Microplastics in industrial wastewater treatment plant: Quantification, identification and ecological risk assessment

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โรงบำบัดน้ำเสียเป็นแหล่งสำคัญเส้นทางหนึ่งในการกระจายไมโครพลาสติกเข้าสู่สิ่งแวดล้อม ซึ่งข้อมูลเกี่ยวกับการกระจายของไมโครพลาสติกนั้น ในปัจจุบันนี้หลายพื้นที่ยังไม่มีข้อมูล และต้องมีการศึกษาและเก็บข้อมูลเพิ่มขึ้นโดยเฉพาะในประเทศไทย ดังนั้นงานวิจัยนี้จึงได้ทำการศึกษาเกี่ยวกับการกระจายของไมโครพลาสติก โดยทำการเก็บตัวอย่างน้ำเสียและตะกอนจากหน่วยบำบัดต่าง ๆ ของโรงบำบัดน้ำเสียอุตสาหกรรมแห่งหนึ่ง ในเดือนกุมภาพันธ์ และกรกฎาคม 2563 โดยมีการจำแนกตัวอย่างไมโครพลาสติกตามขนาด รูปร่าง และชนิดของพอลิเมอร์ด้วยกล้องจุลทรรศแบบสเตอริโอ และเครื่องฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโคปี (FT-IR) จากการศึกษาและสำรวจพบว่า ในตัวอย่างฤดูแล้งมีการพบไมโครพลาสติกมากที่สุดหลังผ่านขั้นตอนของระบบเติมอากาศ (134.35 ± 20.79 ชิ้น/ลิตร) นอกจากนี้ยังพบว่าปริมาณไมโครพลาสติกในตัวอย่างตะกอนของตัวอย่างฤดูแล้งอยู่ที่ 2.27 ± 0.08 ชิ้น/กรัม และในตัวอย่างฤดูฝนอยู่ที่ 1.86 ± 0.28 ชิ้น / กรัม ซึ่งเป็นปริมาณที่ค่อนข้างต่ำ และพบว่าในตัวอย่างตะกอนนั้นส่วนใหญ่มีขนาดมากกว่า 300 ไมครอนของตัวอย่างทั้งสองฤดูกาล ทั้งนี้ช่วงเวลาที่เก็บตัวอย่างมีผลอย่างมีนัยสำคัญต่อขนาดและรูปร่างของไมโครพลาสติก โดยความแตกต่างที่เกิดอาจเป็นผลมาจากการเปลี่ยนแปลงของฤดูกาลหรือการจัดการระบบบำบัดในช่วงที่มีการแพร่ระบาดของโรค ในส่วนของผลการวิเคราะห์พอลิเมอร์ของตัวอย่างที่ได้สุ่มมานั้น ตรวจพบว่าเป็นพลาสติก 71.13% ซึ่งส่วนใหญ่เป็นชนิดพอลีโพรพีลีน (PP) และพอลีเอทิลีน (PE) ในส่วนของประสิทธิภาพในการกำจัดไมโครพลาสติกของระบบบำบัดน้ำเสียกำจัดได้ 93.86% แต่ยังพบไมโครพลาสติกจำนวนมากถูกปล่อยลงสู่แม่น้ำ โดยมีค่าประมาณ 10^8 ชิ้น/วัน ซึ่งเป็นผลจากอัตราปล่อยน้ำภายหลังการบำบัดในปริมาณมากในแต่ละวัน โดยจากการประเมินความเสี่ยงต่อระบบนิเวศ พบว่ามีค่าดัชนีความเสี่ยงของพอลิเมอร์ (H) เท่ากับ 230.38 และ 203.49 ในตัวอย่างของน้ำออกของฤดูแล้งและฤดูฝนตามลำดับ ซึ่งเป็นค่าที่อยู่ในระดับสูง นอกจากนั้น ค่าความเสี่ยงของพอลิเมอร์ (RI) ของตัวอย่างน้ำออกอยู่ในระดับที่อันตรายอย่างมาก เนื่องจากมีพอลิเมอร์ชนิดที่มีความเป็นพิษสูง คือ พอลีเมทิลเมทาคริเลต (PMMA) ในส่วนของการวิเคราะห์ความอ่อนไหวของสปีชีส์ (SSD) กำหนดให้ค่าความปลอดภัย (Safety value) อยู่ที่1.143 ชิ้นต่อลิตร แต่น้ำที่ผ่านกระบวนการบำบัดแล้วมีปริมาณไมโครพลาสติกเกินค่ากำหนดดังกล่าว ซึ่งชี้ให้เห็นว่าอาจมีความเสี่ยงต่อระบบนิเวศ ดังนั้นการบำบัดน้ำเสียในส่วนของการกำจัดไมโครพลาสติกของโรงบำบัดน้ำเสียอุตสาหกรรมในประเทศไทยจึงควรให้ความสำคัญในการลดปริมาณไมโครพลาสติกของน้ำที่ผ่านกระบวนการบำบัดของโรงบำบัดน้ำเสีย เนื่องจากเป็นแหล่งสำคัญในการปล่อยไมโครพลาสติกเข้าสู่สิ่งแวดล้อม และยังเป็นเหตุให้เกิดความเสี่ยงต่อระบบนิเวศได้

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Wastewater treatment plants (WWTPs) are one of the significant pathways of microplastics (MPs) entering the environment. Thus, information regarding this issue is still requisite in Thailand. In this study, wastewater and sludge samples were collected from various treatment units of industrial WWTP in dry season (February 2020) and wet season (July 2020). The MP particles were identified by size, shape, and polymer types. From the wastewater, MPs were detected in the highest amounts after the aeration unit (134.35 ± 20.79 particles/L) in dry season sample. In addition, sludge contained relatively low MPs. The size fraction in wastewater varied, but in sludge was >300 μm commonly in both seasons sample. Fragments and pellets were identified as the most common shape of wastewater and sludge in both seasons sample. The treatment units and sampling period had a significant effect on MP abundance.The distinction might occurred by temporal variation or system operation during the pandemic. Form FTIR result, plastic polymer mainly identified as polypropylene (PP) and polyethylene (PE). The overall removal efficiency was 93.86%, which still discharge to the ocean 10^8 particles per day. For risk assessment of the effluent, polymer risk index (H) was 230.38 and 203.49 of dry and wet seasons, verify as high value. Moreover, the potential ecological risk (RI) of effluent considered as extreme danger level due to high toxicity polymer from Polymethacrylate (PMMA). SSD method showed that the MP abundance was exceeded the limit value derived from SSD model (HC_5 1.143 particles/L), which is relatively low due to selected data. Therefore, the attention must be on WWTPs in Thailand, as they act as the greatest source of MP contamination in the environment and main cause of risk which can affect the ecosystem.

Field of Study:	Industrial Toxicology and Risk Assessment	Student's Signature .....
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CHAPTER I
INTRODUCTION

1.1 Background and significant of research

Plastic implements play an essential role in industry today. Worldwide plastic production has increased continuously from year to year, leading to plastic pollution in environment. These plastics, typically with a diameter less than 5 mm., are defined as microplastics (MPs) (Hidalgo-Ruz, Gutow, Thompson, & Thiel, 2012; Lares, Ncibi, Sillanpaa, & Sillanpaa, 2018; R. C. Thompson, Swan, & Moore, 2009). There are several adverse effects of MPs, not only in naturally persistent and enormous abundance in the environment but also chemically and physically. Regarding the chemical effects of MPs on the high sorption capacity of plastics with hydrophobic organic contaminants, MPs act as carriers permeated with toxic organic pollutants (Lee, Shim, & Kwon, 2014). It was found that MPs can adsorb heavy metals such as lead, manganese, etc. (Gao et al., 2019), which might harm aquatic ecosystems.

MPs have been detected in numerous environments, including oceans (Eriksen, Mason, & Wilson, 2013; Zhu et al., 2018) freshwater (Rodrigues et al., 2018; Shruti, Jonathan, Rodriguez-Espinosa, & Rodriguez-Gonzalez, 2019), soil sediments (Borges Ramirez, Dzul Caamal, & Rendon von Osten, 2019), and aquatic life forms (Desforges, Galbraith, & Ross, 2015; Peters et al., 2018). MPs enter by several pathways, such as direct dumping, drainage water, and a wastewater treatment plant (WWTP). WWTPs are not only the endpoint of the anthropogenic water cycle but also receive many MPs through multiple ways, such as textiles from washing (E. Hernandez, Nowack, & Mitrano, 2017), cosmetics and personal care products, (Eriksen et al., 2013), abrasion of plastic composite products, and pipes and landfill leachates (Kole, Lohr, Van Belleghem, & Ragas, 2017).

WWTP is the bypass of great amounts of water before discharge into the surface water. Nevertheless, the general purposes of treatment process are mainly designed to remove organic matter, nutrients, and heavy metals from wastewater (Magni et al., 2019). This means WWTP are not properly designed to remove MPs from wastewater. Although, the high removal efficiency of MPs in almost all WWTPs

can still be found, at higher than 95% (Sun, Dai, Wang, van Loosdrecht, & Ni, 2019). However, its contribution to the environment is still worrisome due to the great volume of treated water coming from each WWTP, which could lead to the trans-fer of large amounts of MPs into aquatic environments. Some research revealed that a WWTP with daily treated 10,000 m^3 of WWTP could release approximately 10,000,000–460,000,000 microplastic particles per day (Lares et al., 2018). Likewise, others have reported that WWTPs could release over 1,000,000 micro-particles per day with various amounts of treated water discharged (Mintenig, Int-Veen, Loder, Primpke, & Gerdts, 2017; Murphy, Ewins, Carbonnier, & Quinn, 2016; Talvitie, Mikola, Setala, Heinonen, & Koistinen, 2017; Ziajahromi, Neale, Rintoul, & Leusch, 2017). However, this number can be diverse due to seasonal variation. (Ben-David et al., 2021) reported that season is an important factor in the effluent which found microplastic higher in winter compared with the other seasons. While the results from Hongkong showed that the abundances and weights were significantly higher in the wet season than in the dry season (Cheung, Fok, Hung, & Cheung, 2018). In contrast some study reported that there was no relation pattern in MP concentration between seasons across the six sites investigated, and no correlation with recent precipitation (Mani & Burkhardt-Holm, 2020). Indeed, several countries' MPs from WWTPs have been studied, except in Thailand.

Furthermore, there are several adverse effects of MPs, not only in naturally persistent and huge abundance in environment but also chemically and physically effects. Regarding to chemical effects of MPs on the high sorption capacity of plastics with hydrophobic organic contaminants (HOCs), MPs act as a carrier that are permeated with toxic organic pollutants (Lee et al., 2014). It was found that MPs can adsorb heavy metals such as lead, manganese, etc. (Gao et al., 2019) which might harm to aquatic ecosystem. Although, other processes might be needed to confirm how severe of these impacts (Adam, Yang, & Nowack, 2019), the available method can be used is ecological risk assessment which are used widespread among chemical toxic effect to ecosystem. However, there are only a few studies applied with MPs in water sources.

Hence, this work aims to verify the performance and effectiveness of MP removal by each treatment unit: influent, grit chamber, aeration, sedimentation, and

effluent of an industrial WWTP in Thailand. The identification of MP type, its polymer types, and the effect of seasonal variation were recognized. Furthermore, the estimation of an ecological risk assessment of MP pollution detected from wastewater treatment effluent to water sources were approached. Two methods of ecological risk assessment are 1) polymer-based toxicity of MPs which risk index H, and potential ecological risk index RI and 2) species sensitivity distribution which using log-normal model to conduct limitation value to provide safety limit to selected species in the aquatic environment. This work provided insights about the scale of MPs pollution and the preliminary quantitative information on the MPs diversity and ecological risks.

1.2 Objectives

1.2.1 To determine MPs removal efficiency of wastewater treatment units.

1.2.2 To identify type of MPs detected from water and sludge in wastewater treatment units.

1.2.3 To represent MP abundance in dry and wet seasons.

1.2.4 To evaluate potential ecological risk of MPs from effluent and sludge.

1.3 Hypotheses

1.3.1 Wastewater treatment plant provides high level MPs removal efficiency.

1.3.2 Distinction of MP abundances is found between MPs abundance, type, and size fraction.

1.3.3 Seasonal variation has an effect on MP abundance.

1.3.4 MPs from effluent and sludge of the treatment plant have potential risk to aquatic species.

1.4 Scope of the study

1.4.1 Wastewater and sludge samples were collected at Bang Plee Industrial Estate Wastewater Treatment Plant in January and August 2020.

1.4.2 Sampling points were influent, after grit chamber, after aeration, after sedimentation, effluent, and sludge.

1.4.3 Laboratory analysis was performed to determine number, size, and type of MPs at Department of Environmental Science, Faculty of Science, Chulalongkorn University.

1.4.4 Data was analyzed in terms of dynamic distribution in each treatment units.

1.4.5 Potential ecological risk of MPs in effluent was estimated by Species Sensitivity Distribution method.

1.5 Benefits

This research provided newly important information about MP pollution discharged by industrial wastewater treatment plant in Thailand, with dynamic distribution among treatment units and also seasonal variation. In addition, the ecological risk assessment was applied with species sensitivity distribution model and hazard index method. The results obtained were essential process to understand the pollution status and to handle with the environmental MP problem properly.

CHAPTER II
LITERATURE REVIEW

2.1 Microplastics (MPs)

Plastic is the important materials that were use widespread around the world. By the way, the definition is varied due to the researcher. Although several definitions of microplastics have been proposed based on their size, the definition currently accepted by the scientific community is a particle size within 0.001–5mm. The term microplastic (MP) generally refers to any piece of plastic smaller than 5 mm to 1 μm in size along its longest dimension (Crawford & Quinn, 2017b). Another opinion from (R. C. Thompson et al., 2009), which is MPs are the plastic with particles size less than 5 mm which similar to the definition from U.S. National Oceanic and Atmospheric Administration (NOAA). MPs can be categorized in two types according to its occurrence: primary and secondary (Kershaw, 2015).

2.1.1 Primary microplastics

Primary MPs are generally small particles and already 5.0 mm in size or lower before entering the environment. It was intentionally manufactured by the plastics industry for use in various purposes such as cosmetics, personal care products and cleaning agents. Apart from those domestic used plastic, it also comes from industrial feedstock that widely use to shape up plastic products (Crawford & Quinn, 2017c). Moreover, primary MPs have also involved in acrylic blasting, melamine, or polyester as MP scrubbers at boat's engines and outside body to remove rust and paint. After the processes, these scrubbers mostly become contaminated with heavy metals such as cadmium, chromium, and lead (Cole, Lindeque, Halsband, & Galloway, 2011). Consequently, these plastic particles can be swept up and carried on the wind and then distribute in the bodies of water (Gregory, 1996).

2.1.2 Secondary microplastics

Secondary MPs are irregular pieces of plastic that have been unintentionally produced, happened as a result of the degradation of larger pieces of plastic, such as plastic bags, bottles, clothes, ropes and fishing nets, and other plastic products (Reisser et al., 2013). Another important source of this type is industrial or habitat's pipes erosive. When the time pass by, these large pieces of plastic litter possibly degrade by natural action such as exposure to ultraviolet from the sun light (Claessens, Van Cauwenberghe, Vandegehuchte, & Janssen, 2013), and by mechanical process such as air pressure, erosion by rain and tidal waves (Cooper & Corcoran, 2010) to form even more smaller pieces of plastic (Crawford & Quinn, 2017a).

2.2 Effects of MPs

Until now, MPs abundance in the environment considered as a critical and challenge problem towards the detection in various locations. MPs enter into marine environment mainly through human activities such as aquaculture, tourism, fishing, industrial and domestic wastewater systems (Duis & Coors, 2016). MPs can persist in the environment for many years with the potential to cause physical and biological harms. MPs can be eaten by a diverse marine species since primary to the highest trophic level. For example, deposit feeders and detritus feeders (i.e. lungworms and sea urchin), filter and suspension feeders (i.e. copepods, sea lilies and oyster), echinoderms (i.e. sea star), plankton are capable to the ingestion of MPs similar as fishes, turtles, fish, birds, and marine mammals (Guzzetti, Sureda, Tejada, & Faggio, 2018).

Moreover, additive chemicals being attached with plastic litter, these particles can accumulate persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides like Dichlorodiphynyl trichloroethane (DDT) from the water (Mato et al., 2001; C. M. Rochman, Hoh, Hentschel, & Kaye, 2013). Nevertheless, not only physical problem that can be block up the digestive tract was concerned. These additive toxic substances can also cause feeding disruption to reproductive performance, disturbances in energy metabolism, etc.(Anbumani & Kakkar, 2018).

2.3 Wastewater Treatment Plant

A wastewater treatment plant (WWTP) is an industrial facility where a combination of mechanical, physical, chemical and biological processes used to eliminate pollutants from the incoming wastewater (Hreiz, Latifi, & Roche, 2015). These pollutants are various as in the case of some industrial wastewaters. The design of wastewater treatment plants is usually based on the need to reduce organic and suspended solids loads to limit pollution before release to the environment. Therefore, other contaminants removal has very rarely been considered an objective, for reusing of effluents in agriculture, but this must be primary concern and processes should be selected and designed accordingly (Hillman, 1988).

Conventional wastewater treatment consists of a combination of physical, chemical, and biological and other processes to remove solids, organic matter and, undesired nutrients from wastewater. Normally, these terms are used to describe different units of treatment for increasing treatment level include preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment. In some countries, disinfection is applied to remove pathogens follows the last treatment step (FAO, 1992). The treatment steps as follows:

2.3.1 Preliminary treatment

Preliminary treatment typically consists of screening to remove large floating objects, scantling, and other things that could damage or clogged plant equipment; flow measurement devices; storage facilities to quantify the flow to the plant; and grit removal to take out the larger gravel, sand, and other, mostly inorganic, components that can leak into the system (Spellman, 2000).

2.3.2 Primary treatment

The objective of primary treatment is the removal of settleable organic and inorganic solids by sedimentation, and the removal of materials that will float by skimming. Some organic nitrogen, organic (McCormick, Hoellein, Mason, Schluep, & Kelly, 2014), phosphorus, and heavy metals associated with solids are also removed during primary sedimentation but colloidal and dissolved constituents are

not affected (FAO, 1992). Sedimentation relatively causes heavy objects settle out and leave buoyant materials float to the top (plastic, fats, greases, and oil). These are mostly organics at this stage, but there may be a few inorganics mixed in with them (Spellman, 2000).

2.3.3 Secondary treatment

The objective of secondary treatment is the further treatment of the effluent from primary treatment to remove the residual organics and suspended solids. Generally, secondary treatment follows primary treatment and involves the removal of biodegradable dissolved and colloidal organic matter using aerobic biological treatment processes. Aerobic biological treatment is the process occurrence of oxygen by aerobic microorganisms which metabolize the organic matter in the wastewater, thereby microorganism's population increase and inorganic end-products (principally CO_2, H_2O, and NH_3) (FAO, 1992).

2.3.4 Tertiary treatment

Tertiary treatment is the wastewater treatment process after secondary treatment. This step removes the remain contaminants that secondary treatment is not able to dispose. Wastewater effluent quality becomes even better in this treatment process through the use of superior and more advanced treatment systems (Mareddy, 2017). For instance, ozone wastewater treatment is growing in popularity, and requires the use of an ozone generator, purifies the water as ozone bubbles percolate inside the tank (Ameta, 2018).

Industrial wastewater treatment plants are required when municipal sewage treatment plants are incapable or failing to treat specific industrial wastewaters. Industrial wastewater plants may reduce raw water costs by converting selected wastewaters to reclaimed water used for different purposes. Industrial wastewater treatment plants may reduce wastewater treatment charges collected by municipal sewage treatment plants by pre-treating wastewaters to reduce concentrations of pollutants measured to determine user fees (Hammer et. al., 1975).

2.4 MPs in Wastewater Treatment plant

Wastewater treatment plant (WWTP) is an important role in releasing MPs to the environment (Browne, Crump, & Niven, 2011). The WWTP may remove some of the MPs depending on the treatment units applied. However, it has been shown that MPs could pass through the treatment plant, entering into the aquatic water bodies and finally accumulated in the environment (Carr, Liu, & Tesoro, 2016; Murphy et al., 2016). Over the last few years, many studies have detected MPs, including polyethylene (PE) and polypropylene (PP) beads and polyester, acrylic, polyamide and nylon fibers in the marine environment have suggested that wastewater effluent is a potential source (Browne et al., 2011; Dris, Imhof, et al., 2015; Eriksen et al., 2013; Gallagher, Rees, Rowe, Stevens, & Wright, 2016; McCormick et al., 2014). Moreover, the detected MPs similar to MPs collected from WWTP effluent, suggesting that a considerable proportion of detected MP fibers may be associated with wastewater effluent (Ziajahromi et al., 2017). Most studies have shown that MPs are effectively removed from wastewater and large amount end up in the sludge (Michielssen, Michielssen, Ni, & Duhaime, 2016; Talvitie et al., 2015; Talvitie, Mikola, Koistinen, & Setala, 2017a) which are not treated the sludge properly. Even though the removal efficiency of MPs in WWTPs is found to be around 99% (Carr et al., 2016; Murphy et al., 2016). Numerous MP particles are continuously being discharged from WWTPs to recipient waters due to the large amount of daily flow (Lares et al., 2018).

2.5 Ecological risk assessment (ERA)

Risk is defined as the probability that an unwanted event will occur. Consequently, ecological risk refers to the probability of the occurrence of an unwanted ecological event. Ecological risk assessment (ERA) typically focuses on the undesired ecological effects of toxic chemicals as shown in Figure 2.1.

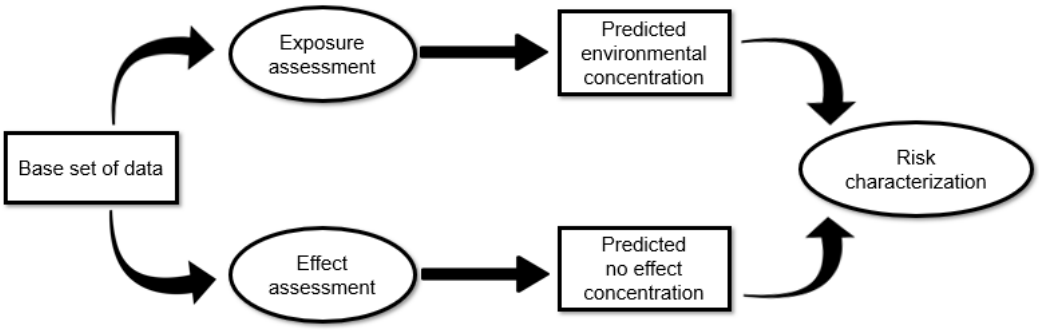

Figure 1 Ecological risk assessment processes

In the environment, the stressors may include physical, geological, hydrological, and biological stressors (Bartell, 2008). In environmental risk assessment, predicted no effects concentration (PNECs) will be compared to predicted environmental concentration (PEC) to determine if the risk of a substance is acceptable or not. If PEC/PNECs < 1, the risk is acceptable. ERA can be estimated by following methods.

2.5.1 Exposure assessment

Exposure characterization describes potential or actual contact or cooperate of stressors with receptors. It is based on measures of exposure and ecosystem and receptor characteristics that are used to analyze stressor sources, their distribution in the environment, and the extent and pattern of contact or co-occurrence (USEPA, 1998). The most valuable data is the field observations (Bartell, 2008). In the practical way, first step is to quantify the emissions of a chemical into environmental components (water, air, soil and solid waste). The estimated emission rates will then be used in exposure models such as EUSES (European Union System for the Evaluation of Substances) to calculate predicted environmental concentrations (PEC) and to further quantify risk quotient (ECHA, 2017).

2.5.2 Effect assessment

This procedure was used to evaluate the severity of the expected ecological response related to the quantity, frequency and duration of the exposure. Effect assessment might show some choices of basic physiological processes (e.g., photosynthesis, respiration) and corresponding lethal or sublethal (i.e., growth

inhibition) effects on individual organisms. This step depends on the quantity and quality of available data. Sources of data that might be used include: the results of toxicity tests (acute, chronic) performed under controlled laboratory conditions, direct measures of exposure and response in controlled field experiments, and the application of statistical relationships (Bartell, 2008).

Canadian Environmental Quality Guidelines recommends the SSD approach for water quality threshold derivations where a sufficient number, quality, and variety of toxicity test data are available. The species sensitivity distribution (SSD) concept is a statistical approach which use endpoint speculate from the most sensitive study using a statistical significance. SSD is the cumulative probability distribution of some measure of toxicity of a certain chemical to a set of animal species (CCME, 2007). At increasing concentrations of a toxicant, the proportion of species affected (at a given level of effect such as 20% growth impairment or 50% reduction of abundance) increases. (FCSAP, 2012)

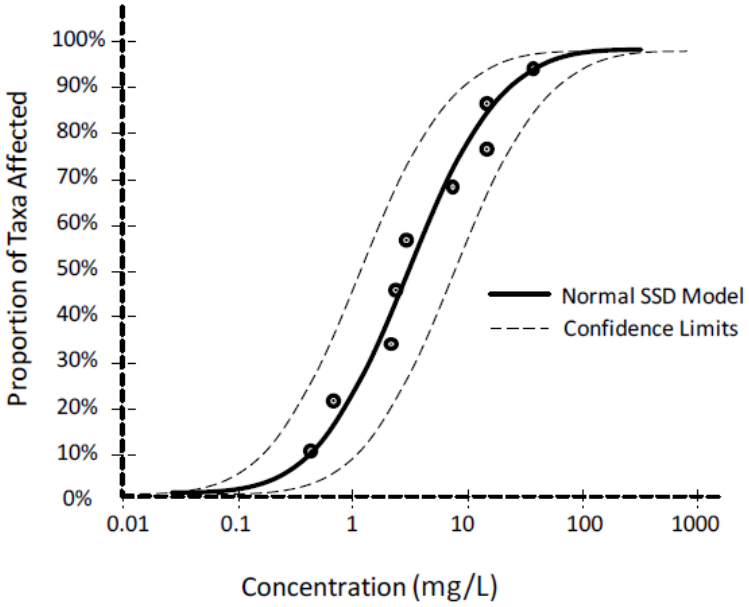

Figure 2 Example of Species Sensitivity Distribution (SSD)

From Figure 2, the circles represent individual species (for the purpose of this example, they may be assumed to be various freshwater benthic fauna). The x-axis is the chemical concentration (in logarithmic scale) at which a threshold response size occurs. The SSD is a solid line curve and dashed line is confidence limits through

the concentration-response curve. This allows an assessment of hazardous concentration at which a given proportion of species is affected (e.g., 35% of benthic fauna affected at 4 mg/L in the example) (FCSAP, 2012).

To derive an SSD, single-species toxicity data (e.g., LC_{50} values, ICX values, or LOAEC / NOAEC data) for many species are fit to a distribution such as the lognormal or log-logistic. From this distribution of species sensitivities, a hazardous concentration (HCp) is identified at which a certain percentage (p) of all species is presumed to be affected (Posthuma, Suter, & Traas, 2001)FCSAP, 2012).

2.5.3 Risk characterization

Risk characterization is the final step of an ecological risk assessment. During this step, represent the overall degree of confidence in the risk estimates, cite evidence supported and interpret the adversity of ecological effects (USEPA, 1998). A various of methods and tools are suitable for risk estimation. For assessing risks posed by toxic chemicals, one simple method simply divides the exposure concentrations by the toxicity reference values (Bartell, 2008). However, the method that widely use in ecotoxicological field for estimate risk in aquatic media mainly based on the determination of predicted environmental concentrations (PECs) and predicted no effect concentrations (PNECs). PECs and PNECS are then used in a risk quotient approach, if the PEC/PNEC ratio is lower than 1, the situation is not considered to be of concern; if the PEC/PNEC ratio is higher than 1, further risk can occur and should be concerned (TGD, 2003).

2.5.4 Hazard index

Other ways to evaluate risk from pollution which will affects the environment is using an ecological risk index in aquatic environmental pollution control i.e., a risk index which provides a fast and simple quantitative value on the potential ecological risk of a given contamination situation in a given lake or fresh water system. (Håkanson, 1980) invented the risk index method, to express the potential ecological risk of a given contamination to penetrate one of many possible pathways towards a potential ecological risk index to be used as a diagnostic tool for

water pollution control purposes, i.e., to figure out which limnic area and substances should be given special attention. The methodology is based on the assumption that the sensitivity of the aquatic system depends on its productivity following four requirements for this method.

a) The concentration requirement

b) The number requirement

$$C_d = \sum_{i=1}^{\infty} c_f^i = \sum_{i=1}^{\infty} \frac{C_{0-1}^i}{C_n^i} \quad (2.1)$$

C_d = the degree of contamination;

C_f = the contamination factor;

$C_{0\text{-}1}$ = the mean content of the substance in question (i) from superficial sediment (0-1cm) from accumulation areas. At least 5 samples, which provide an even area cover of the lake/basin should be taken.

C_n = the standard preindustrial reference level determined from various European and American lakes to be (in ppm): PCB = 0.01, Hg = 0.25, Cd = 1.0, As = 15,Cu = 50, Pb = 70, Cr = 90, and Zn = 175.

c) The toxic factor requirement

d) The sensitivity requirement

$$RI = \sum_{i=1}^{\infty} Er^i = \sum_{i=1}^{\infty} Tr^i \cdot C_f^i \quad (2.2)$$

RI = the requested potential ecological risk index for the basin/lake;

Er = the potential ecological risk factor for the given substance (i);

Tr^i = the "toxic-response" factor for the given substance, i.e., PCB = 40 × BPI/5, Hg = 40 × 5/BPI, Cd = 30 × √(5/BPI), As = 10, Pb = Cu = 5 × √(5/BPI), Cr = 2×√(5/BPI) and Zn = 1×5/√BPI, where BPI = the bioproduction index.

2.6 Microplastics in Wastewater treatment plant

Magnusson & Norén, 2014 evaluated the charge of wastewater treatment plants as entrance routes for MP particles to the marine environment in Sweden. Analyzing were performed on MP particles collected by a 300 μm mesh filter, both in the WWTP (influent and effluent water, and sewage sludge) and in the sea water. They found that MP concentration in the final effluent was higher than recipient area (8.25 plastic particles/m^3 were found in the effluent compared to 1.14, 1.29, 1.97 and, 0.45 particles/m^3 in the lake distance 20 m, 50 m and, 200 m from the recipient area and reference area, respectively). Higher particle concentrations were found close to the mouth of the tube compared to 200 m downstream.

Dris, Gasperi, et al., 2015 gave attention to MP contamination in an urban area of Greater Paris. Wastewater was collected at the Seine-Centre wastewater treatment plant. Three sampling on 3 consecutive days (8, 9 and 10 April 2014) were carried out. Raw wastewater after pretreatment, settled wastewater and treated water were collected. MPs were found in high concentrations (from 260 x 10^3 - 320 x 10^3 particles/m^3) in raw wastewater. With the exception of one spherical particle, the MPs observed were fibrous. The presence of numerous fibers may be explained by washing machine effluent because it has been reported that up to 1,900 fibers per wash can be discharged. In the final effluent, the contamination decreases from 14 x 10^3 to 50 x 10^3 particles/m^3. No observed fiber was in the size class of 1001–5000 μm. Size distribution was globally similar to that after the primary treatment. These first results suggested that WWTPs remove a large amount of the MP contamination (from 83 to 95 %), which is probably transferred to sludge. As highlighted by the size pattern changes, water treatment seems to be more effective at removing longer fibers, which are absent from treated water.

Carr et al., 2016 studied about fate of MP particles in wastewater treatment plants in Southern California from effluent discharged of seven tertiary plants and one secondary plant. Over 0.189 million liters of effluent at each of the seven tertiary plants were filtered using a stack of sieves with 45 μm as finest mesh sizes. The results suggested that tertiary effluent is not a significant source of MPs and plastic particles are effectively removed during the skimming and settling treatment

processes. However, at a downstream secondary plant, an average of one micro-particle in every 1.14 thousand liters of final effluent was counted.

Murphy et al., 2016 studied about MPs releasing by wastewater treatment plant in Scotland. The influent contained on average 15.70 (±5.23) particles/L. This was reduced to 0.25 (±0.04) particles/Lin the final effluent, efficiency of 98.41%. Despite this large reduction, they calculate that this treatment plant is releasing 65 million MPs into the recipient water every day. A significant proportion of the MP accumulated in and was removed during the grease removal stage (19.67±4.51 particles/2.5 g). This study showed that despite the efficient removal rates of MP achieved by this modern treatment plant when dealing with such a large volume of effluent even a modest number of MPs being released per liter of effluent could result in significant amounts of MPs entering the environment.

Talvitie, Mikola, Setälä, Heinonen, & Koistinen, 2017 explore on the removal of microlitter in a tertiary level of a largest wastewater treatment plant in Finland. The samples were collected from the plant influent, after pre-treatment, after the activated sludge (AS) process, plant effluent, excess sludge, reject water and dried sludge. Most of the microlitter was removed already during the pre-treatment and activated sludge treatment further decreased the microlitter concentration. The microlitter content of excess sludge, dried sludge and reject water were also examined. According to the balance analyses, approximately 20% of the microlitter removed from the process is recycled back with the reject water, whereas 80% of the microlitter is retained in the dried sludge.

Dyachenko, Mitchell, & Arsem, 2017 had quantified the MP particles from treatment plant's effluent in United States. The use the extraction method was adapted from the National Oceanic and Atmospheric Administration's published in July, 2015. In Catalytic Wet Peroxidation Oxidation (CWPO), hydroxyl radicals generated upon decomposition of hydrogen peroxide, oxidize the majority of the natural organic matter to carboxylic acids, aldehydes, CO_2 and H_2O. The presence of a catalyst ($FeSO_4$) allows for rapid digestion of organic matter under mild conditions. Other extraction methods have been discussed and rejected due their potential to degrade MPs. Density separation with saturated sodium chloride solution was not effective in separating the adhere substances due to their similar density to MPs.

Leslie, Brandsma, van Velzen, & Vethaak, 2017 presented the MP concentration data in wastewater treatment plant in Netherland. The sample was collected on raw sewage influents, effluents and sewage sludge from seven municipal WWTPs contained mean particle concentrations of 68–910 particles/L, 51–81 particles/L and 510–760 particles/kg wet weight (ww), respectively and particle sizes occurred between 10 and 5000 μm. MPs distribute in the water column have the potential to be discharged into the sea with other riverine suspended particulates.

Mintenig et al., 2017 studied about MP abundance in effluents of wastewater treatment plants. This study investigated MP in the effluents of 12 WWTPs in Lower Saxony, Germany. In density separation process, zinc chloride solution was used. Polyethylene was the most frequent polymer type in both size classes. Quantities of synthetic fibers ranged from 9×10^1 to 1×10^3 particles/m^3 and were predominantly made of polyester. Considering the annual effluent of WWTPs, total discharges of 9×10^7 to 4×10^9 particles and fibers per WWTP could be expected. Interestingly, one tertiary WWTP had an additionally installed post-filtration that reduced the total MP discharge by 97%. In addition, the sewage sludge of six WWTPs was inspected majority of polyethylene.

Lares et al., 2018 studied MPs concentration in different treatment units of a municipal WWTP at Mikkeli, Finland. In this study, collected wastewater and sludge samples once in every two weeks during a 3-month sampling period. Most of the MP fraction was removed before the activated sludge process. The results of the 3-month sampling campaign show that 98.3% of MPs were removed during the treatment process of the studied WWTP. According to the MPs occurrence data, MP fibers (82%) are posing a more severe problem than MP particles (18%).

E. A. Gies et al., 2018a studied about retention of MPs in a major secondary wastewater treatment plant in Vancouver, Canada. Wastewater samples (influent, primary effluent, and secondary effluent) were collected on September 16, September 29, and October 28, 2016 and sludge samples (primary sludge and secondary sludge) were collected on September 14, September 27, and October 11, 2016. Suspected MP particles, including fibers, were counted and categorized using light microscopy in influent, primary effluent, secondary effluent, primary sludge and secondary sludge. Fourier Transform Infrared Spectroscopy (FTIR) confirmed that just 32.4% of the

suspected MPs were plastic polymers. Using FT-IR corrected data, they presume that 1.76 ± 0.31 trillion particles enter the WWTP annually, with 1.28 ± 0.54 trillion particles settling into primary sludge, 0.36 ± 0.22 into secondary sludge, and 0.03 ± 0.01 trillion particles released into the recipient environment.

Magni et al., 2019 looked into MPs in the biggest North Italian wastewater treatment plant by evaluating the amount at the inlet, the removal efficiency after the settler and at the outlet, and their transfer to sludge. Samples were collected in three days of a week and plastic debris was characterized in terms of shape and size. NaCl was used for MP separation by sediments, similar to sewage sludge, is recommended by the Marine Strategy Framework Directive (MSFD) and suggested when a huge number of samples needs to be processed. Indeed, this method is cheap, widely available and eco-friendly, despite the extraction performance of high-density MPs, as plasticized polyvinylchloride (1.3–1.7 g/cm^3) or polytetrafluoroethylene (2.1–2.2 g/cm^3), could be lower than other synthetic polymers.

X. M. Lv et al., 2019 investigated MPs at a WWTP, Eastern China, compared of oxidation ditch (OD) and membrane bioreactor (MBR). Sampling campaign was performed in February 2018. The influent MPs mainly consisted of polyethylene terephthalate (PET, 47%). MP morphotypes dominated in fragments (65%) and fibers (21%), which mainly were PET. Typical plastic microbeads were not observed. The dominant size of MPs was >500 mm (40%) and 62.5 -125 mm (29%). MP concentrations increased across the treatment systems depends on facility of treatment process. Influent MPs were removed by 99.5% in MBR system versus 97% in OD system on the basis of plastic mass while 82.1% versus 53.6% on MP number.

X. Liu, Yuan, Di, Li, & Wang, 2019 quantify the number of MPs particles in one WWTP of Wuhan China based on the conventional activated sludge process. The results showed that the abundance of MPs in wastewater declined sharply, from 79.9 particles/Lin the influent to 28.4 particles/Lin the effluent, with a removal rate of 64.4%. MPs removed were mostly transferred and stored into the sludge. Larger size fraction of MPs in the effluent was reduced compared to that in the influent due to mechanical erosion and sedimentation into sludge. An interesting finding is that the particles with the size ranged from 100 to 800 μm (average size of 348.1 μm), were

plenty observe in the influent with a percentage of 4.4%, but not observed in the effluent. A higher fraction of microbead and foam in sludge (17.1% and 12.9%) indicates MPs with the smaller size (average size of 90.3 and 240.1 μm, respectively) in wastewater are possibly adsorbed and transferred into sludge.

Table 1 Method of microplastic sampling

Year	Location	Flow rate	Wastewater sampling	OM removal	Density Separation	Finest mesh	Detection method	References
2012	Russia	960,000	Pump			20	Visual	Hidalgoruz et al. (2012)
2013	Germany	31	Container	30% H_2O_2		40	Visual	Dubaish and Liebezeit (2013)
2014	Sweden	5,200	Container/Pump			300	Visual/FTIR	Magnusson and Noren (2014)
2015	France	243,000	Autosampler			100	Visual/FTIR	Dris et al. (2015)
2016	United States	153,0000	Pump		Deionized water	100	Visual/FTIR	Carr et al. (2016)
2016	Scotland	264,000	Container			65	Visual/FTIR	Murphy et al. (2016)
2016	United States	254,000	Container			20	Visual	Michielssen et al. (2016)
2016	United States		Container	30% H_2O_2		20	Visual	Michielssen et al. (2016)
2016	United states	1,730	Container	30% H_2O_2		20	Visual	Mason et al. (2016)
2016	Finland	274,000	Pump			20	Visual	Talvitie et al. (2016);
2017	Australia	310,000	Pump	30% H_2O_2	Nal (1.49 /cm^3)	25	Visual/FTIR	Ziajahromi et al. (2017)
2017	United States	291,000	Container	30% H_2O_2		125	Visual/FTIR	Dyachenko et al. (2017)
2017	Australia	17,200	Pump	30% H_2O_2	Nal (1.49 /cm^3)	25	Visual/FTIR	Ziajahromi et al. (2017)
2017	Netherlands	5,600	Container			0.7	Visual	Leslie et al. (2017)
2017	New Zealand	200,000	Pump	30% H_2O_2		125	Visual/FTIR	Dyachenko et al., 2017
2017	Finland	270,000	Pump			20	Visual/FTIR	Talvitie et al. (2017)
2018	Finland	10,100	Container	30% H_2O_2		250	Visual/FTIR/Raman	Lares et al. (2018)
2018	Damark		Autosampler Container	30% H_2O_2		10	FTIR	Simon et al. (2018)
2018	Vancouver	490,0000	Container	30% H_2O_2	Canola oil	63	Visual/FTIR	Gies et al. (2018)
2019	Italy	400,000,000	Container	15% H_2O_2	NaCl (1.2 g/cm^3)	63	FTIR	Magni ei al. (2019)
2019	China	50,000	Pump	30% H_2O_2	Nal (1.49 g/cm^3)	25	Visual/FTIR	Lv et al. (2019)
2019	China	20,000	Container	30% H_2O_2	NaCl, NaI	47	Visual/Raman	Liu et al. (2019)
2020	Thailand	24,000	Container	30% H_2O_2		300	Visual/FTIR	Hongprasith et al. (2020)

2.7 MP effects on aquatic living

Prata, da Costa, Lopes, Duarte, & Rocha-Santos, 2019 has reviewed Effects of MPs exposure to microalgae (Freshwater: *Chlorella* sp., *Scencedesmus* sp., *Amphora* sp. And Saltwater; *Dunaliella tertioleecta*, *Thalassiosira pseudonana*, *Skeletonema costatum*) based on several effect criteria. MPs at concentrations in the low ppm range had negative effects on microalgae by inhibiting growth, reducing chlorophyll and photosynthesis, inducing oxidative stress, causing changes in morphology and promoting the production of heteroaggregates. However, microalgae seemed to recover from these changes through adaptative responses and current environmental concentrations are unlikely to cause harm. Nonetheless, MPs may disturb microalgae populations by reducing the available nutrients, by inhibiting primary consumers or by acting as a substrate. All these changes are dependent on specific properties of MPs, such as polymer type, size and surface charge, that are still not well understood and thus require further research.

P. ma, Wei Wang, Liu, Feng Chen, & Xia, 2019 revealed that MPs ingestion can affect aquatic life in many ways. Majority by physical damage, clogging the intestinal tract of organisms, reducing the absorption of nutrients. These affect the growth and development of organisms, and even causing death (J. Li, Zhang, & Zhang, 2018). Experiments on freshwater aquatic living with *Hyalella azteca* exposure to PP fiber and PE particles showed that MPs can affect the digestive function, reducing growth and reproduction (Au, Bruce, & Bridges, 2015). Experiments also found that the toxicity of MP fibers is greater than other types, which may be related to the longer duration of fiber in the intestinal tract. In addition, the accumulation of MP particles in zebrafish (*Danio rerio*) and nematode (*Caenorhabditis elegans*) (Lei et al., 2018), both can cause intestinal damage, including cracking of villi and splitting of enterocytes. Small size MPs adsorbed on *Chlorella* and *Scenedesmus* form physical blockages on light and air, which obstruct photosynthesis of algae (Bhattacharya, Lin, & Turner, 2010). In the presence of MPs, the chlorophyll concentration and population growth rate of the *Scenedesmus obliquus* significantly decreased, indicating potential chronic effects (E. Besseling, Wang, & Lurling, 2014). Moreover, physical damage of MPs can also release toxic of

substances (additives) or combination form with other pollutants in the water. Researches on freshwater animals show the combined pollution of MPs and organic pollutants can not only damage the liver cells of Japanese medaka (*Oryzias latipes*) (C. M. Rochman et al., 2013) but also affect the gene expression of medaka fish from the genetic level (C. M. Rochman, Kurobe, & Flores, 2014) similar to the inhibition of proteins synthesis in *Clarias gariepinus* (Karami, Romano, Galloway, & Hamzah, 2016). For *Daphnia magna*, the toxicity of MPs adsorbing phenanthrene is higher than that of single MPs (Y. N. Ma, Huang, & Cao, 2016). This study suggested that more studies about the cellular gene level are needed to provide information for the further understanding of the toxicity mechanisms.

2.7 MPs ecological risk assessment

Everaert et al., 2018b had performed risk assessment of MPs in the ocean by Modelling approach. They performed an environmental risk assessment for MPs (<5 mm) in the marine environment by estimating the MPs of the past, present and future concentrations based on global plastic production data. For the effect assessment, they complied the available scientific literature for effect data that expose marine organisms to MPs. NOEC and LOEC were apply according to European Union (EU) legislation (EC, 2006). If several chronic NOEC or LOEC values for different endpoints were available for a single species, the lowest value was used. LOEC values were converted to NOEC values by dividing them by 2 (OECD,1997). The species sensitivity distribution (SSD) of the NOEC values was developed using a lognormal model as described by Aldenberg and Jaworska (2000) and implemented by Szöcs (2015) using the fitdistrplus package in the free statistical software R (R Development Core Team, 2015). The mean HC_5 (hazardous concentration for 5% of the species) and a confidence interval around the HC_5 were derived using 1000 random parameter iterations of the distribution. As stipulated in the EU legislation, the safe concentration, also known as predicted no effect concentration (PNEC), was calculated from the HC_5 using an assessment factor (AF) of 1-5 (EC, 2006). Such AFs are often applied to effect data to yield a dose or concentration to which humans or organisms may be exposed that is expected to be safe. Their risk assessment

(excluding the potential role of MPs as chemical vectors) suggests that on average, no direct effects of free-floating MPs in the marine environment are to be expected up to the year 2100.

P. Xu et al., 2018 conducted risk assessment in surface waters of the Changjiang Estuary by using data on both the concentration and chemical hazard of MP polymers. The results indicated that PVC caused a critical concern for MP risk. Especially, areas around aquaculture farms were regarded as “hotspots” of MP pollution due to the accumulation of MPs and the presence of hazardous MP.

R. Li, Yu, Chai, Wu, & Zhu, 2020 explored the mangroves of Southern China, by collecting surface sediments to explore MP concentration and to evaluate ecological risk. Based on the results, the higher MP concentration in the Futian mangrove was mainly related to inputs from the Pearl River, the third largest river in China. MPs concentration in mangrove sediments increased with more social-economic development of surrounding districts, which indicated the clear influence of anthropogenic activity on MP pollution in these mangroves. Based on a comprehensive evaluation using the potential ecological risk factor (Ei), potential ecological risk (RI), polymer risk index (H) and pollution load index (PLI), MPs were found to present ecological risks in these mangroves, with the highest risk occurring in the Futian mangrove.

Wang et al., 2021 stated that, this study investigated the characteristics of MP pollution during dry (April) and wet (July) seasons in surface water of the Manas River Basin, China. The average abundance of MPs in April (17 ± 4 items/L) was higher than that in July (14 ± 2 items/L). The range in the abundance of MPs in April and July were 22 ± 5–14 ± 3 items/L and 19 ± 2–10 ± 1 items/L, respectively. Highly hazardous polymers such as Polyvinyl chloride (PVC) and Polycarbonate (PC) have a significant impact on the results of the evaluation of the presence of MPs.

CHAPTER III

MATERIAL AND METHODS

3.1 Study area and sample collection

Central wastewater treatment plant is located at Bang Plee Industrial Estate, Bang Sao thong, Samut Prakarn. This plant receives pre-treatment wastewater from 1 6 7 industrial factories include plastic materials, frozen food, paper, cosmetic products, stainless metals, mechanic shatters, motor parts, paint, electroni cs, tire, rubber, and clothes etc. The treatment capability of this plant is approximately 4,000 - 8,000 m^3/day. Due to the dominant influent is from food manufacturing, the major parameter needed to treat is organic matter. Thus, this plant work mainly on activated sludge system which include grit chamber, aeration, and sedimentation. Sludge from sedimentation pond is recirculated to aeration pond and the excess sludge is disposed by the waste management company. The effluent is directly discharge into a public canal named Khlong Hua Kluea, Khlong Mai, Khlong Bang Pu and then go through inner gulf of Thailand.

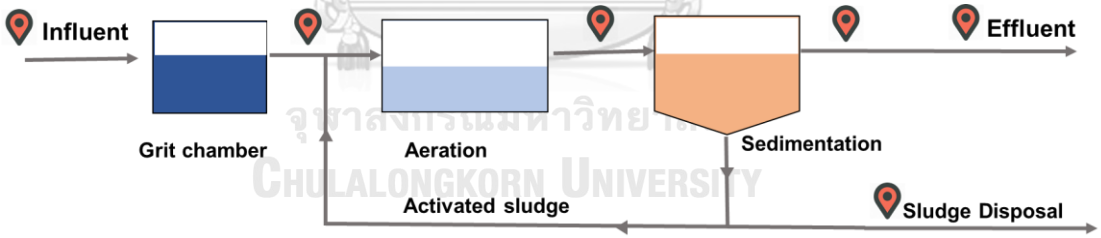

Figure 3 Wastewater treatment plant and sampling point

Samples were collected in February and July 2020 to represent dry and wet seasons. The sampling points were at influent, after the grit chamber, after the aeration, after the sedimentation, effluent, and sludge. Moreover, 2−15 L of water and 60 g of sludge were collected by bulk sampling with three replications. The wastewater samples were sieved through an 8-inch-diameter steel sieve of four size fractions (300 µm, 212 µm, 100 µm, 20 µm) in the field.

3.2 Materials

3.2.1 Stainless steel sieves, an 8-inch-diameter steel sieve (300 µm, 200 µm, 100 µm, 20 µm)

3.2.2 Distilled water bottle

3.2.3 250-mL glass beaker

3.2.4 Metal spatula

3.2.5 Drying oven (Binder Series ED Avantgarde.Line)

3.2.6 Iron (Fe (II)) solution

3.2.7 30% Hydrogen peroxide

3.2.8 Stir bar

3.2.9 Hot plate

3.2.10 Watch glass

3.2.11 Sodium chloride

3.2.12 Standard metal forceps

3.2.13 Density separator funnel

3.2.14 4-mL glass vials

3.2.15 Stereo microscope (NSZ-405J3)

3.2.16 FT-IR (FTIR PerkinElmer Spectrum IR 10.6.2)

3.3 Sample Processing

Processes were based on the NOAA Marine Debris Program method with some adjustment.

3.3.1 Rinsing

Transferred the sieved sample to 250 ml beaker. Rinsed sample bottle with distilled water 2-3 times until no organic matters detected.

3.3.2 Drying sieved solids

Placed beaker in 60 ^{o}C drying oven for 24 hours, cover with aluminum foil.

3.3.3 Digested samples via Wet peroxide oxidation (WPO) process

a) Added 20 mL of 0.05 M Fe (II)NO_3 solution and 20 mL of 30% hydrogen peroxide with a stir bar to the beaker and covered with a watch glass. Heated up to 65ºC on a hotplate, left for approximately 45 minutes. If natural organic material was visible, added more 20 mL of 30% hydrogen peroxide and then repeated until no organic material detect.

b) Added approx. 6 g of NaCl (1.2 g/cm^3) per 20 mL of sample to generate aqueous solution (5M NaCl) and heated mixture to 65ºC until the salt dissolves.

3.3.4 Density Separation

a) Rinsed the sample beaker with distilled water to transfer all remaining solids to the density separator, covered loosely with aluminum foil and settle overnight.

b) Discarded settled solids from the separator.

c) Rinsed glass funnel with NaCl to wash out the particles that attached to the glass and left for settle down 1 hr. Repeated this step 2-3 times.

3.3.5 Filtration

a) Transferred sample from glass funnel directly to vacuum filtrated through microfiber filter with 0.45 µm pore size (Cellulose nitrate filter).

3.4 Sample Identification

3.4.1 Microplastic identification

The physical shape of MP particles was characterized using stereo microscope (NSZ-405J3) as fiber, film, pellet, and fragment. For the definition, fibers have a relatively even or consistent thickness along their entire length and illustrate three-dimensional bending (Dris, Gasperi, et al., 2015). Fragments are hard and jagged plastic particles, pellets are hard round plastic particles, and film are thin plane flimsy plastic (Free et al., 2014)

3.4.2 Polymer characterization

The polymer composite was examined using Fourier Transform infrared spectroscopy (FTIR PerkinElmer Spectrum IR 10.6.2) with 24 scans to incur wavelength region of 450-4000 cm^{-1}. Spectra were compared to the libraries provided by PerkinElmer. About 10% of the potential particles were randomly picked for identification (Mahon et al., 2017).

3.5 Quality control

3.5.1 Background blank

To estimate the potential for airborne contamination, background blanks are taken whenever samples are processed. For this, a membrane filter was placed in a petri dish without a cover in the area where work was performed. At the final of each eight-hour period, the petri dish was examined (E. A. Gies et al., 2018b)

3.5.2 Field blank

Another test is field blank, which the samples were treated exactly in the same manner as the other samples, using distilled water instead of wastewater and sludge (Lares et al., 2018).

3.5.3 Spiked recovery

Evaluation of the potential for loss during sieving or transfer from glassware, spiked samples with known amounts of MPs as described earlier in ‘sample collection’ was conducted. Recovery of particles is determined using:

$$\text{Recovery } (\%) = \frac{\text{Known MPs - Counted MPs}}{\text{Known MPs}} \times 100\% \quad (3.1)$$

In order to avoid external plastics contamination, almost the vessels used during the experiment were glassware or stainless steel. To minimize contamination, the sample processing was performed under a fume hood and all laboratory glassware were covered with aluminum foil (Nuelle, Dekiff, Remy, & Fries, 2014). During flotation process, the flasks were covered by aluminum foil to prevent MPs contamination from atmosphere (X. Li et al., 2019).

3.6 Ecological risk assessment

3.6.1 Hazard index assessment

In order to estimate the potential risk occurred by MPs, both number and polymer composition effects have to be considered. The risk was calculated separately. To evaluate potential risk of polymer toxicity which might harm aquatic ecology, this study applied Lithner's hazard scores (Lithner, Larsson, & Dave, 2011) to evaluate the risk by the following formula:

$$E_i = T_i \times \left(\frac{C_i}{C_0}\right) \quad (3.2)$$

$$RI = \sum_{i=1}^{n} E_i \quad (3.3)$$

where E_i is the potential ecological risk factor

RI is the potential ecological risk

T_i is the chemical toxicity coefficient for the constituent polymer (Lithner et al., 2011)

C_i/C_0 is the observed MP concentration (C_i) divided by background level (C_0).

Due to a lack of available background data, the lowest MPs concentration measured in this study was adopted as the background value. *(*R. Li et al., *2020)*

Acquiring values of E_i and RI were not important points, but it can be used to compared with other researches. Another assumption that can be used to indicate the risks of MPs (Lithner et al., 2011), as follows:

$$H = \sum P_n \times S_n \quad (3.4)$$

where H is the polymer risk index

P_n is the percent of MPs polymer composition

S_n is hazard score from Lithner et al. (2011) for each type of polymer (PE = 11, PP = 1, PMMA = 1021, PET = 4).

3.6.2 Risk characterization method

Another way to conduct risk assessment is to compare the detected concentration at effluent and sludge to the derived PNEC (Predicted no effect concentration). If the observed concentrations are above the PNEC value, it is indicated that the sample can further cause risk in environment. On the other hand, if the observed concentrations are below the PNEC value, it can be concluded that there are no ecotoxicological effect.

This study derived PNEC by SSD (Species Sensitivity Distribution) method, which is a cumulative probability function based on ecotoxicity tests from multiple species representing a range of taxa (Posthuma et al., 2001).

a) Exposure assessment

Predicted environmental concentration (PEC) deprived by average microplastics concentration from treatment plant effluent in both dry and wet seasons.

b) Effect assessment

1) Review of the literature on effect thresholds

First of all, various literature could present many types of data. The endpoints most frequently used for deriving PNECs are mortality (LC_{50}: Median Lethal Concentration), growth (ECx: Effective concentration or NOEC: No Observed Effect Concentration) and reproduction (ECx or NOEC). LOEC (lowest observed effect concentration) can be obtained, in which case NOEC not presented, by calculated as LOEC/2. (ECHA, 2017)

Assessment factors (AFs) are used to address the differences between laboratory data and natural conditions, taking into account of interspecies differences and intraspecies differences. Assessment factors applied for long-term tests are smaller because the uncertainty of the extrapolation from laboratory to natural environment is reduced. More data on more species in the same environmental compartment can also reduce uncertainties, thus further decreasing assessment factors.

2) Conducting species sensitivity distributions (SSDs)

According to ECHA guidance, Species Sensitivity Distributions (SSD) are a very important technique used in ecological risk assessment. It is primarily used to derive predicted no effect concentrations (PNECs) for environment risk assessment. SSD properly shows different sensitivities of various species (fish, invertebrates and plants) to the same chemical substance and the variation among those species can be described by a statistical distribution using the Log-normal model and derive HC_5 (hazardous concentration for 5% of species) (ECHA, 2017).

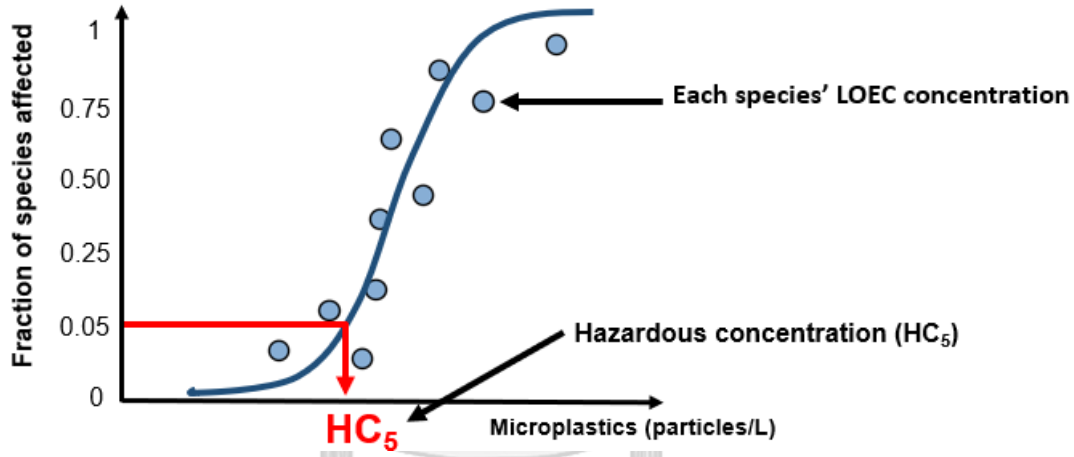

Figure 4 Example of species sensitivity distribution

3) Calculate PNEC by derived HC_5

Derived HC_5 can then be used to calculate PNEC by dividing it with an additional safety factor (1-5) as PNEC = HC_5/AF. If there is a small dataset (i.e. algae, daphnid and fish), the SSD approach will not be applicable. In that case, it usually needs to divide the lowest NOEC value with larger safety factor (typically 10) (ECHA, 2017).

c) Risk characterization

$$\text{Risk quotient (RQ)} = \frac{\text{Predicted Environmental concentration (PEC)}}{\text{Predicted No Effect Concentration (PNEC)}} \quad (3.5)$$

RQ < 1: no immediate risk

RQ >1: environmental contamination exceeds the safe concentration.

3.7 Statistical analysis

The average numbers and standard deviations of three replicates were calculated and presented as mean ± standard deviation. To evaluate the influence of season variational (dry and wet season) and treatment units (influent, after-grit chamber, after aeration, after sedimentation, effluent, and sludge), size fraction, and shape on MP abundances, a two-way ANOVA was performed. Regarding the differences in MP content in each unit and season, a paired t-test was conducted. The statistically significant value was $p < 0.05$.

CHAPTER IV
RESULT AND DISCUSSION

4.1 Method validation and efficiency

Since there is no standard procedure to identify microplastics, then many methods were applied. Every method provided different quality. For instance, to understand the quality of procedure, method efficiency test must be performed. In this work, spiked recovery was performed using 30 green column-shaped PP and 10 red column-shaped PET spiked into the sample, treating similar to the collecting sample to observe method efficiency.

$$\text{Recovery (\%)} = \frac{\text{Known MPs - Counted MPs}}{\text{Known MPs}} \times 100\% \quad (4.1)$$

$$\text{Recovery (\%)} = \frac{40 - 4}{40} \times 100\% = 90\% \quad (4.2)$$

From the test, 36 particles were detected, accounted as 90% of spike microplastics. Although, it is not all MPs were discovered but more than 90% recovery indicated satisfactory result.

According to the field blank test, 20 particles were found. While in background blank was undetected. These amounts were lower than 10% of the MPs' average found, considered good contamination control, as suggested by (Lusher, 2015). While background blank did not contain any microplastic particles.

4.2 Microplastics abundance

From Table 2 and Figure 5, it was found that average microplastics in this central wastewater treatment plant was 414 particles which was 247 particles for dry season sample and 168 particles for wet season sample. The influent sample was presented only in the wet season due to inconvenience in the dry season. MPs detected in influent was 103.13 ± 59.48 particles/L.

Table 2 Microplastics abundance in wastewater treatment units

	Detected MPs	
Treatment unit	**Dry season**	**Wet season**
Influent	-	103.13 ± 59.48
Grit chamber	80.11 ± 33.04	19.32 ± 4.75
Aeration	134.35 ± 20.79	31.38 ± 10.36
Sedimentation	13.98 ± 4.50	7.49 ± 3.08
Effluent	18.17 ± 7.87	6.33 ± 1.36
Sludge	2.27 ± 0.08	1.86 ± 0.28

*particles/L for wastewater, particles/g dry weight for sludge

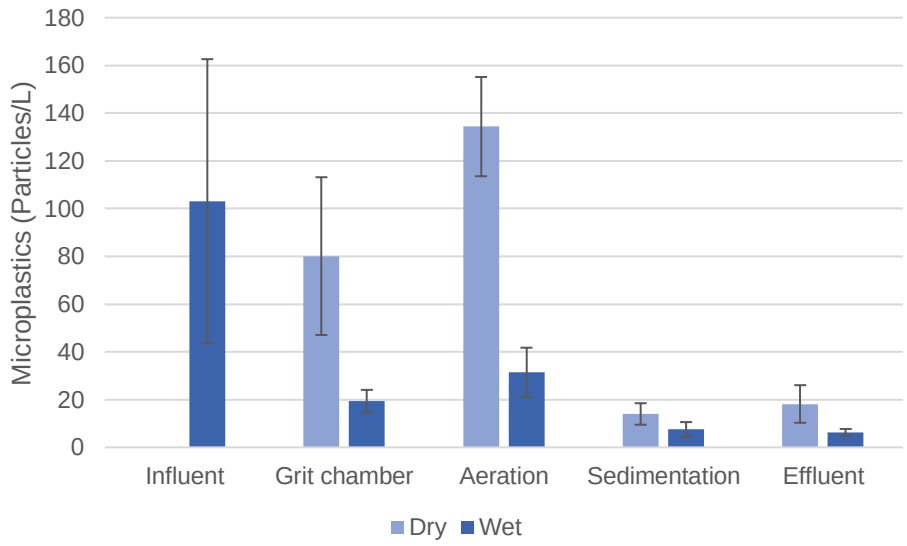

Figure 5 Microplastic abundance in wastewater treatment units in dry and wet seasons

The previous researches presented that the number of MPs found in different WWTPs was very variety. Some WWTP found only small number of MPs e.g., 0.25 particles/L (Murphy et al., 2016), 0.48 particles/L in Australia (Ziajahromi et al., 2017), on the other hand, others showed high number with 31.1 particles/L from Vancouver (Esther A. Gies et al., 2018) and 126.0 particles/L from China (Jiang et al., 2020) as in Table 3. Nevertheless, they were reported that municipal WWTPs deal with microplastic ranging from 0.28 – 6.10 x 10^2 particles/L, while in industrial WWTPs the microplastic abundances ranged from 1.60 – 3.14 x 10^4 particles/L. This

evidence can be roughly concluded that industrial WWTPs receive higher amount of microplastics. Being the cause of higher MP abundance found. (W. Liu et al., 2021)

Table 3 Microplastics compared with other studies

Country	**MP final effluent (particles/L)**	**Removal efficiency (%)**
China (Jiang et al. 2020)	126.00	75.7
Netherland (Leslie et al. 2017)	51.00-81.00	72.0
Denmark (Simon et al. 2018)	54.00	99.3
France (Dris et al. 2015)	14.00-50.00	95.0
China (Liu et al. 2019)	28.40	64.4
Thaliand (This study)	24.50	93.9
Turkey (Gűndoğdu et al. 2018)	7.02	73.0
Finland (Lares et al. 2018)	1.05	99.4
Australia (Ziajahromi ei al. 2017)	0.48	99.4

From the statistical analysis, the result indicated that the treatment units caused distinction on MPs abundance (p=4.83E-07). The highest number of MPs was found in the aeration unit, where the dry and wet season samples were 134.35 ± 20.79 and 31.38 ± 10.36 particles/L, respectively. This result was like WWTPs in eastern China (Lv, Dong, Zuo, Liu, & Wu, 2019) that also reported the highest value of MPs was presented in the aeration unit, as an air generator might cause broken MP particles to be distributed thoroughly in the following treatment units.

The lowest MP numbers were found in the sedimentation unit of the dry season sample (13.98 ± 4.50 particles/L) and in the effluent of the wet season sample (6.33 ± 1.36 particles/L). The sediment unit was designed to remove organic matter, and MPs were affected by this process through adsorption and fouling by bacteria. Other physical processes could cause sinking (Magni et al., 2019), according to the wet season sample. However, the dry season sample acted in a dissimilar way; the effluent MPs were higher than in sedimentation because the increased contact time of sludge in the treatment system may lead to higher MPs found in the effluent (Carr et

al., 2016). Hence, there is no significant distinction between sedimentation MPs and effluent in both the dry and wet seasons (P=0.426, P=0.695). Previous same-size limitation studies (20–25 μm) reported effluent MP abundance as 0.005–13.5 particles/L (Michielssen et al., 2016; Mintenig et al., 2017; Talvitie et al., 2015; Talvitie, Mikola, Koistinen, & Setala, 2017b; Ziajahromi et al., 2017) which was quite similar to this study.

For instance, the distinction happened between microplastic abundances due to several factors, such as flow rate, population serve, construction site, treatment management. Moreover, the socio-economic development area was significantly linked to MP pollution in mangrove (R. Li et al., 2020). While the technologies were quite important, WWTP with membrane bioreactor seems to be appropriate for preventing MPs discharged into the environment which provided around 99% removal efficiency. w(Lares et al., 2018; X. Lv et al., 2019; Ziajahromi et al., 2017)

The MPs in sludge abundance were 2.27 ± 0.08 and 1.86 ± 0.28 particles/g dw in the dry and wet season samples. It was a higher number than WWTP from the Netherlands (0.37-0.95 particles/g dw) (Brandsma, Nijssen, Van Velzen, & Leslie, 2013)) and Sweden (1.7± 1.96 particles/g dw) (Magnusson & Norén, 2014)). In contrast, compared to a study from Spain (165 ± 37 particles/g) (Edo, González-Pleiter, Leganés, Fernández-Piñas, & Rosal, 2020) and Finland (170.9 ± 28.7 particles/g dw (Lares et al., 2018), their number was much higher than this study. Some studies have shown that microplastics are effectively removed from wastewater, and most particles end up in sludge (Magnusson & Norén, 2014; Michielssen et al., 2016; Talvitie et al., 2015; Talvitie, Mikola, et al., 2017b). It is also possibly dependent on the effectiveness of sedimentation units or MP particle properties. The issue that has to be concerned is sludge utilization like incineration, landfill, soil compost, and agriculture, because microplastics contaminate in soil affect the agricultural crops. (Corradini et al., 2019)

According to the wet season sample, the most effective MP removal unit was from the grit chamber (81.27%). This result is similar to (X. Liu, Yuan, Di, Li, & Wang, 2019a) who reported the highest reduction rate of MPs at 40.7%, which originated from the grit chamber and primary settling tank. (Iyare, Ouki, & Bond, 2020) report that preliminary and primary wastewater treatment removed 92–93% of

microplastic particles. (Talvitie, Mikola, et al., 2017b) found that fibers were largely removed during primary sedimentation due to its physical shape. Most MPs could be removed during the primary treatment stages through settling processes. The high reduction in this stage possibly caused many MP particles to be adsorbed by suspended solids in wastewater (Carr et al., 2016; Lares et al., 2018).

Secondary treatment is usually designed to remove suspended solids and dissolved solids remaining in wastewater by mostly biological treatment (Patel, 2015). Almost treatment plants mainly use activated sludge in this stage. The flocculation is the core process to aid the removal of microplastic through accumulation of particulate matter (Murphy et al., 2016). By the way, there were not confirmed that microplastics were trapped effectively with flocs, but at least the MP particles were reduced.

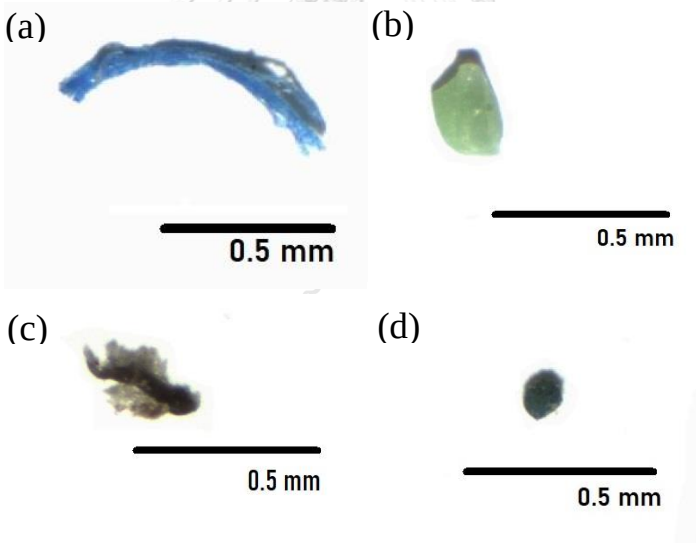

Figure 6 Microplastic from stereomicroscope; (a) fiber (b) fragment (c) film (d) pellet

This plant's treatment units do not contain any tertiary or additional treatment technologies, i.e. various membrane filtration or dissolved air flotation system. The additional processes mostly provided high removal rate, because the residual microplastic will be trapped once again after tertiary treatment. In the plant in Finland with gave 99.4% - 99.9% and 99.9% MPs removal efficiency by MBR (Lares et al., 2018; Talvitie, Mikola, et al., 2017b) and gravity filtration (Carr et al., 2016).

The final removal efficiency of this treatment plant was 93.86%, which was rather high compared to the similar size limitation study. Therefore, some MPs still released into the environment. The studies in Asia reported 53.7–83.60% efficiency (Hongprasith et al., 2020; Jiang et al., 2020; X. Liu et al., 2019a; X. Lv et al., 2019). However, in Europe, researchers reported higher efficiency ranging from 75−99% (Esther A. Gies et al., 2018; Lares et al., 2018; Leslie, Brandsma, Velzen, & Vethaak, 2017; Murphy et al., 2016; Ziajahromi et al., 2017). This is possibly attributed to lower population densities and better waste management systems in highly developed countries (Jambeck et al., 2015; Lebreton, van der Zwet, & Damsteeg, 2017).

Although approximately 10 MP particles/L found in effluent seems does not matter, but the effluent from WWTP discharges with high flow rate per day (8,000 m^3/day in dry season sample and 4,000 m^3/day in wet season sample) natural. Although, this study released MPs with effluent approximately 1.44×10^8 particles per day in dry season and 2.5×10^7 in wet season sample. This number considered as high compared to other studies e.g., 4.25×10^4 particles/day from Sweden (Magnusson & Norén, 2014), 4.65×10^6 particles/day from Australia (Ziajahromi et al., 2017), quite similar compared to 8.40×10^9 particles/day from France (Dris, Gasperi, et al., 2015), 1.97×10^8 particles/day from Finland (Talvitie et al., 2015), and lower compared to 1.48×10^{10} particles/day from USA (Michielssen et al., 2016). In addition, several factors can drive this variation such as different site, sources of microplastic, treatment units, and the temporal variation.

4.3 Microplastic characteristics distribution

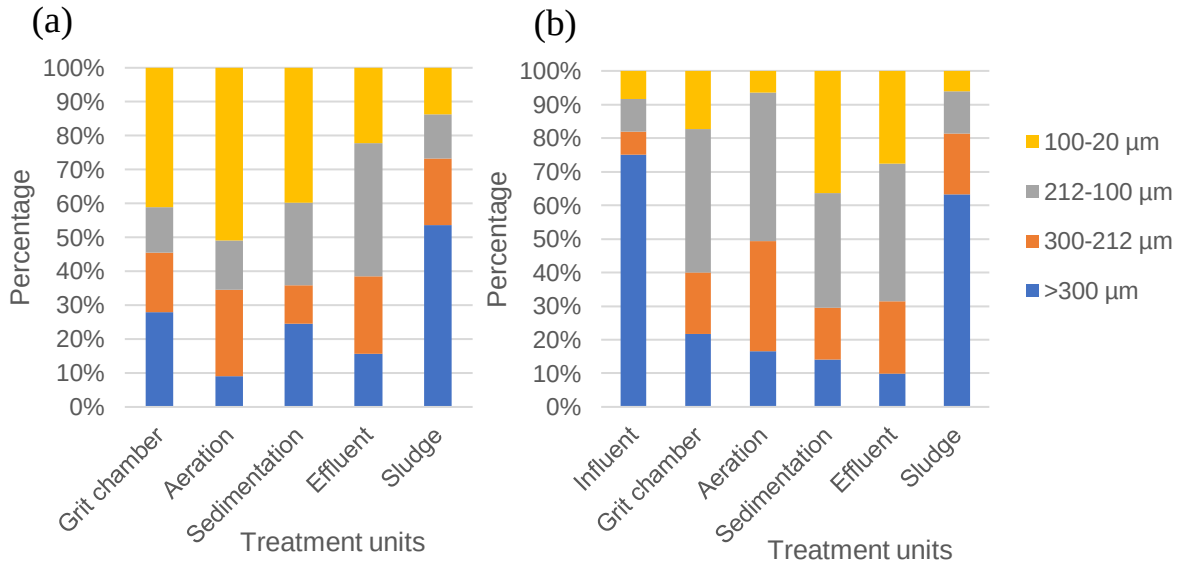

Figure 7 Microplastic percentage (a) size fraction in dry season, (b) size fraction in wet season

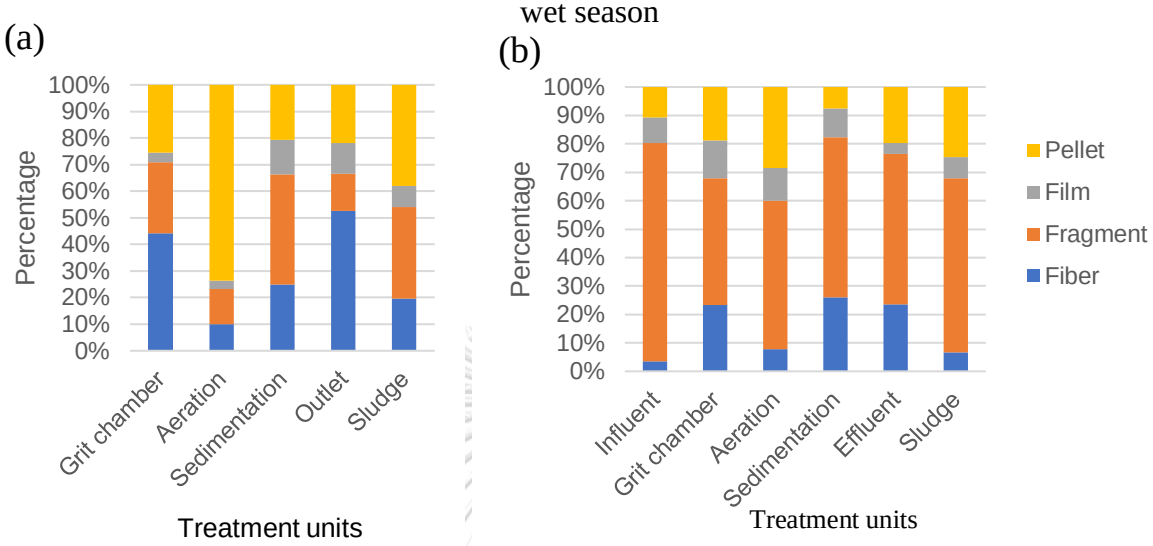

Figure 8 Microplastic percentage (a) shape fraction in dry season, and (b) shape fraction in wet season

The largest scale of MPs in the influent was >300 µm. When the water passed through the grit chamber, the large-scale MPs were removed (Fig 4.3). Grit chambers could effectively prevent large debris and cause the significant reduction of MPs in wastewater (X. Liu et al., 2019a). In the aeration unit, there was a differentiation between dry and wet seasons; the dominant size in the dry season sample was 100–20 µm and in the wet season sample was 212–100 µm. The large MPs (>300 µm) were significantly removed during the processes (P=0.006). Hence, the number of smaller MPs increased, and larger MPs decreased from influent wastewater, just as (X. Liu et al., 2019a), found that the smaller-sized fraction (300–20 µm) becomes higher throughout treatment units Some MP particles were broken by mechanical contact in the grit chamber and aeration unit. However, (Ziajahromi et al., 2017), with the same size limitation (20 µm), stated that only 20% of microliters were larger than 300 µm, due to various site and sample procedures.

In the sedimentation unit, the size fraction was the same as in the aeration unit and effluent, which most of the large size MPs were removed significantly. The

majority size of MPs in sludge in both dry and wet season samples was >300 μm. The larger particles showed heavier weight, leading to settling in the sludge.(X. Liu et al., 2019a; X. Lv et al., 2019). Likewise, (Murphy et al., 2016) concluded the size of MPs in the sludge was higher than in the wastewater because the smaller particles might still remain in the water mass. However, there were some reports explored different pattern, they found smaller microplastic particles (up to 60–70 μm) retained in the activated sludge, while the larger particles found in the effluent. This might be due to its process and treatment units. This highlighted the harm of the issue, smaller size microplastic particles were more probable to be ingested by aquatic livings such as plankton, invertebrate organisms, and fishes, which will lead to ecotoxicological effects to these ecosystem (Qiao et al., 2019).

The shape of microplastics is also an important factor which has an impact on their removal performance in WWTPs treatment units (McCormick et al., 2014). The dominant shape of MPs in wastewater in the dry season samples was pellet (51%), while in the wet season samples (Figure 7d), it was fragment (67%). Similarly, the dominant shape of sludge MPs in the dry season samples was pellet (38%) and fragment (34%), while in the wet season samples, it was fragment (61%). Film shape considered as low number in both seasons. (Murphy et al., 2016) also reported that mainly fragments (67.3%) were found in wastewater. (X. Lv et al., 2019) accounted for fragments as the dominant morphotype (65%), with the same result as rural areas of China (Wei et al., 2020). Incidentally, other researchers have reported that fibers and fragments are the dominant shape. (Carr et al., 2016; Mason et al., 2016). Suspected MP particles in sludge were dominated by fragments (Esther A. Gies et al., 2018) due to an opportunity in which larger MPs can crack down throughout the treatment processes.

On the other hand, dry season samples showed that pellet MPs were majority, which this plastic shape were widely used in personal care products and cosmetics. They were still found in surface water as their incomplete removal from WWTPs (Cheung & Fok, 2017; Chelsea M. Rochman et al., 2015).

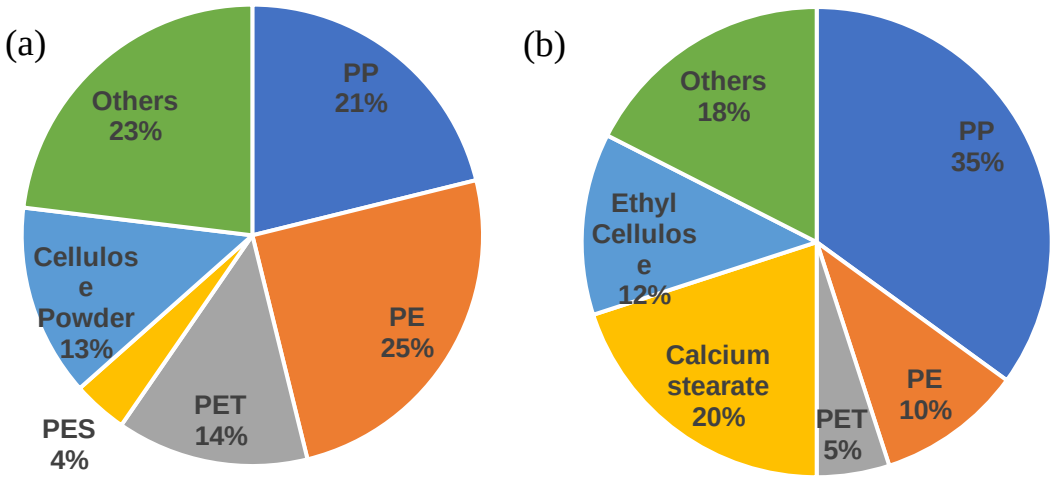

Figure 9 Polymer percentage: (a) dry season sample (b) wet season sample

97 MP particles were randomly selected from dry season (n=44) and wet season samples (n=53) to verify the polymer type. Moreover, 69 MP particles were confirmed as plastic polymers (71.13%). As shown in Figure 4.4, polypropylene (PP) was the majority polymer type in the wet season sample (35%), and polyethylene (PE) was common in the dry season sample (25%). (Edo et al., 2020) reported PE followed by PP as a majority polymer in wastewater and sludge. In Valencia, Spain, MP fragments were characterized as PE and PP as the main polymer (63% and 25%, respectively) (Alvim, Mendoza-Roca, & Bes-Piá, 2020). (Lares et al., 2018) found similar results, with 65.9% of MP fragments like PE in a WWTP in Finland. Polyethylene accounted for most plastics used in flexible packaging, plastic bags, film, and plastic bottles (R. Hernandez & Selke, 2001) Polypropylene also is popular for film usage. Both polymers generally originated from everyday products.

According to the FTIR results, the transparent fiber found in the dry season sample was related to PE (0.92-0.97 g/cm^3) and cellulose fiber. Fragments and film were characterized as PP (0.85 to 0.94 g/cm^3) and PE. The pellet MPs, which had the highest abundance in the dry season sample, were verified as polymethacrylate (PMMA 1.17–1.20 g/cm^3). In the wet season sample, fragments were considered PP and PE, but some fibers were PET, PP, and calcium stearate. Cellulose was found to be a mainly natural polymer from the results. Some particles taken from the same sample physically look alike under a stereomicroscope, showing different types of

polymers due to the adhesive interfering with the scanner, leading to the misinterpretation of results. (Dyachenko et al., 2017) stated that cellulose fiber was found to be a major interferent and most natural polymers were not digested by the catalytic WPO process by only one-time digestion. The most frequently found MPs are considered low-density particles (<1.2 g/cm^3) (Hidalgo-Ruz et al., 2012). Some high-density MPs were also

detected, but in a low number (i.e., PET with 1.37–1.45 g/cm^3). It indicates organic digestion is an important process, as well as the proper chemical substances to remove natural polymer and density separation to be effective. Otherwise, the effect of hazardous polymer will take an action on aquatic livings.

4.4 Effect of seasonal variation on MPs abundance and characterization

This study found that seasonal variation mostly influenced on size fraction , and shape as shown in Table 4. The difference of MP abundance between seasons was found on the aeration unit (p=0.029) in Table 5. Moreover, in Table 6, pellet shape and 100–20 μm size fraction were significantly affected by seasonal variation with p=0.012 and p= 0.013, relatively. The others found no significant distinction. As the run-off water system was separated from the wastewater treatment system, only small effects were observed.

Table 4 Result from Two-way ANOVA test; Seasonal variation of treatment units in each category (size fraction, shape) (*represent statistically significant P<0.05)

Treatment unit	Size fraction	Shape
Grit chamber	***0.001****	***0.001****
Aeration	***4.71E-05****	***1.95E-06****
Sedimentation	***0.042****	***0.017****
Effluent	***0.007****	***0.003****
Sludge	0.114	0.168

Table 5 Result from t-test between MP in each treatment unit of both seasons.
(*represent statistically significant P<0.05)

Size fraction			
> 300 µm	**300 - 212 µm**	**212 -100 µm**	**100 - 20 µm**
0.060	0.096	0.201	***0.013****
Shape			
Fiber	**Fragment**	**Film**	**Pellet**
0.073	0.180	0.148	***0.012****

Table 6 Result from t-test show distinction of MPs in each categories of both seasons
(*represent statistically significant P<0.05)

Treatment unit	**MP abundance**
Grit chamber	0.065
Aeration	***0.029****
Sedimentation	0.277
Effluent	0.111
Sludge	0.129

The other researches have shown greatly effect of season on microplastic abundance. The number of MPs in Nakdong River, South Korea, increased 10-fold from 1,410 items m^{-3} during dry season to 15,560 items m^{-3} during rainy season (Kang, Kwon, Lee, Song, & Shim, 2015). The higher rainfall during the wet season likely explains these differences because surface runoff and leachate can transport plastic debris from inland areas to streams and rivers (Shimizu et al., 2008) (Cunningham & Wilson, 2003). The study from Israel found that the MPs abundance in winter season is the highest number among other seasons (Ben-David et al., 2021) according to 45% increase in run off flow rate a higher usage of washing machines (Browne et al., 2011). This evidence supports the influence of run off pathway which was separated from this treatment plant.

Rainfall and storm events are regarded as major periods of contaminant input to recipient water (A. L. Andrady, 2011; Hitchcock, 2020). Mason et al. (2016) reported that a WWTP with a combined sewer system (i.e., including stormwater

runoff), after a storm event, could increase fragmented particles in effluent. (X. Li et al., 2018) stated that rainfall has a significant influence on the temporal variability of MP concentrations in sewage sludge. However, the researchers who paid attention to the effect of weather on MP abundance mostly collected the environment sample base.

By the way, the result maybe not represent the effect of seasonal variation perfectly, because the COVID-19 crisis which cause impact on many industrial factories in the area, half of it were closed, cause a reduction on flow rate (from 8,000 m^3/day to 4,000 m^3/day). Accordingly, the flow rate also played an important role with seasonal effect and must be analyzed concurrently. This might cause lower MP concentration and various MP characterizations. In addition, this study did not focus on storm events, rainy days, or prolonged sampling periods. Thus, this is an important factor in evaluating the effect of seasonal variation.

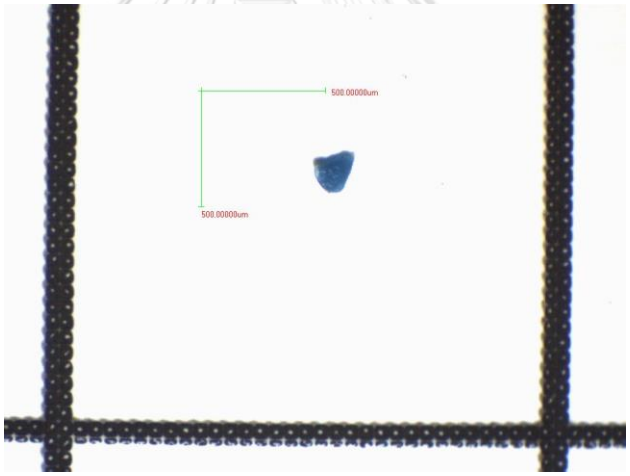

Figure 10 Nylon fragment MP particle with 200 µm diameter from 30x magnification

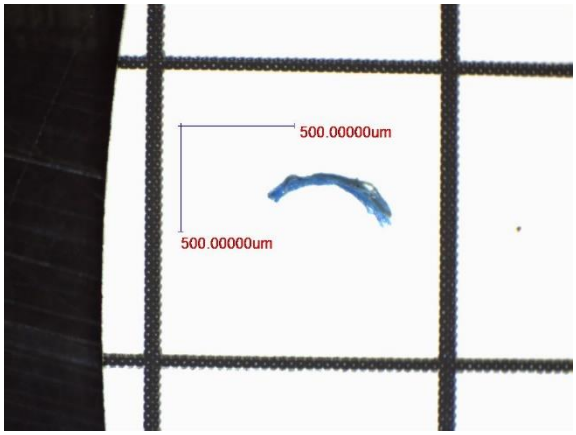

Figure 11 Polyethylene fiber MP particle with 500 µm length from 30x magnification

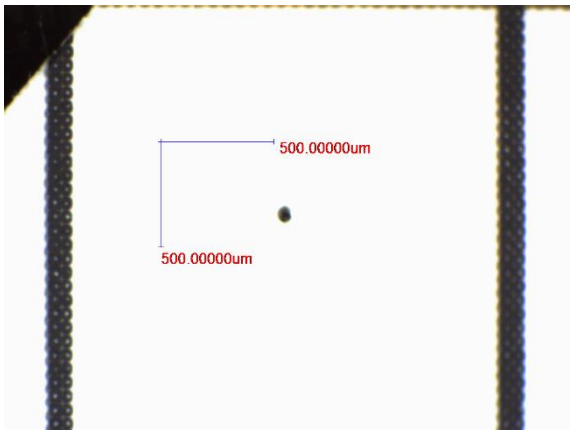

Figure 12 Asphalt pellet MP particle with 50 μm diameter from 30x magnification

4.5 Ecological risk assessment

4.5.1 Hazard scores index assessment

Plastics is a polymer which occurred by combination of monomers. It is produced to employed with diverse purposes. Unfortunately, aside from the benefits, it could produce toxicity pollution in the environment area. In the former times, study on the ecological risks caused by microplastics focused on the harm that may affect to organisms, bioavailability and the additives attached within them (Au et al., 2015; Lei et al., 2018; J. Li et al., 2018) which lacked of estimating the level of pollution. However, there are still no standardized method to assess the ecological risks caused by microplastics. Recently, there are some methods adapted to estimate the risk. Some works choose risk assessment models which normally were developed for the evaluation of chemical toxicity of plastic monomers (Lithner et al., 2011). This method was use along with microplastics which were found in environment by some researchers such as mangrove in china (R. Li et al., 2020), Changjiang Estuary, China (Pei Xu et al., 2018), the Dongshan Bay of China, (Pan et al., 2021), and freshwater river sediments in Shanghai (Peng, Xu, Zhu, Bai, & Li, 2018). Mostly, the microplastics sample were collected in the ocean, river, and other environments. This study is one of the early works to adapt the risk model from sample collected from wastewater treatment units which is the significant sources of pollution due to its various pathway from the beginning.

According to ecological risk model, the variables that were used in this study are E_i (Potential ecological risk factor), which is quotient for the observed MP concentration versus the background level ((Ci/Co) multiple by Polymer Hazard index), RI (the potential ecological risk) which is the summary of Ei from all monomers, either risk level will be presented in minor to extreme danger, and H (Polymer risk index) which is the multiple result from hazard score of MPs polymers and the proportion of each individual MPs polymer type. The risk categories represent in levels (I-IV). All method were already described intensively in Section 3.6. (Lima, Costa, & Barletta, 2014; R. Thompson et al., 2004)

Table 7 Ecological risk level and categories

Ei	**Risk category**	**RI**	**Risk category**	**H**	**Risk category**
<40	Minor	<150	Minor	<10	I
40-80	Medium	150-300	Medium	10-100	II
80-160	High	300-600	High	100-1,000	III
160-320	Danger	600-1,200	Danger	>1,000	IV
≧320	Extreme danger	≧1,200	Extreme danger		

Table 8 Potential ecological risk estimation of microplastics in effluent and sludge of wet and dry seasons

Season	Sample	Ei ((Ci/Co)*Polymer Hazard index)				RI (SumEi)	H (Sum Pn*PHI)
		PE	PP	PET	PMMA		
Dry	Effluent	59.540	1.439	4.787	2,306.6	2,375.4	230.38
Wet		66.121	13.427	4.000	5,089.9	5,173.5	203.49
Dry	Sludge	26.551	4.207	4.000	4,752.9	4,787.7	390.01
Wet		11.000	9.142	4.411	3,746.6	3,771.2	252.85

The results were categorized into effluent and sludge of wet and dry seasons (Table 6). The only main polymers from sampling were calculated i.e., PE PP PET and PMMA. Considering RI (the potential ecological risk) which based on the concentration of MPs polymer, the higher risk was found in effluent of wet season samples over dry season samples. This indicates that risk may occur the most in this period. The reason behind this is that the higher water surface temperatures during the summer season which are affected to the atmosphere temperatures, are assumed to urge the rate of plastic degradation which lead to shedding of toxicity or pollution (A. Andrady, 2015). However, all RIs were in extreme danger case (>1,200) either dry or wet season.

For polymer risk index (H) which based on percentage of MP polymer, the risk of microplastics in effluent either dry or wet season was in high level (100-1,000), and in dry season was higher. The different result occurs from two different calculations. One depends on concentration of MPs polymer, and another depends on percentage of polymer. This means the result is responsible to calculation methodology and the researchers have to choose this properly.

For sludge, dry season samples provided the highest RI value, stipulating to the extreme danger level. H value of sludge in dry and wet season samples were categorized in high level (100-1,000) and dry season samples showed higher value than wet season sample. In contrast to water sample, sludge sample showed similar risk level in both dry and wet seasons from both calculation method.

As mentioned previously that this study is an early study which collecting sample from wastewater and calculate risk level, so there is no comparable study. Nevertheless, there were several results from in-situ sampling. In the South of China, they studied the MP risk from mangroves. The risk index (H) was lower than 10 which indicated that there is a low level of chemical risk. It is due to the MP polymers found in this report were mainly low hazard scores such as PP (1), PE (11) and PS (30) and slightly higher scores, for example, polymethacrylates (PMMA) (1,021), styrene-acrylonitrile (6,788) and polyvinyl chloride (10,551) (R. Li et al., 2020). The value of H for the entire Dongshan Bay (estuary) was estimated as 12.94 based upon the average MPs polymer composition as Hazard Level II. While all values of RI were lower than 150, it indicated that potential ecological risk were minor in the Dongshan Bay (Pan et al., 2021). Comparing with this study, both values were high mainly due to its high hazard score of the PMMA polymer. Importantly, the calculation may underestimate the toxicity of MPs in aquatic environment owing to a lack of polymer toxicity that has not been observed.

According to the results, PMMA was the most significant polymer driving the risk values due to its hazard score (1,021), which is the highest among polymer types that found in this study. PMMAs are non-shape material thermoplastics with high transparency, which can be simply transformed and converted into many products like films, tubes, and sheets. Nevertheless, this PMMA has a quite high coefficient of thermal expansion, leading to tissue necrosis. PMMA can absorb water over several weeks at body temperature and properties such as tensile strength and fatigue strength, decrease upon water absorption. However, PMMA has poor stability, but it degrades very little in aqueous environment (Samavedi, Vaidya, Gaddam, Whittington, & Goldstein, 2014). By the way, in the ecosystem, the recipient area was not bear only one type of polymer. The chemical toxicity of the others MP polymers found in this study cannot be neglected. Since these additives may be easily released from MPs into ecosystem due to degradation by environmental condition i.e.,
sunlight, heat, wave etc. by river and ocean conditions (Capolupo, Sørensen, Jayasena, Booth, & Fabbri, 2020).

4.5.2 Risk characterization method

For comprehensive evaluation, this study chose another method to estimate ecological risk from microplastics which is a Species Sensitivity Distribution method (SSD) as a primarily used to derive predicted no effect concentrations (PNECs) for environment risk assessment. Normally, conducting risk from toxicity of polymer is not efficient because there are living things in the environment that will suffer from microplastic pollution. So, this method was adapted in this study. Since different species in aquatic environment (fish, invertebrates and plants) show various effect to the same chemical substance leading to many eco-toxicity endpoints, then to describe the variation between those species is to use a statistical distribution, the SSD will be appropriate statistical process, and derive HC_5 (hazardous concentration for 5% of species) from this process (EU, 2006). However, this method relies on the laboratory test of specific species. If the test results are sufficient, the method will have more quality.

This study refers to the ecological toxicity endpoint from literatures review, which were discover by Scopus search engine. The effect data with particles per liter unit were selected. Although most of the toxicity test conduct via mass per liter unit, cause less data founding. There were some studies suggest that the unit can be converted by dividing with a factor {Connors, 2017 #3503}, but this method might cause some distorted. In addition, marine species which can be found in Thailand gulf with no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) were included. The endpoints were mortality, growth rate, reproduction, and ingestion. The polymer type and other data presented in Table 7 to conduct SSD.

Table 9 Marine species polymer toxicity endpoint

Species	Concentration	Polymer	Endpoint	Reference
Crassostrea gigas	96.8	PS	Reproduction	(Sussarellu et al., 2016)
Perna viridis	600,000	PVC	Filtration and respiration rates	(Rist et al., 2016)
Brachionus koreanus	730,000,000	PS	Reproduction and life span	(Jeong et al., 2016)
Scrobicularia plana	2	PS	Antioxidant capacity and DNA damage	(Ribeiro et al., 2017)
Parvocalanus crassirostris	5,000	PET	Reproduction	(Heindler et al., 2017)
Tripneustes gratilla	100	PE	Growth	(Kaposi, Mos, Kelaher, & Dworjanyn, 2014)
Paracentrotus lividus	500	PS	Fertility	(Martínez-Gómez, León, Calles, Gomáriz-Olcina, & Vethaak, 2017)
Arenicola marina	110,000	PE	Increase of energy consumption	(Van Cauwenberghe & Janssen, 2014)
Tripneustes gratilla	300,000	PE	Larval growth and development	(Kaposi et al., 2014)
Lytechinus variegatus larvae	200	PE	Larval growth and development	(Nobre et al., 2015)
Calanus helgolandicus	75,000	PS	Decrease in survival and fecundity	(Cole, Lindeque, Fileman, Halsband, & Galloway, 2015)
Pomatoschistus microps	100	PE	Reduction of the predatory performance and efficiency	(de Sá, Oliveira, Ribeiro, Rocha, & Futter, 2018)

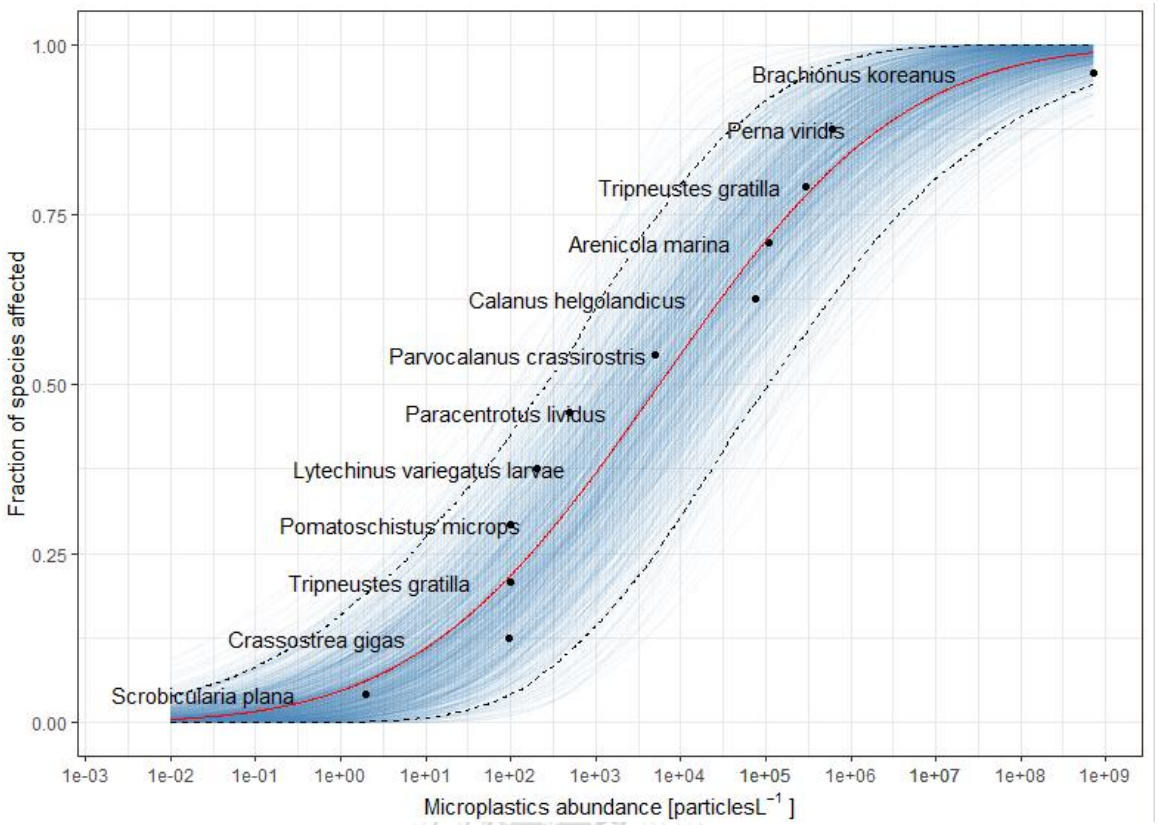

Figure 11 Log-normal species sensitivity distribution

A log-normal distribution with the species tested in laboratory and microplastics found in this work were presented. Black dots represent the NOECs of each species. Redline is the species sensitivity distribution, bordered by a confidence interval which is black dotted lines obtained using 1,000 random parameter repetitions (blue lines) of the lognormal distribution. The developed SSD based on the selected toxicity data of 12 marine species from Table 7 provided HC_5 of 1.143 particles/L (95% confidence interval: 3.56×10^{-3} - 1.32×10^{3} particles/L). From Table 8, when compared with the PNEC from the study of microplastics in the ocean, PNEC of 33.3 particles/L (95% confidence interval: 0.36 - 13,943 particles/L), it considered as a low number (Everaert et al., 2018a).

In consist, the study from (Ellen Besseling, Redondo-Hasselerharm, Foekema, & Koelmans, 2019; Emily E. Burns & Alistair B.A. Boxall, 2018) provided 640, 6.4 x 10^4 particles/L PNEC relatively. This distinction caused by different sources of sampling, methodology to derived PNEC and criteria on data selection. For example, the range of effect values used for HC_5 derivation in this study

(2-730,000,000 particles/L) was wider than those from (Jung et al., 2021) (340-4,000,000), (E. E. Burns & A. B. A. Boxall, 2018)(100,000-10,000,000), and (Ellen Besseling et al., 2019)(1000-100,000,000 particles/L).

Table 10 Comparison of PNEC

Location	Exposure assessment	PNEC (particles/L)	Reference
Global	Model prediction	6.4×10^4	(Emily E. Burns & Alistair B.A. Boxall, 2018)
Global	Model prediction	640	(Ellen Besseling et al., 2019)
Global	Model prediction	33.3	(Everaert et al., 2018a)
South Korea	Sampling	12	(Jung et al., 2021)
Thailand (This study)	Sampling	1.143	

The effluent contained microplastics 18.16 particles/L in dry season and 6.33 particles/L in wet season, both were much exceeding the PNEC value (1.14 particles/L). It indicated that there were 5% of selected aquatic species affected and the ecological risk occurred from released effluent. Even though, this wastewater treatment plant has a high microplastics removal efficiency (94%), but the results showed that it still possesses ecological risk to the ecosystem. However, as we known that there are many wastewater treatment plants around the world show inadequate MP removal efficiencies, it will cause severely effect to the living things.

Importantly, this method capability is depending on the quality and quantity of data. If the extensive eco-toxicity data available, it will enhance the effectiveness and provide closely actuality estimation. The microplastics limitation value calculated in this research might include some uncertainty because it was conducted from effluent which releasing from treatment plant, the concentration of microplastics might be underestimation from the realistic aquatic area. The sample from additional environmental site will be proper to confirm the results.

The expansive in-situ sampling must need to perform in order to confirm the results. Therefore, the problems of microplastics ecological risk assessment study including a lack of systematic and standardized models and acceptable background values. The ecological risk assessment in this study is to provide an initial understanding the potential ecological risks and to grant an information to rely on for plastic waste management (Pan et al., 2021).

CHAPTER IV

CONCLUSION AND RECOMENDATION

5.1 Conclusion

The MP abundance from an industrial wastewater treatment plant in Thailand during dry and wet seasons varied. Most MPs detected on the aeration unit in the dry season sample were 134.35±20.79 particles/L with a pellet shape (51%) and in influent was 103.13± 59.48 particles/L with a fragment shape (67%) in the wet season sample. Statistically significant differences were also found among the treatment units in two sampling times. The distinction might occur by not only the temporal variation but also system operation during the pandemic. The most common size fraction in the dry sample of the sludge sample contained 2.27 ± 0.08 and 1.86 ± 0.28 particles/g dw, which was relatively low. The most common size fraction in sludge was >300 μm in the shape of fragments (34% in dry, 61% in wet season sample) and pellets (38% in dry, 25% in wet season sample), due to the settling down of larger sizes and wrecking by mechanical processes that cause fragmentation. In addition, the 93.86% removal efficiency of this study was discovered, which was quite a high percentage, even though there are still some MPs discharging into the environment, accounting for millions of particles per day.

According to FTIR results, 71.13% was confirmed as a plastic polymer. Polypropylene (PP) was the majority polymer type in the wet season sample (35%), and polyethylene (PE) was common in the dry season sample (25%). However, the cellulose also detected from the sample indicated that additional digestion was required. The common polymers found in this study originated from plant production processes, that is, everyday products. Seasonal variations also influence MP abundance. Therefore, the result needs to be confirmed by prolonged sampling and atmospheric data for a better understanding of MPs during different seasons. Moreover, microplastics from WWTPs in Thailand must be considered, as they can be a major source of MP contamination in the environment.

The hazard index method was evaluated to estimate ecological risk. RI (the potential ecological risk) was in extreme danger category, and H value (polymer risk

index) was in high level. This method influenced by the polymer type mainly PMMA which attributes high hazard score and provides high risk index also. The risk might be underestimate due to others polymer types that was not include in the calculation. The ecological risk assessment results conducted by SSD method showed that the aquatic livings might be affected by microplastics. The limitation value from the 12 species stimulate calculation was 1.14 particles/L. While effluent found microplastics 18.16 particles/L in dry season sample and 6.33 particles/L in wet season sample, both were much exceeding the PNEC value. By the way, in-situ sampling was required to confirm the result.

5.2 Recommendation

1. To support the results, more WWTPs sampling will be appropriate. Thailand has 181 treatment plants around the country (Department of Industrial work, 2020). Some of them have a very large treatment capacity, but they have not been investigated on microplastics abundance. Hence, microplastic pollution situation in Thailand was negligent.

2. The seasonal variation effect has to be supported by long-period sampling. The trend of microplastics throughout the year will inform us how the dynamic distribution is moving. Whereas the consequence needs to be analyzed together with atmospheric data or meteorological information out of any factors i.e., system operation.

3. According to the results of ecological risk assessment, this study focused on sample from wastewater. However, the results need to be supported from environmental based sampling such as site in the river stream or ocean. Because of during the leakage into ecosystem, there are many processes that can cause microplastic sinking, breaking, or floatation, which will affect the microplastics abundance. It might lead to misinterpretation of risk assessment.

4. Microplastics detection has various methodologies which has been conducted. For example, changing the solution from NaCl to NaI or $ZnCl_2$, to use the invented separatory funnel, induced aeration, or centrifuge. Because different methods can cause different results. Although, the standard method is very important for comparation of the microplastics amount that has been found.

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APPENDIX A

Table A1 Microplastic abundances in dry season sample (1)

	Plastic avg (MP/L)											
Grit chamber	Fiber	Fragment	Film	Pellet	Overall	Red	Blue	Black	White	Transparent	Others	Overall
>300 µm	20.22222	0.666667	1	0.555556	22.44444	0.777778	0.777778	2.666667	0.555556	17.44444	0.111111	22.33333
300-200 µm	8.888889	3	1	1.111111	14	1.444444	1.555556	2.555556	1.777778	6.111111	0.444444	13.88889
200-100 µm	2.555556	5.222222	0.555556	2.333333	10.66667	1.222222	3.555556	1.333333	2.222222	1.555556	0.555556	10.44444
100-20 µm	3.666667	12.66667	0.333333	16.33333	33	3.666667	18.66667	2	4	3.333333	0.333333	32
Aeration												
>300 µm	3.8	5.288889	1.511111	1.511111	12.11111	1.155556	1.511111	3.622222	2.444444	3.377778	0.222222	12.33333
300-200 µm	3.644444	5.177778	1.622222	23.75556	34.2	4.311111	2.866667	21.57778	3.088889	3.088889	0.688889	35.62222
200-100 µm	2.288889	4.955556	0.844444	11.42222	19.51111	3.022222	5.311111	6.266667	2.4	1.688889	1.2	19.88889
100-20 µm	3.755556	2.177778	0.333333	62.26667	68.53333	2.022222	10.31111	51.53333	1.2	3.377778	0.333333	68.77778
Sedimentation												
>300 µm	0.977778	1.911111	0.133333	0.422222	3.444444	0.266667	2.755556	0.177778	0.022222	0.844444	0.022222	4.088889
300-200 µm	0.666667	0.622222	0.2	0.088889	1.577778	0.244444	0.733333	0.066667	0.155556	0.333333	0.044444	1.577778
200-100 µm	1.333333	1.111111	0.644444	0.311111	3.4	0.177778	1.466667	0.866667	0	0.866667	0.044444	3.422222
100-20 µm	0.491667	2.175	0.808333	2.083333	5.558333	1.458333	1.358333	2.5	0.125	0.125	0.083333	5.65

Table A2 Microplastic abundances in dry season sample (2)

	Plastic avg (MP/L)											
Effluent	Fiber	Fragment	Film	Pellet	Overall	Red	Blue	Black	White	Transparent	Others	Overall
>300 µm	1.922399	0.454145	0.16843	0.340388	2.885362	0.02381	1.12963	0.121693	0.02381	1.467372	0.047619	2.813933
300-200 µm	2.373898	0.74515	0.929453	0.145503	4.194004	0.07231	1.368607	0.047619	0.561728	2.143739	0	4.194004
200-100 µm	4.996473	0.912698	0.914462	0.429453	7.253086	0.309524	2.108466	0.458554	0.16843	3.847443	0.313933	7.206349
100-20 µm	0.375	0.458333	0.125	3.125	4.083333	0.833333	2.833333	0.25	0.083333	0.083333	0	4.083333
Sludge												
>300 µm	0.11274	0.513591	0.081423	0.488538	1.196292	0.100213	0.476012	0.062633	0.338219	0.081423	0.119003	1.177502
300-200 µm	0.081423	0.137793	0.062633	0.156583	0.438432	0.050106	0.169109	0.062633	0.09395	0.043843	0.025053	0.444695
200-100 µm	0.087686	0.062633	0.01879	0.119003	0.288112	0.012527	0.07516	0.03758	0.087686	0.07516	0.006263	0.294376
100-20 µm	0.375	0.4583333	0.125	3.125	4.0833333	0.8333333	2.8333333	0.25	0.0833333	0.0833333	0	4.0833333

Table A3 Microplastic abundances in wet season sample (1)

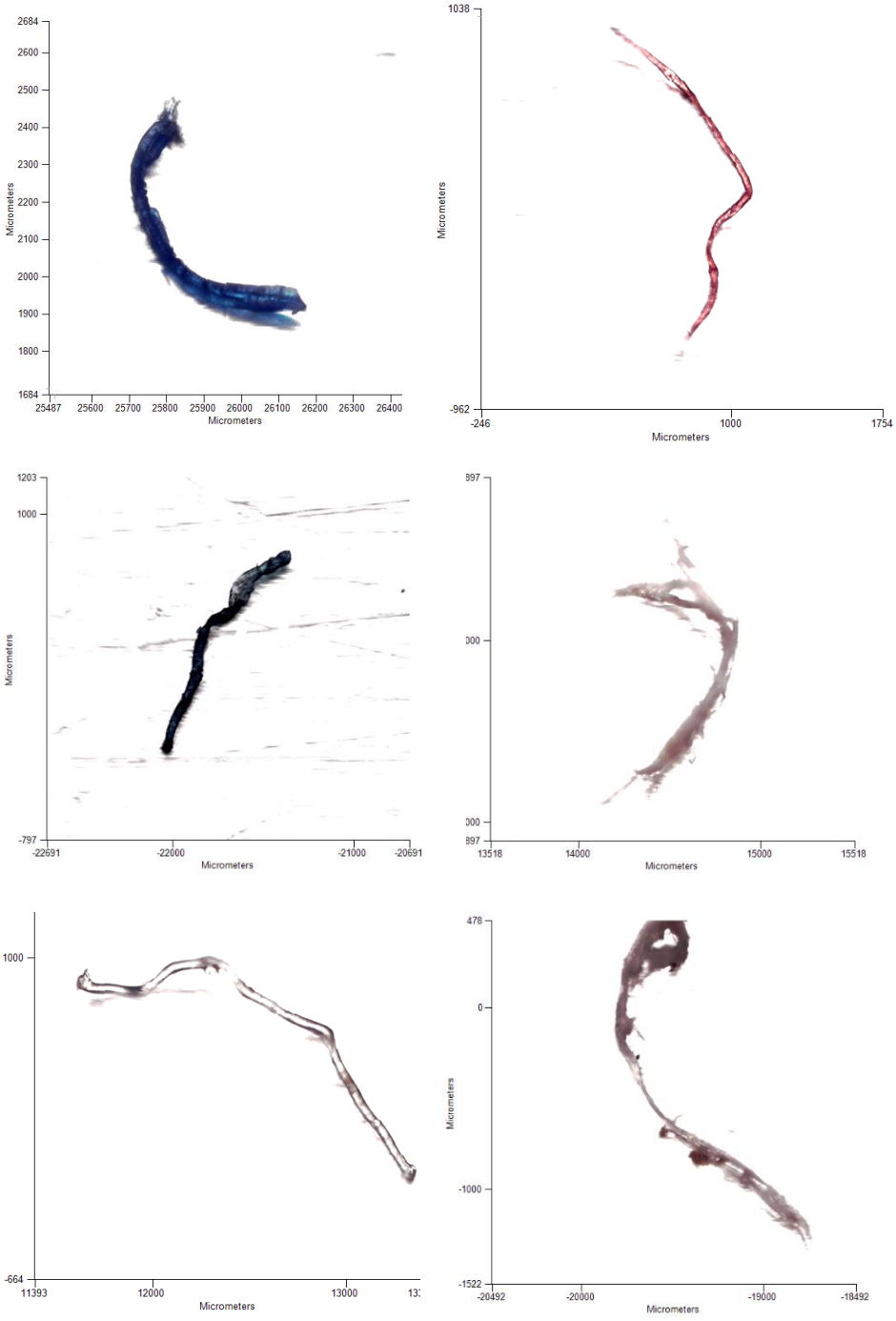
	Plastic avg (MP/L)											
Influent	Fiber	Fragment	Film	Pellet	Overall	Red	Blue	Black	White	Transparent	Others	Overall
>300 µm	0.3	80.8	5.366667	0	86.46667	0	6.966667	4.933333	74.13333	0.166667	0.266667	86.46667
300-200 µm	0.733333	3.933333	2.1	1.166667	7.933333	0.8	0.933333	4.433333	0.8	0.633333	0.5	8.1
200-100 µm	1.133333	3.233333	2.733333	4.033333	11.13333	0.666667	4.2	5.033333	0.166667	0.466667	0.6	11.13333
100-20 µm	1.8	0.5	0.266667	7.033333	9.6	0.166667	7.166667	0.833333	0.666667	0.766667	0	9.6
Grit chamber												
>300 µm	1.733333	1.733333	0.733333	0	4.2	0.133333	0.533333	1.066667	0.933333	1.533333	0	4.2
300-200 µm	0.933333	1.466667	0.6	0.533333	3.533333	0.066667	0.533333	0.8	1.2	0.733333	0.2	3.533333
200-100 µm	1	4.583333	0.666667	2	8.25	0.166667	2.666667	3.75	0.5	0.666667	0.5	8.25
100-20 µm	0.833333	0.833333	0.583333	1.083333	3.333333	0	1.25	0.416667	1	0.666667	0	3.333333
Aeration												
>300 µm	1.2	2.533333	1.4	0.066667	5.2	0.133333	0.666667	1.8	1.6	0.666667	0.333333	5.2
300-200 µm	0.466667	8.866667	0.466667	0.466667	10.26667	0.666667	0.8	4.333333	3.933333	0.2	0.333333	10.26667
200-100 µm	0.5	4.5	1.583333	7.333333	13.91667	0.583333	2.416667	10.33333	0.333333	0	0.25	13.91667
100-20 µm	0.25	0.5	0.166667	1.083333	2	0.083333	0.916667	0.666667	0.166667	0	0.166667	2
Sedimentation												
>300 µm	0.730864	0.17679	0.125432	0.026667	1.059753	0	0.209383	0.024691	0.25284	0.550123	0.049383	1.08642
300-200 µm	0.635062	0.390123	0.08	0.051358	1.156543	0	0.258765	0.024691	0.338765	0.534321	0	1.156543
200-100 µm	0.310123	1.722469	0.441481	0.08	2.554074	0	1.511111	0.443457	0.264691	0.281481	0.053333	2.554074
100-20 µm	0.281481	1.924938	0.100741	0.414815	2.721975	0.053333	2.409877	0.106667	0	0.152099	0	2.721975

Table A4 Microplastic abundances in wet season sample (2)

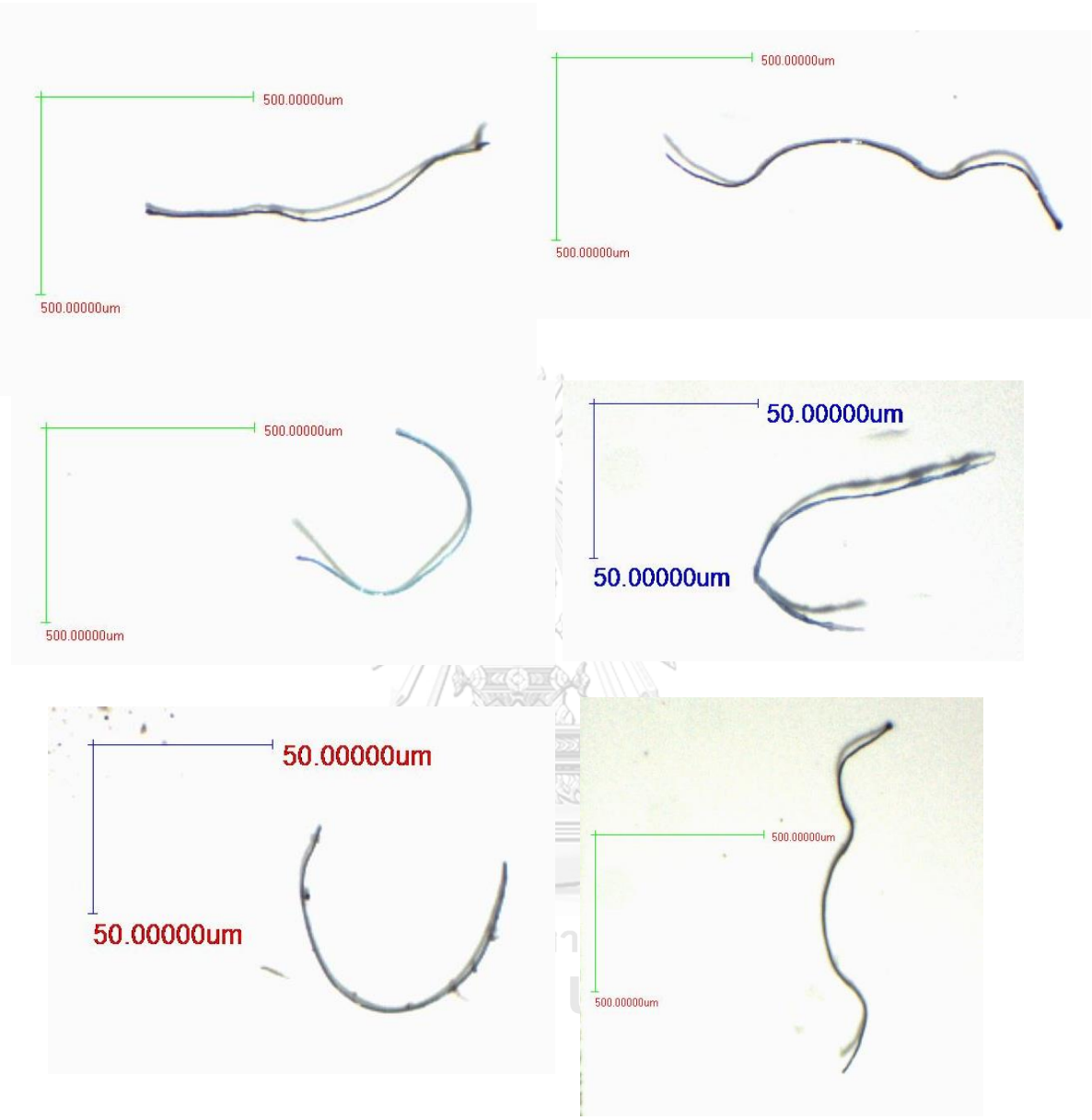
	Plastic avg (MP/L)											
Effluent	Fiber	Fragment	Film	Pellet	Overall	Red	Blue	Black	White	Transparent	Others	Overall
>300 µm	0.379365	0.223316	0.026667	0	0.629347	0	0.249982	0	0.026667	0.299365	0.053333	0.629347
300-200 µm	0.55351	0.705644	0.098977	0	1.358131	0.02381	0.82843	0	0	0.45739	0.048501	1.358131
200-100 µm	0.48843	2.113122	0	0	2.601552	0.024691	1.996049	0.167549	0.024691	0.388571	0	2.601552
100-20 µm	0.076049	0.302646	0.123457	1.241834	1.743986	0.07231	1.349383	0.246243	0	0.051358	0.024691	1.743986
Sludge												
>300 µm	0.074871	0.677615	0.106544	0.319965	1.178995	0.118037	0.149485	0.509091	0.265639	0.049477	0.081181	1.17291
300-200 µm	0.031192	0.281561	0.018766	0.006341	0.33786	0.055623	0.069388	0.10647	0.06856	0	0.018797	0.318838
200-100 µm	0.006085	0.14474	0.012426	0.069795	0.233046	0.057218	0.082386	0.043678	0.025168	0.006085	0.018511	0.233046
100-20 µm	0.012742	0.037849	0	0.062189	0.11278	0.037563	0.031478	0.031539	0.018797	0	0.012426	0.131803

APPENDIX B

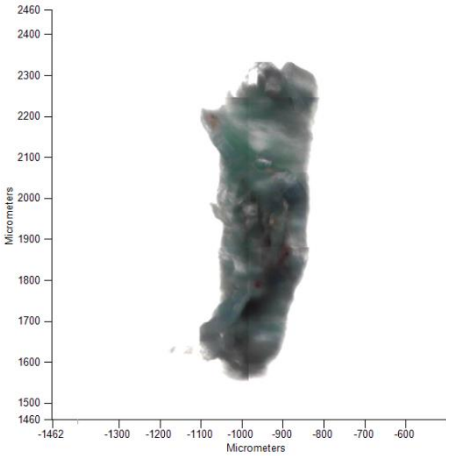
B1 Microplastic in fiber shape (1)

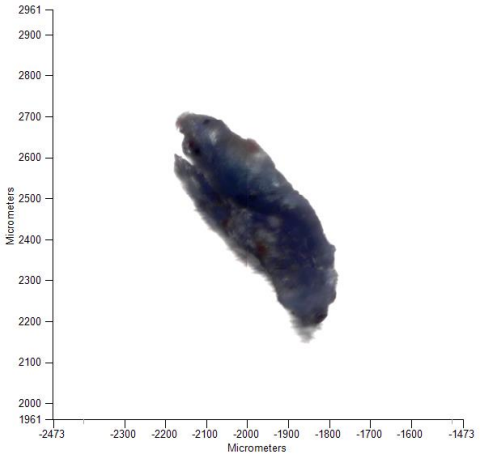


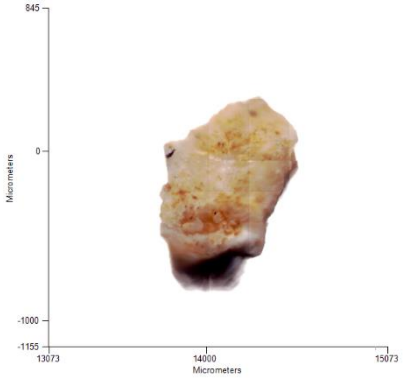
B1 Microplastic in fiber shape (2)

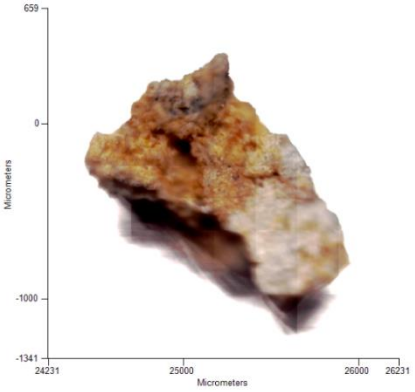


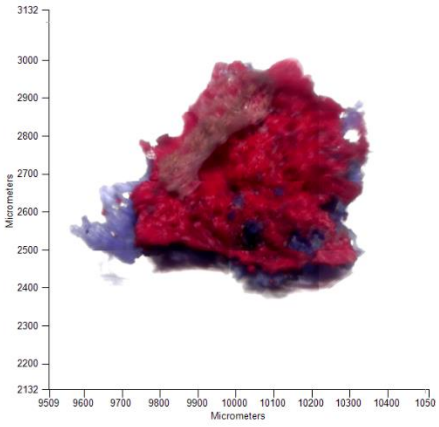
B2 Microplastic in fragment shape (1)

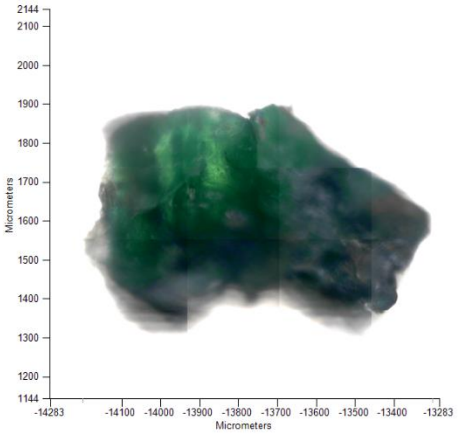




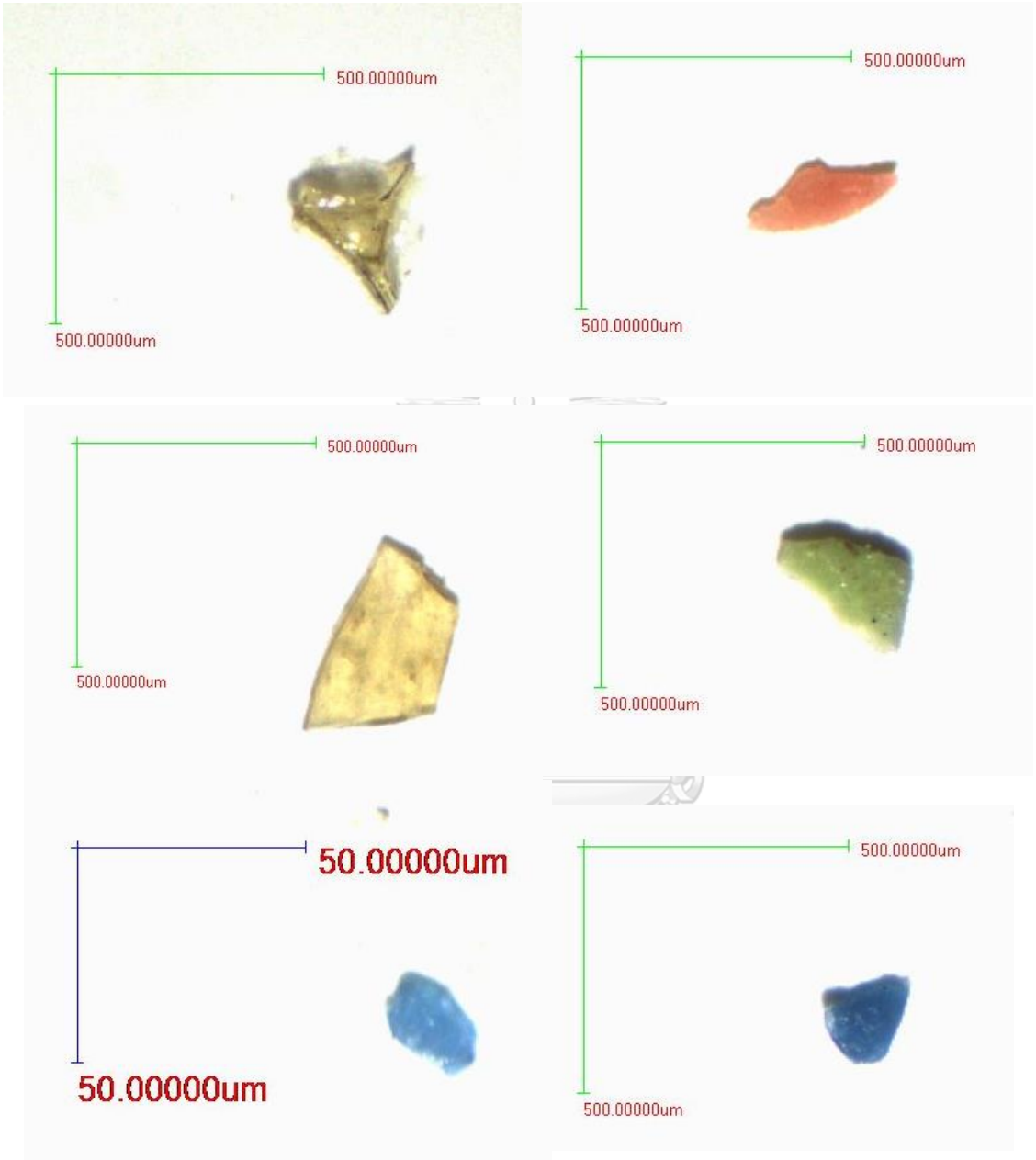




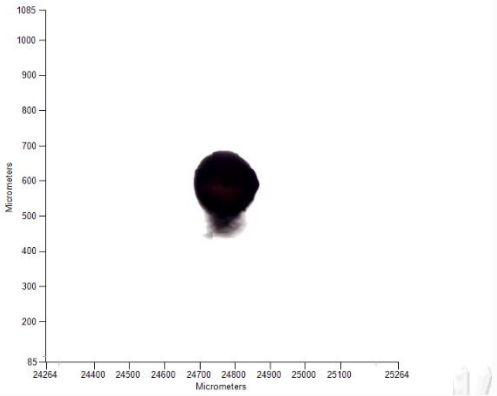


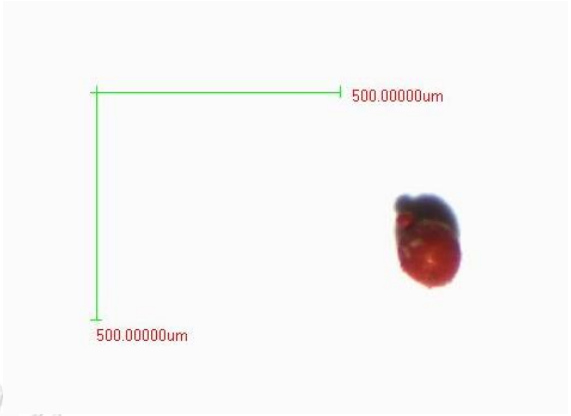


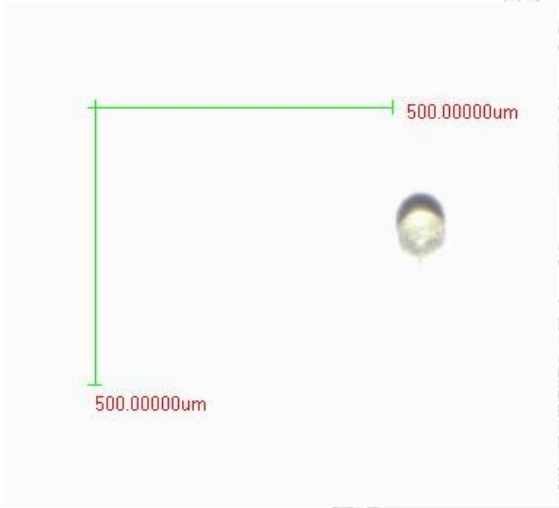
B2 Microplastic in fragment shape (2)

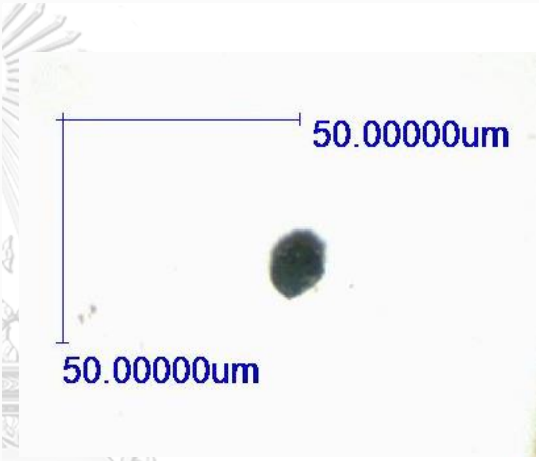


B3 Microplastic in pellet shape

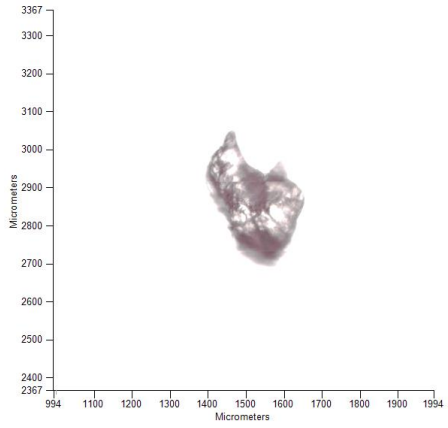


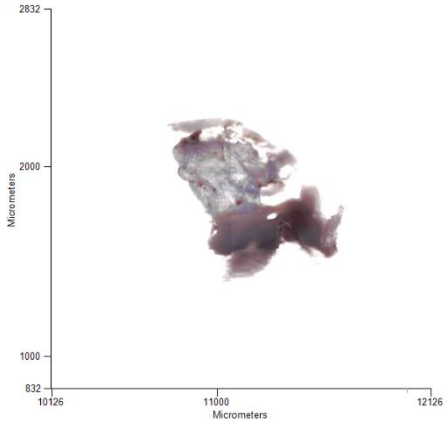


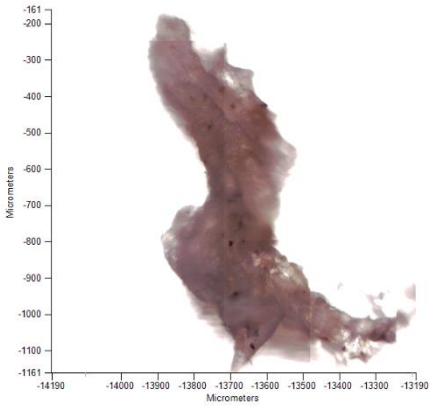


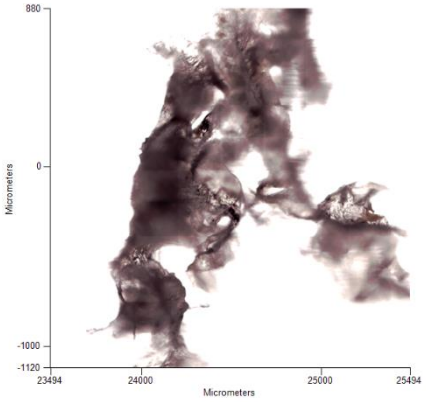


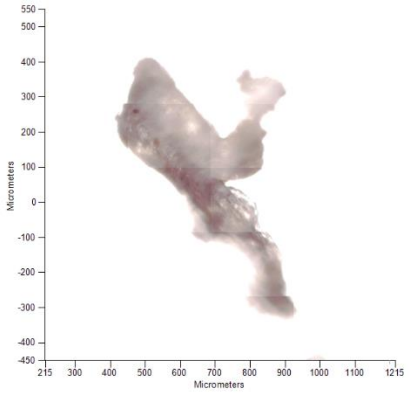
B4 Microplastic film shape

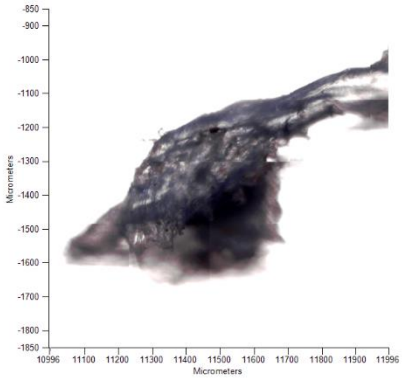








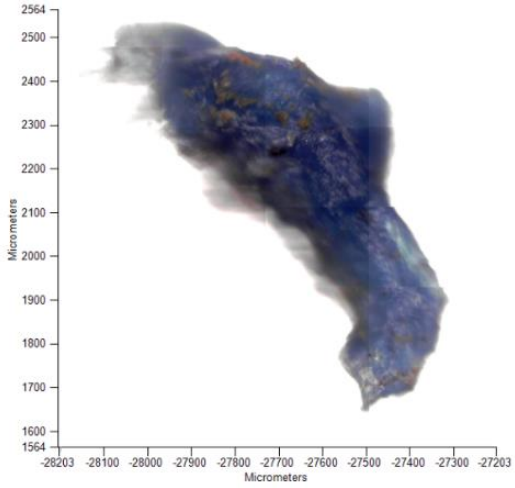


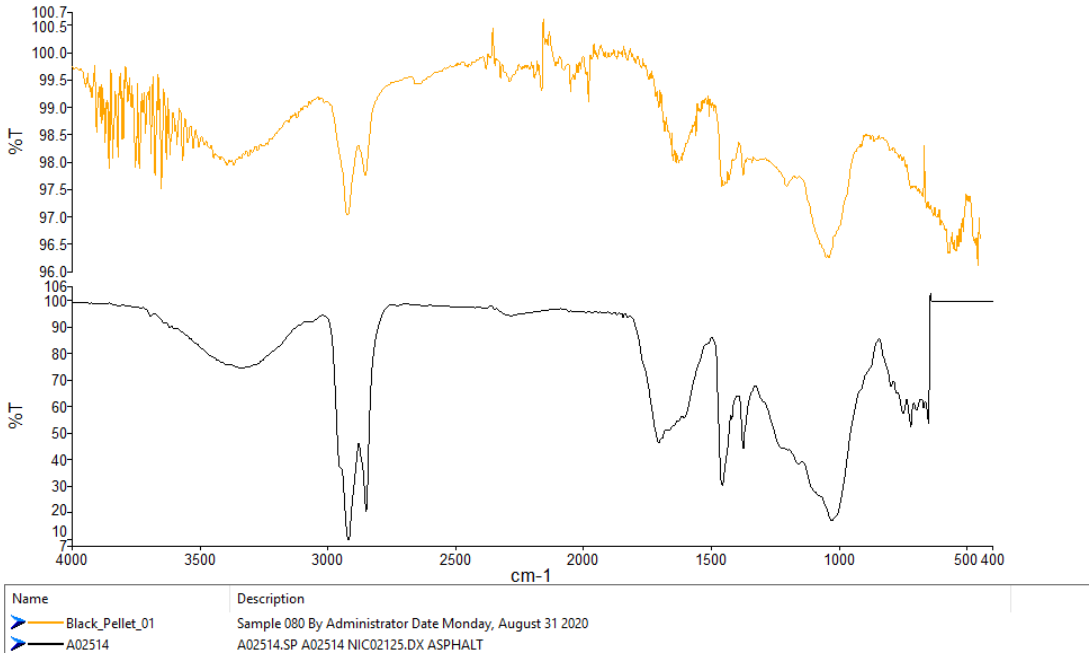


APPENDIX C

C1 Asphalt

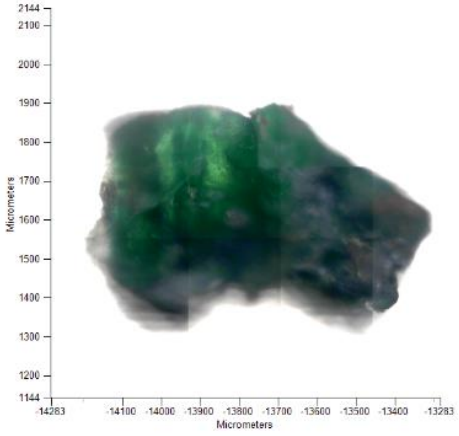
Asphalt

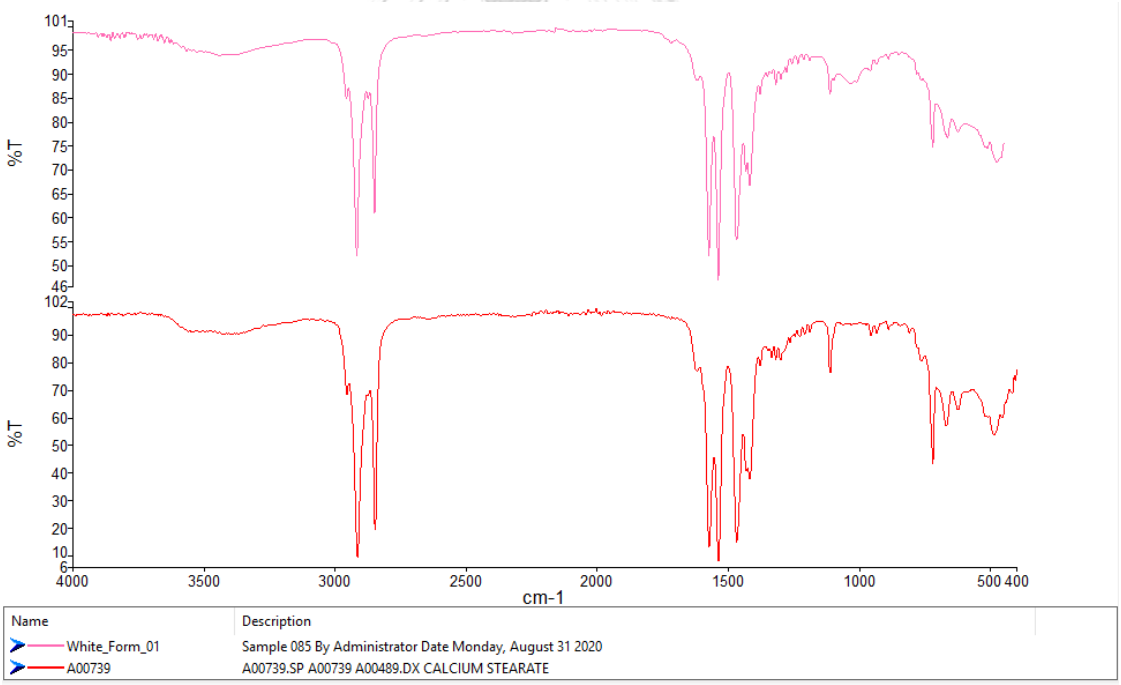




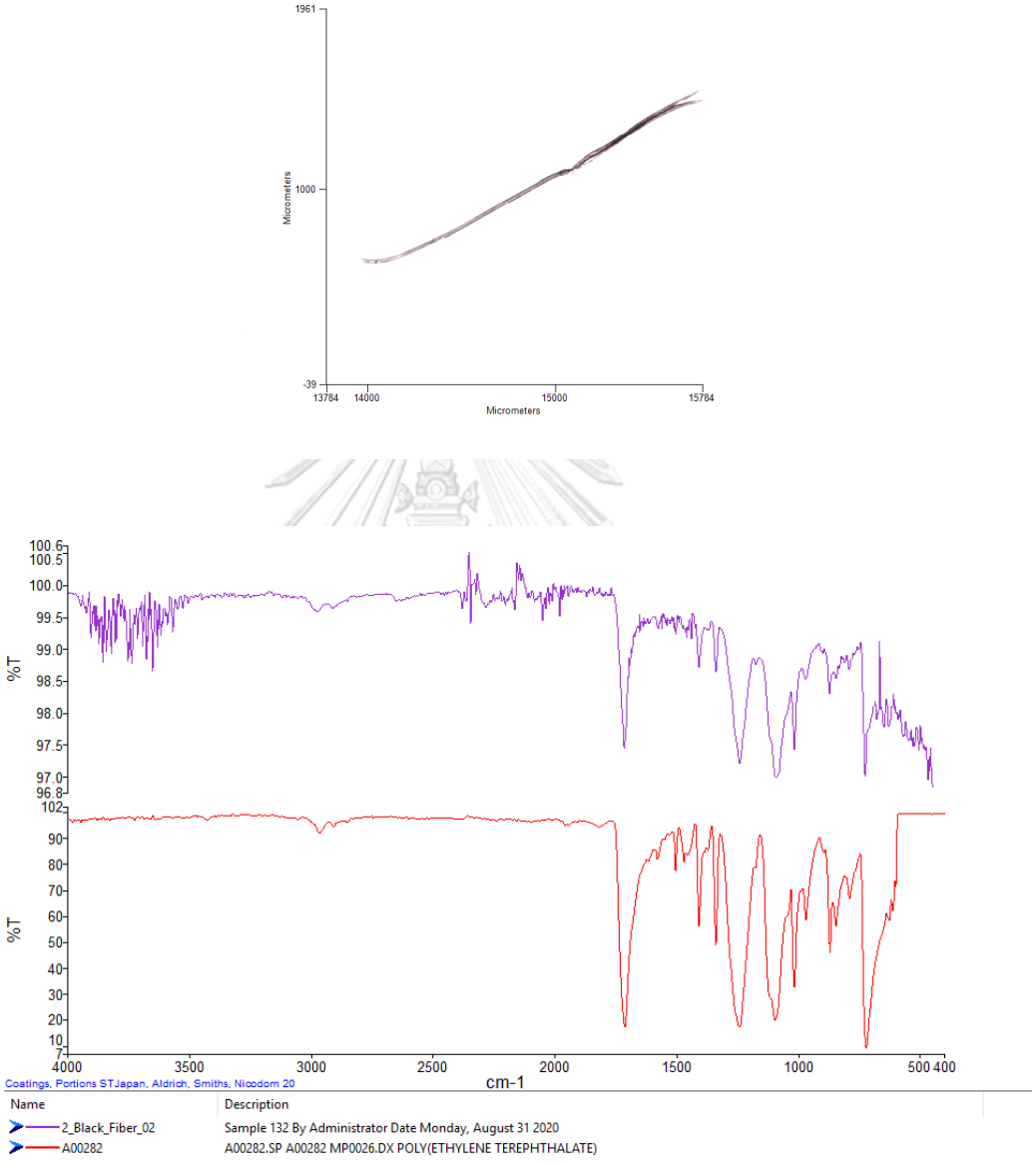
C2 Calcium Stearate

Calcium Stearate

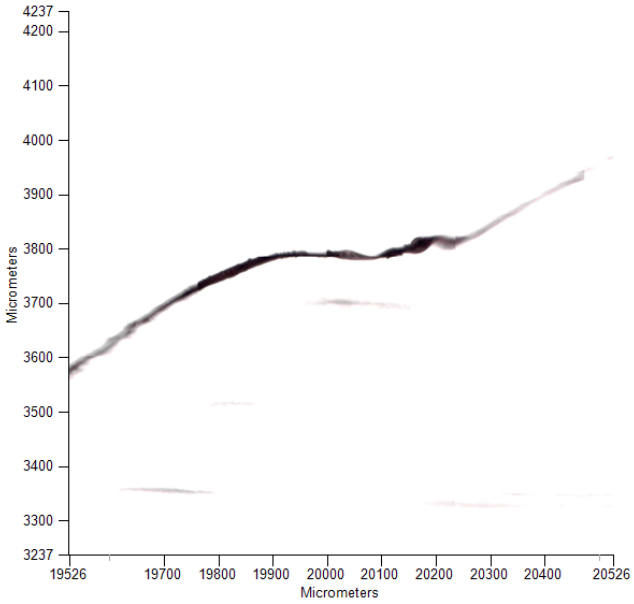


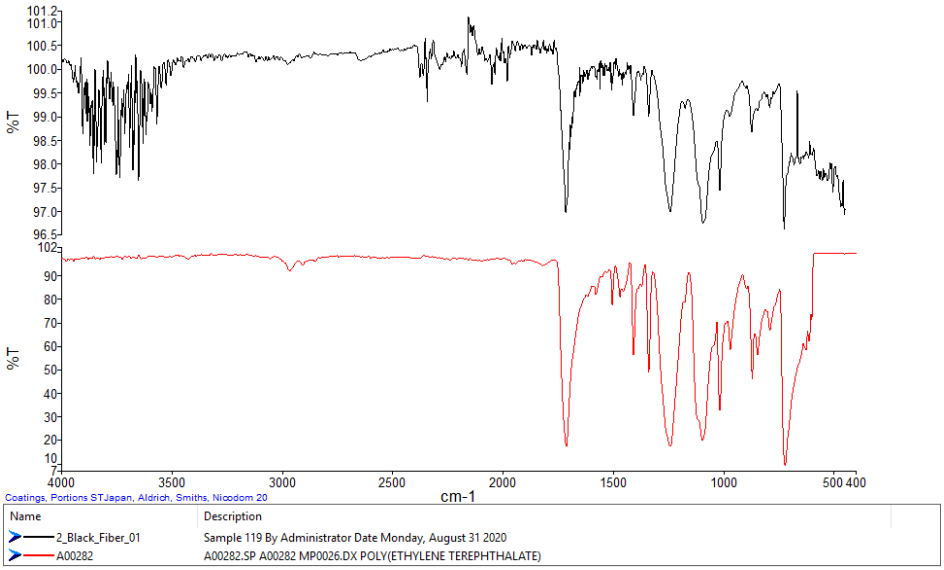


C3 Polyethylene Terephthalate

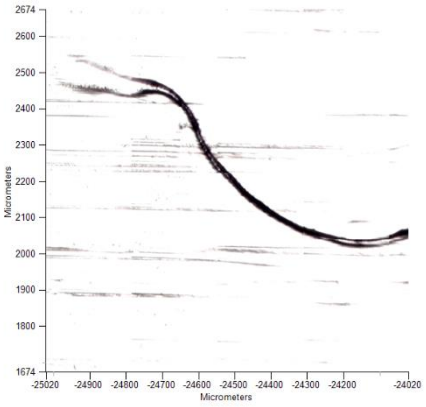


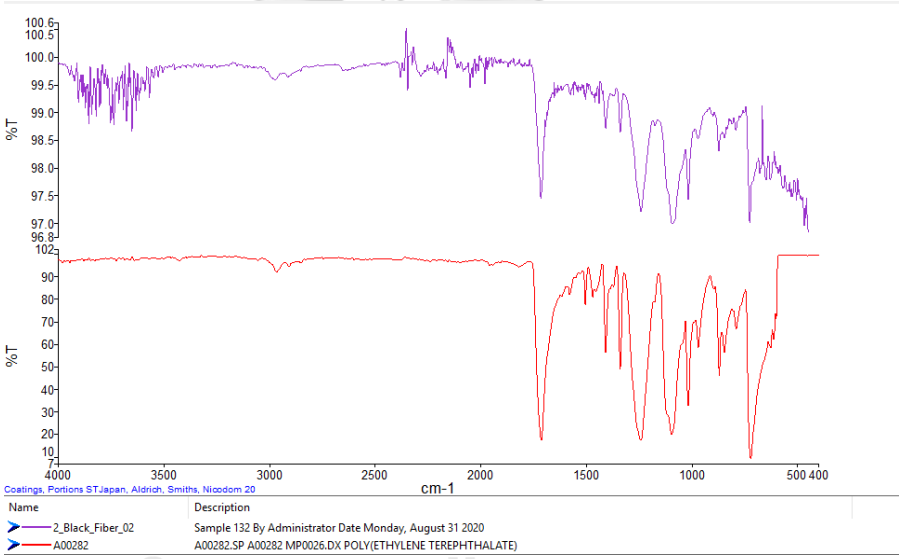
Polyethylene Terephthalate



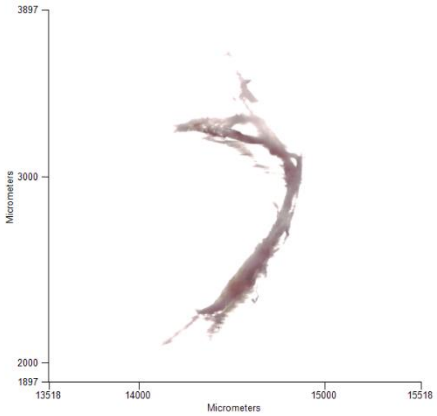


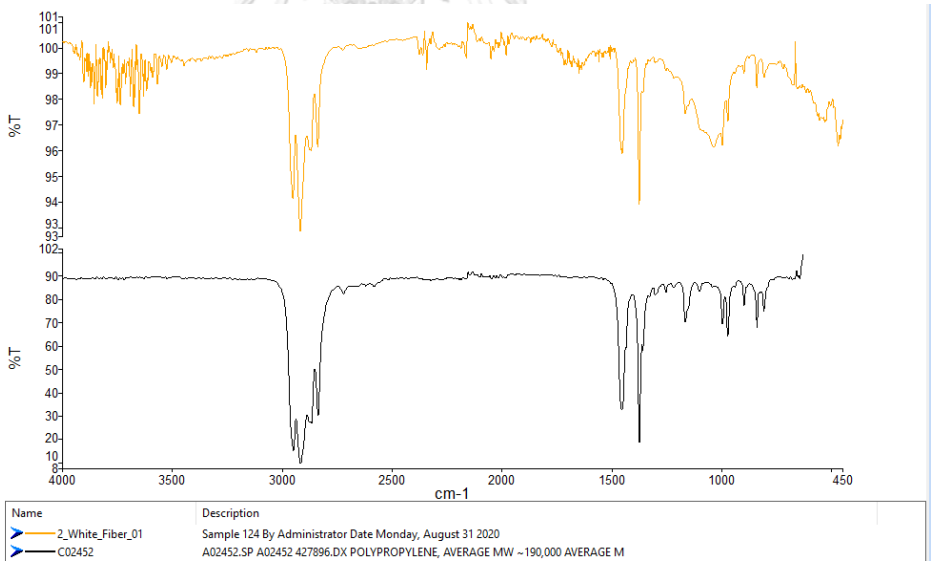
Polyethylene Terephthalate



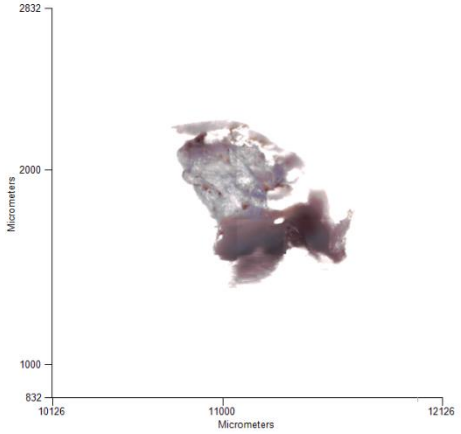


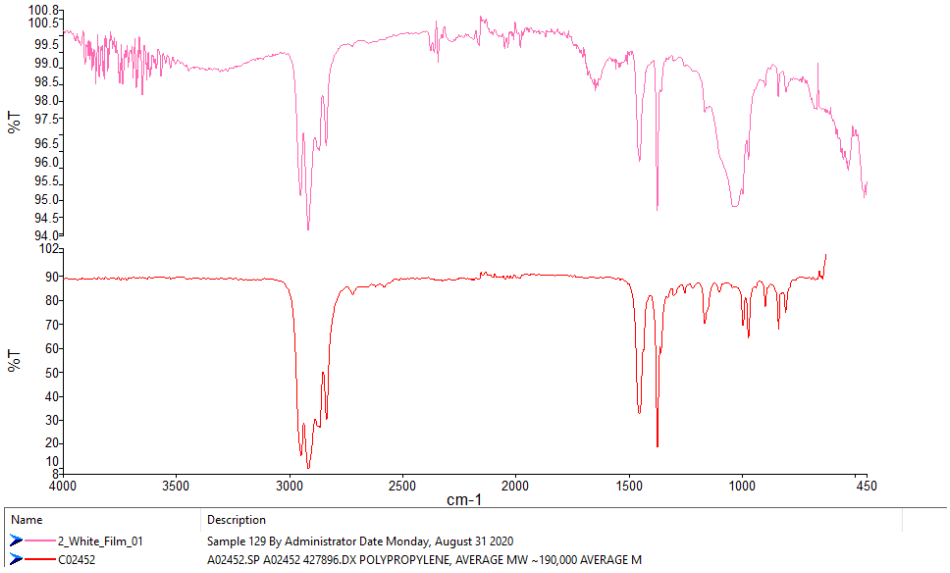
C4 Polypropylene



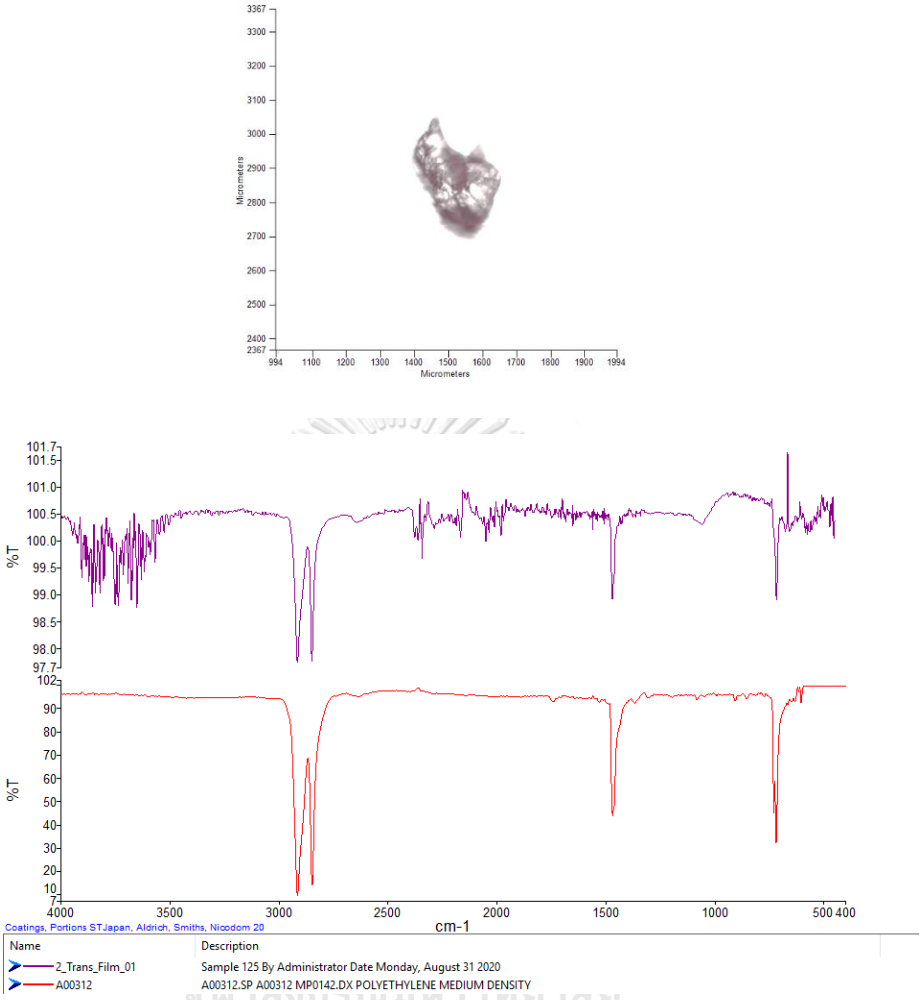


Polypropylene

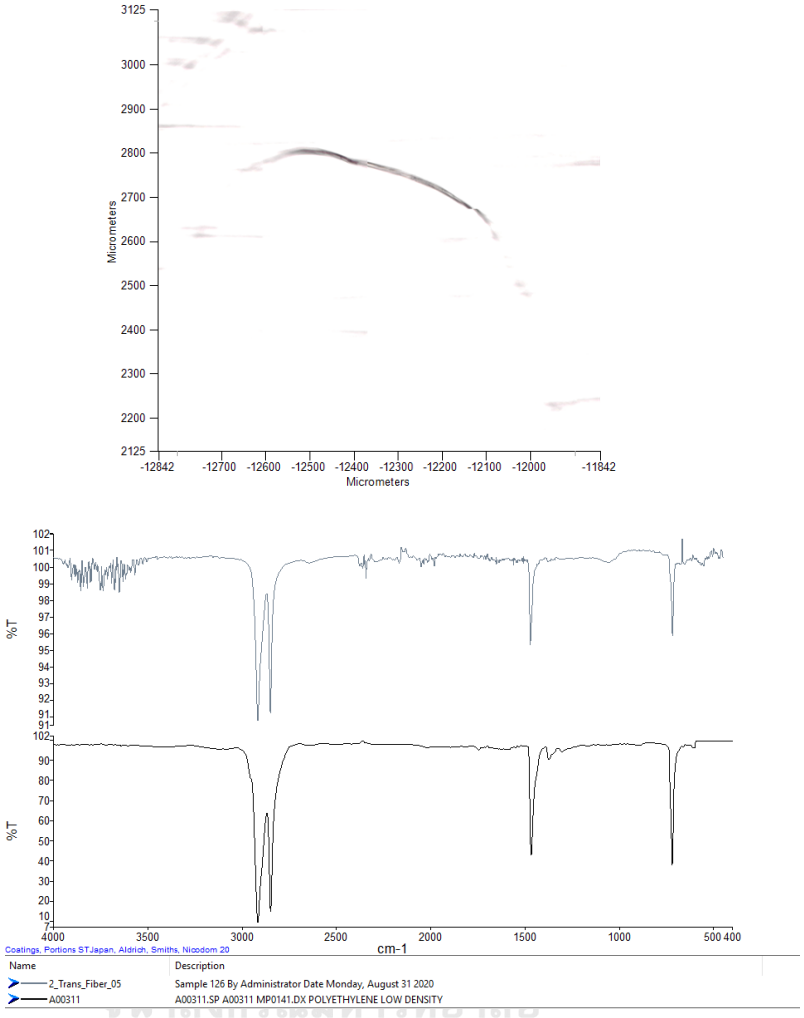




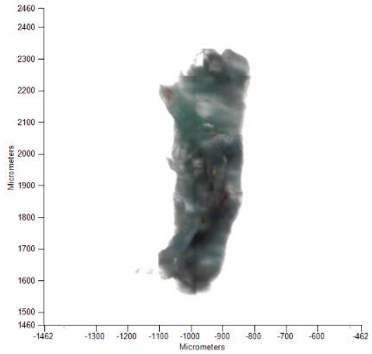
C5 Polyethylene

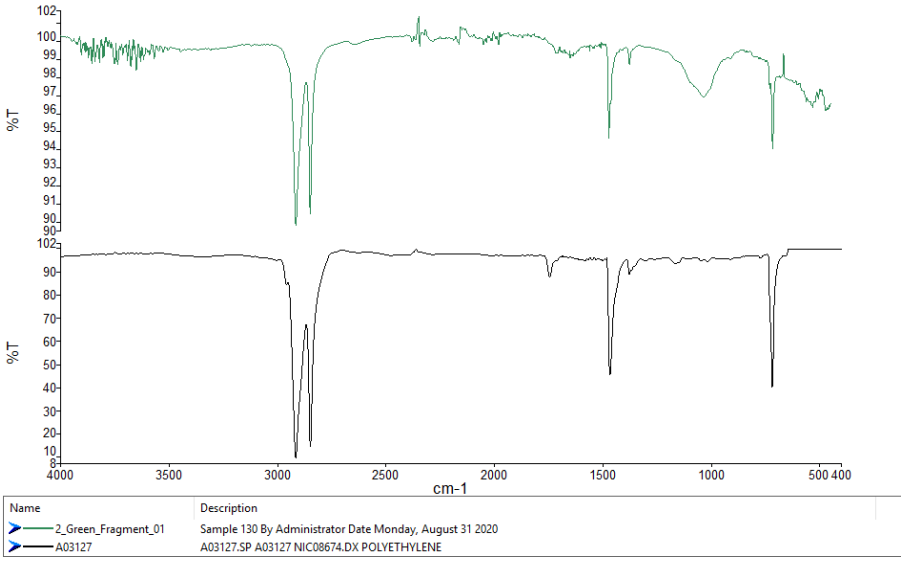


Polyethylene

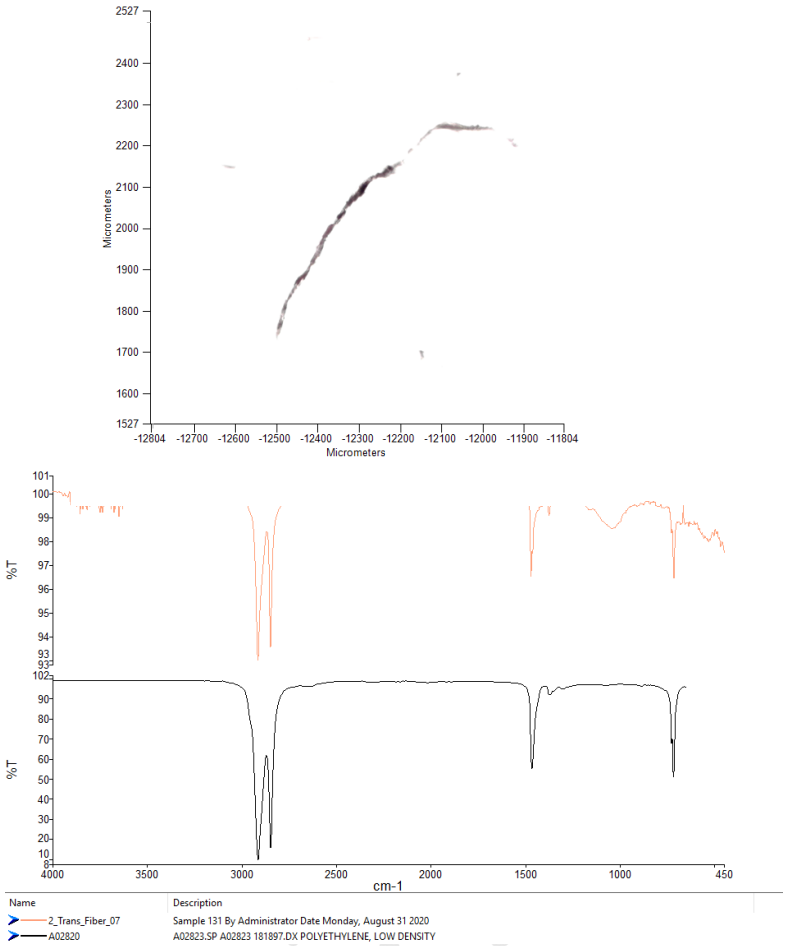


Polyethylene

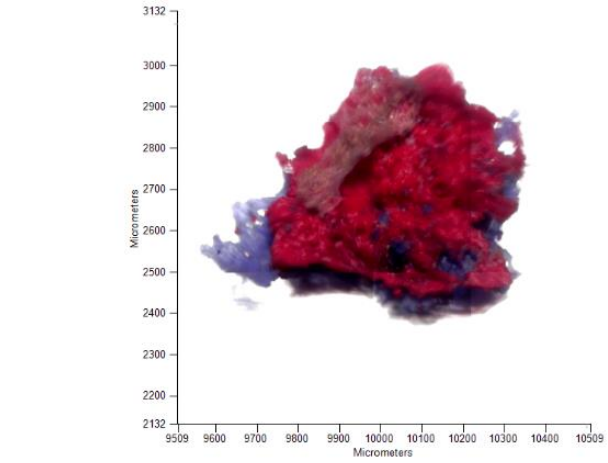


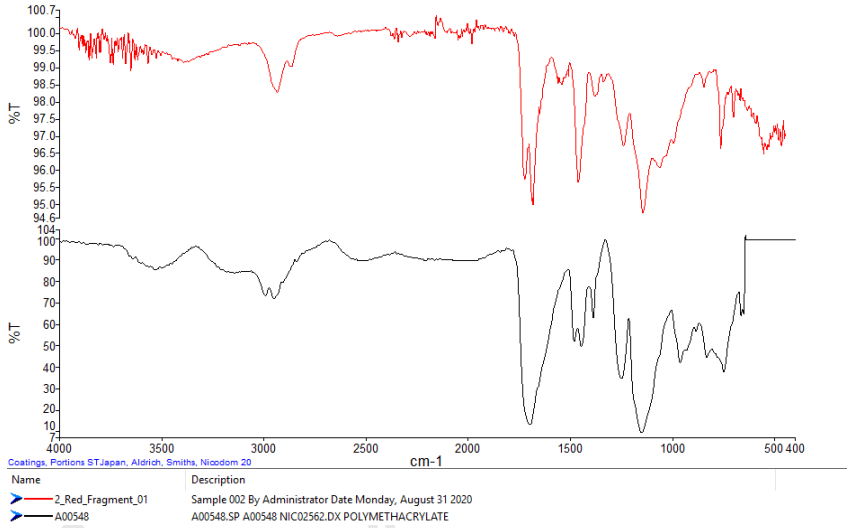


Polyethylene

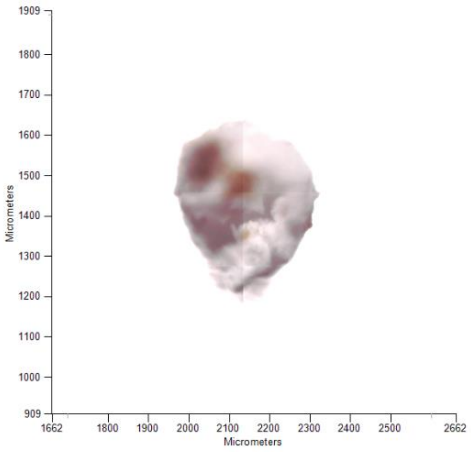


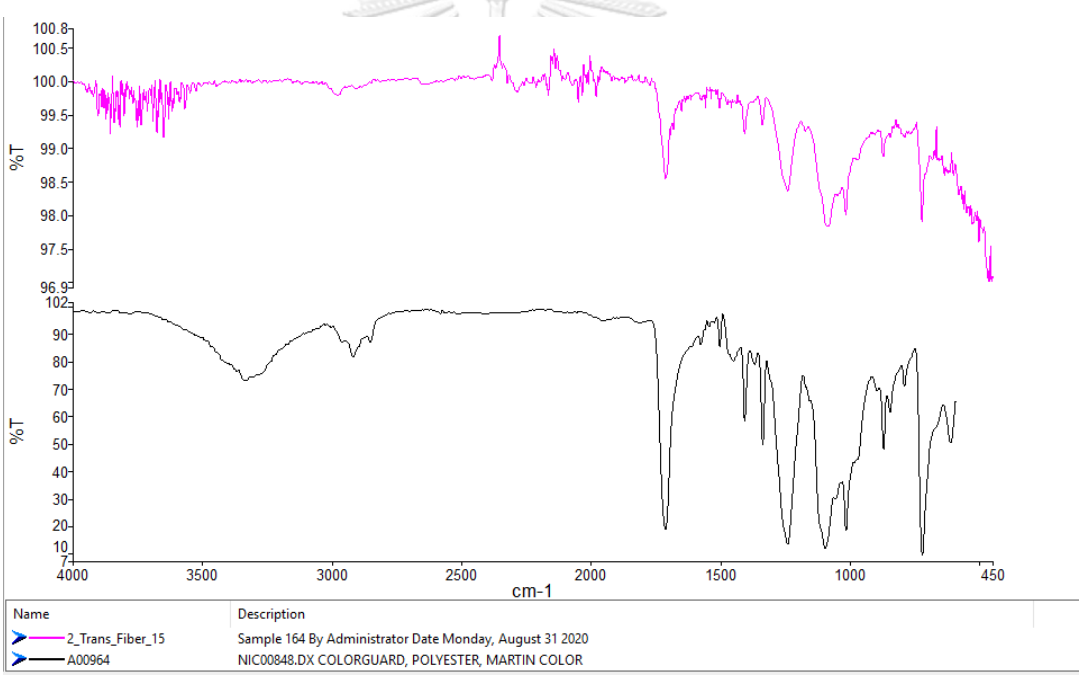
C6 Polymethacrylate



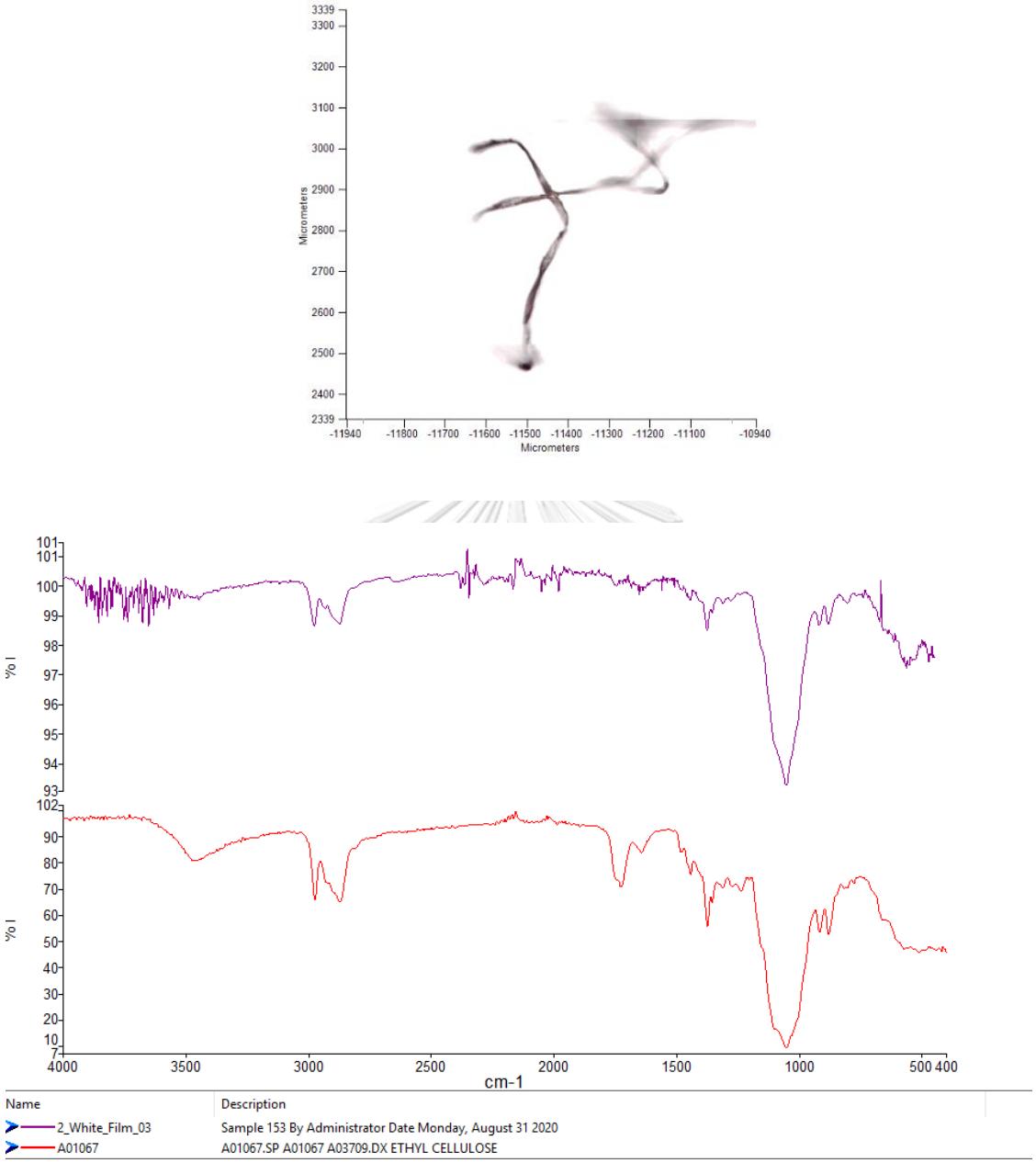


C7 Polyester





C8 Ethyl Cellulose



APPENDIX D

Figure D1 Influent

Figure D2 Influent and grit chamber

Figure D3 Aeration lagoon

Figure D4 Aeration lagoon

Figure D5 Sedimentation pond

Figure D6 Sedimentation pond

Figure D7 Sedimentation pond 2

Figure D8 Sedimentation pond 2

Figure D9 Effluent

Figure D10 Effluent

Figure D11 Sludge drying pond

Figure D12 Sludge drying pond

APPENDIX E

Table E1 Atmospheric information in February 2020

Time	Temperature (° F)			Dew Point (° F)			Humidity (%)			Wind Speed (mph)		
Feb	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min
1	91	81.1	72	70	64.1	59	78	58.4	34	14	6.6	2
2	91	82.7	75	73	68.3	61	89	64.7	36	13	6.0	0
3	91	82.4	77	73	71.8	66	89	72.0	46	13	7.1	3
4	91	83.5	79	75	72.6	68	84	71.4	49	14	8.7	3
5	91	83.4	77	75	71.8	64	89	69.9	43	15	9.0	6
6	90	82.9	77	75	72.8	70	89	72.9	52	13	8.5	3
7	93	83.7	77	75	72.5	66	89	71.2	43	10	6.2	3
8	93	83.4	77	77	72.9	66	94	73.8	41	12	6.7	3
9	93	83.4	75	75	68.2	61	94	63.8	34	14	7.7	2
10	91	84.3	77	66	63.4	61	61	49.8	38	17	9.8	6
11	95	86.4	81	73	66.2	63	79	52.1	34	12	6.9	2
12	93	84.7	79	77	74.2	70	94	72.4	49	21	11.2	3
13	91	84.4	79	75	73.8	70	89	71.7	52	17	11.7	5
14	91	85.0	81	75	73.5	70	84	69.5	49	18	13.7	7
15	93	84.4	77	75	67.2	54	84	60.1	26	17	9.7	3
16	95	84.9	79	75	65.8	50	84	57.4	22	14	8.3	1
17	95	84.3	75	75	71.9	63	94	68.9	34	17	7.7	5
18	90	84.1	79	75	65.8	61	79	55.5	40	17	10.8	7
19	93	84.0	75	72	61.4	59	66	47.4	32	17	8.6	3
20	95	86.0	79	73	63.8	57	79	50.1	28	20	9.3	3
21	93	85.3	75	63	59.0	55	61	42.2	28	17	11.8	6
22	93	84.6	77	61	59.2	55	54	42.8	32	22	12.5	6
23	93	84.8	77	63	59.9	57	57	43.8	30	17	10.2	2
24	95	84.8	73	70	60.9	54	74	46.6	26	15	6.9	0
25	93	83.9	75	75	69.7	59	83	64.8	32	13	7.1	0
26	93	84.4	77	75	72.6	68	89	69.8	44	14	7.3	3
27	90	84.3	79	77	73.4	68	89	71.1	52	15	6.9	1
28	91	84.5	81	75	73.0	68	84	69.3	49	20	9.8	3
29	91	83.9	79	75	73.0	70	89	71.1	52	14	8.5	3

Table E2 Atmospheric information in July, 2020

Time	Temperature (° F)			Dew Point (° F)			Humidity (%)			Wind Speed (mph)		
Jul	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min
1	91	84.4	79	79	74.0	72	94	72.2	58	22	7.5	0
2	91	83.7	77	79	76.8	73	100	81.5	59	18	7.0	0
3	90	83.0	75	77	74.6	73	100	78.1	59	18	7.5	2
4	90	82.8	77	79	75.7	72	100	80.3	55	12	6.0	1
5	93	85.7	81	79	74.5	72	94	70.5	52	14	6.4	0
6	95	86.8	81	77	73.4	70	79	64.9	47	13	6.6	0
7	91	84.2	79	81	77.9	75	100	81.5	62	18	6.5	1
8	91	85.9	81	79	77.6	73	94	76.8	55	14	8.6	2
9	95	87.5	82	81	76.3	73	89	70.3	49	14	8.2	2
10	95	86.9	81	81	77.3	70	100	74.2	46	17	7.4	1
11	93	86.8	81	81	75.8	72	94	70.8	49	10	5.7	0
12	93	87.0	82	81	77.1	72	89	72.8	52	13	7.3	2
13	91	84.2	77	79	76.7	75	94	79.2	59	18	8.6	0
14	90	81.8	75	79	76.1	73	100	84.0	62	12	5.5	1
15	91	83.0	77	79	76.5	73	100	81.9	62	13	5.3	2
16	93	84.0	79	77	75.5	73	94	77.4	55	18	6.0	1
17	93	86.0	79	79	75.6	70	94	72.3	49	9	5.5	0
18	91	85.1	81	81	78.0	75	94	79.4	63	12	6.2	2
19	93	87.0	82	81	78.6	75	89	76.1	59	13	8.9	5
20	91	84.2	79	81	78.1	72	100	81.7	62	17	6.0	1
21	90	83.4	79	79	77.5	73	94	82.6	59	12	5.3	1
22	91	83.3	77	79	76.5	75	94	80.5	59	13	5.5	1
23	93	84.8	77	79	76.8	72	100	78.1	49	13	6.6	0
24	91	83.7	75	81	77.0	73	100	81.2	55	18	6.3	1
25	90	82.4	79	79	77.2	75	100	84.7	62	17	6.1	0
26	91	84.5	77	79	76.9	73	100	79.0	55	16	7.4	0
27	91	86.5	81	81	77.1	73	94	74.6	55	15	8.1	0
28	91	84.5	73	81	77.2	73	100	79.4	59	14	6.1	0
29	95	84.8	79	81	76.7	72	100	78.6	49	17	6.1	2
30	93	85.7	79	79	74.9	72	100	72.0	49	12	6.2	1
31	93	86.1	81	79	74.0	72	84	67.5	52	12	7.6	3

VITA

NAME Sujarat Saiwaree

DATE OF BIRTH 1 December 1995

PLACE OF BIRTH Songkhla

INSTITUTIONS ATTENDED

HOME ADDRESS