen and Samaesarn of the eastern coasts of Thailand. The abundance of microplastics in the invertebrates at rates of 0.2–0.6 counts/g which periwinkle samples collected from Bangsaen were not contaminated. The shape of microplastic that found were rod shaped and fragmented synthetic fibers also, colors that found were red, brown, blue, white and transparent.

CHAPTER 3

MATERIALS AND METHODS

The present study is supported from 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). Therefore, the surface sediment samples of this study were already collected. However, the remaining analysis is mainly part of this research.

3.1 Study Areas and Sampling Points

The Mae Klong, Tha Chin, Chao Phraya and Bangpakong rivers were carried excess sediment, nutrient and various pollutants runoff from land-based human activities. This phenomenal are considered serious threats to coastal and marine ecosystems by most conservation practitioners, resource managers, fishers, and other “downstream” resource users. Therefore, river estuaries are chosen to study on contamination status and characterization of microplastics. Because, the river estuaries are a source of microplastics due to river runoff, subsequently microplastics can be distributed and contaminated in the river mounts and entire the inner Gulf of Thailand.

Due to the rivers as a sources of microplastic contamination, sampling points were selected from the previous study (Faculty of Fisheries, 2019). Station MK-R, TC-R, CP-R and BK-R were selected as the results of river influences due to various activities and pollutants particularly microplastics. While, station MK-W, TC-W, CP-W and BK-W were selected because these stations were located in the western area of the river mounts. Finally, station MK-E, TC-E, CP-E and BK-E were selected because these stations were located in the eastern area of the river mounts. Both western and eastern stations are located in zone of sedimentation, which was relative high accumulation of pollutants in the surface sediment (Figure 3.1).

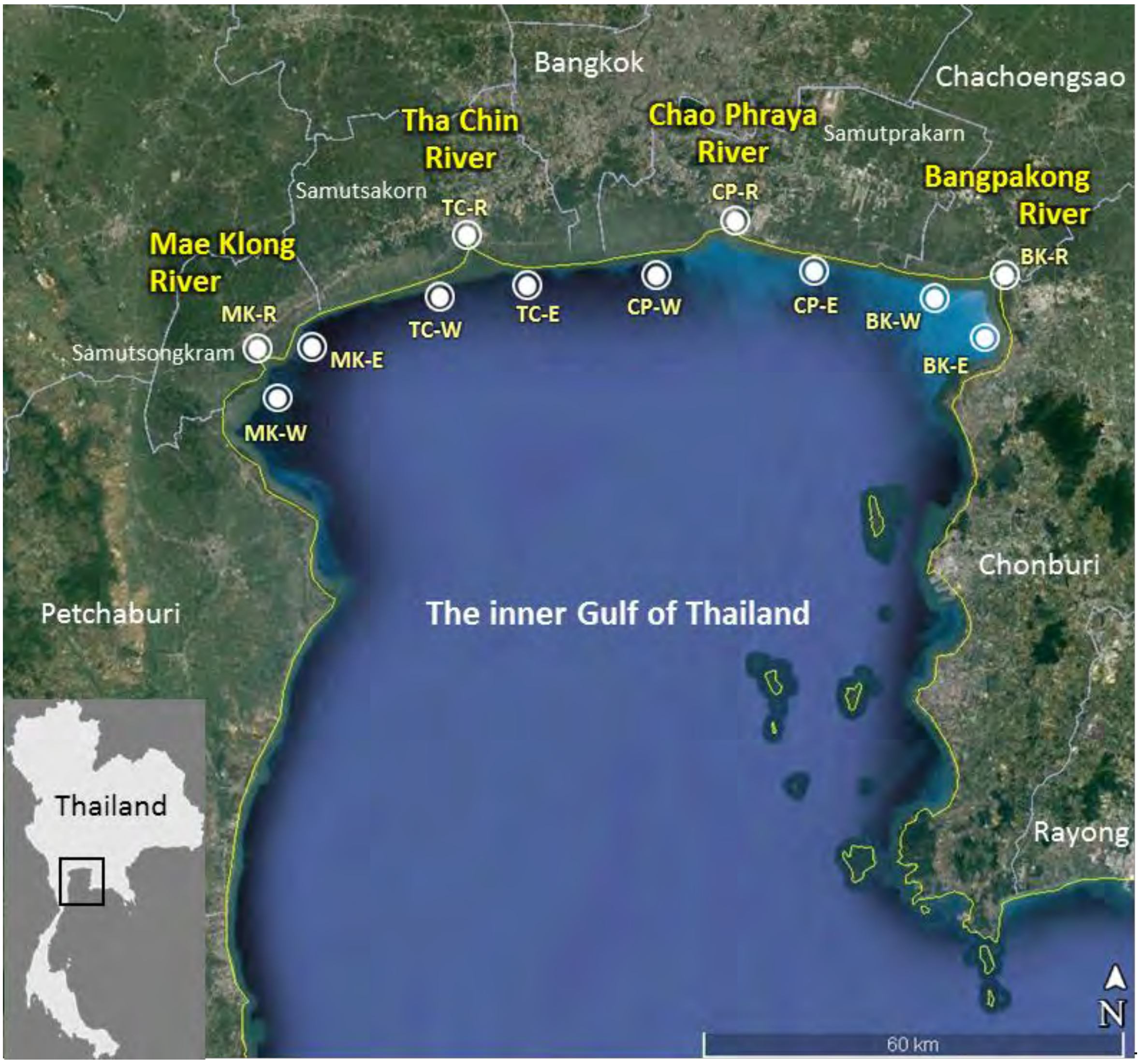


Figure 3.1 Study areas and sampling points, where were located in the Mae Klong (MK-W, R, E), Tha Chin (TC-W, R, E), Chao Phraya (CP-W, R, E), and Bangpakong (BK-W, R, E) river estuaries.

3.2 Research Materials

3.2.1 Laboratory instruments

1) Digital balance 5 digits (Mettler Toledo, MS204S, Switzerland)
2) Digital balance 2 digits (Mettler Toledo, ML1602/01, 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

The top layer of sediment was considered for microplastic contamination, which was sink and accumulated in this layer. Sediment samples were collected from all sampling points of the river estuaries using the Ekman grab sampler. Subsequently, surface sediments (0-5 cm) were sliced off using a wooden ladle. Then, sediment samples were put into a Ziplock plastic bag (precisely identified) and kept in a cooling box at 4 °C for later laboratory analysis.

3.4 Laboratory Analysis

3.4.1 Water content

Firstly, water content of sediment was determined from the weight loss after drying wet sediment at 105 ºC until constant weight (approximately 24 hour). Then, water content was calculated according to 3.1 equation.

$$\text{Water content (\%)} = \frac{\text{Wet sediment (g)} - \text{Dry sediment (g)}}{\text{Wet sediment (g)}} \times 100 \quad (3.1)$$

3.4.2 Microplastic analysis

1) Subsequently, the wet sediment was weighed about 100 g and transferred into the beaker, then dried in an oven at 60 to 80 °C.
2) After that, 100-200 ml of Calgon 5% are added to the beaker and the sample was blended thoroughly by laboratory mixer for 30 minutes.
3) Then, samples are separated into different sized by using ASTM test sieves for 4 mm to 1 mm sediment size and using dip net into the following sediment size: 1000 to 300 μm and 300 to 100 μm (Figure 3.2).
4) Secondly, the sample in each size was dried in an oven at 60 °C. If there is a high volume of sediment samples, the NaCl solution with a density 1.17 g/cm^3 was added. Then, put the sample into the separation unit and leave it overnight. In this phase, all sediments were settled, while the microplastics were floated above the NaCl solution. After that, settled sediment was drained from the separator. If there is a low volume of sediment sample, the sample was conducted as following next step.
5) $FeSO_4$, H_2O_2 and NaCl were added in collected microplastics, and then heated in order to remove organic material from microplastic surface.
6) After that, the sample was poured into the separation unit for collect the floating microplastics. Then, microplastics sample was filtered with dried membrane (polycarbonate membrane was dried in an oven at 60 °C until constant weight and then weighted the membrane).
7) Next, the membrane was dried at 60 °C and weighted for the calculation of microplastic concentration in unit of mg/kg sediment dry weight.

8) Finally, shapes and colors of microplastics were identified under the stereomicroscope for the calculation of microplastic concentration in unit of pieces/kg sediment dry weight.

3.4.3 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

Potential differences of microplastic concentrations, sizes, shapes and colors between Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries were evaluated using one-way ANOVA and Post Hoc multiple comparison.

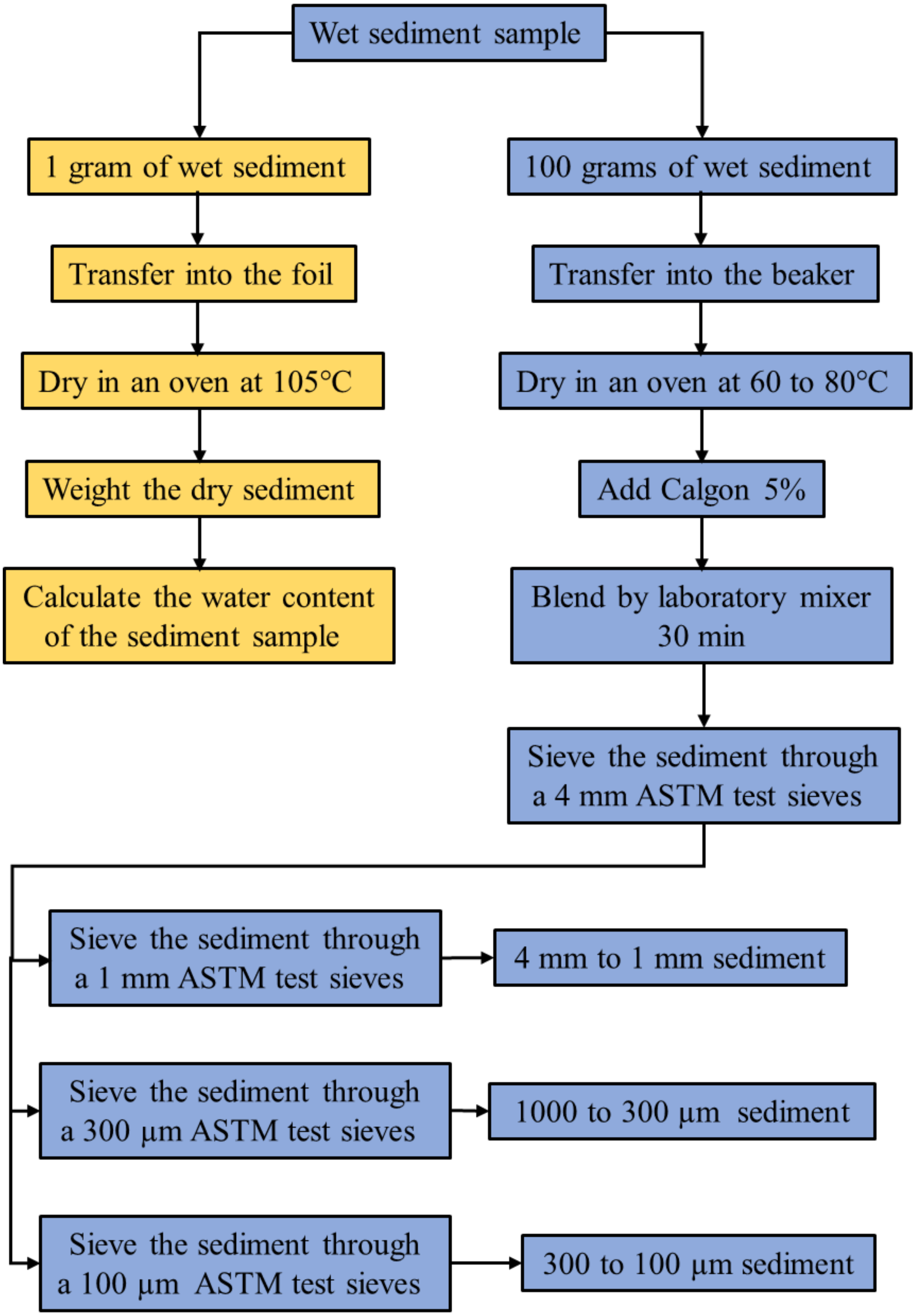


Figure 3.2 Diagram of water content examination and sample preparation.

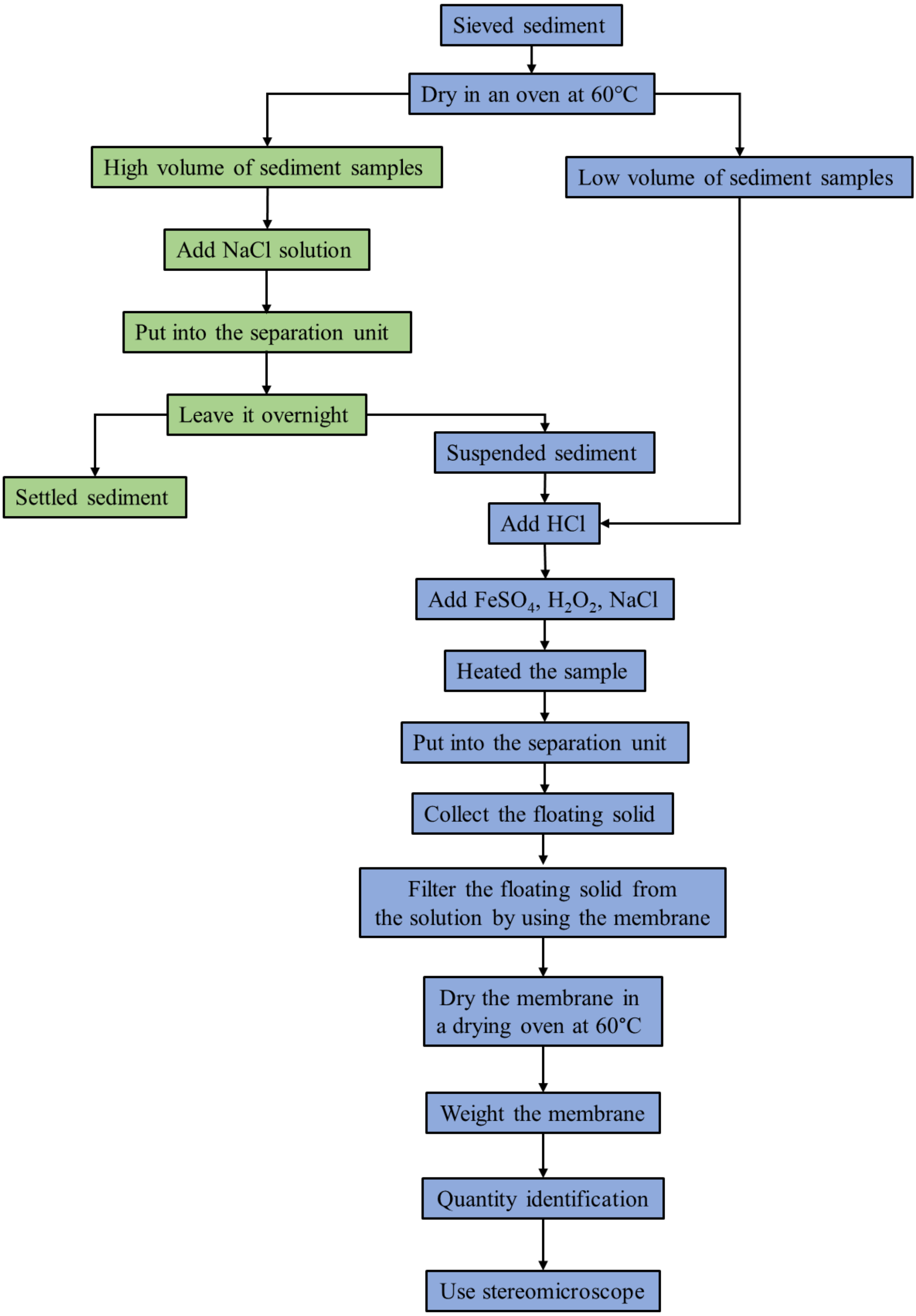


Figure 3.3 Diagram of surface sediment analysis including microplastic extraction and quantity identification.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overviews of Microplastic Analysis

Microplastics analysis in different surface sediments of 4 main river estuaries of the inner Gulf of Thailand, which including the Mae Klong, Tha Chin, Chao Phraya and Bangpakong rivers were demonstrated that microplastics were contaminated in various concentrations (981-11,920 pieces/kg SDW), diverse sizes (4000-1000 μm, 1000-300 μm and 300-100 μm) and different shapes (pellet, fragment, fiber, film and foam) in all sampling sites (Table 4.1). For color identification including brown, black, white, transparent, blue, Green, red and others, the Chao Phraya river estuary was found all the colors, while the Mae Klong, Tha Chin river estuaries were found all the colors accepted green, while green and red were not found in the Bangpakong river estuary.

Table 4.1 Overall detection of microplastics analysis in the present study

Abundance/ characteristics	Categories	River estuaries			
		MK	TC	CP	BK
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	Brown	✓	✓	✓	✓
	Black	✓	✓	✓	✓
	White	✓	✓	✓	✓
	Transparent	✓	✓	✓	✓
	Blue	✓	✓	✓	✓
	Green	×	×	✓	×
	Red	✓	✓	✓	×
	Others	✓	✓	✓	✓

4.2 Total Microplastic Concentration

The results of study contamination and characterization of microplastics in different sediments of 4 main river estuaries from the microscopic analysis were found various concentrations of total microplastic with the ranges from 981 to 11,920 pieces/kg SDW. The highest concentration of total microplastic was found in riverine area (station CP-R) of the Chao Phraya river estuary. According to microplastic analysis, total concentration of the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries was in the range of 3,586-4,848, 1,159-7,403, 2,631-11,920 and 981-5,302 pieces/kg SDW, respectively (Figure 4.1). The average concentration of total microplastics was 4,022±715, 3,498±3,404, 6,119±5,061 and 3,815±2,456 pieces/kg SDW for the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries, respectively. However, mean comparison using one-way ANOVA indicated that no significant different was found between river estuaries ($p=0.765$) (Figure 4.2).

The concentration of total microplastic from the gravimetric analysis were also found various mass concentrations of total microplastic with the ranges from 18.1 to 436.7 mg/kg SDW. The highest concentration of total microplastic was also found in riverine area (station CP-R) of the Chao Phraya river estuary. According to microplastic analysis, total concentration of the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries was in the range of 39.15-69.57, 18.06-78.83, 79.93-436.70 and 35.04-70.81 mg/kg SDW, respectively (Figure 4.1). The average concentration of total microplastics was 49.52±17.37, 56.51±18.93, 284.36 ±184.00 and 50.99±30.70 mg/kg SDW for the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries, respectively. Although mean comparison using one-way ANOVA indicated that significant different was found between river estuaries ($p<0.05$), the Tukey HDS analysis showed no significant different between river estuaries (Figure 4.2).

The number and weight of microplastic particles, which could be separated from the sediments, strongly differed between 3 sampling sites, particularly in the Chao Phraya river estuary (Figure 4.1 and 4.2). This result indicated that strong heterogeneity distributions were occurred in the sediment samples of the Chao Phraya river estuary. Additionally, a good linear correlation ($r^2=0.4011$; $p<0.05$) of the total microplastic number and the total microplastic mass underlined the same tendencies of piece and weight of microplastic particles (Figure 4.3). This result showed that the numerical abundance and the mass fraction can possibly be used interchangeable to describe the microplastic pollution in sediments of the river estuaries in the inner Gulf of Thailand.

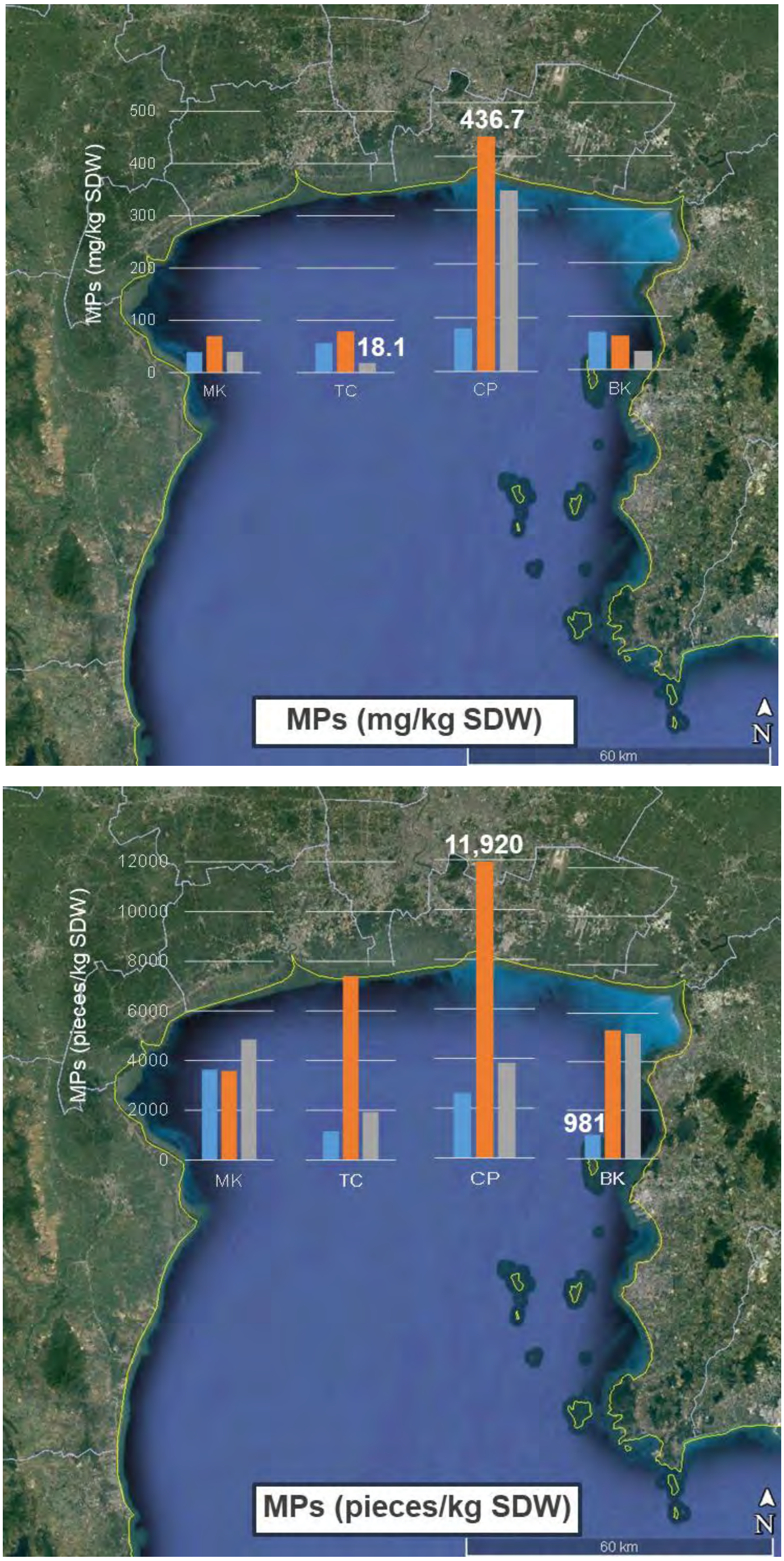


Figure 4.1 Total concentration of microplastics in surface sediment of the Mae Klong (MK), Tha Chin (TC), Chao Phraya (CP) and Bangpakong (BK) river estuaries

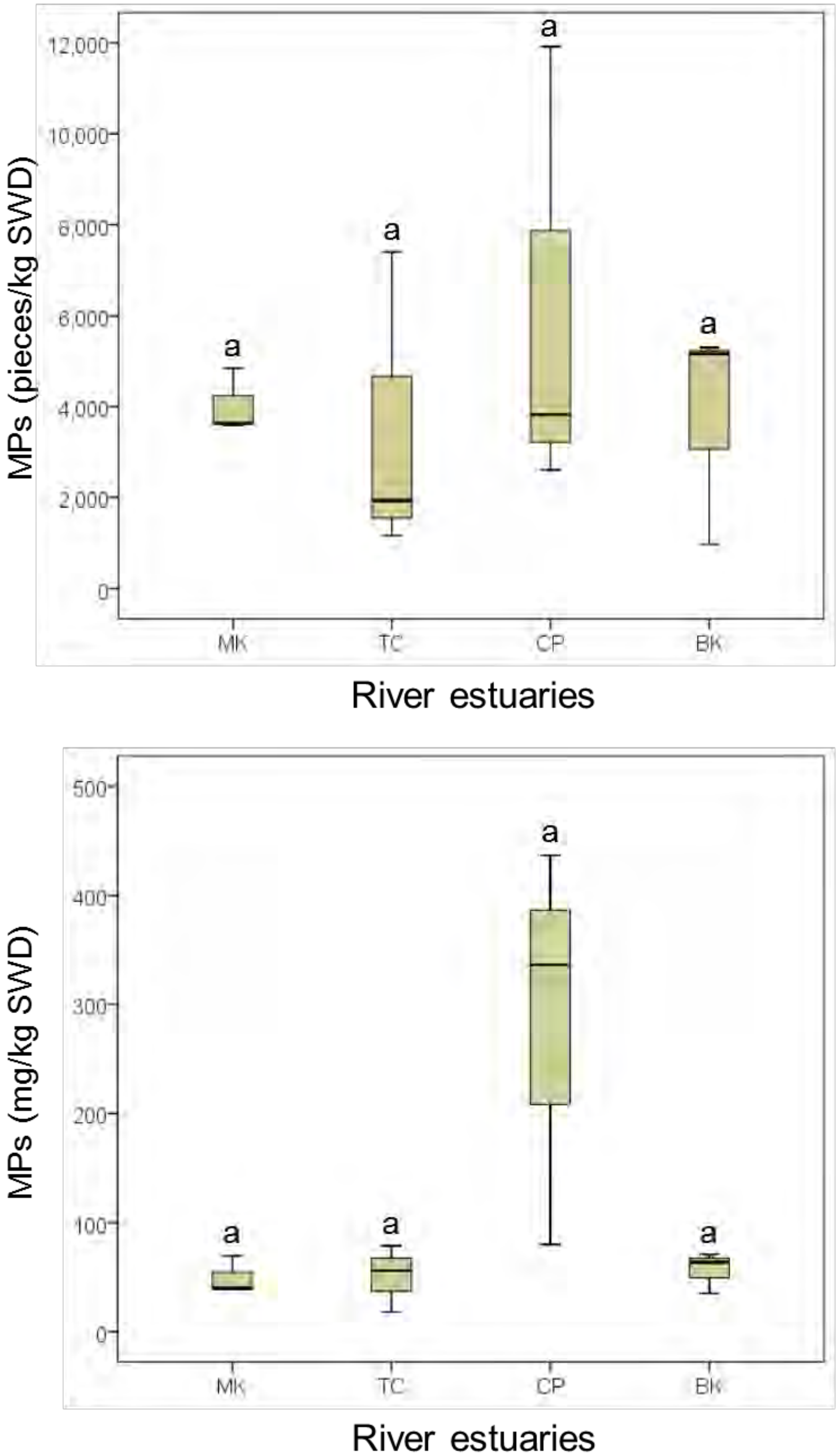


Figure 4.2 The statistical comparisons of microplastics in surface sediment of the Mae Klong (MK), Tha Chin (TC), Chao Phraya (CP) and Bangpakong (BK) river estuaries

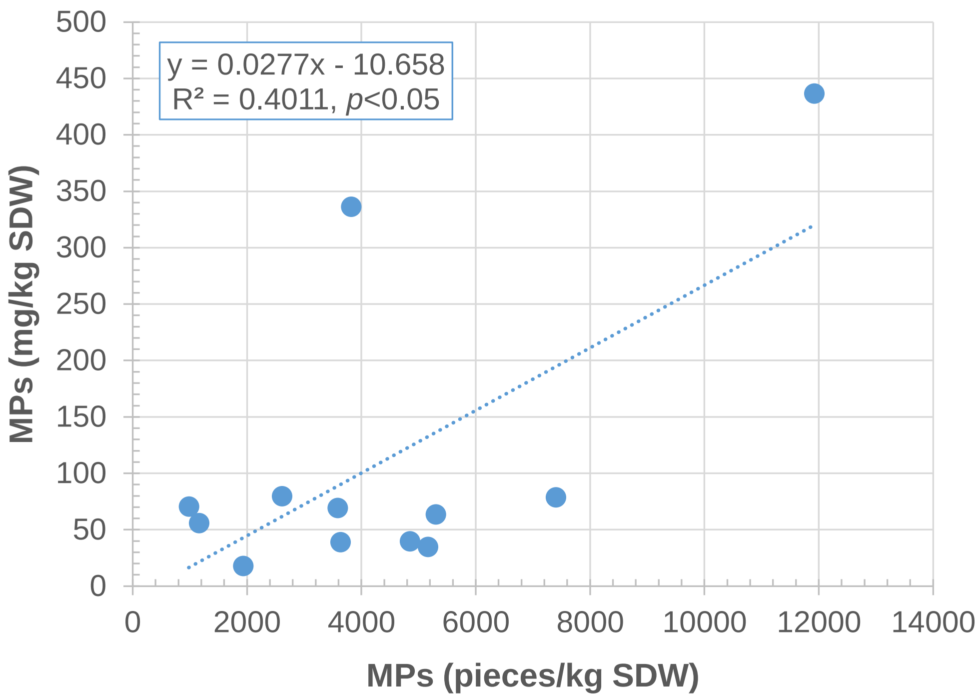


Figure 4.3 Pearson correlation analysis of total microplastics in pieces/kg SDW and mg/kg SDW of the river estuaries

4.3 Microplastic Sizes

Microplastics of the present study was separated into size of 4000-1000 µm, 1000-300 µm and 300-100 µm (Figure 4.4). As a result, size composition of microplastic contamination in surface sediment were found all sampling points, which size 300-100 µm (56, 47 and 59%) was the most dominant size follow by 1000-300 µm (24, 41 and 23%) and 4000-1000 µm (20, 12 and 18%) in the Tha Chin, Chao Phraya and Bangpakong river estuaries, respectively. While the Mae Klong river estuary was found the most common size with 1000-300 µm (52%) follow with in size 300-100 µm (41%) and 4000-1000 µm (7%), respectively. From observed have shown that the 4 main estuaries mostly contaminated with small microplastics, which indicates the ability to cause ecological risks from the absorption of various toxic substances such as POPs, heavy on the surface of the microplastic (Wu et al., 2019). Also, microplastic have low density able distribute to other areas (He et al., 2020) and able aggregate with organic matter result in accumulates in the sediment, which may affect aquatic organisms through ingestion, such as zooplankton, invertebrates, and fish which may receive toxic chemicals and transmitted through the food web (Vermeiren et al., 2016). Similarly, a microplastic investigated in sediment of Brisbane River, most commonly found that the size is less than 3 mm (He et al., 2020).

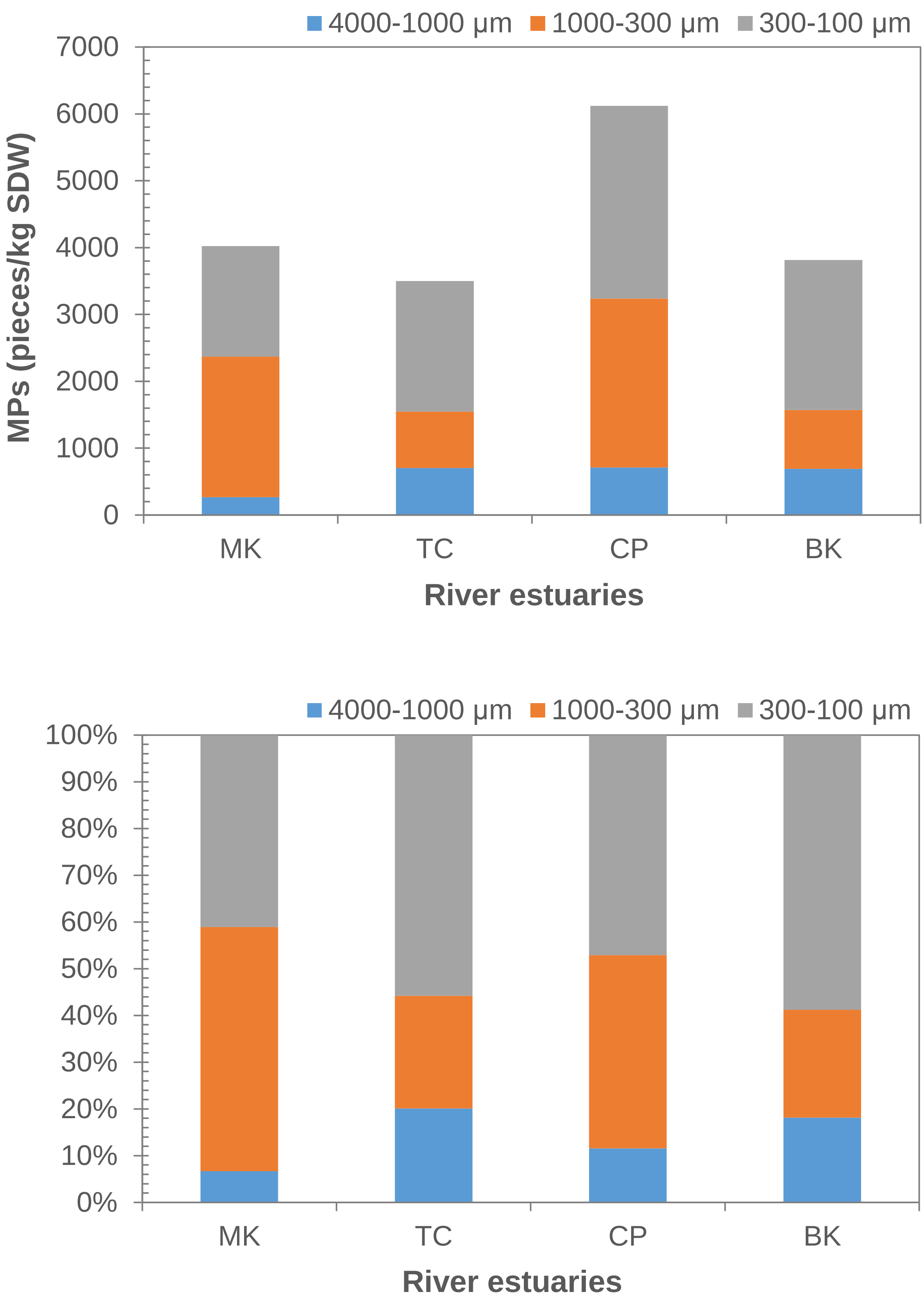


Figure 4.4 Size compositions of microplastics in surface sediment of the Mae Klong (MK), Tha Chin (TC), Chao Phraya (CP) and Bangpakong (BK) river estuaries

4.4 Microplastic Shapes

The result of shape composition of microplastic in 4 main river estuaries were classified in 5 shape as follow: pellet, fragment, fiber, film and foam (Figure 4.5). The shape composition of microplastic in the Mae Klong river estuary were found in arrangement of fragment (2,682 pieces, 67%), foam (704 pieces, 18%), film (275 pieces, 7%), pellet (204 pieces, 5%) and fiber (157 pieces, 4%), The Tha Chin river estuary were found in arrangement of fragment (2,385 pieces, 68%), film (483 pieces, 14%), fiber (342 pieces, 10%), foam (219 pieces, 6%) and pellet (69 pieces, 2%). While, the Chao Phraya river estuary were found in arrangement of fragment (4,596 pieces, 75%), foam (690 pieces, 11%), fiber (574 pieces, 9%), film (130 pieces, 2%) and pellet (129 pieces, 2%). Finally, the Bangpakong river estuary were found in arrangement of fragment (1,805 pieces, 47%), foam (1,113 pieces, 29%), fiber (488 pieces, 13%), film (376 pieces, 10%) and pellet (33 pieces, 1%).

The most common shape of microplastic of 4 main river estuaries were fragment (66%) follow by foam (16%), fiber (9%), film (7%) and pellet (2%). Microplastic shape, of the fragment was most commonly found in the Chao Phraya river estuary followed by Mae Klong river Tha Chin and Bangpakong river estuaries, respectively. From the observed of microplastic shape, it can be seen that the fragment has the highest concentration of contamination, which can be identified origin as secondary microplastic, large plastic fracture process caused by ultraviolet light (Jiang, 2018). The abundance of fragments was found in this study higher than Chagjing Estuary of China (Peng et al., 2017) which Chagjing estuary and the river of the Tibet Plateau Jiang et al. (2019) reported were found the most fibers in the investigated. While, Bangpakong river estuary was found more foam shape than Mae Klong, Tha Chin, and Chao Phraya river estuaries whereas pellet and fragment shape was found at the lowest between the 4 river estuaries. From this study, the lowest pellet shape was found which the same as Wu et al. (2019) reported in the HaiHe Estuary and Yongdingxinhe Estuary in Bohai Bay with the lowest abundance of pellet shape.

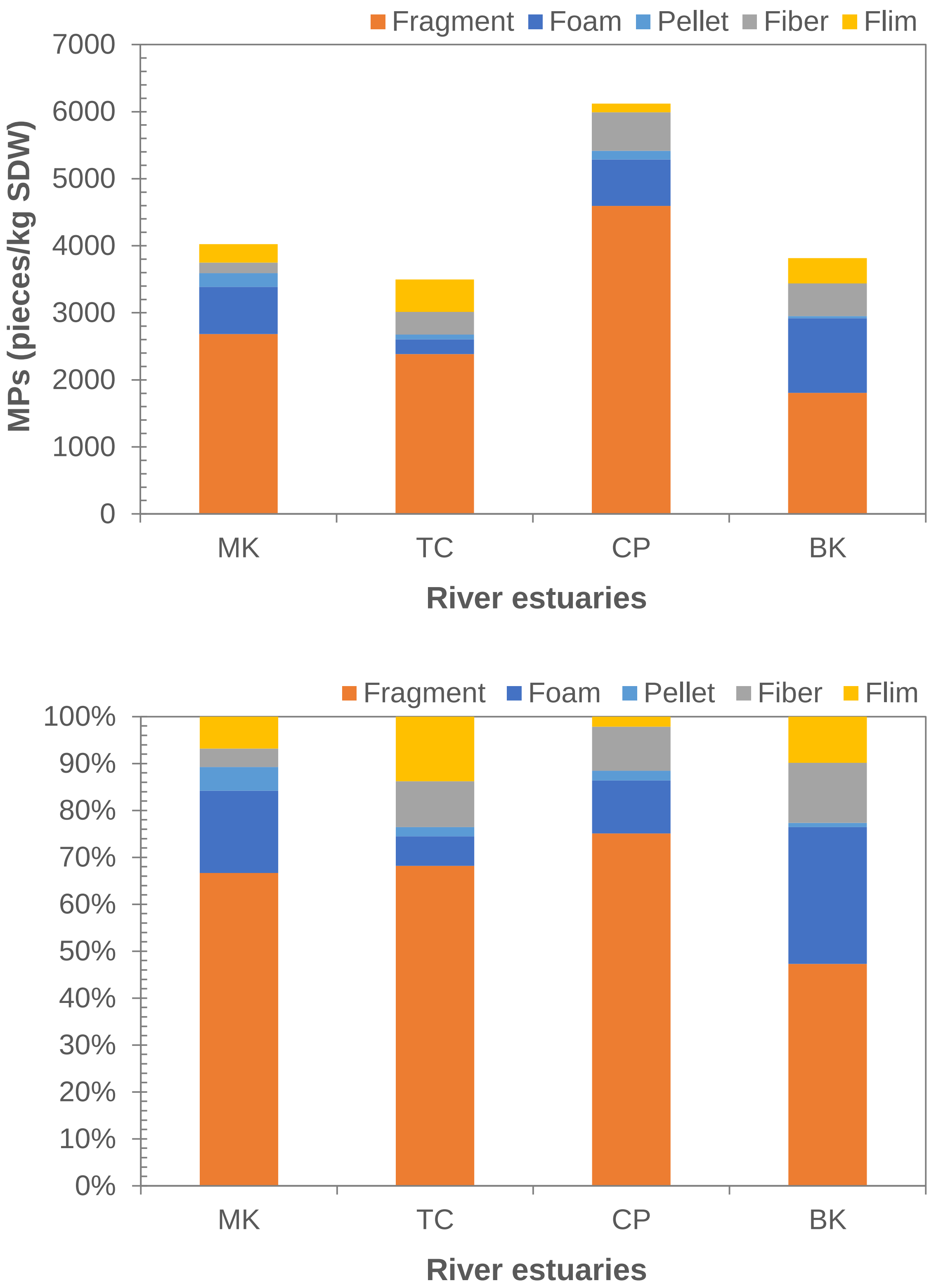
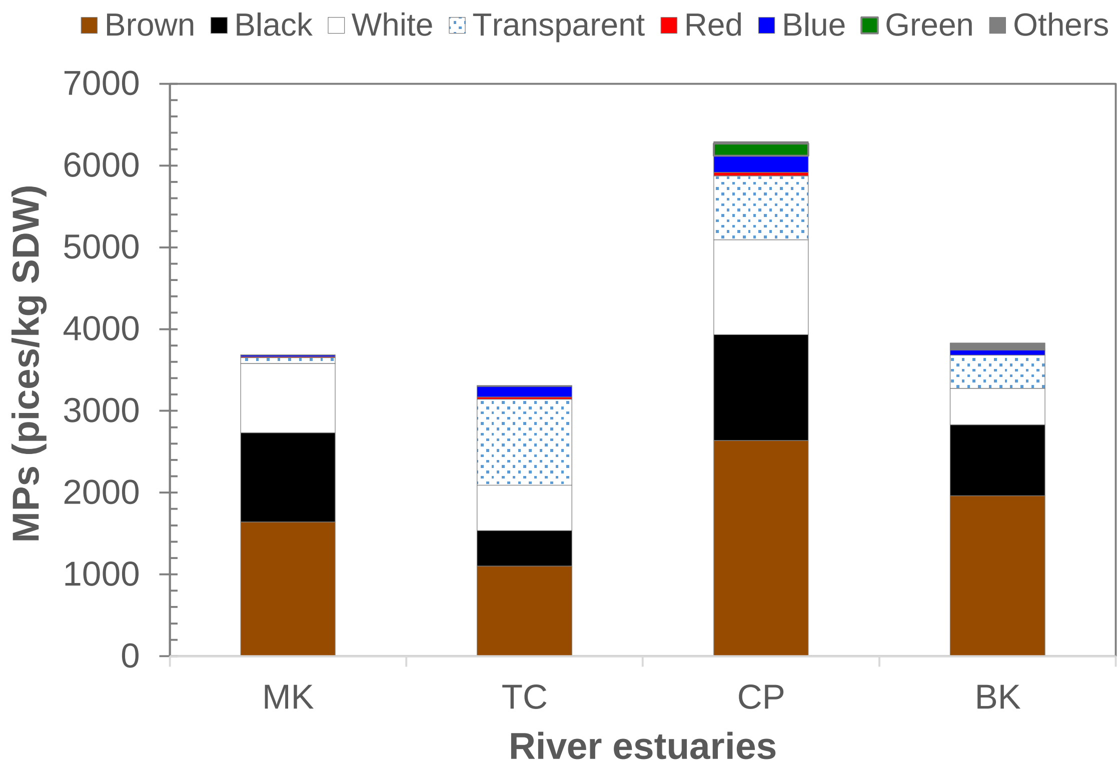


Figure 4.5 Shape compositions of microplastics in surface sediment of the Mae Klong (MK), Tha Chin (TC), Chao Phraya (CP) and Bangpakong (BK) river estuaries

4.5 Microplastic Colors

The result of color composition of microplastic was found various colors (Figure 4.6). The Mae Klong river estuary were found in arrangement of brown (1,640 pieces, 44%), black (1,092 pieces, 30%), white (847 pieces, 23%), transparent (72 pieces, 2%), blue (17 pieces, 0.46%), red (11 pieces, 0.30%) and the others (6 pieces, 0.15%). The Tha Chin river estuary were found in arrangement of brown (1,103 pieces, 33%), transparent (1,053 pieces, 32%), white (554 pieces, 17%), black (431 pieces, 13%), blue (129 pieces, 4%), red (27 pieces, 0.83%) and the others (11 pieces, 0.33%). While, the Chao Phraya river estuary were found in arrangement of brown (2,637 pieces, 42%), black (1,296 pieces, 21%), white (1,161 pieces, 18%), transparent (780 pieces, 12%), blue (204 pieces, 3%), green (150 pieces, 2%), red (42 pieces, 0.66%) and the others (23 pieces, 0.37%). Finally, the Bangpakong river estuary were found in arrangement of brown (1,960 pieces, 51%), black (868 pieces, 23%), white (448 pieces, 12%), transparent (406 pieces, 11%), the others (86 pieces, 2%) and blue (63 pieces, 2%).

The most common color composition of microplastic in 4 main river estuaries were brown (43%) follow by black (22%), white (18%), transparent (13%), blue (2%), green (1%), red and others (1%). Which brown, black, white, transparent, blue and other were found in all 4 main river estuaries whereas, green was found only one area at the Chao Phraya river estuaries. Also, red was found all river estuaries except the Bangpakong river estuary. While the most common color composition of microplastic in Brisbane River was found white (He et al., 2020). Investigation at the HaiHe Estuary and Yongdingxinhe Estuary of Bohai Bay found that the most common color composition of red (Wu et al., 2019) and the investigation in the Changjiang Estuary were found the most transparent (Peng et al., 2017). Which was different from this study, the most common color was brown. The survey found that contaminated microplastic in the sediment has various colors that may cause misunderstandings of feeding organisms such as zooplankton or benthic animals.



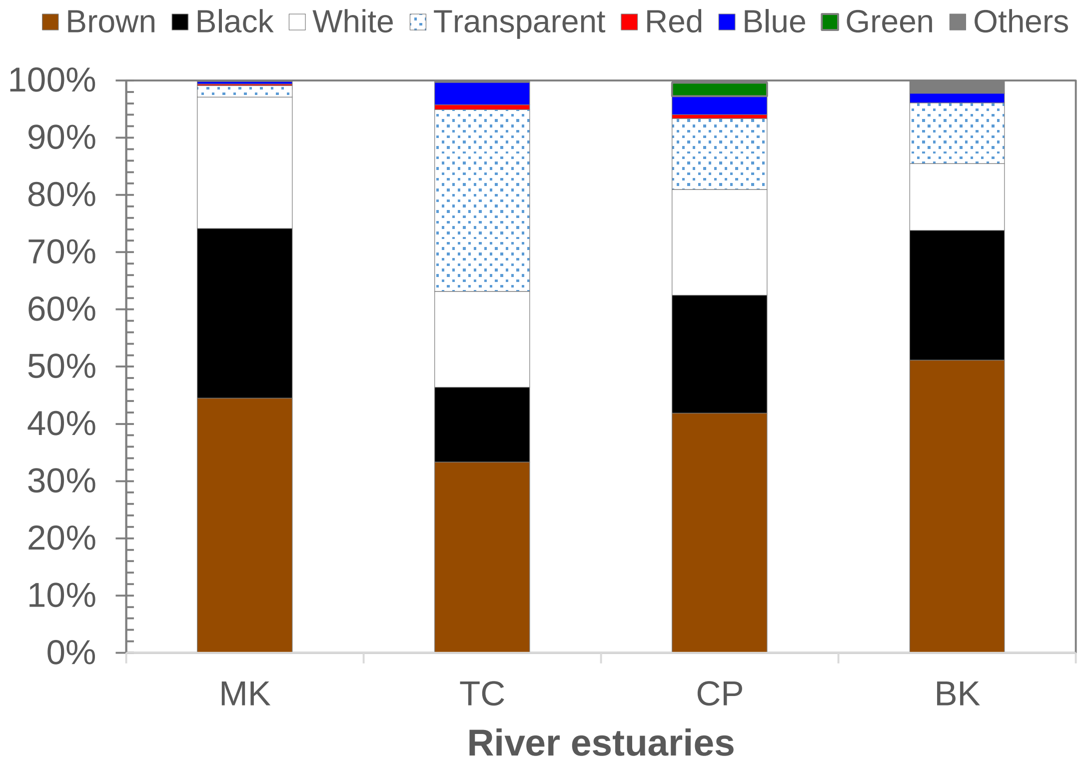


Figure 4.6 Color compositions of microplastics in surface sediment of the Mae Klong (MK), Tha Chin (TC), Chao Phraya (CP) and Bangpakong (BK) river estuaries

4.6 Status of Microplastic Contamination in the River Estuaries of Thailand

From the study of microplastic in different sediments of 4 main river estuaries including Mae Klong, Tha Chin, Chao Phraya and Bangpakong river which the averages concentration of microplastic was 4022±715, 3498±3404, 6119±5061 and 3815±2456 pieces per kg dry sediment. Which has a higher average than surveyed in other areas for example, Peng et al. (2017) investigates microplastic of Changjiang estuary was found 121±9 pieces per kg dry sediment, Jiang et al. (2019) investigates in surface sediment of Lhasa River was found 195±64 pieces per kg dry sediment, Wang et al. (2017) investigates in Beijiang River littoral zone was found microplastic concentration ranges from 178±69 to 544±107 pieces per kg dry sediment, He et al. (2020) investigates in Brisbane River sediment of Australia was found microplastic concentration ranges from 10 to 520 pieces per kg dry sediment and Peng et al. (2018) investigates in river sediment in Shanghai of China was found 802±594 pieces per kg dry sediment. While the river Rhine and Main of Germany were found microplastic concentration ranges from 228 to 3,763 pieces per kg dry sediment and 786 to 1368 pieces per kg dry sediment, respectively (Klein et al., 2015).

Table 4.2 Literature on the comparison of microplastic abundance in global surface sediment of river estuaries

Study area	Size (mm)	Average concentration (piece/kg)	Reference
Changjing Estuary, China	0.1–5	121±9	Peng et al. (2017)
Lhasa River, Tibet Plateau	1-5	195±64	Jiang et al. (2019)
Beijiang River littoral zone, China	<5	178±69-544±107	Wang et al. (2017)
Brisbane River, Australia	<5	10-520	He et al. (2020)
Freshwater river sediment in Shanghai, China	<5	802±594	Peng et al. (2018)
The river Rhine and Main, Germany	<5	228-3763 and 786-1368	Klein et al. (2015)
The Mae Klong river estuary	**0.1–4**	**4022±715**	**This study**
The Tha Chin river estuary	**0.1–4**	**3498±3404**	**This study**
The Chao Phraya river estuary	**0.1–4**	**6119±5061**	**This study**
The Bangpakong river estuary	**0.1–4**	**3815±2456**	**This study**

CHAPTER 5

RESEARCH CONCLUSIONS

5.1. Conclusions

In the present study, contamination and characterization of microplastics in different sediments of the river estuaries of the inner Gulf of Thailand were investigated. Four main conclusions were drawn from our data.

1. In the surface sediment in the Mae Klong, Tha Chin, Chao Phraya and Bangpakong river estuaries were found total microplastic concentration ranges from 981 to 11,920 pieces per kg dry sediment and 18.06 to 78.83 mg per kg dry sediment.
2. The most common size of microplastic was 300-100 µm in the Tha Chin, Chao Phraya and Bangpakong river estuaries while the Mae Klong river estuaries were found with size 1000-300 µm. Also, the most commonly microplastic shape and color in 4 main river estuaries was fragment (66%) and brown (43%), respectively.
3. The mean difference concentration of total microplastic between river estuaries were not significant.
4. This study the contamination and characteristics of microplastics in the estuary of Thailand to be used as a database in order to develop a plastic waste management and environmental policies in Thailand.

5.2 Research Suggestions

From this study, it can be identified that 4 main river estuaries of Thailand have high microplastic contamination compared to studies in estuaries of other areas. This is a problem that may affect aquatic organisms, environmental values, human health, including the economy. Therefore, there should be a policy to manage plastic waste that can be achieved to prevent the effects that may occur.

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