Ecological risk assessment of microplastics and heavy metals in central industrial wastewater treatment plant

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การประเมินความเสี่ยงทางนิเวศวิทยาของไมโครพลาสติกและโลหะหนักในโรงบำบัดน้ำเสียอุตสาหกรรมส่วนกลาง

น.ส.ธมนวรรณ ภู่ประเสริฐ

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ไมโครพลาสติกกำลังเป็นมลภาวะสำคัญต่อสิ่งแวดล้อม ด้วยขอนุภาคที่มีขนาดเล็กและความสามารถในการดูดซับสารมลพิษต่าง ๆ จากสภาพแวดล้อมใกล้เคียงโดยเฉพาะอย่างยิ่งโลหะหนัก โรงบำบัดน้ำเสียอุตสาหกรรมส่วนกลางเป็นแหล่งสำคัญในการปล่อยไมโครพลาสติกออกสู่สิ่งแวดล้อม งานวิจัยนี้ทำการเก็บตัวอย่างน้ำเสียและตะกอนจากโรงบำบัดน้ำเสียส่วนกลาง 2 แห่ง ความเข้มข้นของไมโครพลาสติก โลหะหนัก และ โลหะหนักบนไมโครพลาสติกจากตัวอย่างทั้งสองชนิดจะถูกวิเคราะห์ก่อนนำไปประเมินความเสี่ยงทางนิเวศวิทยา ผลการสำรวจพบว่า น้ำหลังผ่านบ่อตกตะกอนและตะกอนจากการโรงบำบัด A ปลดปล่อยไมโครพลาสติก 11.04 ± 0.08 ชิ้นต่อลิตร และ 2,398 ± 11.37 ชิ้นต่อกิโลกรัม ตามลำดับ ในขณะที่โรงบำบัด B ปลดปล่อยไมโครพลาสติก 33.53 ± 0.55 ชิ้นต่อลิตร 1,930 ± 7.57 ชิ้นต่อกิโลกรัม ตามลำดับ ไมโครพลาสติกที่พบมีรูปร่างแบบชิ้นส่วน สีขาว ขนาดในช่วง 100 – 500 ไมโครเมตร และเป็นพอลิเมอร์ชนิดโพลีโพรพิลีน นอกจากนั้นยังพบสังกะสีและเหล็กในน้ำเสียตะกอน และบนไมโครพลาสติก การประเมินความเสี่ยงทางนิเวศวิทยาของไมโครพลาสติก โลหะหนัก และโลหะหนักบนไมโครพลาสติก พบว่า ผลการศึกษาพบว่า ดัชนีความเสี่ยงของไมโครพลาสติก (H index) ในโรงบำบัด A มีค่า 35,835 ในขณะที่โรงบำบัด B มีค่า 45,652 แสดงถึงความเป็นพิษในระดับสูง ขณะที่ดัชนีภาระมลพิษ (PLI) ของโรงบำบัด A มีค่าเท่ากับ 97.82 อยู่ในความเสี่ยงระดับ 4 และโรงบำบัด B มีค่า 12.76 อยู่ในความเสี่ยงระดับ 2 ดัชนีการสะสมเชิงธรณี (I_{geo}) ของโลหะหนักทั้งหมดอยู่ในระดับที่ต่ำ การประเมินความเสี่ยงต่อระบบนิเวศ (PER) ของไมโครพลาสติกนั้นอยู่ในระดับอันตรายมาก โดยในโรงบำบัด A มีค่า 20,089 และในโรงบำบัด B มีค่า 49, 761 ในทำนองเดียวกัน ค่าการประเมินความเสี่ยงต่อระบบนิเวศของโลหะหนักนั้นอยู่ในระดับอันตราย โดยมีค่าอยู่ที่ 49,875 และ 16,660 จากโรงบำบัด A และโรงบำบัด B ตามลำดับ และการประเมินความเสี่ยงต่อระบบนิเวศของโลหะหนักบนไมโครพลาสติกมีค่า 49,875 และ 16,660 จากโรงบำบัด A และโรงบำบัด B ตามลำดับ ค่าการประเมินความเสี่ยงต่อระบบนิเวศเหล่านี้แสดงให้เห็นความเป็นพิษในระดับที่อันตรายมาก

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Thamonwan Phuprasert : Ecological risk assessment of microplastics and heavy metals in central industrial wastewater treatment plant . Advisor: Asst. Prof. VORAPOT KANOKKANTAPONG, Ph.D. Co-advisor: Asst. Prof. SARAWUT SRITHONGOUTHAI, Ph.D.

Microplastics become a big concern in environment. Their small particle size can absorb a variety of pollutants from the nearby environment when discharged into the environment, especially heavy metals. Central industrial wastewater treatment plant (WWTP) is an important source of microplastics in the environment. In this study, wastewater and sludge were collected from two industrial estates in Thailand. Wastewater was collected from 4 points i.e., influent, post grit chamber, post aeration tank, and post sedimentation tank while sludge was collected from sludge storage. Concentration of microplastics, heavy metals and heavy metals on microplastics were examined to evaluate ecological risk assessment. Based on the results, microplastics concentration in effluent and sludge were 11.04 ± 0.08 particles/L, 2,398.00 ± 11.37 particles/kg from WWTP A, while 33.53 ± 0.55 particles/L, 1,930.00 ± 7.57 particles/kg from WWTP B, respectively. In addition, main shape, size, colors, and polymer were pellet, range 100 – 500 µm, white/clear, and polypropylene polymer from both sites, respectively. In terms of heavy metals, zinc was the most detected in wastewater, sludge, and on microplastics. The risk assessment was carried out. The result found that polymer risk index (H) showed 35,835 in WWTP A and 45,652 in WWTP B, which high toxic level. High risk of pollution load index (PLI) with category IV (97.82) was showed in WWTP A, while PLI of WWTP B were presented in category II (12.76). For geo-accumulation Index (I_{geo}), all heavy metals presented in low degree level, except Cd and Se from WWTP A presented as low toxic (0.7) and moderate toxic (1.2), respectively. Potential ecological risk (PER) of microplastics presented extreme danger (48,893 and 20,087 from WWTP A and WWTP B, respectively.), while PER of heavy metals also showed low toxic level with 70.71 and moderate toxic with 96.61 from WWTP A and WWTP B, respectively. Furthermore, PER of heavy metals on microplastics exhibited 48,936.63 and 20,183.61 from WWTP A and WWTP B, respectively, which extreme danger level.

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

Background

Plastic is the biggest problem in the world due to the large number of usages in the past and have increased every year. Most problems are caused by the improper disposal and are directly released into nature. Microplastic is a particle caused by plastic degradation and has a particle size of less than 5 micrometres. The type of microplastic depends on the type of polymer, such as polyethylene made from the combination of ethylene monomers. Microplastics can be classified into two categories: primary microplastic originated from plastic industrial or personal care products such as scrub with beads of plastic components and secondary microplastic resulting from the large plastic breaking. Because of their small particle, microplastics can be released into the environment and easily moved to the different phase. Microplastics have been transferred and accumulated in a wide range of aquatic organisms including bivalve, seahorse, crustaceans, and fish by mistake and also ingested through food web (Akhbarizadah et al., 2017 and Jinhui et al., 2019) . In addition, microplastics can act as carriers for pollutants by continuously through the aquatic system, which direct physical and chemicals effects to aquatic life.

Effluent from wastewater treatment plant (WWTP) is an important source, which can be directly discharged microplastics to the environment. Lares et al. (2018) collected effluent water from a lake that discharged by a municipal WWTP in Finland. The result showed that a fraction of microplastics can pass out of the treatment plant. There are variety of chemicals used in industrial WWTP. One of them is heavy metal that remains widely used. Therefore, microplastics and heavy metals can be contaminated and heavy metals absorb on the surface of the microplastics. Both will accumulate and enter to the organism through trophic level and finally transfer into the human, which will induce effect to the human health. Akhbarizadeh et al. (2019) showed that the microplastic trophic transfer and implications for human health by seafood. This means the ecological risk of microplastics should be evaluated.

From previous works, several studies on ecological risks of microplastics focus on the harmful organisms, without heavy metals and evaluating the degree of pollution. Hence, this research will study the concentration and their risk of microplastics, heavy metals and heavy metals adsorbed on the microplastics in two central WWTPs from industrial estates in Chonburi and Bangkok provinces in Thailand.

Objectives

1.2.1 To identify the size, type and polymer of microplastics detected in the central industrial WWTPs.

1.2.2 To analyse the number of heavy metals from microplastics, wastewater, and sludge in the central industrial WWTPs.

1.2.3 To evaluate potential ecological risk assessment of heavy metals and microplastics.

1.2.4 To compare the effectiveness of the microplastics and heavy metals treatment in the central industrial WWTPs.

Hypotheses

1.3.1 Most microplastics appeared in the central industrial WWTPs are 20-100 μm, fiber, and polyethylene (PE) polymer.

1.3.2 The number of microplastics from sludge in the sedimentation tank is higher than other treatment units.

1.3.3 Microplastics from sludge has highest potential risk level.

1.3.4 Central industrial WWTP in Bangkok has effective treatment than in Chonburi.

Scopes of the study

1.4.1 Wastewater and sludge samples were collected from two central WWTPs in industrial estates located in Bangkok and Chonburi provinces, Thailand.

1.4.2 Sampling points of wastewater were from influent and treatment units i.e., after grit chamber, after aeration, after sedimentation tank and effluent. Sampling point of sludge is from sedimentation tank.

1.4.3 Laboratory analysis was performed to determine size (20-100 µm, 100-212 µm, 212-500 µm, and >500 µm), shape (fiber, pellet, film and fragment) and polymer type (PP, PE, PS and etc.) of microplastics and concentration of heavy metals along with total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total solids (TS), total suspended solids (TSS), and total dissolved solids (TDS) at Department of Environmental Science, Faculty of Science, Chulalongkorn University. The other parameters (temperature and pH) were analyzed in each treatment units.

1.4.4 Potential ecological risk assessment of heavy metals and microplastics method was estimated by Hakanson (1980).

CHAPTER II
LITERATURE REVIEW

2.1 Plastic waste

The global problem of plastic waste has various social, economic and environment impacts. Ever since the 20th century, plastic is an important invention as significantly changed our lifestyle (Dong et al., 2020). Plastic use in various of application in packaging, outfit domestic, electrical and electronic components, and industrial products (Akhbarizadeh et al., 2019 and Rajendran et al., 2020). With convenience, plastic is an essential part of the daily routine. Based on polymer, plastic is a processable material originate from the polymerization of crude oil which widely synthetic polymers used in various industries (Xu et al., 2018). Plastic has several properties include their flexibility, lightness, cheapness, and persistence. The plastic waste can be accumulated in both terrestrial and aquatic ecosystems by anthropogenic. In 2018, the global production reached 360 million tons, but only 47.1% properly disposed of waste by recycling and landfill. Up to 80% of plastic have been discharged to river networks and transferred into the oceans. Plastic debris has been found from seashore to ocean, from surface to abyssal depth of ocean and from polar to the equator also freshwater such as surface water and ground water (Zhao et al., 2018 and Zhang et al., 2019). The negative effects of plastic debris include causing chemical and physical harm to organism, accumulate through trophic level and enter to human body.

Global microplastic pollution has become a serious concern, and plastic pollution has increased significantly in recent years because of incompetent management of the COVID-19 epidemic. The exponential increase in plastic waste has a direct impact on the life and ecological effects of the emitted particulate matter and the leaching of hazardous chemicals.

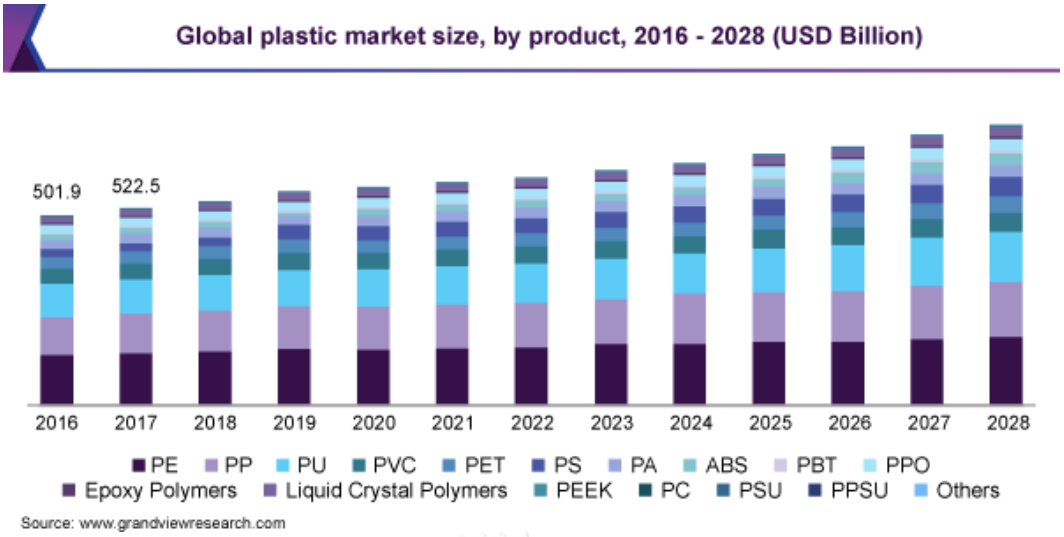


Figure 2-1 *trend of the world plastic production from 2016 to 2028.*

2.2 Microplastics

The debris plastic waste is cracking, pitting, abrasion, and surface erosion under physical and chemical actions e.g., wave, wind, UV radiation and bacteria (Bandow et al., 2017; Kokalj et al., 2019 and Min et al., 2020). GESAMP (2016) defines that any plastic debris smaller than 5 mm in diameter are “microplastic” as a new type of pollutant that received considerable attention in recent year. They are commonly classified occurrence and distribution of microplastics in the environment include: (i) primary source are derived from intentionally produced e.g., constituents of personal care products (microbeads from scrub, toothpaste, or detergent), scrubbers in air-blasting and waste product from plastic processing plants; (ii) secondary source are derived from fragmentation of larger plastic (Garrido et al., 2019). Over time, microplastics are fragmentation by sunlight oxidize the polymer matrix and changing the shape of the particles. New surface is created during fragmentation and the debris can be seen as long-term source of toxic compound. The parallelepipeds particles degrade slower than cubic particles (Bandow et al., 2017). The small debris plastic can be ingested to organism by mistakes and possible transfer to trophic level (Bradney et al., 2020).

There are different sources of microplastics pollution release to the environment included untreated wastewater, runoff of several origins e.g., agriculture,

industrial, urban, and atmospheric deposition (Edo et al., 2020, and Pico et al., 2021). The undeniable presence of microplastics (MPs) in soil, air and, especially, in the aquatic environment has revealed them to be an emerging pollutant. Liu et al. (2019) gave attention to source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Result showed approximately 120.7 kg of suspended atmospheric microplastics are transported through Shanghai air and approximately 21 particles are inhaled daily by people from outdoor in Shanghai. Year later, Velasco et al. (2020) found microplastics in a remote mountain, alpine, and uninhabited lake in Switzerland. The most polymer types were PE and PP. Estuaries and other coastal ecosystems are productive. Patterson et al. (2020) studied about microplastics in an Indian coral reef ecosystem. Average of abundance of MPs varied from 60 ± 54 to 126.6 ± 97 items/L in water and from 50 ± 29 to 103.8 ± 87 items/kg in sediment. At the same year, Li et al. (2020) was attention on microplastics in mangroves. Microplastics were detected 227 – 2,249 particles per kg. In terms of main shapes, size, color, and polymer were fiber, 500 – 500 µm, white-clear, and PP, respectively.

As a kind of emerging and persistent environmental pollutants, microplastics have recently been detected on a variety of substrates in the world. In addition, size of microplastics is similar with food for marine organism therefore they can be ingested as food by various aquatic organism by mistake and transfer through humans by their consumption. Hu et al. (2022) evaluated microplastics effect and toxicity on growth, liver damage, and gut microbiome composition of crucian (*Carassius carassius*) by feed polyethylene microplastics for 30 days. Result showed that MPs adversely affect crucian growth and health, with increased disease risk. Likewise, effects of microplastics and heavy metals accumulation in the yellow seahorse (*Hippocampus kuda*) were studied by Jinhui et al. (2019). After exposure, physical (body length and body weight), growth rate, and survival rate were decreased. These results suggested that the effect of microplastics on seahorse growth is caused by the accumulation of heavy metals, rather than by the microplastics themselves. Akhbarizadeh et al. (2019) investigated microplastics bioaccumulation in seafood from the Persian Gulf. Gills of five popular commercial species i.e., 3 fish, 1 crab, and 1 prawn from the Persian Gulf were detected. The highest number of microplastics was found 0.931 particles per

gram of gill in fish (*Liza klunzingeri*). The microplastics presented in seafood assess that human can be intake microplastics through seafood consumption.

The most chemical used for producing plastic polymers are derived from crude oil and several are hazard. It is expected that microplastic adsorption and release more potential harmful and they have toxic in their own, and difference in their chemical components (Pico et al., 2021). Lithner et al. (2011) identified and compiled the environment hazard of polymer (Table 2-1). The polymers were ranked based on monomer hazard classification. The initial assessments of hazard ranking model were developed for the hazard classes and categories in the EU classification and labelling (CLP) regulation which is based on the UN Globally Harmonized System. Amato-Lourenço et al. (2021) confirms that microplastics are present in the air and may be inhaled by humans. Their studied determined the presence of microplastics in human lung tissues obtained at autopsies with 33 particles and 4 fibers from 13 of 20 tissue sample. All polymers were smaller than 5.5 μm with mainly PE and PP. Kannan and Vimalkumar (2021) studied about the occurrence of microplastics in human food. The microplastics present and their additives can be decreased energy and lipid metabolism that increased the prevalence of overweight and obesity in human populations. Besides, Huang et al. (2021) shown that microplastics have harmful effects on living organisms, especially on the gut barrier. The exposure to MPs could cause oxidative damage and inflammation in the gut by reduction of the mucus layer, microbial disorders, and immune cell toxicity.

2.3 Microplastics from WWTP

Wastewater treatment plant (WWTP) is the place were design to accommodate both wastewater and other toxic substance before discharged to environment. Lares et al. (2018) presented the microplastics from municipal WWTP in Finland. Wastewater and sludge sample were collected every two weeks during period study. The result showed that most of the microplastics was removed before the activated sludge and 98.3% of microplastics were removed during this process. According to the MPs occurrence data, microplastic fibers are posing a more severe problem than microplastic particles.

Bayo et al. (2020) studied about the abundance, concentration, and variability of microplastic in an urban WWTP located in Spain. The most prominent microplastics from effluent were fragments and fibers with range 400–600 μm. Influent wastewater with high concentrations of suspended due to hetero-aggregation with particulate matter, mostly from agriculture near the sewage plant and single plastic bags.

Funck et al. (2021) looked into microplastics release to aquatic via WWTP. Three WWTPs were compare and investigated the impact of sand filter as tertiary treatment. In this study, four common polymers were detected i.e., PE, PS, PP, and PET by using thermal extraction desorption gas chromatography mass spectrometry (TED-GC-MS). Result showed that total microplastics load for tertiary effluents were at most 2.1 mg per year. However, this study clearly shows high removal efficiency of microplastic during tertiary sand filter.

Shen et al. (2021) present aluminosilicate filter media and their surfactant-modified products as the potential low-cost integrated material for removal microplastic in wastewater before discharge to environment. The obtained scanning electron microscopy (SEM) were used to capture and determine the fate of microplastics in the filters. the removal efficiency of microplastics was higher than 96%.

Blair et al. (2019) evaluated the average daily flow of microplastic through a tertiary WWTP on ten-month, with an average inflow of 8.1×10^8 items per day in all sampling period. Microplastics concentration were decreased from influent to final effluent on average by 96%. Here, the presence of MPs was confirmed by ATR - FTIR analysis, with PP as the most abundant polymer type, fiber was the most detected and secondary of microplastics were mainly observed as secondary types.

Petroody et al. (2021) explore on transport and accumulation of microplastics through sludge from WWTP in northern Iran. Sludge process is an important role in trap and reducing the number of microplastics. Their sludge produced from WWTP may be used for agriculture as fertilizer due to its high nutrient content along with bacteria, virus, and other pollution as well as organic compounds and heavy metals. More than 129 particles/ dry weight of microplastics were detected. Polyester fibers and polyethylene particles were the main type. However, more than 100 billion of

microplastic particles enter the environment per year through sludge produced.

Table 2-1 *Detailed information for microplastic polymers including monomer, density, usage, and score. Lithner et al. (2011)*

Polymer	**Abbreviation**	**Monomer**	**Main application**	**Score**	**density**
Polyethylene	PE	Ethylene	Toy, bottles, etc.	11	0.87-0.97
Polypropylene	PP	Propylene	Food packaging, etc.	1	0.85-0.88
Polyvinyl Chloride	PVC	Vinyl chloride	Pipe, etc.	10,551	1.38-1.39
Polyamide (nylon)	PA	Adipic acid	Bearing	47	1.13-1.41
Polyurethane	PUR	Propylene oxide	Sports mats, packaging	7,384	0.05-1.72
Polystyrene	PS	Styrene	Plastic ups, packaging	30	0.96-1.05
Polycarbonate	PC	Bisphenol A	Storage dish	1,177	1.10-1.15
Polyethylene terephthalate	PET	Terephthalic acid and Ethylene glycol	Packaging, fabrics	4	1.37-1.38

2.4 Adsorption of pollutant on microplastics

Plastics have a variety of properties that are dependent on the polymer type and can change over time, determining their fate in the environment. Furthermore, they absorb a variety of pollutants from the nearby environment when discharged into the environment.

Holmes et al. (2012) collected plastic from beaches of southwest England. Their particles contain considerable concentration of trace metals (Cr, Co, Ni, Cu, Zn, Cd and Pb). Experiment was set for examine the mechanisms and kinetics of trace metal uptake by virgin and beaches plastics. Result showed greater metal were accumulate in beaches particles. This was because the reaction of surface of aged particle has changes from itself and physic-chemical characteristics i.e., charged or polar regions of plastic surface, accumulations of biofilm, roughness, hydrogenous precipitates increase the charge, and porosity.

Garrido et al. (2019) reviewed effect of microplastics on the toxicity of chlorpyrifos exposure to microalgae (*Isochrysis galbana*). This study showed the chlorpyrifos effect on microalgae growth in concentration over 2.4 mg/L and was slight affected by exposure to microplastics. The particle size of microplastics and microalgae was similar (4-5μm), therefore microplastics cause shading on algae and hinder photosynthesis. However, inhibition of microalgae exposed to chlorpyrifos depending on the presence or absence of microplastics was tested. All cases, lower inhibition was obtained when presence of microplastics. More than 80% of chlorpyrifos was adsorb onto microplastic surface by two factors: hydrophobicity and physical features of microplastics particles. Thus, the role of microplastic was vectors of pollutants for aquatic system.

Wang et al. (2020) studied about the adsorption of metals ion (Cu^{2+} and Zn^{2+}) in aqueous solution by the virgin and aged microplastics (PET) under UV radiation. The result showed that Cu^{2+} and Zn^{2+} ions can be accumulated by microplastics and the age microplastics had higher adsorption capacity than the virgin. This phenomenon could be explained by UV radiation could contribute to the increase of oxygen-containing functional group on microplastics. Meanwhile, higher the temperature and pH were also showed influence the adsorptive performance.

Effects of microplastics and heavy metals accumulation in the yellow seahorse (*Hippocampus kuda*) were studied by Jinhui et al. (2019). After exposure, physical (body length and body weight), growth rate, and survival rate were decreased. These results suggest that the effect of microplastics on seahorse growth is caused by the accumulation of heavy metals, rather than by the microplastics themselves.

2.5 Heavy metals

Heavy metals are well known for harmful to organism. Heavy metals are in periods 4-7 of the periodic table with atomic number in range 23-92. They are high toxic and carcinogenic even at low concentration. Heavy metals are widely appeared in the surface runoff, ground water, sediment and atmospheric by natural geological and anthropogenic source e.g., excess fertilizers-pesticide application on agriculture, combustion of fossil fuels and industrial wastes. After entering rivers, most heavy metals deposit into the sediment which serves as both sink and source. On the other hand, the sediment may desorb or resuspend to river as secondary pollution with optimum conditions. The accumulation of heavy metals are influences to aquatic ecosystem and human through food web. There are different types of dissolved heavy metals, and their proportions vary depending on the pH value. Heavy metals from WWTP are an important source that can be discharged to environment

2.6 Heavy metals from WWTP

Wastewater treatment plant were designed to remove solid suspensions containing biota and organic compound. In industrial area, wastewater has a smaller share of the sewage mix compare with municipal wastewater treatment plant. However, the development of industrial is main reason of pollution in environment with a variety of substances include pesticides, antibiotics, and heavy metals (Hubeny et al., 2021). In addition, the WWTPs are also not complete to eliminate completely the substances during the treatment process. Therefore, the removal of substances through accumulation in sewage sludge can be regarded as a by-product of the treatment process.

Principi et al. (2006) studied the toxicity of three heavy metals (copper, zinc and nickel) on an activated sludge biomass located in Italy. This studied was measuring several parameters of microbiological activity and the dynamics of microbial composition. Principal component analysis was evaluated the relationship between biological effects and chemical measurement. The impact of heavy metals was displayed by biomass deflocculating phenomena.

Iloms et al. (2020) studied industrial effluent impact on municipal WWTP in river at Africa. Analysis of the results from the study indicates that the WWTP was effective in maintaining most of the wastewater parameters within standard. However, the type of industry and activity undertaken therein influence the pH and elemental composition of the effluent.

Du et al. (2020) presented the occurrence and fate of heavy metals in municipal wastewater in China. Wastewater was collected to quantified heavy metals (Pd, Cd, Cr, As and Hg) releases into the environment. The removal ratios of five heavy metals ranged from 50% to 67% and there were detected in influent and effluent with concentrations up to 940 and 170 μg/L, respectively.

2.7 Ecological risk assessment

The basic preconditions for prevention and control of pollution are ecological risk assessment. Multivariate methods are conducted to identify potential pollution sources and to indicate relationships pollutant. Hakanson ecological risk index and ecological risk factor models were widely utilized to identify the ecological risk of heavy metals (Varol et al., 2020) . Currently, there are no standardized models to assess the ecological risk assessment of microplastics. Xu et al. (2018) and Li et al. (2020) have been developed the models to evaluate the ecological risk of microplastics in water and sediment.

Ecological risk assessment of microplastics and heavy metals Li et al. (2020) presented the ecological risk of microplastic in the mangroves of Southern China. Sediment was collected to explore microplastics concentration. Microplastics were found to present ecological risks base on a comprehensive using the potential ecological risk factor (E_i), potential ecological risk (PER), polymer risk index (H) and

pollution load index (PLI). Overall, the higher microplastic concentration was mainly related to sediment from the river, which indicated the influent of anthropogenic.

Xu et al. (2018) performed ecological risk assessment of microplastics at the estuary in China. In this study, both of concentration and chemical composition of microplastics were developed to evaluate the potential risks of microplastic in surface water. An initial assessment was performed adopting the hazard score of plastic polymers and the pollution load index. The accumulation of microplastics and the presence of hazardous microplastic around aquaculture farms were regarded as "hotspots" of microplastic pollution.

Patel et al. (2018) presented the risk assessment of heavy metals contamination in river water and sediments at India. Potential ecological risk provided the cumulative information about all analysed heavy metals. Various sediment indices and water index values showed the lithogenic and anthropogenic influences in controlling heavy metal content.

Varol et al. (2020) studied the ecological of heavy metals in soil sources in Turkey. Hakanson ecological risk index and ecological risk factor were widely utilized to identify the ecological risk. Enrichment factor, geoaccumulation index and contamination factor were frequently used for environmental risk assessment. The samples were examined to evaluate possible sources, pollution status and environmental, ecological and health risks of heavy metals.

Pico et al. (2021) studied ecological risk assessment of microplastic detected in mixed surface and treated wastewater in Saudi Arabian. The ecological risks of microplastic were divide into two indexes: the hazard index (HI) and the pollution load index (PLI). Microplastics polymers were identified by FTIR and they have toxic in their own, and difference in their chemical components. The average percentage of each polymer were assessed HI index and microplastic concentration at each sampling point were calculate followed PLI. Their risk was considered risk category III which a loss of biodiversity in the aquatic environment.

Table 2-2 *Reported microplastics concentrations (items/L) in water and sediment around the world.*

Location	Sample type	Concentration	Main size	Main shape	Main polymer type	Reference
WWTP, Finland	Final effluent wastewater	1.0 items/L	>5.0 mm	Fiber and fragment	Rayon, PES, PET, PE, PA, and PP	Lares et al. (2018)
	Sludge sediment	27.2 items/g	0.25 – 0.5 mm			
Mangroves, China	Sediment	2.3 items/g	500 μm – 5mm	Fiber	PES and PE	Li et al. (2020)
Urban tap water and water source, China	Urban tap water	1.6 items/L	10 - 5000 μm	Fiber	Rayon and PET	Zhang et al. (2020)
	water source	0.7 items/L				
Coastal, Iran	Sediment	1.2 items/g	≤100 μm	Fiber and fragment	PES and PET	Akhbarizadeh et al. (2017)
Estuary, China	Surface water	0.4 items/L	0.07 – 1.0 mm	Fiber, fragment and film	PE and PP	Xu et al. (2018)
Urban WWTP, Spain	Final effluent wastewater	4.6 items/L	400 - 600 μm	Fiber, film and fragment	LDPE	Bayo et al. (2020)

CHAPTER III
METERIAL AND METHODS

3.1 Study area and sample collection

Wastewater and sludge samples were collected from two central industrial estates, located in Chonburi and Bangkok provinces, Thailand (WWTP A and WWTP B) in October 2020. WWTP A has a capacity of 20,500 m^3/day and serves wastewater from approximately 200 factories, mainly 54 factories of automotive, 44 factories of electronics, and 40 factories of plastic industries. Note that 40 factories of plastic industries in plant A comprise mainly of automotive parts and packaging. WWTP B can service wastewater up to 18,600 m^3/day from approximately 146 factories which are 22 factories of automotive, 8 factories of transportation, 6 factories of electronics and 4 factories of plastics packaging which might be a main source of microplastics. Both WWTPs are operated based on an activated sludge system. WWTP A, in particular, has reverse osmosis (RO) unit to recycle some treated wastewater.

All collected samples have been conducted follows the processes developed and supported by the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program. Samples were then separated into three types for analysis i.e., microplastics, heavy metals and heavy metals on microplastic analysis. For WWTP A, water samples were collected from 5 points i.e., influent, post grit chamber, post aeration tank, effluent, and RO. Water samples from WWTP B were collected from 4 points i.e., influent, post grit chamber, post aeration tank, and effluent (Fig.3-2). All water samples for microplastics and heavy metals on microplastic analysis were kept in 15 L containers at the depth of 12-15 cm from the surface. At each station, samples were sieved through an 8-in diameter steel sieve with 500, 200, 100, and 20 µm of mesh size and rinsed with deionized water into glass bottles and 1 L of wastewater was kept into polypropylene bottle for heavy metals analysis. Nitric acid was added until pH less than 2 to preserve heavy metals in water samples. To prevent further microbial growth, samples were stored in a container at 4°C and dark until further analysis. To avoid contamination, equipment was washing with deionized water in advance.

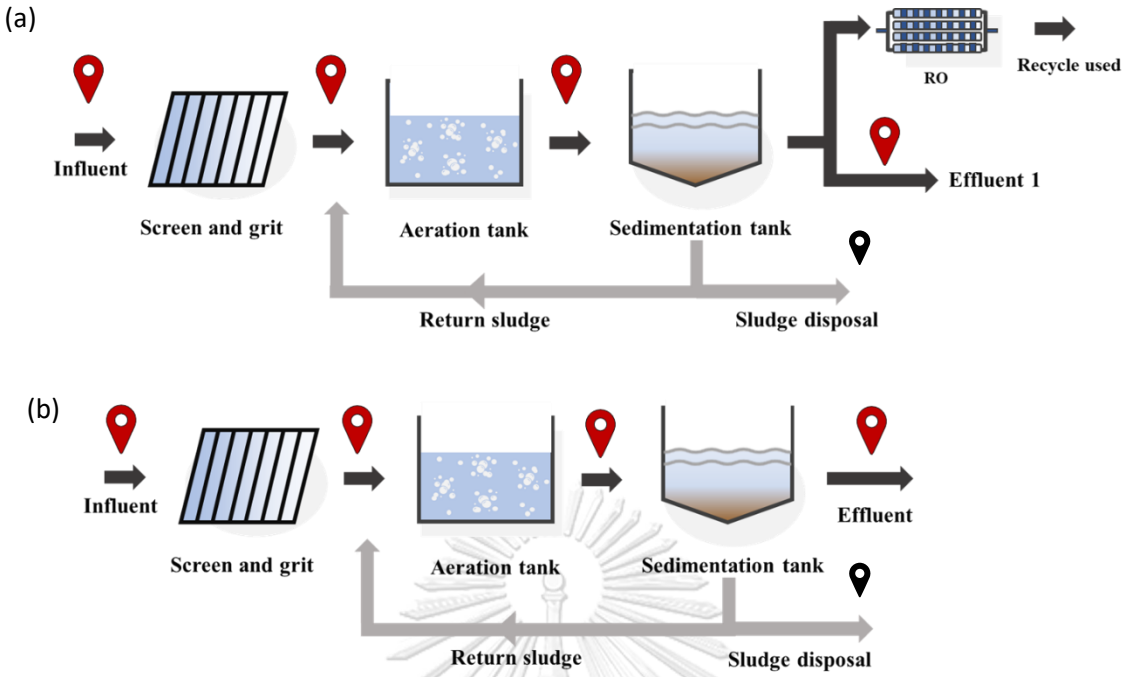


Figure 3-2 *Graphical presentation of the central industrial wastewater treatment process*
in a) Chonburi province and b) Bangkok province, Thailand with sampling point for water ( ) and sludge ( ).

Around 0.5-1 kg of sludge samples were collected at depth of 10 cm from sludge storage point with stainless steel bucket and packed in zip-locked bag for separately analyzed of microplastics, heavy metals and heavy metals on microplastics.

3.2 Material

3.2.1 Equipment for microplastics analyze

1) Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR - FTIR) (PerkinElmer Spectrum IR 10.6.2)
2) Stereomicroscope (NSZ-405J3 Olympus)
3) Stainless steel sieves, an 8-inch-diameter steel sieve (500 µm, 200 µm, 100 µm, and 20 µm)
4) Drying oven (Binder Series ED Avantgarde.Line)
5) Density separator funnel
6) Vacuum filtration
7) 0.45 µm-cellulose nitrate filter (Sartorius filter)

8) Hot plate with stirrer bar
9) Stainless steel bucket
10) Glassware
11) Standard metal forceps
12) Distilled water bottle

3.2.2 Equipment for heavy metals analyze

1) Inductively Coupled Plasma Optical Emission Spectrometer (ICP- OES) (Analytik Jena PQ 9100)
2) Freeze Drying (Labconco)
3) Microwave digestion (Ethos one)
4) Auto pipette
5) Glassware
6) polypropylene bottle
7) Balance
8) Dropper
9) 2.5 µm Whatman™ Qualitative Filter Paper - Grade 5

3.2.3 Equipment for parameters in wastewater analysis

1) pH meter with multi-function
2) DO meter
3) Dropper
4) Polypropylene bottle
5) Nitrogen analyzer (Buchi)

3.2.4 Chemicals

1) ICP mixed standard solution
2) Nitric acid (HNO_3)
3) Hydrochloric acid (HCl)
4) 30% Hydrogen peroxide (H_2O_2)
5) Iron (Fe (II)) solution (Fenton's solution)
6) Sodium chloride (NaCl)

7) Sodium iodide (NaI)
8) Deionized water

3.3 Sample processing

3.3.1 Quality control

To avoid contamination, all equipment for microplastics analysis was washed with tap water and twice with deionized water before use. The use of plastic lab materials was limited. Laboratory benches were wiped with deionized water and cellulose tissue before each measurement. Field blank samples for both sites were collected during sampling while laboratory blank was taken by nitrocellulose membrane placed in a petri dish without cover for 8 h. For the relevant part of heavy metals analysis, glassware set was cleaning with tap water and soaked with 10% HNO_3 for 24 h. In laboratory analysis, the operator wore a laboratory coat and gloves during sampling or analysis.

3.3.2 Analysis of parameters in wastewater

Each water sample will be analyzed for total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total solids (TS), total suspended solids (TSS), and total dissolved solids (TDS), temperature, and pH that may affected on solubility, distribution and mobility of heavy metals. Analytical methods of wastewater are illustrated in Table 3-3.

Table 3-3 *Detail of wastewater analysis.*

Parameter	Method
Total organic carbon (TOC)	High temperature oxidation
Chemical oxygen demand (COD)	Closed-Reflux, Titrimetric
Total Kjeldahl nitrogen (TKN)	Titrimetric Kjeldahl standard
Total suspended solids (TSS)	Gravimetric
Total dissolved solids (TDS)	Gravimetric
Temperature	pH meter
pH	pH meter

3.4 Microplastics analysis

3.4.1 Identify the characteristics of microplastics

Processes were developed and supported by the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program. Microplastics in glass bottles were transferred to beaker by rinsed with distilled water 2-3 times until clear then dried by oven at 60 ± 5 ºC for 24 hours. 30% H_2O_2 solution and 20 mL of 0.05 M Fe (II) solution, known as Fenton's reagent, were added to a beaker and accelerated the reaction by heating and stirring in a hot plate at 60 ± 5ºC until the solution was clear. Samples were obtained through the density separation method with 20 mL of NaCl (1.2 g/cm^3) and settled in a separatory funnel for 24 h. Supernatants were filtered onto a 0.45 µm nitrocellulose membrane by vacuum filtration. Residual sediment was added to 10 mL of NaI (1.69 g/cm^3) to ensure effective separation and filtered after the mixed solution settled in the separatory funnel. The funnel was then washed with distilled water and filtered. Residual microplastics on filters were dried in glass petri dishes at room temperature for identification. Sludge samples were dried

and powdered by freeze-dried before size separation. The residual on stainless steel sieves was rinsed to beaker and then removal organics process followed above method.

In addition, the particles were classified into four shapes i.e., fiber, pellet, film, and fragment along with colours and number by stereomicroscope. During the analysis, attenuated total reflectance – Fourier Transform Infrared Spectroscopy (PerkinElmer Spectrum IR 10.6.2) was used to determine the polymer types of microplastics for all samples with a resolution of 4 cm^{-1} in the scanning range of 800 – 4000 cm^{-1}. Spectra were then compared to the libraries provided by PerkinElmer.

3.4.2 Data analysis

Microplastic particles were reported as particles/L. The particle was characterized as triplicate and presented as mean ± standard deviation. A paired t-test with a p-value < 0.05 was conducted for the differences in microplastics content in each unit.

3.5 Heavy metals analysis

All sample were digested and analysed by microwave digestor and inductively coupled plasma emission spectroscopy (ICP-OES) at Department of Environmental Science, Faculty of Science, Chulalongkorn University. In this study, the various heavy metals included barium (Ba), arsenic (As), cobalt (Co), cadmium (Cd), iron (Fe), chromium (Cr), manganese (Mn), copper (Cu), selenium (Se), zinc (Zn), nickel (Ni), and lead (Pb) were analyzed at the same time. Concentration of heavy metals was presented in milligram per liter (mg/L) in water and milligram per kilogram (mg/Kg) in sludge.

3.5.1 Preparation of standard solutions

In this study, the quantification of various heavy metals (Ba, As, Co, Cd, Fe, Cr, Mn, Cu, Se, Zn, Ni, and Pb) concentration was determined using ICP-OES to increase the accuracy of results analysis and reduce the limitation of the analytes. Preparation of aqua regia solution as a dissolving agent by mixed-well of HCl and

HNO_3 with ratio 3:1 and then adjust volume by deionize water. The ratio of volume adjust was 4 ml of aqua regia per 100 ml of deionize water.

Intermediate mixed standard solutions were prepared by pipette 5 ml from stock solution (100 mg/L) and diluted with aqua regia solution until a total volume of 50 ml with an initial concentration 10 mg/L and using serial dilution by aqua regia solution until measure concentration 2, 1, 0.5, 0.3, and 0.1 mg/L, respectively. All standard solutions were loaded into clear vials for further analysis with ICP-OES. The correlation coefficients of each heavy metals were greater than 0.99.

3.5.2 Microwave digestion

The heavy metals on environment media were determined. Before heavy metal analysis, wastewater sample were extracted organic matter and interference matrix by microwave digestor follow by EPA 3015 method. In brief, 40 ml of wastewater sample were added to Teflon vessels then add 4 ml of aqua regia solution, which was mixed-well of HCl and HNO_3 with ratio 3:1, using a microwave digestion system at temperature 170±5°C. The vessel allowed to cool at the end of digestion process. Solutions were filtered through 2.5 µm filter and adjust volume to 50 ml by deionized water. Samples were stored at 4°C prior to analysis.

Freeze-dried sludge and microplastics for heavy metals analytical sample were digest followed EPA 3051 method. In brief, 4 ml of aqua regia were add to 0.5 g powered sludge in Teflon vessel and using a microwave digestion system at temperature 175±5°C. Likewise, residual microplastics on filter were cut into small pieces by plastic scissors prior to digestion process. At the end of digestion process, all solutions were cool and filtered through 2.5 µm filter before adjusted volume to 50 ml by deionized water. Samples were stored in a container at 4°C further analysis.

3.5.3 Analytical Performance Characteristics

Limit of detection (LOD) defines as 3 times the standard deviation of the blank which is detected a range of concentration.

Limit of quantification (LOQ) defines here as 10 times the standard deviation which is the lowest concentration that gives greater confidence that the reported values are quantifiable.

3.6 Ecological risk assessment

3.6.1 Microplastic risk assessment

1) Polymer risk index (H)

The hazard scores of plastic polymer and polymer type as an important index to evaluate its ecological harm was used to assess the risks of microplastics based on polymer properties as follows:

$$H = \sum P_n \, X \, S_n \qquad \text{eq. 1}$$

Where

P_n is the percentage of each MP polymer type at each sampling site

S_n is the score for the polymers comprising the microplastic from Lithner *et al.* (2011).

Hazard ranking model has been developed in order to categorize hazardous ingredients and compared the different polymers, based on risk of affecting the environment and human health. All substances which are identified as used in the production of each polymer type are classified hazard data that reflected the intrinsic hazardous properties of a substance. The procedure for calculating the sum of hazard score for the polymer is based on the classifications of the monomer that the polymer is made of.

2) The pollution load index (PLI)

For monitoring the degree of pollution in the area, The PLI is regarded as a standardized rule which refers to the microplastics concentration as follows:

$$CFi = Ci/C0i \qquad \text{eq.2}$$

$$PLI = \sqrt{CFi} \qquad \text{eq.3}$$

Where

CF_i is microplastic concentration factors

C_i is the microplastic concentration in each station

C_{oi} is the minimal microplastic concentration

3.6.2 Heavy metals risk assessment

1) Contamination factor (CF)

The CF was used to determine heavy metals contamination in soil base on this factor compared to their natural and soil contamination rate. The hazard scores of plastic polymer and polymer type as an important index to evaluate its ecological harm was used to assess the risks of microplastics as follows:

$$CF = \frac{C_{sample}}{C_{background}} \quad \text{eq.4}$$

Where

C_{sample} is heavy metals concentration in samples

$C_{background}$ is heavy metals concentration in crustal

Sediments were classified based on the Table 3-5 to discriminate obtained contamination factor.

2) Geo-accumulation index (I_{geo})

According to Muller (1969), the I_{geo} is used to evaluate heavy metals contamination in soil by comparing pre-industrial. Pre - industrial WWTP background values were compared with the recent heavy metal concentration for quantitative measure of heavy metal pollution

$$I_{geo} = \log_2\left(\frac{C_n}{1.5B_n}\right) \quad \text{eq. 5}$$

Where

C_n is heavy metal concentration in sediment samples (μg/g)

B_n is background concentration of the heavy metals in field value (μg/g)

1.5 is a matrix correction factor due to lithogenic effluents.

The index consists of seven grades are classified in Table 3-5.

3.6.3 The potential ecological risk (PER) of microplastics and heavy metals

Both of microplastics and heavy metals were found to present ecological risk assessment based on a comprehensive evaluation as follows:

$$C_f^i = \frac{C^i}{C_n^i} \quad \text{eq. 6}$$

$$E_r^i = \mathrm{T}_r^i \times \mathrm{C}_f^i \quad \text{eq. 7}$$

$$PER = \sum_{n=1} E_r^i \quad \text{eq. 8}$$

Where

C_f^i is the single element pollution factor of microplastics or heavy metals

C^i is the observe microplastics or heavy metals concentration in samples

C_n^i is the background level of microplastic concentration or reference value of heavy metals. Due to a lack of available background data of microplastics, in this study was adapt minimal of microplastic as the background value, while heavy metal were using reference value of element

E_r^i is the potential ecological risk index of an individual microplastic or heavy metals

T_r^i is the chemical toxicity coefficient for the constituent polymer from Lithner et al. (2011) or biological toxic factor of an individual heavy metals

The different categories of PER of microplastics and heavy metals are shown in Tables 3-4 and 3-5.

3.6.4 The risk assessment of heavy metals on microplastics

In this study, the model to evaluate heavy metals on microplastic risk assessment was developed from:

$$\mathrm{E_r} = (\sum E_r)_{microplastics} + (\sum E_r)_{heavy\ metals} \quad \text{eq. 9}$$

$$\boldsymbol{E_{r\,(microplastics)} = H \times \frac{C^i}{C_n^i}} \quad \text{eq. 9.1}$$

Where

H is Polymer risk index

C^i is concentration of microplastics at each station

C^i_n is background level of microplastic concentration (minimum concentration)

$$\boldsymbol{E_{r\,(heavy\ metals)} = T_r^i \times \frac{C^i}{C_n^i}} \quad \text{eq. 9.2}$$

Where

H is Polymer risk index

T_i is biological toxic factor of an individual element

C_i is reference value of heavy metals

The different categories of H, PLI, E^i_r and PER for microplastics and CF, I_{geo}, E^i_r and PER for heavy metals are shown in Tables 3-4 and 3-5.

Table 3-4 *Pollution categories of potential ecological risk posed by microplastics.*

H	**Risk category**	**PLI**	**Risk category**	**E^i_r**	**Risk category**	**PER**	**Risk category**
<10	I	<10	I	<40	Minor	<150	Minor
10-100	II	10-20	II	40-80	Medium	150-300	Medium
100-1000	III	20-30	III	80-160	High	300-600	High
>1000	IV	>30	IV	160-320	Danger	600-1200	Danger
				≥320	Extreme danger	≥1200	Extreme danger

Table 3-5 *Pollution categories of potential ecological risk posed by heavy metals.*

CF	Risk category	I_{geo}	Class	Risk category	PER	Risk category
<1	Low degree	≤0	0	Practically uncontaminated	<150	Low grade
1 -3	Moderate degree	0 -1	1	Uncontaminated to moderately contaminated	150 – 300	Moderate grade
3 – 6	Considerable degree	1 – 2	2	Moderately contaminated	300 – 600	Severe grade
>6	Very high degree	2 – 3	3	Moderately to heavily contaminated	>600	Serious grade
		3 - 4	4	heavily contaminated		
		4 - 5	5	Heavily to extremely contaminated		
		≥ 5	6	Extremely contaminated		

CHAPTER IV
RESULT AND DISCUSSION

4.1 Analysis of parameters in wastewater

In this study, wastewater from each treatment unit from 2 WWTPs were considered. The following water quality parameters include total organic carbon (TOC), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total Solids (TS), total suspended solids (TSS), total dissolved solids (TDS), temperature, and pH were measured. The result is illustrated in Table 4-6. In general, influent had a relatively high concentration of organic matter. In plant A, the physical parameter includes pH and temperature were presented in range of 6.94-7.23 and 28.30-34.40 °C, respectively. Meanwhile, the chemical parameter includes TOC, COD, and TKN were showed in range of 4.83-36.02 mg/L, 16.00-320.00 mg/L, and 6.23-22.82 mg/L, respectively. In terms of solids in water, the result presented in range 275-887 mg/L of TS, while TSS and TDS were in range 0.00-50.00 mg/L and 275.00-837.00 mg/L, respectively. The highest values of each parameter were normally showed in influent samples and less value were presented in effluent and least through RO. For plant B, the physical parameters include pH and temperature were presented in range 6.98-7.38, and 28.70-33.20 °C, respectively. Meantime, the chemical parameters include TOC, COD, and TKN were showed in range of 18.53-125.06 mg/L, 40.00-560.00 mg/L, and 1.81-50.89 mg/L, respectively. The range 1,420.00-3,129.50 mg/L of TS were revealed, while TSS and TDS were presented in range 10.00-60.00 mg/L and 1,4100-3,077.50 mg/L, respectively. Almost of highest values of each parameter were also shown in influent samples and least values of all parameters were presented in effluent. In case of TOC, COD and TS, the high values were presented in post grit chamber and post aeration tank which variety and variability to inorganic and organic. In general, WWTP located in open area and exposed to sunlight that may easily vary of temperature. Compared between both plants, WWTP B had organic loading over than other one. However, effluent remains within the standard before discharge to environment. Moreover, relevant relationships among wastewater parameters, microplastics, and heavy metals needs to be further attention.

Table 4-6 *Physicochemical parameters in the WWTPs.*

Parameters	WWTP A					WWTP B			
	Influent	Post grit chamber	Post aeration tank	Effluent	Post RO	Influent	Post grit chamber	Post aeration tank	Effluent
pH	7.23	7.06	6.94	7.22	7.10	7.38	7.08	7.09	6.98
Temp (°C)	34.40	32.40	29.90	28.30	29.8	30.70	33.20	28.70	28.70
TOC (mg/L)	22.35	28.50	36.02	12.78	4.83	19.57	125.06	30.95	18.53
COD (mg/L)	320.00	80.00	48.00	32.00	16.00	400.00	560.00	560.00	40.00
TSS (mg/L)	37.50	50.00	50.00	2.50	n.d.	52.00	60.00	34.00	10.00
TDS (mg/L)	640.00	827.50	837.50	802.50	275.00	3,077.50	1,597.50	1,552.50	1,410.00
TS (mg/L)	677.50	877.50	887.50	805.00	275.00	3,129.50	1,657.50	1,586.50	1.420.00
TKN (mg/L)	22.82	12.63	13.37	13.27	6.23	50.89	22.54	10.81	1.81

4.2 Microplastics

4.2.1 Microplastics abundance

Wastewater and sludge samples were collected at each unit for possibility of encountering microplastics. After organic extraction, the microplastic particles were counted under stereomicroscope. Result of microplastics at each unit shows in Table 4-7.

Table 4-7 Microplastics abundance in wastewater treatment unit

Treatment unit	Number of microplastics (particles/L)		Removal efficiency (%)	
	WWTP A	**WWTP B**	**WWTP A**	**WWTP B**
Influent	101.87 ± 0.47	148.44 ± 0.91		
Post grit chamber	113.49 ± 0.71	150.56 ± 1.81		
Post aeration tank	87.89 ± 0.62	80.89 ± 0.41		
Effluent	11.04 ± 0.08	33.53 ± 0.55	89.16	77.41
Post RO	0.44 ± 0.04	-	99.57	
Sludge	2,398.00 ± 11.37	1,930.00 ± 7.57		

Microplastics were found in every unit of both WWTPs. From Table 4-7, the abundances of microplastics in influent, effluent, and RO from WWTP A were 101.87 ± 0.47, 11.04 ± 0.08 and 0.44 ± 0.04 particles/L, respectively. The microplastic removal efficiency was 89.16% through effluent and 99.57% through RO. On the other hand, microplastics found in WWTP B from influent and effluent were higher than that of WWTP A which were 148.44 ± 0.91 and 33.53 ± 0.55 particles/L, respectively, with a removal efficiency of 77.41%. The heightened removal percentage of microplastic particles was 87% and 58% through the sedimentation tank from WWTP A and WWTP B, respectively. This is probably because microplastic

particles may attach with microorganisms and tend to settle at the bottom of the unit. From WWTP A, RO performed microplastic removal efficiency of 99.54%.

Microplastic particles slightly increased from influent and post grit chamber in both WWTPs (WWTP A from 101.87 ± 0.47 and 113.49 ± 0.71 particles/L, respectively and WWTP B from 148.44 ± 0.91 and 150.56 ± 1.81 particles/L, respectively) and obviously decreased in the aeration tank. Hongprasith et al. (2020) also found a similar concentration of microplastic particles in the influent and grit chamber. It was possible that primary treatment processes had no ability to remove microplastics. However, it may attach high density and large size of particles on sand and grit (Zhang et al., 2021) and the light particles float before entering the next process (Bilgin et al., 2020 and Yang et al.,2021).

The removal efficiency within the secondary treatment process showed that WWTP A was higher than WWTP B and the highest in tertiary treatment (Ben-David et al., 2021, Lares et al., 2018, and Lv et al., 2019). Magni et. al 2019 studied about the fate of microplastics from municipal WWTP. The result showed 84% of microplastics decrease between influent and effluent and the greater removal was 64% within the secondary treatment. In contrast, compared to a study from Talvitie (et al 2017) showed that most of the microplastics was removed during the pre-treatment. Ziajahromi et. al (2021) also found that 69-70% of microplastics was removed by screening and grit removal. Alvim (et. al. 2020) reported that 74.8% of microplastic removal was observed from primary to secondary treatment by related to the activated sludge. According to removal percentage of both WWTPs within the secondary treatment process, some part of microplastics was reduced. The one possible way that they were settled and transferred to sludge, however, removal efficiency possibly depended on the effectiveness of sedimentation units or density properties of microplastics. In addition, they also found that a high concentration of microplastics was detected in the sludge (112.0 particles/ dry weight). The sludge is widely reused in agriculture as fertilizer that can lead to soil contamination. Alvim et.al (2021) presented an innovative to remove microplastics in sludge using ultrasound. The result showed that more than 38% of microplastics were removed. This may be another option for reducing new hot spot of microplastics.

This study investigated the abundance and characteristics of microplastics in central industrial wastewater treatment plants in Thailand however, there were a large number of microplastics released into the environment with the effluent. It can be estimated that around 226 million and 624 million microplastics per day are discharged from WWTP A and WWTP B, respectively. Even more than 70% removal efficiency, the microplastics concentration also leads to observation much higher. This number considered as high compared to other studies. Many researchers studied microplastics in municipal wastewater treatment plant. Despite high efficiency of removal, microplastics still released 22.1 x 10^6 to 133x 10^6 particles per day from Australia (Ziajahromi et. al 2021) and 9.1 x 10^{10} particles per day from China (Tang et. al. 2020).

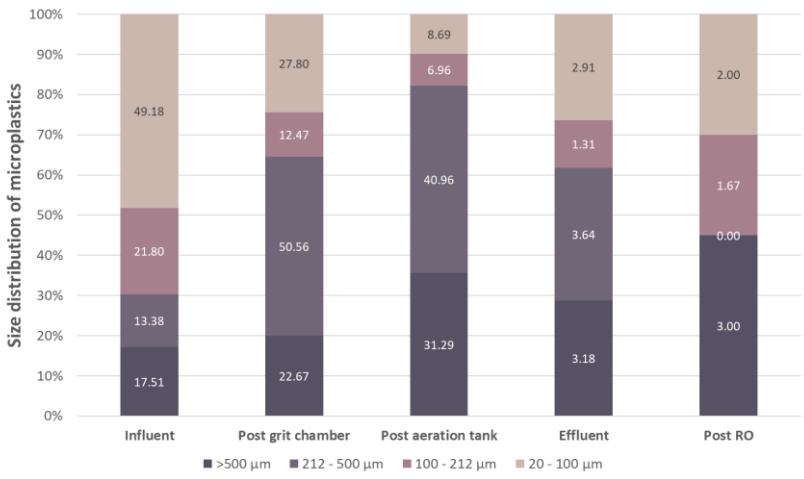
Note that, microplastics were compared the relationships with wastewater parameters. From Table 4-6, TSS from RO in WWTP A showed not detected (n.d.) while microplastics were detected 0.44 ± 0.04 particles/L. 50 ml of wastewater were used for analyzed TSS, while 15 L of wastewater were used for analyzed microplastics concentration. This was because the different volume of wastewater for analysis. Also, TSS parameter was analyzed using weight of suspended solids and the detected microplastic was small and light. This may the reasons that can be support found microplastics in RO and found nothing by TSS.

4.2.2 Characteristic of microplastics

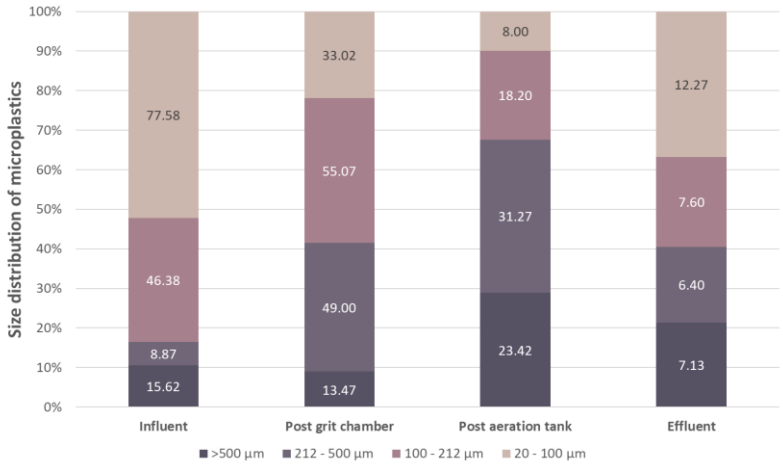
4.2.2.1 Size distribution

During visual analysis, a various of morphological characteristics of microplastics were detected in wastewater and sludge samples. The size separation first makes it easier to classify other details. This study, all samples were separated in four size fraction ranges which is greater than 500 µm, 200–500 µm, 100-200 µm, and 20-100 µm. Result shows in Fig.4-3.

(a)



(b)



(c)

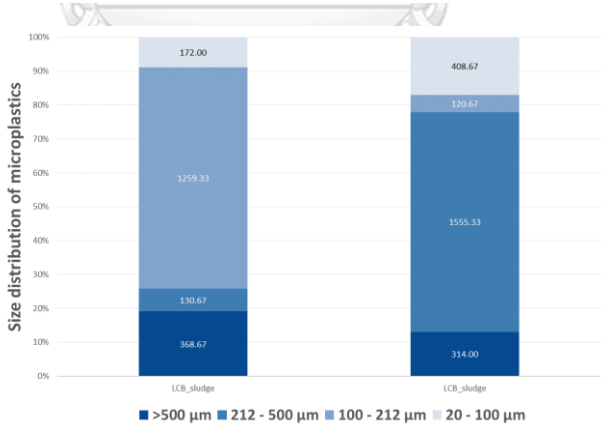


Figure 4-3 Size distribution of microplastics in (a) wastewater from WWTP A, (b) wastewater from WWTP and (c) sludge from both plants.

As shown in Figs. 4-3(a) and 4-3(b), the most detected size of microplastics found in WWTP A was 212-500 µm followed by 20-100 µm, > 500 µm, and 100–212 µm. In contrast, 20-100 µm was the size of most microplastics detected from WWTP B followed by 100-212 µm, 212-500 µm, and >500 µm. However, there was no significant difference in size distribution from both plants. The breakdown of large plastics leads to many sizes of microplastics. The size of most microplastics found in WWTP was different. For example, Tadsuwan et al. (2021) found that the main size was 0.05-0.5 mm whilst Franco et al. (2020) reported that the major size was 100–355 µm. in WWTP.

From both sites, trends in the microplastic distribution of various sizes were similar. At the influent, post grit chamber, and post aeration tank, the number of small particles was decreased while larger particles were increased in order. This could be indicated that the flotation of small particles with low density and wide surface area was removed. In contrast, in the post aeration tank from both sites, smaller microplastic particles were increased and the larger was decreased. In addition, microplastics can crack and pit under physical and chemical actions such as wave, wind, and UV radiation bacteria (Bandow et al. (2017), Kokalj et al. (2019), and Min et al. (2020)). Some larger particles can also be broken by the friction force of the aeration tank (Yang et al. (2021)).

Size distribution of microplastics in sludge showed in Fig.4-3(c). There is difference of last size scale. The most size in WWTP A was 100-212 µm followed by > 500 µm, 20-100 µm, and 212-500 µm, respectively, while 212-500 µm were the most size from WWTP B followed by 20-100 µm, > 500 µm, and 100-212 µm. However, there were some reports explored different pattern e.g., 100-150 µm from Spain (Alvim et. al. 2020), 0.5-5 mm from Italy (Pittura et al 2021), and 0.25-0.5 mm from Finland (Lares et. al. 2018).

Sludge storage was one part that microplastics that can be transferred from aeration tank, so size distribution of aeration tank and sludge from both sites were similar. This study showed higher concentration of small particles in sludge from both sites. This was probably the cracking particles from post aeration tank led to their aggregation with activated sludge by microorganism, contact time, and chemical

concentration (Pittura et al 2021). However, the larger particles still remain (Pittura et al 2021, Lares et. al. 2018) but the importance is the monitoring smaller size for confirmed the importance of physical processes in the removal of microplastics.

4.2.2.2 Shape

The length, width and dimension can be explained about materials source and fate of microplastics. In terms of shape, microplastics were categorized in four main typologies: pellet, fragment, fiber, and film according to characteristics given. The characterization of microplastics from FTIR is shown in Fig.4-4.

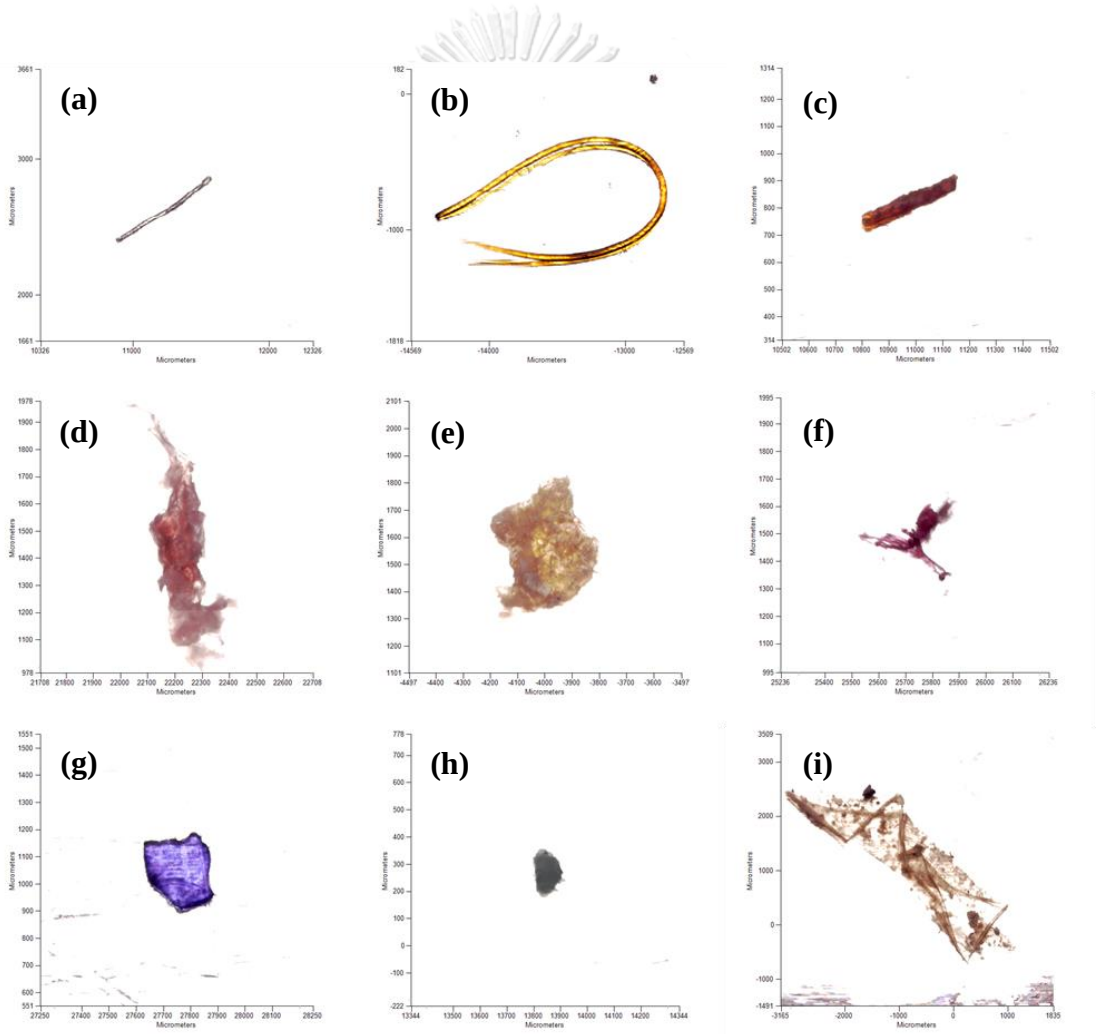
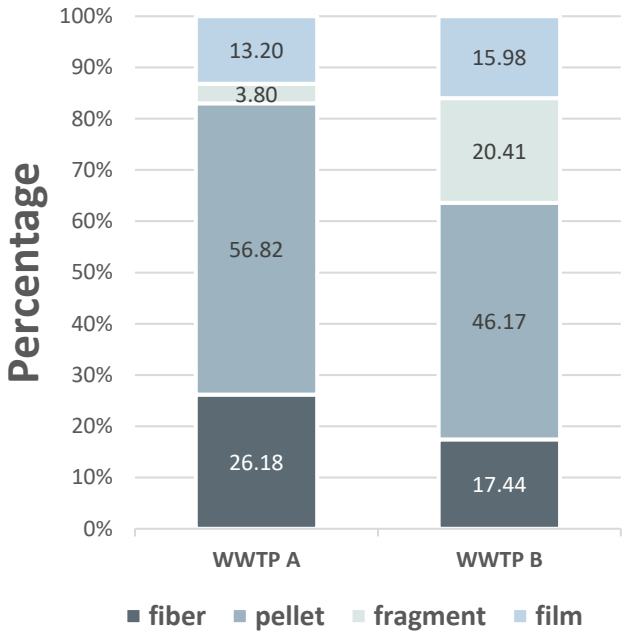


Figure 4- 4 Characterization of microplastic particles
(a) – (c) fiber, (d) - (f) fragment, (g) - (h) pellet, and (i) film from both WWTPs by FTIR.

(a)



(b)

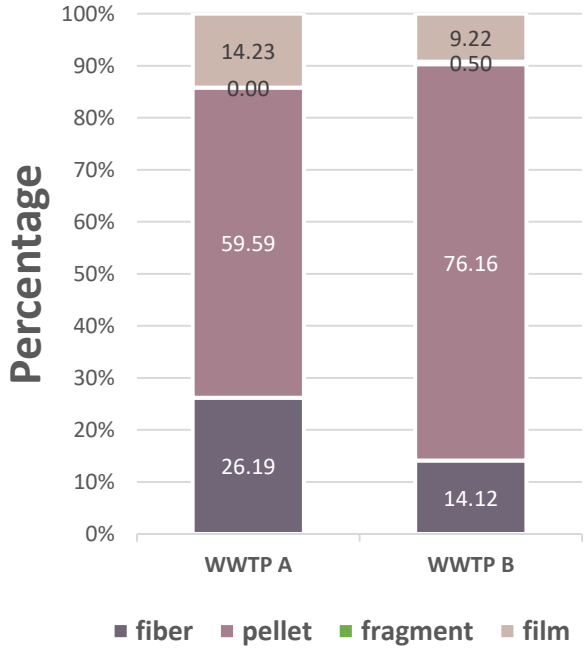


Figure 4-5 Percentage of microplastics shape
in (a) wastewater from both WWTPs and (b) sludge from both WWTPs.

The shape of microplastics derived from FTIR classified as pellet, fragment, fiber, and film (Fig. 4-5(a)). Pellets presented as the main shape found in wastewater of both study sites (56.82% from WWTP A and 46.17% from WWTP B) followed by fiber (26.18%), film (13.20%), and fragment (3.80%) from WWTP A and fragment (20.41%), fiber (17.44), and film (15.98) from WWTP B. In sludge (Fig. 4-5(b)), pellet also the most found from both sites (59.59% from WWTP A and 76.16% from WWTP B) follow by fiber and film (26.19% and 14.23% from WWTP A and 14.12% and 9.232% from WWTP B, respectively). It is worth noting that, fragment was found so rarely from WWTP B (0.5%) and found nothing from WWTP A.

Pellet is a secondary microplastic broken down from consumer products including construction materials, container, and decorating materials used in a variety of industrial processes. Fiber was the most frequently observed size of microplastics from several studies (Ben-David et al., 2021, and Lares et al., 2015). However, Lv et al (2019) indicated that among the type of microplastics, fragment has the highest percentage (65%) in wastewater. Tang et al. (2020)'s study found that in the comparison of two WWTPs, less fiber and film were detected in one WWTP, while more microbead and fragments were found in another one.

4.2.2.3 Colours

Colour is an outstanding manner to be able to immediately identify irregularities and easily separated from the organic material.

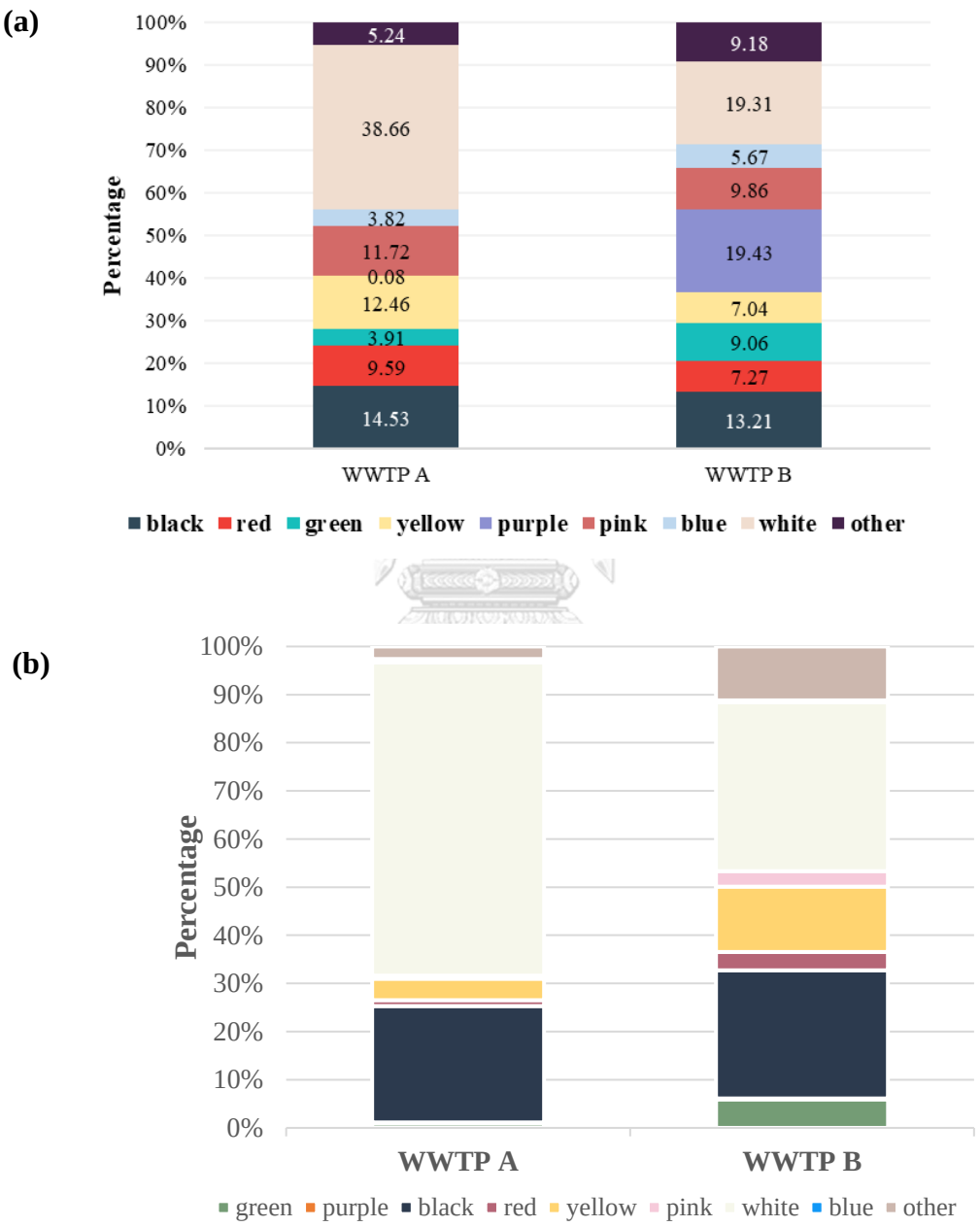


Figure 4-6 *Percentage of microplastics color in (a) wastewater from both WWTPs and (b) sludge from both WWTPs.*

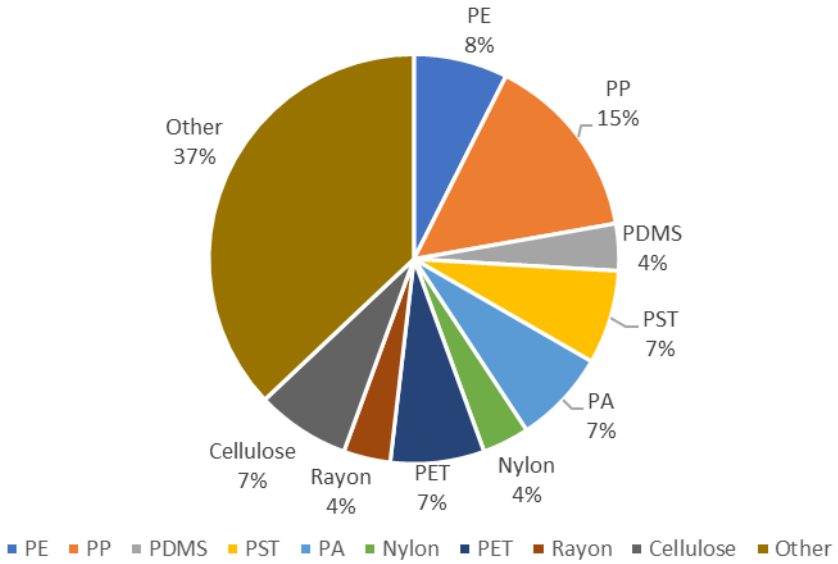
As shown in Fig. 4-6(a), the colours of microplastics in wastewater from WWTP A were mostly composed of white/clear (38.66%) > black (14.53%) > yellow (12.46%) > pink (11.72%) > red (9.59%) other (5.24) > green (3.91%) > blue (3.82%) > purple (0.08%). In WWTP B, the colours in wastewater included purple (19.43%) > white/clear (19.31%) > black (13.21%) > pink (9.86%) > other (9.18%) > green (9.06%) > red (7.27%) > yellow (7.04%) > blue (5.67%). Whilst the colours of microplastics in sludge from WWTP A (Fig. 4-6(b)) were mostly composed of white/clear (65.08%) > black (24.17%) > yellow (14.44%) > other (2.58%) > red (1.24%) > green (0.93%). In WWTP B, the colours in sludge included white/clear (35.09%) > black (26.61%) > yellow (13.60%) > other (11.17%) > green (5.91%) > red (3.81%) > pink (3.22%) > blue (0.39%).

The observed microplastics were mostly white/clear and black and that same as the study of Tang et al. (2020) that found more than 80% of total microplastic particles were white/clear and black. Conley et al. (2019) also reported that the most common colors were white/clear (60%) and black (22%). Color can be used to identify the sources of microplastics. White/clear and black microplastics are from a wide range of sources such as packaging, plastic bag, and bottle. In addition, the original bright color of microplastics can be changed to white/clear and black due to oxidation, aging of the dye by UV irradiation, and soaking in the WWTP. Regardless of the different research, the results of microplastic color were similar (Yang et al., 2021).

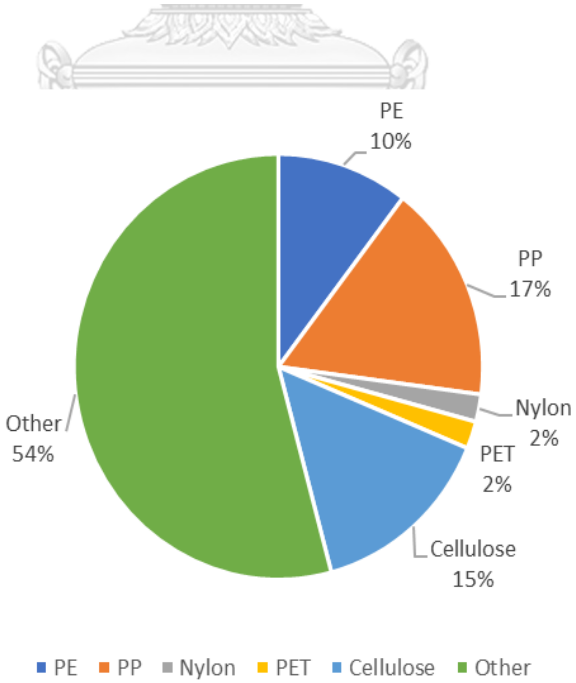
4.2.2.4 Polymer

The occurrences of microplastics in WWTP have been link to source. FTIR were used to confirm their plastics and identify polymer typologies. Result shows in Fig. 4-7.

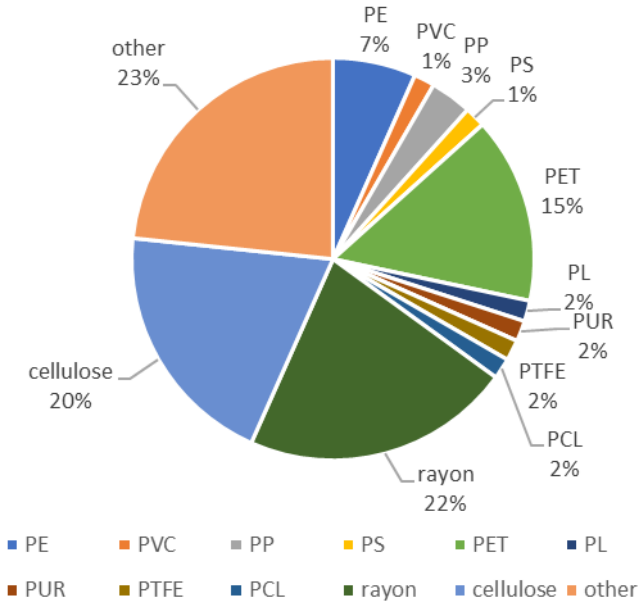
(a)



(b)



(c)



(d)

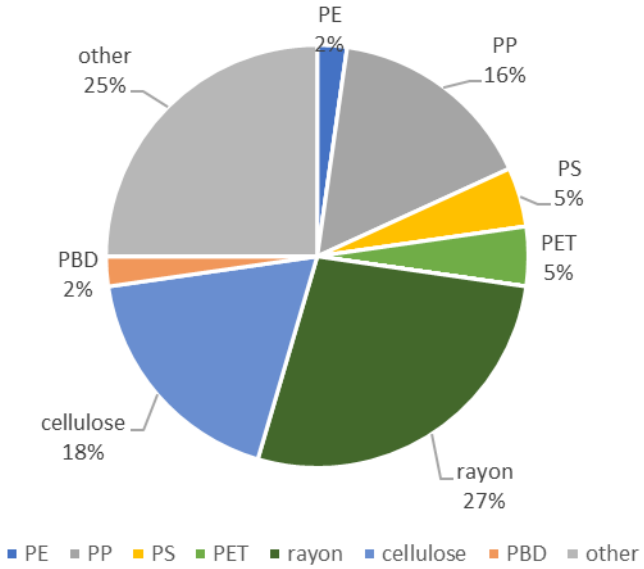


Figure 4- 7 *Proportion of microplastics in (a) wastewater from WWTP A, (b) wastewater from WWTP, (c) sludge from WWTP A, and (d) sludge from WWTP B.*

The suspected microplastic particles were randomly analyzed for identification by FTIR, as shown in Fig. 4-7. 157 samples of suspected particles were detected. A total of 10 detected polymer types were polyethylene (PE), polypropylene (PP), polydimethylsiloxane (PDMS), polystyrene (PST), polyamide (PA), polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE), polybutadiene (PBD), Nylon, and Rayon. From 90 particles, the percentages of polymer in wastewater from WWTP A (Fig.4-7(a)) were PP (14.8%) followed by PE (7.4%), PST (7.4%), PA (7.4%), PET (7.4%), PDMS (3.7%), nylon (3.7%) and rayon (3.7%). Over 37% were identified as others and cellulose detected at 7.4% from another group. The most percentage in wastewater in plant B (total 67 particles) was others (54.2%). However, the most percentages of polymer in wastewater from WWTP B was PP (16.7%) followed by PE (10.4%), PET (2.1%), and Nylon (2.1%) with cellulose 14.58% (Fig.4-7(b)).

On the other hand, the highest percentage of polymer in sludge from WWTP A was rayon (21.7%) followed by PET (15%), PE (6.7%), PP (3.3%), PST (1.7%), and PTFE (1.7%) (Fig.4-7(c)). Rayon (27.3%) was the most percentages of polymer in sludge from WWTP B followed by PP (15.9%), PST (4.5%), PET (4.5%), PE (2.3%), and PBD (2.3%) (Fig.4-7(d)). 14% and 25% were identified as others and cellulose showed 20% and 18.2% from both plants, respectively.

Note that, some of microplastic polymer were detected in sludge but not detected in wastewater. These reasons possible that samples were collected in COVID-19 pandemic period, therefore the industries were not operated full scale, especially the reduction of exports of plastic factories, resulting in water samples that are not homogeneous. In addition, some early microplastics from the previous wastewater before COVID-19 pandemic may settle and accumulate in each unit through wastewater treatment by density. Moreover, the use of ATR - FTIR which was an instrument used to identify microplastics in this research has to sampling the pieces of microplastics from filter paper by manual. This can occur some error in the experiment. The researcher recommends further study by using μ-FTIR which is automated algorithm to applied with reference spectra in database.

PP, PE, and PET were detected in both sites. There are commonly and widely used in packaging material. It has been suggested that some portions of these particles

are likely to escape the drainpipe by clean surface. The density range of microplastics can be classify polymer type with lower relative density. PP (0.85 g/cm^3) and PE (0.87 g/cm^3) are easily found in wastewater because their lower density than water. This relative could be the main efficiency removal because of buoyancy effect (Wang et. al. 2021). Moreover, the high density of microplastics i.e., PET (1.33 g/cm^3), PST (1.05 g/cm^3) is slightly heavier than water, giving its ability to behave as a colloid and can be accumulation in sludge (Franco et. al. 2020). In addition, non-microplastic particles were identified as paint, rubber, additives, cellulose, and others (Tang et. al. 2020, Franco et. al. 2020, Bayo et.al. 2020, Gies et. al. 2018, and Wang et. al. 2021).

4.3 Heavy metals

Wastewater and sludge samples collected from two study sites were analyzed for heavy metals. Table 4-8 shows performance of quality control for heavy metals using ICP-OES. It was found that manganese and lead were detected from both cellulose nitrate and GF/C filter papers while nickel was found only in cellulose nitrate filter. However, the amount of these heavy metals found were very low that means no significant in heavy metals analysis. In addition, concentration of heavy metals in tissue paper and blank was lower than LOD.

Table 4-9 presents the comparison of metals in this study with world and international guideline. All heavy metals in wastewater detected were lower than other researches and within water quality standards of industries. Almost heavy metals in sludge showed lower than average shale values and crustal average, except Cd and Fe. Compare with average shale values and crustal average, Cd from WWTP A show higher concentration while WWTP B showed lower. On the other hand, Fe was lower than average shale values and higher than crustal average.

Table 4-8 *Performance of quality control for heavy metals in this study (mg/L).*

Heavy metals	LOD	LOQ	Cellulose nitrate filter	GF/C filter	Tissue paper
Ba	0.0009	0.0023	-	-	-
As	0.0216	0.0648	-	-	-
Co	0.0003	0.0010	-	-	-
Cd	0.0001	0.0003	-	-	-
Fe	0.0031	0.0092	-	-	-
Cr	0.0007	0.0021	-	-	-
Mn	0.0002	0.0007	0.0017	0.0003	-
Cu	0.0003	0.0009	-	-	-
Se	0.0033	0.0098	-	-	-
Zn	0.0003	0.0009	-	-	-
Ni	0.0003	0.0008	0.0002	-	-
Pb	0.0009	0.0026	0.0003	0.0002	-

Table 4- 9 *Comparison of average metals in this study (mg/l) with world and international guideline.*

Reference	Location	Media	Ba	As	Co	Cd	Fe	Cr	Mn	Cu	Se	Zn	Ni	Pb
Turekian and Wedepohl 1961	average shale values	soil	580	13	19	0.3	47200	90	850	45	0.6	95	68	20
Taylor 1964	Crustal average	soil	425	1.8	25	0.2	5.63	100	950	55	0.05	70	75	12.5
Liu et al 2021	China	soil	-	8.8	-	0.16	-	57.66	-	24.57	-	59.84	25.6	22.15
Ampai et al 1981	Thailand	soil	-	-	-	0.3	-	-		192	-	1000	-	20
Waewtaa et al. 2004	Thailand	Sediment	-	-	-	0.048	11333	-	254	18.45	-	26.22	8.43	18.26
Petpiroon and Petpiroon 1996	Severn Estruary (U.K)	sediments	-	-	-	-	4.5	-	1820	38	-	280	-	119

Reference	Location	Media	Ba	As	Co	Cd	Fe	Cr	Mn	Cu	Se	Zn	Ni	Pb
Chalanya and Suwannee 1981	Thailand	seawater	-	-	-	1.5	-	-		5.28	-	3.44	-	5.58
Suthanaruk 1991	Upper Gulf of Thailand	seawater	-	-	-	0.0476	-	-	1.99	2.89	-		-	5.32
Waewtaa et al. 2004	Thailand	seawater	-	-	-	5	300	-	100	50	-	100	-	50
This study	Thailand	wastewater	0.00	0.00	0.00	0.01	0.30	0.01	0.62	0.07	0.00	0.92	0.04	0.04
			0.00	0.00	0.00	0.01	0.41	0.02	0.16	0.01	0.00	0.16	0.04	0.03
		Sludge	8.76	0.08	0.25	0.43	266.8	24.59	7.97	10.23	0.13	94.00	7.00	0.61
			6.50	0.04	0.06	0.01	91.17	1.00	2.17	18.85	0.02	59.25	0.58	0.32

4.3.1 Heavy metals in wastewater

Wastewater and sludge samples were removed organic matter by microwave digestor before analysis by ICP-OES. In this study, the quantification of various heavy metals (Ba, As, Co, Cd, Fe, Cr, Mn, Cu, Se, Zn, Ni, and Pb) concentration was determined as shown in Figure 4-8.

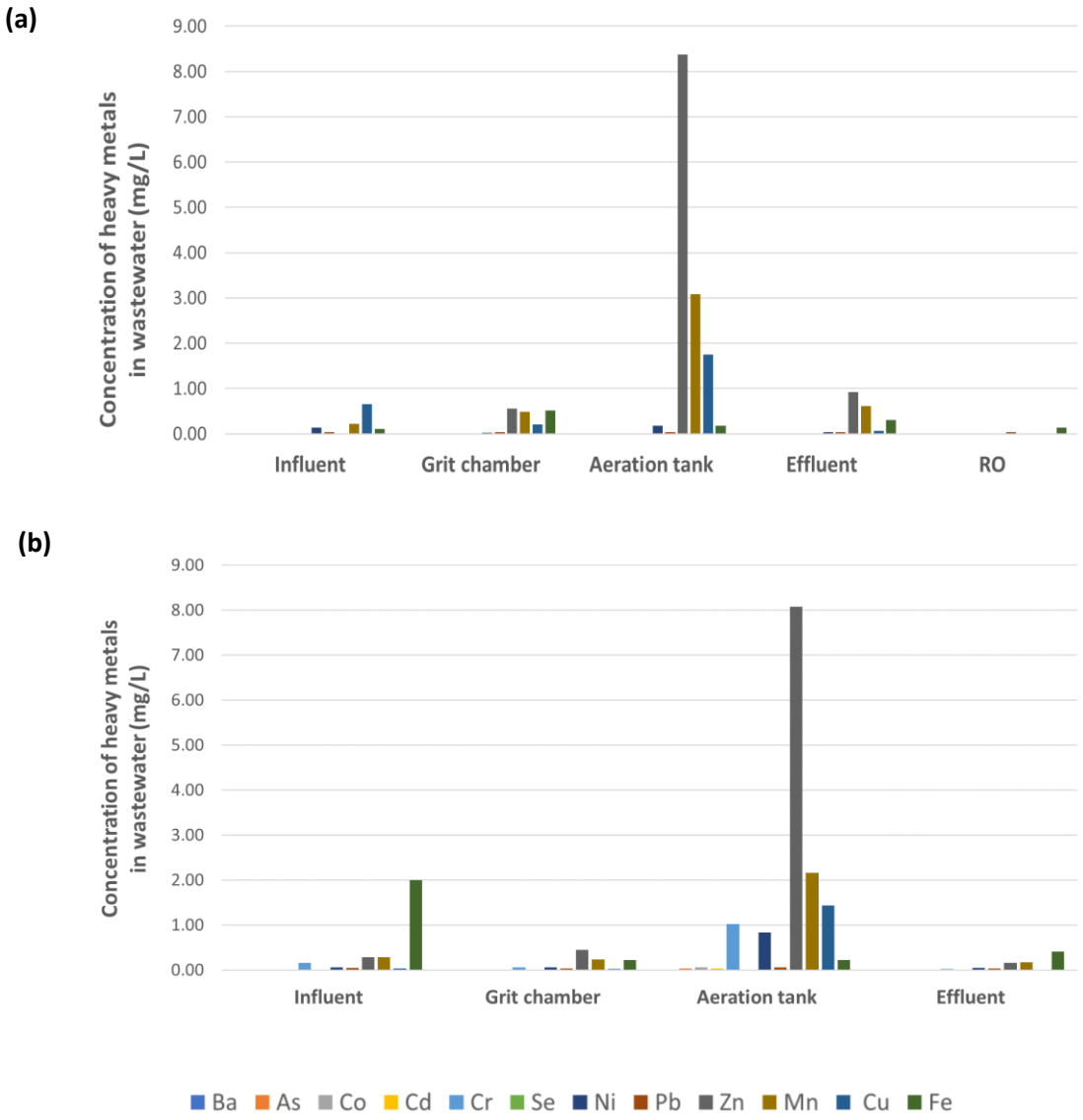


Figure 4-8 *Centration of heavy metals in wastewater from (a) WWTP A and (b) WWTP B.*

The various of heavy metals were still detected event after preliminary treatment process from each factory prior to input into central WWTP. From plant A, Zn, Mn, and Cu were the most detected from every unit, especially, post aeration tank. In this unit, Zn showed the highest value with average concentration of 8.3 mg/L followed by Mn (3.08 mg/L), and Cu (1.75 mg/L). In comparison with influent, effluent was detected lower concentrate and normally lowest through tertiary treatment process. However, this study presented Fe (0.14 mg/L) and Pb (0.03 mg/L) which were detected from post RO process. Likewise, Zn also detected as highest concentration in post aeration tank from plant B (8.08 mg/L) followed by Mn (2.16 mg/L) and Cu (1.43 mg/L). On the other hand, Ba presented below detection limit for both sites. However, effluent remained within the standard before discharged to environment. From both plants, extreme concentration of zinc presented in aeration tank might be transferring from activated sludge in aeration tank to aqueous phase along with transformation particulate zinc to aqueous phase in this unit (Yamagata et al 2010). Zn concentration was decreased after aeration tank with related to the decrease of TKN level. Morikubo et al (2021) found that the presence of zinc due to zinc oxide can be afforded ammonia adsorption. Iloms et al. (2020) reported that almost Zn and Cu concentration were detected from effluent of automotive and electronics industries. Consistent with our results, automotive and electronics are mainly factories in this studies sites. Similarly, Zn, Cu, and PB also found with high concentration from urban wastewater, but in this case, these metals might be attributed to leach from stagnant water remaining in the pipework (Rule et al., 2006). Therefore, Zn and Cu could also be related to the geological sources in addition to anthropogenic source. In contrast, Hammoudani et al (2021) studied effluent from domestic wastewater treatment plant and found Fe was the most concentration, followed by Zn and Cu while Hg were the lowest concentration detected in wastewater from biological WWTP. In South Africa, Agoro et al (2020) studies about distribution of five heavy metals (Cd, Pb, Cu, Zn, and Fe) from Municipal Treatment Plants. The result found that Cd was the higher in effluent and Zn was below detection.

4.3.2 Heavy metals in sludge

Next, heavy metals from sludge sample were determined from both study sites as shown in Figure 4-9.

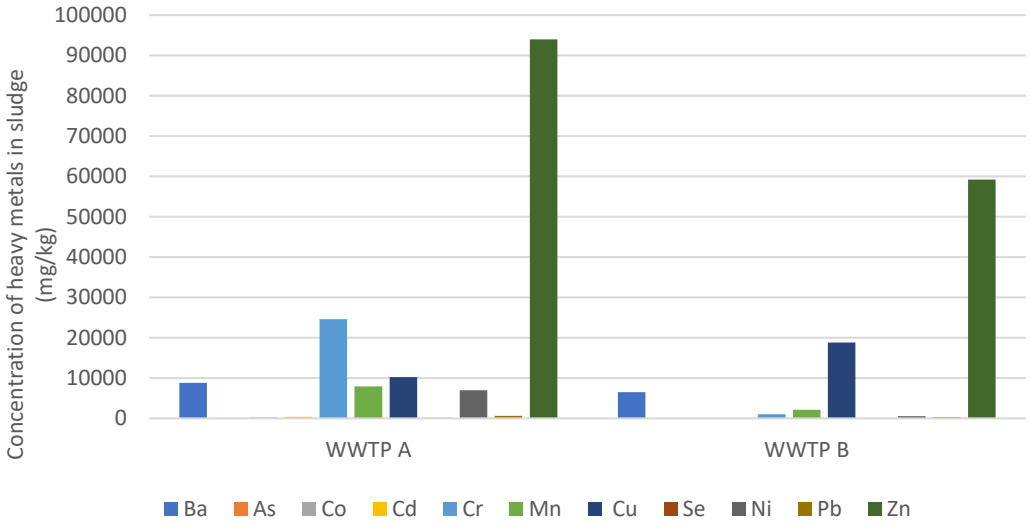


Figure 4-9 Concentration of heavy metals from both WWTPs.

From both sites, Zn, Cu, Cr and Ba showed higher concentration. In Plant A, Zn was the highest concentration followed by Cr and Ba. Zn also showed the highest concentration from plant B but slightly different in Cu followed by Ba. However, As was almost detected from both sites. Comparison of sludge between two sites, almost heavy metals from plant A showed higher concentration than plant B. In addition, Ba presented in sludge sample. As known that sludge from WWTP is the source to accumulate colloidal, soluble organic and heavy metals that present in wastewater process. Hence, heavy metals concentration is accumulated in sludge over than wastewater. In contrast, Kowalik et al (2021) studied heavy metal accumulated from sewage sludge and found the highest concentration of Zn and lowest concentration of Cd. Hammoudani et al (2021) found another heavy metal that is Hg in high level in sludge sample followed by Cd, Fe, Cr, and Ni and this heavy meal can be accumulated in sludge after treatment. Normally, sludge is used as fertilizers. Sharma et al (2016) showed trend of heavy metals in soil that uptake to vegetable from five

heavy metals which are Fe, Co, Cu, Cd, and Pb. Fe exhibited high uptake on plant. Therefore, risk assessment of heavy metals should be concerned.

4.4 Heavy metals on microplastics

4.4.1 Heavy metals on microplastics from wastewater

Due to the surface properties of microplastics that can carry pollutions and contaminations to aquatic system. After microplastics were counted, the residual filler that carried microplastics samples were extracted organic matter using microwave digestor. Various heavy metals include Ba, As, Co, Cd, Fe, Cr, Mn, Cu, Se, Zn, Ni, and Pb were determined by ICP-OES.

At each unit, heavy metals on microplastics were analyzed from each size fraction to explain effect of adsorbent size with adsorption efficiency. From WWTP A, Zn presented highest concentration followed by Cu (Fig. 4-10). From WWTP B, Cr presented highest concentration followed by Ba (Fig. 4-11).

Highest heavy metals concentration was detected at post aeration tank. This result was the same as heavy metals in wastewater. However, from plant A, heavy metals concentration decreased after sediment unit, and lowest detected after through RO. Likewise, lower heavy metals were detected at post sedimentation from plant B. Khalid et al. (2021) found that microplastic can be adsorption and desorption heavy metals depend upon pH of the external solution. Moreover, Fan et al. (2021) found that aging particles of microplastic also can be adsorption-desorption of heavy metals ion after UV ageing process. However, the removal of heavy metals has several technologies available such as chemical precipitation, ion exchange, adsorption, membrane filtration and coagulation-flocculation (Heiderscheidt et al., 2020). This might be one of possible to decreases heavy metals on microplastics.

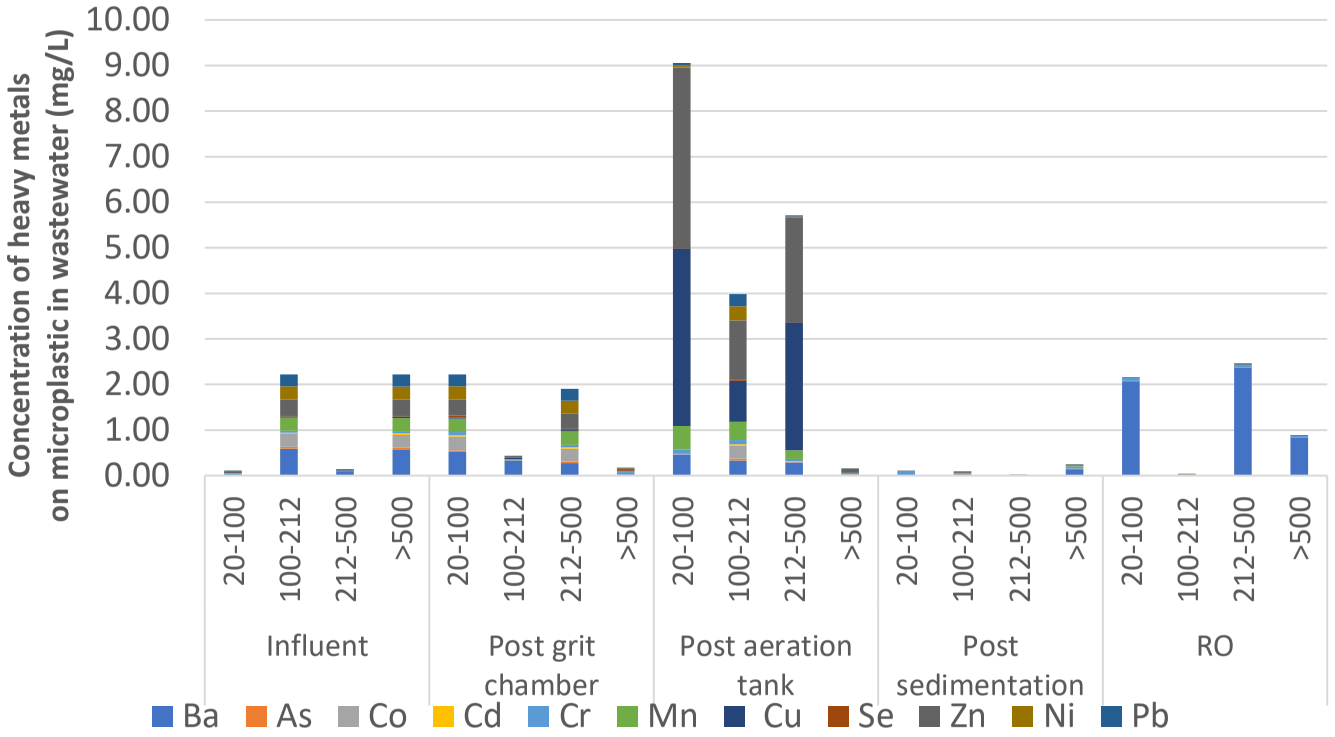


Figure 4-10 *Heavy metal concentration in wastewater from WWTP A divided into four fraction sizes.*

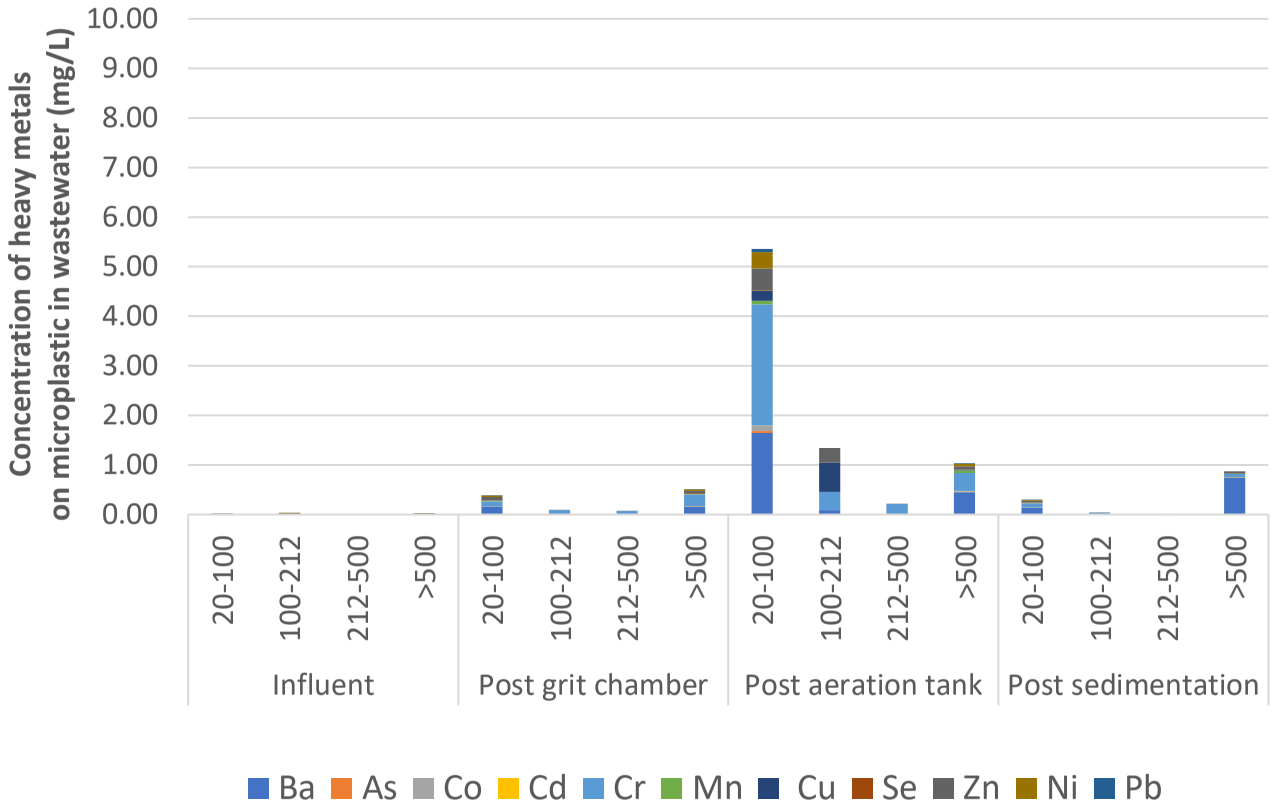


Figure 4-11 *Heavy metal concentration in wastewater from WWTP B divided into four fraction sizes.*

4.4.2 Heavy metals on microplastics from sludge

Sludge samples were also determined for heavy metals absorbed on microplastics. Samples were separated into 4 size fractions before extraction and analyzed by ICP-OES.

From Fig. 4-12, The higher heavy metals concentration in microplastics were present in 212-500 μm of fraction size. Zn, and Ba were also mostly detected in sludge from both sites. There was a slight difference of heavy metals concentration between both sites. Moreover, Pb from plant A presented higher concentration than other metals especially in 500 μm sizes, while Cr showed the highest concentration from plant B in 20 μm. The high concentration of Pb in this part related with high concentration of Pb in sludge. However, smaller size of microplastics was detected high concentration of heavy metals except microplastics with greater than 500 μm.

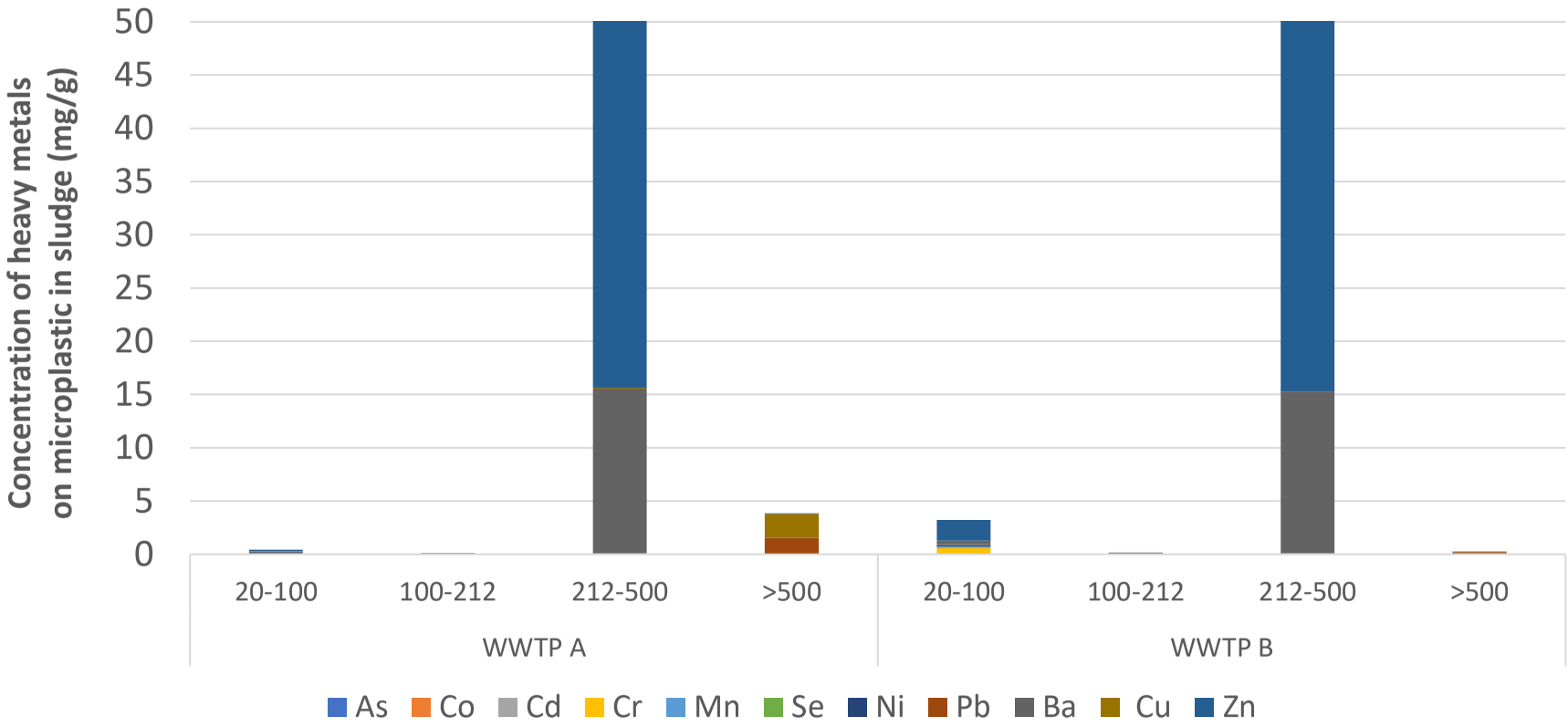


Figure 4-12 *Heavy metal concentration in sludge from both sites divided into four fraction sizes.*

The concentration of heavy metals on microplastics from wastewater showed higher detected than heavy metals in wastewater. From both study sites, the highest concentration of heavy metals was detected from post aeration unit and decreases in order. Zn, Mn and Co showed high concentration in wastewater, while Fe found high concentration on microplastics and not presented in wastewater. This might be due to the Fenton's reagent in digestion step which used iron (II) solution as a catalyst in the reaction to digest organic matter. In contrast, heavy metals in sludge presented higher concentration than on microplastics in sludge. Fe and Zn were the most found in sludge sample only, and also found on microplastics.

In addition, smaller size of microplastics was detected high concentration of heavy metals than bigger size. This might be due to microplastics properties i.e., surface, polar, aged. Study of Wang et al (2020) presented adsorption of heavy metal by microplastics effected by UV radiation. PET was used to sorption Cu^{2+} and Zn^{2+}. The result showed high performance between aging level of microplastics and sorption capacity of heavy metals. This was because the increased surface area and presented of oxygen containing function after UV radiation in aged microplastics. At the same time, those changed properties of microplastics can release harmful additives in microplastics to environment (Bandow et al., 2017).

4.5 Ecological risk assessment

4.5.1 Microplastics risk assessment

As no standardized method for microplastics risk assessment, the application of ecological risk from sediments was conducted. In this study, after identifying microplastics by ATR-FTIR, polymer risk index (H), pollution load index (PLI), potential ecological risk factor (E_i), and potential ecological risk (PER) were evaluated.

4.5.1.1 Polymer risk index (H)

The hazard score of plastics polymers was used as indicator to assess the risk following polymer risk index (H) which based on percentage of microplastics polymer for every treatment unit. At each unit, percentage of microplastics multiplied by score of polymers compound from Lithner et al (2011) that comprised of microplastic particles. This index is applied to evaluate the chemical toxicity of microplastics polymer to ecosystem harm.

The percentage of each polymer in wastewater is shown in Table 4-10. From plant A, every unit showed the average H index upper than 150 (except RO) that would be in risk category III due to moderate toxic characteristics of polymer in environment. Especially, the average H index from post aeration tank (HI = 1,040) showed highest level which is risk category IV as high toxic characteristics of polymer. However, tertiary treatment process showed higher removal efficiency of microplastics and also reduced toxic characteristic of polymer at the same time, therefore low toxic was shown after RO (HI = 0). Meanwhile, slightly fewer toxic characteristics of polymer was shown from plant B with the average H index mostly in risk category III at post aeration tank (HI = 292.3) and decreased to category II in effluent (HI = 20). From both study sites, the risk tendency of H index was in the same which increased in post aeration unit and decreased in effluent.

For sludge, this study using polymer categorized the average H index into two groups i.e., common and others (Table 4-11). Common polymer group includes the main types which are PE, PP, PS, and PET, while other polymer group consists of PVC, PL, PUR, PTFE, and PBD as high polymer toxic level. The assessment of the

pollution load index of microplastics in sludge units showed that both areas were moderate toxic in common group (HI = 186.67 and 195.45 for plant A and B, respectively) and high toxic in other group (HI = 35,648.33 and 45,456.82 for plant A and B, respectively). However, various microplastic polymer was accumulated in sludge that may reused in agriculture as fertilizer and can lead to soil contamination, therefore H index should be concerned in the overview. Total HI in plant A showed 35,835 while plant B showed 45,652.27 that can be categorized both in high toxic level. Detection of some particle of microplastics i.e., PVC, PL, PUR, PTFE, and PBD increased high risk. In this study, PVC showed the most harmful with HI = 17,585. Xu et al. (2018) also indicated that PVC exhibited a critical concern for microplastic risk in estuary. Some additives are mixed to maintain the properties of product (Canesi et al, 2015). However, the polymerization reaction is not always completed during the production process (Lithner et al., 2011), therefore, this additive may release from microplastic into environment. Moreover, number of detected microplastics did not calculate in this model, therefore more risk assessment models should be estimated.

Table 4-10 *Polymer risk index (H) of microplastics polymer in wastewater from both sites.*

Polymer	Polymer risk index (H)								
	WWTP A					WWTP B			
	Influent	Post grit chamber	Post aeration tank	Post sediment tank	RO	Influent	Post grit chamber	Post aeration tank	Post sediment tank
PE	366.67	0.00	0.00	550.00	0.00	122.22	0.00	253.85	0.00
PP	0.00	16.67	13.33	50.00	0.00	27.78	8.33	7.69	20.00
PS	0.00	0.00	400.00	0.00	0.00	0.00	0.00	0.00	0.00
PET	0.00	133.33	0.00	0.00	0.00	0.00	0.00	30.77	0.00
PA	0.00	0.00	626.67	0.00	0.00	0.00	0.00	0.00	0.00
Total	366.67	150.00	1,040.00	600.00	0.00	150.00	8.33	292.31	20.00
Risk category	III	III	IV	III	I	III	I	III	II

Note: I = low toxic, II = moderate toxic, III = considerable toxic, and IV = high toxic.

Table 4-11 *Polymer risk index (H) of common and other groups of microplastics polymer in sludge from both sites.*

Group	Polymer	WWTP A			WWTP B		
		HI	Risk category	Total	HI	Risk category	Total
Commons	PE	73.33	186.67 (III)	35,835 (IV)	25	195.45 (III)	45,652.27 (IV)
	PP	3.33			15.91		
	PS	50			136.36		
	PET	60			18.18		
Others	PVC	17,585	35,648.33 (IV)		0	45,456.82 (IV)	
	PL	2,356.67			0		
	PUR	12,306.7			0		
	PTFE	3,400			0		
	PBD	0			45,456.82		

Note: I = low toxic, II = moderate toxic, III = considerable toxic, and IV = high toxic.

4.5.1.2 Pollution load index (PLI)

The PLI was used for monitoring the degree of microplastic concentration from both study sites followed equations 2 and 3, and their result illustrates in table 4-12. Before PLI assessment, contamination factor (C_f) was analysed. In this study, lowest concentration of microplastics at each study sites were used as background (C_{0i}). The trend for contamination was similar to microplastics abundance that slightly increased at post grit chamber and decreased in order. The highest microplastic contamination was presented in sludge from both sites (C_f = 2,174.17 from WWTP A, and C_f = 35.77 from WWTP B), however, based on C_{0i}, the lowest concentration in plant A was 0.44 particles, while plant B showed 33.53 particles.

For PLI, influent from plant A presented in risk category II (15.15), while influent from plant B showed category I (2.10). However, the PLI was slightly increased at post grit chamber (15.99 (II) from plant A, and 2.12 (I) from plant B) and decreased in order of treatment process, and effluent presented in category I (PLI = 1.00 from both sites). Moreover, sludge from WWTP A presented the highest risk category (46.63(IV)). The detection of high concentration of microplastic particles led to high pollution load in environment. In addition, plant A indicated that microplastic concentration would be within the category IV with PLI = 97.82 (wastewater and sludge) as high risk to the aquatic biota, while plant B presented in PLI = 12.76 with category II. Note that the PLI of microplastics concentration in this study should be focused on two units, which is effluent and sludge unit. However, only effluent can be directly discharged to environment, while sludge can be illegally dumped, therefore risk assessment of both sources is required.

Table 4-12 Pollution load index (PLI) of microplastics concentration in sludge in both sites.

Units	Contamination factor (C_f)		Pollution load index (PLI)	
	WWTP A	WWTP B	WWTP A	WWTP B
Post influent	229.43	4.43	15.15 (II)	2.10 (I)
Post grit chamber	255.61	4.49	15.99 (II)	2.12 (I)
Post aeration	197.95	2.41	14.07 (II)	1.55 (I)
Post sediment	24.87	1.00	4.99 (I)	1.00 (I)
RO	1.00	-	1.00 (I)	-
Sludge	2,174.17	35.77	46.63 (IV)	5.98 (I)
PLI_{zone}			97.82 (IV)	12.76 (II)

Note: I = low toxic, II = moderate toxic, III = considerable toxic, and IV = high toxic.

4.5.1.3 Potential ecological risk (PER)

Potential ecological risk (PER) refers to total concentrations of microplastics and response of the environment. This model developed based on Hakanson (1980). In this study, PER values were relatively within the extreme risk category in both study sites, especially, microplastics polymer from sludge. From WWTP A (Table 4-13), common microplastic polymers (PE, PP, PS, and PET) were detected. The E_r from influent and post grit chamber were 25.00 and 20.45, respectively, and increase to 140.91 in post aeration tank due to considerable toxic E_r level, while others microplastic polymers (PVC, PL, PUR, PA, PTFE, and PBD) was not detected in this plant, except PA in post aeration tank, which E_r = 213.64 (high toxic). However, the E_r of all treatment units was decrease in effluent. In the same way, the risk level from common microplastic polymer presented high level in WWTP B (Table 4-14). The E_r from influent and post grit chamber were 0.81 and 0.03, respectively, and increase to 1.13 in post aeration tank due to low toxic E_r level, while others microplastic polymers was not detected in this plant, however, the E_r of all treatment units was decrease in effluent. Presented of PBD in sludge, which 20,001 (very high toxic). PER of both plants presented 48,893.19 and 20,087.03, respectively. In addition, presents of other microplastic polymers (PVC, PL, PUR, PA, and PTFE) were grouped under the extreme danger risk categories. Almost other microplastic polymers were presented in sludge sample with high density of microplastics. Moreover, microplastics can adsorb various pollutions include heavy metals, therefore, the complex toxicity of microplastics and heavy metals remains to investigation.

Table 4-13 *Potential ecological risk (PER) of microplastics in WWTP A.*

Polymer	WWTP A					
	Influent	Post grit chamber	Post aeration tank	Effluent	RO	Sludge
PE	25.00	0.00	0.00	25.00	0.00	100.00
PP	0.00	2.27	4.55	2.27	0.00	4.55
PS	0.00	0.00	136.36	0.00	0.00	68.18
PET	0.00	18.18	0.00	0.00	0.00	81.82
Er	25.00	20.45	140.91	27.27	0.00	254.55
PVC	0.00	0.00	0.00	0.00	0.00	23,979.55
PL	0.00	0.00	0.00	0.00	0.00	3,213.64
PUR	0.00	0.00	0.00	0.00	0.00	16,781.82
PA	0.00	0.00	213.64	0.00	0.00	0.00
PTFE	0.00	0.00	0.00	0.00	0.00	4,636.36
PBD	0.00	0.00	0.00	0.00	0.00	0.00

Er	0.00	0.00	213.64	0.00	0.00	48,611.37
∑Er	25.00	20.45	354.55	27.27	0.00	48,865.92
PER = 48,893.19						

Note: For Er, <40 = Minor, 40-80 = Medium, 80-160 = High, 160-320 = Danger, ≥ 320 = Extreme danger. For PER, < 150 = Minor, 150-300 = Medium, 300-600 = High, 600-1,200 = danger, ≥ 1,200 = Extreme danger.

Table 4- 14 *Potential ecological risk (PER) of microplastics in WWTP B.*

Polymer	WWTP B				
	Influent	Post grit chamber	Post aeration tank	Effluent	Sludge
PE	0.66	0.00	0.98	0.00	11.00
PP	0.15	0.03	0.03	0.03	7.00
PS	0.00	0.00	0.00	0.00	60.00
PET	0.00	0.00	0.12	0.00	8.00
Er	0.81	0.03	1.13	0.03	86.00
PVC	0.00	0.00	0.00	0.00	0.00
PL	0.00	0.00	0.00	0.00	0.00
PUR	0.00	0.00	0.00	0.00	0.00
PA	0.00	0.00	0.00	0.00	0.00
PTFE	0.00	0.00	0.00	0.00	0.00
PBD	0.00	0.00	0.00	0.00	20,001.00

Er	0.00	0.00	0.00	0.00	20,001.00
∑Er	0.81	0.03	1.13	0.03	20,087.00
PER = 20,087.03					

Note: For Er, <40 = Minor, 40-80 = Medium, 80-160 = High, 160-320 = Danger, ≥ 320 = Extreme danger. For PER, < 150 = Minor, 150-300 = Medium, 300-600 = High, 600-1,200 = danger, ≥ 1,200 = Extreme danger.

For effluent, H index value in WWTP A was higher than WWTP B (H = 600 (III) and 292 (III), respectively), while WWTP B showed higher value of H index (45,652 (IV)) than WWTP A (35,648 (IV)) in sludge. PLI value from WWTP A (97.82 (IV)) was also higher than that of WWTP B (12.76 (II)). However, it is difficult to decide toxicity area because one was considered about component of polymer in microplastics particles and other one discussed on number of detected microplastics particles, therefore the development of PER was used to assess. Considering the overall composition, even plant B presented in low danger value, the result of WWTP A was more than remarkable (PER of WWTP A = 97.82 > PER of WWTP B = 12.67).

Based on properties of microplastics, many researchers applied the ecological risks to estimate on occurrence. PER was considered risk category III from mixed surface and treated wastewater from Riyadh, Saudi Arabia (Pico et al, 2021). In addition, H index would be in category III also as PLI value. Different study sites, Xu et al. (2018) studied about microplastics in surface water at Changjiang Estuary, China, based on the PLI and H index. The PLI of East China Sea and Changjiang Estuary showed 20.4 and 18.4, which was according to WWTP B in this study. PVC also considered to be the most harmful polymer in this study. In mangrove, the PLI value fell within categories II and III, but did not have the most hazardous polymer. H index indicated lower than 10. The RI value was relatively higher within extreme danger risk category (Li et al., 2020). In addition, some plastic polymers are biologically inert and have less of an impact on the aquatic environment (Matlack, 2001). This ecological risk of microplastics became serious issue and the potential harm to human health, therefore, the health risk assessment should be concerned.

4.5.2 Heavy metals risk assessment

The concentration of heavy metals was analyzed at each treatment unit, then risk assessment was estimated. The Geo-accumulation Index (I_{geo}) is used to compare the recent heavy metal concentration with pre-industry background, while potential ecological risk (PER) is used to evaluated degree of heavy metals pollution. In this

study, only heavy metals in sludge were calculated. Due to the limitations of background data, the analysis of some heavy metals type may need to be reduced.

4.5.2.1 The Geo-accumulation Index (I_{geo})

The Geo-accumulation Index (I_{geo}) value as shown in Table 4-15 were used to assess the pollution level of heavy metals without toxic impact in sludge compared with crustal value. Before I_{geo} assessment, contamination factor (C_f) was analyzed. In this study, average shale values were compared with the recent heavy metal concentration for quantitative measure of heavy metal pollution. From both study sites, all heavy metals presented in low degree level. Cd from WWTP A presented as low degree (0.7) and Se from WWTP A presented as moderate degree (1.2). This result showed that heavy metals were contaminated by an anthropogenic.

According to Muller (1969), the I_{geo} is used to evaluate heavy metals contamination in soil by comparing average crustal. Mostly I_{geo} was practically uncontaminated, except for uncontaminated to moderately with Cd (0.65, class 1) and moderately contaminated with Se (1.19, class 2) from WWTP A. Fe presented heavily to extremely contaminated from both sites. From WWTP A, I_{geo} of Fe presented heavy to extreme contamination (4.98, class 5), while heavily contamination in WWTP B with 3.43 (class 4).

The high value of Fe in terms of C_f and I_{geo} assessment was presented in both plants. Fe is general occurred in high concentration in water and sediment. However, Cd and Se only presented in WWTP A assess by I_{geo} model. Cadmium is strong attached to soil surface and low detected in wastewater due to less dissolution ability, while Se is used as a preliminary approach to assess soil contamination (Roca-Perez et al., 2010). The results confirmed the influence of anthropogenic activity in both WWTPs. In contract, Zn found the highly contamination in sewage sludge, while Pb showed the lowest (Tytla 2019). Heavy to extreme contamination has been observed with Cu and Cr in suspended and bed sediment in river (Patel et al., 2018). Varol et al. (2020) showed that the I_{geo} value of Ni was positive as moderately polluted in soil. However, I_{geo} focused on the accumulation level of heavy metal without toxicity impact, therefore the model to consider about total quantity and toxicity of heavy metal should be continued.

Table 4- 15 *Contamination factor (C_f) and geo-accumulation Index (I_{geo}) of heavy metals concentration in sludge in both sites.*

Heavy metals	Contamination factor (C_f)		**Geo-accumulation Index (I_{geo})**	
	WWTP A	WWTP B	WWTP A	WWTP B
Ba	0.016 (L.D.)	0.012 (L.D.)	-6.143 (0)	-6.559 (0)
As	0.010 (L.D.)	0.006 (L.D.)	-4.416 (0)	-5.032 (0)
Co	0.034 (L.D.)	0.024 (L.D.)	-5.841 (0)	-6.333 (0)
Cd	1.570 (M.D)	0.167 (L.D.)	0.651 (1)	-2.579 (0)
Fe	0.006 (L.D.)	0.002 (L.D.)	4.983 (5)	3.435 (4)
Cr	0.274 (L.D.)	0.011 (L.D.)	-2.607 (0)	-7.186 (0)
Mn	0.010 (L.D.)	0.003 (L.D.)	-7.413 (0)	-9.122 (0)
Cu	0.228 (L.D.)	0.420 (L.D.)	-3.007 (0)	-2.128 (0)
Se	0.285 (L.D.)	0.104 (L.D.)	1.187 (2)	-0.268 (0)
Zn	0.993 (L.D.)	0.628 (L.D.)	-0.154 (0)	-0.816 (0)
Ni	0.109 (L.D.)	0.015 (L.D.)	-3.926 (0)	-6.834 (0)
Pb	0.049 (L.D.)	0.035 (L.D.)	-4.244 (0)	-4.739 (0)

Note: For CF, L.D. = low degree, M.D. = moderate degree, C.D. = considerable degree, and V.D. = very high degree. For Igeo, class 0 = practically uncontaminated, class 1 = uncontaminated to moderately contaminated, class 2 = moderately contaminated, class 3 = moderately to heavily contaminated, class 4 = heavily contaminated, class 5 = heavily to extremely contaminated, and class 6 = extremely contaminate

4.5.2.2 Potential ecological risk (PER)

Analysis of the potential ecological risk (PER) of heavy metal concentration in sludge has been developed from Hokinson (1980). Due to the limitations of background data, this study was estimated only five heavy metals (As, Cd, Pb, Zn, and Cu) in sediment from both study sites. The result illustrates in Table 4-16. To calculate PER, contamination factor (C_f) has to analyze by compared with background pre-industry value (C_n). Pb exhibited high value of contamination factor in both study sites (54.13 in WWTP A, and 94.23in WWTP B), followed by Cr which was 5.93 in plant A and 2.03 in plant B. However, both Pb and Cr also found in higher concentration in bed sediment (Patel et al., 2018). These two study areas are located in industrial estates, therefore it is possible to find a high number of heavy metals.

PER provides the cumulative information of heavy metals in different site. From Table 4-16, PER from plants A and B showed extreme value of heavy metals (PER = 70.71 and 96.61, respectively). One reason is that Cr and Cu which have high value of pollution and they are often used in industrial factories. Patel et al (2018) also found very high degree contamination of Cr, Cu, Pb, and Zn in sediment. Hg and Cd posed a considerable to very high environmental risk in sewage sludge from municipal wastewater treatment plant (Tytle 2019). Liu et al (2021) showed the ecological risk levels of all heavy metals were much higher in the upstream.

Table 4-16 *Potential ecological risk (PER) of heavy metals concentration in sludge in both sites.*

Polymer	Cn	Ti	Contamination factor (Cf)		E^i_r	
			WWTP A	WWTP B	WWTP A	WWTP B
As	15	10	0.01	0.00	0.06	0.03
Cd	1	30	0.43	0.01	12.90	0.27
Cr	90	2	2.96	1.01	5.93	2.03
Pb	70	5	0.15	0.27	51.13	94.23
Zn	175	1	0.00	0.00	0.00	0.00
Cu	50	5	0.14	0.01	0.70	0.06
		PER			70.71	96.61

Note: For Er, <40 = Minor, 40-80 = Medium, 80-160 = High, 160-320 = Danger, ≥ 320 = Extreme danger. For PER, < 150 = Minor, 150-300 = Medium, 300-600 = High, 600-1,200 = danger, ≥ 1,200 = Extreme danger.

4.5.3 The risk assessment of heavy metals on microplastics.

Heavy metals that attached on microplastics' surface is one source of risk in water. In this study, PER was used to applying as diagnostic tool to evaluate microplastics and heavy metal characteristics together in sludge. Both study sites exhibited extreme danger of heavy metals on microplastics in sludge samples. The result shows in Table 4-17 that summation of potential ecological factor (E_r) in polymer from plant A was 48,865.92, while 20,087 was in plant B. Meanwhile, E_r of heavy metal presented 70.71 and 96.61 from both plants, respectively. WWTP A showed higher PER than WWTP B, that was 48,936.63 and 96.61, respectively. It was worth noting that both WWTP carries high toxicity of polymer (e.g., PBD, PVC, PL, PUR, and PDFE) and hazardous heavy metal (Cr), which inevitably enhance their toxicity. The number and type of industrial plants can be a major cause of increasing ecological risks, especially in industrial estates that consist of plastic and electronics and automotive factories. There are approximant 200 factories in WWTP A while 146 factories in WWTP B. This may be a reason for high value of PER from WWTP A.

Nowadays, microplastics become a hot issue, however there are no standardized models to assess the ecological risk assessment of microplastics. Moreover, microplastics can be adsorbed a variety of pollutant. This is difficult and challenging to develop and assess risk of heavy metal and other substances, which deserves further attention.

Table 4-17 *The risk assessment of heavy metals on microplastics by develop potential ecological risk (PER) in sludge in both sites.*

WWTP A				**WWTP B**			
Polymer	E_r	Heavy metal	E_r	Polymer	E_r	Heavy metal	E_r
PE	100.00	As	0.06	PE	11.00	As	0.03
PP	4.55	Cd	12.90	PP	7.00	Cd	0.27
PS	68.18	Cr	5.93	PS	60.00	Cr	2.03
PET	81.82	Pb	51.13	PET	8.00	Pb	94.23
PVC	23,979.55	Zn	0.00	PVC	0	Zn	0.00
PL	3,213.64	Cu	0.70	PL	0	Cu	0.06
PUR	16,781.82			PUR	0		
PA	0			PA	0		
PTFE	4,636.36			PTFE	0		
PBD	0.00			PBD	20,001		
$\sum E_r$ = 48,865.92		$\sum E_r$ = 70.71		$\sum E_r$ = 20,087.00		$\sum E_r$ = 96.61	

PER = 48,936.63	**PER = 20,183.61**

Note: For Er, <40 = Minor, 40-80 = Medium, 80-160 = High, 160-320 = Danger, ≥ 320 = Extreme danger. For PER, < 150 = Minor, 150-300 = Medium, 300-600 = High, 600-1,200 = danger, ≥ 1,200 = Extreme danger.

CHAPTER V
CONCLUSION AND RECOMENDATION

5.1 Conclusion

The abundance and characteristics of microplastics from two central industrial wastewater treatment plants (WWTPs) were conducted. The abundances of influent, effluent, and sludge from WWTP A were present 101.87 ± 0.47 particles/L, 11.04 ± 0.08 particles/L, and 2,398.00 ± 11.37 particles/kg, respectively, while WWTP B present 148.44 ± 0.91 particles/L, 33.53 ± 0.55 particles/L, and 1,930.00 ± 7.57 particles/kg. Moreover, tertiary treatment process from WWTP A showed highest performance which found 0.44 ± 0.04 particles/L of microplastic particles after RO process. The study also recorded a removal efficiency of 89.16% for WWTP A and 77.41% for WWTP B within the secondary process and the highest in the tertiary process (99.57%). From both sites, trends in the microplastic distribution of various sizes were similar. At the influent, post grit chamber, and post aeration tank, the number of small particles was decreased while larger particles were increased in order. The most detected size of microplastics found in WWTP A was 212-500 µm, and 100-212 µm, while 20-100 µm, and 212-500 µm from WWTP B, in wastewater and sludge, respectively. The observed microplastics from both sites were mostly white/clear. The shape and polymer of microplastics derived from FTIR found that pellets presented as the main shape in wastewater and sludge. PP, PE, and PET were detected in both sites. However, there were a large number of microplastics released into the environment with the effluent during the treatment process. We estimated that around 624 million and 226 million microplastics per day were discharged from effluent and 2 million and 1.9 million per ton from WWTP A and WWTP B, respectively, were released from sludge to environment.

The analysis of total heavy metals concentrations from both WWTPs is an important topic. Heavy metals attach on the surface of microplastics and enter the organism. In this study, various heavy metals included Ba, As, Co, Cd, Fe, Cr, Mn, Cu, Se, Zn, Ni, and Pb concentration were determined by ICP-OES. Zn, Mn, and Cu were the most detected in wastewater from both sites, especially highest in post

aeration tank. Also, Zn found high concentration in sludge from both sites. Due to the surface properties of microplastics that can be carried pollutions and contaminants to aquatic system. Heavy metals on microplastics were paid attention from both wastewater and sludge. Zn showed high concentration of heavy metal on microplastics from both sites. However, smaller size of microplastics was detected high concentration of heavy metals over than bigger size with microplastics properties i.e., surface area and aged.

The evaluation of the ecological risk is possible to identify the pollution status of the WWTPs and estimate the microplastics in the environment. Overall, the results provided extreme damage on microplastics and heavy metals in WWTPs. Total H index in effluent from plant A showed considerable toxic (600), while effluent from plant B showed moderate toxic (20). In addition, the H index from both plant showed high toxic which 35,835 and 45,652, respectively. PLI showed 97.82 (category IV) and 12.76 (category II) from WWTP A and WWTP B, respectively. All heavy metals presented in low degree level, except Cd and Se from WWTP A presented 0.7 as low toxic and 1.2 as moderate toxic, respectively, by I_{geo}. PER values of microplastics, heavy metals, and heavy metals on microplastics, which higher than 48,893 from WWTP A and 20,087 from WWTP B for microplastic, 70.71 as low toxic from plant A and 96.61 as moderate toxic from plant B for heavy metals, and extreme danger for heavy metals on microplastics which 48,936 and 20,183 from both plants, respectively. Moreover, microplastics can be adsorbed a variety of pollutant. This is difficult and challenging to develop and assess risk of heavy metal and other substances, which deserves further attention.

5.2 Recommendation

1. The method of microplastics detection has variation and is developing for accurate detected, therefore µ-FTIR is suggested identifying polymer type for high performance. However, the different method or equipment can be led to different results, therefore the standard methods for analyses have been discovered.

2. Long-period sampling was suggested to inform trend of microplastics distribution from WWTPs within different operating system condition. A year trend in microplastic particles will inform us how the dynamic distribution is evolving.

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APPENDIX A

Table A1 The size distribution and abundance of microplastics in wastewater from WWTP A.

	Size	Influent	Post grit chamber	Post aeration tank	Effluent	RO	Total
NaCl	>500	13.07	18.27	26.80	2.00	0.07	60.13
	212-500	10.93	9.33	33.60	1.93	0.00	55.80
	100-212	19.00	10.13	5.13	0.87	0.13	35.13
	20-100	42.93	24.60	5.40	0.80	0.13	73.73
	Total	85.93	62.33	70.93	5.60		
Nal	>500	4.60	4.27	4.67	1.20	0.13	14.73
	212-500	2.47	41.60	7.93	1.60	0.00	53.60
	100-212	2.93	2.20	1.80	0.47	0.00	7.40
	20-100	6.13	3.20	3.27	2.13	0.00	14.73
	Total	16.13	51.27	17.67	5.40		
Total	>500	17.67	22.53	31.47	3.20	0.20	74.87
	212-500	13.40	50.93	41.53	3.53	0.00	109.40
	100-212	21.93	12.33	6.93	1.33	0.13	42.53
	20-100	49.07	27.80	8.67	2.93	0.13	88.47
	Total	102.07	113.60	88.60	11.00	0.47	

Table A2 Colors of microplastics in wastewater from WWTP A.

Units	Colors	NaCl				NaI				Total			
		>500	212-500	100-212	20-100	>500	212-500	100-212	20-100	>500	212-500	100-212	20-100
Influent	green	0.00	2.00	2.60	3.00	0.00	0.27	0.00	0.00	0.00	1.33	4.07	3.47
	purple	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
	black	0.00	0.60	1.67	4.47	0.07	1.73	0.00	0.00	3.13	1.87	2.13	5.40
	red	0.00	0.73	0.47	2.73	0.07	2.27	0.00	0.00	2.07	2.67	0.47	4.13
	yellow	0.00	1.00	2.13	5.60	0.80	0.13	0.00	0.00	4.13	0.87	1.87	6.93
	pink	0.00	1.60	5.53	7.80	0.00	0.60	0.00	0.00	0.00	1.67	6.20	10.20
	white	0.00	4.53	5.87	13.60	2.07	1.47	0.00	0.00	5.40	4.73	6.80	18.93
	blue	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
	other	0.00	0.13	0.40	0.00	0.87	0.00	0.00	0.00	2.87	0.13	0.40	0.00
Post grit chamber	green	0.40	0.33	0.67	0.40	0.00	0.00	0.40	0.00	0.40	1.20	0.67	0.40
	purple	0.07	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.07	0.00	0.07	0.00
	black	2.20	1.87	0.67	5.20	0.27	0.00	0.73	0.47	2.47	8.27	0.87	5.67
	red	0.33	2.20	0.33	0.07	0.27	0.00	0.60	0.20	0.60	10.87	0.73	0.27
	yellow	1.47	1.33	0.53	1.00	1.40	0.00	1.53	0.80	2.87	1.67	1.60	1.80
	pink	12.00	0.27	1.40	2.93	0.13	0.00	0.47	0.00	12.20	2.40	0.80	2.93
	white	0.13	4.80	4.73	8.93	1.87	0.00	5.13	0.93	2.00	23.53	5.47	9.33
	blue	0.13	0.07	0.07	6.07	0.00	0.00	0.07	0.07	0.13	1.40	0.13	6.13
	other	1.47	0.27	1.80	0.53	0.33	0.00	0.33	0.73	1.80	1.60	2.00	1.27
Post aeration tank	green	0.13	0.07	0.20	0.00	0.13	0.07	0.00	0.00	0.27	0.13	0.20	0.00
	purple	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
	black	6.13	0.20	0.93	0.07	1.40	0.20	0.00	0.00	4.20	8.40	0.47	0.47
	red	10.73	0.00	0.13	0.00	0.27	0.00	0.00	0.00	7.67	0.00	0.13	0.00
	yellow	0.73	0.93	2.27	0.13	0.87	0.73	0.00	0.00	1.60	11.67	1.60	1.07

	pink	0.20	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.33	0.00
	white	15.07	6.07	0.00	0.20	1.67	6.27	0.00	0.00	13.93	21.33	0.00	4.87
	blue	0.07	0.00	6.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	4.00	0.00
	other	3.13	0.00	0.13	0.80	0.40	0.00	0.00	0.00	3.53	0.00	0.13	2.27
Post sediment	green	0.07	0.07	0.00	0.00	0.00	0.07	0.00	0.00	0.07	0.13	0.00	0.00
	purple	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	black	0.13	0.33	0.60	0.60	0.00	0.07	0.20	0.00	0.87	0.80	0.33	0.53
	red	0.13	0.00	0.13	0.27	0.00	0.00	0.13	0.00	0.33	0.00	0.07	0.27
	yellow	0.07	0.20	0.80	0.87	0.00	0.00	0.00	0.00	0.20	0.47	0.20	0.80
	pink	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.07	0.00
	white	0.40	1.00	0.47	0.87	0.00	0.73	1.27	0.00	1.73	1.93	0.60	1.00
	blue	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00
	other	0.00	0.13	0.20	0.33	0.00	0.00	0.13	0.00	0.00	0.13	0.07	0.33
RO	green	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	purple	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	black	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	red	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	yellow	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	pink	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	white	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.13	0.13
	blue	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	other	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table A3 Shape of microplastics in wastewater from WWTP A.

Units	fiber	pellet	fragment	film
Influent	27.27	47.13	11.87	15.80
	27.20	47.07	11.93	15.73
	27.33	47.20	11.80	15.87
Post grit chamber	21.00	72.67	0.00	19.93
	20.93	72.60	0.00	20.00
	21.07	72.73	0.00	19.87
Post aeration	28.33	55.40	0.07	4.80
	28.27	55.47	0.13	4.73
	28.40	55.33	0.00	4.87
Post sediment	5.73	4.07	0.07	1.13
	5.80	4.13	0.00	1.20
	5.67	4.00	0.13	1.07
RO	0.33	0.13	0.00	0.00
	0.27	0.07	0.00	0.00
	0.40	0.20	0.00	0.00

Table A4 The size distribution and abundance of microplastics in wastewater from WWTP B.

	Size	Influent	Post grit chamber	Post aeration tank	Effluent	Total
NaCl	>500	6.533	11.933	21.933	5.933	46.333
	500 - 212	6.267	43.200	30.000	4.800	84.267
	212 - 100	21.533	30.333	7.933	5.933	65.733
	100 - 20	70.867	15.333	4.733	10.133	101.067
	Total	105.200	100.800	64.600	26.800	
Nal	>500	9.00	1.67	1.87	1.20	13.73
	500 - 212	2.53	5.40	1.40	1.60	10.93
	212 - 100	24.93	23.80	10.20	1.67	60.60
	100 - 20	7.20	17.20	3.27	2.13	29.80
	Total	43.67	48.07	16.73	6.60	
Total	>500	15.53	13.60	23.80	7.13	60.07
	500 - 212	8.80	48.60	31.40	6.40	95.20
	212 - 100	46.47	54.13	18.13	7.60	126.33
	100 - 20	78.07	32.53	8.00	12.27	130.87
	Total	148.87	148.87	81.33	33.40	

Table A5 Colors of microplastics in wastewater from WWTP B.

Units		NaCl				NaI				Total			
		>500	212-500	100-212	20-100	>500	212-500	100-212	20-100	>500	212-500	100-212	20-100
Influent	green	0.87	1.13	1.33	2.73	0.00	0.53	0.00	0.27	1.53	1.33	3.33	3.00
	purple	0.40	0.67	13.20	30.00	0.67	0.00	0.00	0.00	1.73	0.67	23.87	30.67
	black	0.27	0.67	0.00	15.80	0.20	0.27	0.00	1.73	1.13	0.93	0.67	18.20
	red	0.20	0.00	0.13	4.00	0.73	0.27	0.00	1.60	1.60	0.27	0.80	5.60
	yellow	0.67	0.33	0.33	1.13	0.07	0.47	0.00	0.13	1.40	0.80	1.00	1.27
	pink	0.67	1.67	4.87	6.80	0.00	0.00	0.00	0.60	1.33	1.40	8.87	7.07
	white	2.73	1.60	1.53	6.87	0.20	2.67	0.00	1.47	4.47	3.40	6.33	9.00
	blue	0.13	0.00	0.20	0.00	0.00	0.00	0.00	0.47	0.80	0.00	0.87	0.47
	other	0.87	0.00	0.07	2.67	0.00	0.00	0.00	0.13	1.53	0.00	0.73	2.80
Post grit chamber	green	0.47	0.00	1.80	0.87	0.33	0.67	7.33	12.07	0.80	4.47	9.13	11.87
	purple	0.00	0.00	5.00	2.40	0.00	2.40	8.67	0.00	0.00	17.87	13.20	2.40
	black	1.40	0.00	6.27	3.33	0.60	1.13	7.80	1.67	1.73	6.93	13.40	5.20
	red	0.13	0.00	0.93	0.40	0.07	0.00	0.47	0.20	0.20	1.20	1.40	0.60
	yellow	0.13	0.00	0.87	1.53	0.07	0.07	0.20	0.93	0.20	2.13	1.07	2.47
	pink	3.40	0.00	3.07	0.60	0.07	0.07	0.00	0.00	3.47	3.73	3.07	0.60
	white	5.13	0.00	10.93	4.33	0.47	0.80	0.67	0.20	5.60	9.80	11.13	4.67
	blue	0.00	0.00	0.00	0.00	0.13	0.20	0.00	2.67	0.13	0.73	0.00	2.67
	other	1.47	0.00	1.73	1.80	0.00	0.07	0.00	0.27	1.47	1.73	1.73	2.07
Post aeration tank	green	3.73	1.33	0.13	0.00	2.07	0.00	0.67	0.33	3.47	1.53	1.47	0.33
	purple	0.13	1.00	0.60	0.07	4.07	0.00	0.00	0.00	2.00	1.00	0.93	0.07
	black	1.93	4.87	1.20	1.27	2.20	0.00	1.00	0.00	1.93	5.53	3.53	1.67
	red	0.27	0.53	0.07	0.00	0.13	0.00	0.00	0.00	0.40	0.53	0.20	0.00
	yellow	0.27	3.07	0.53	0.27	0.07	0.00	1.07	0.00	0.33	3.07	2.27	0.27

	pink	1.13	3.67	0.60	0.07	0.00	0.00	0.00	0.00	1.20	4.33	0.87	0.07
	white	13.00	12.27	3.73	2.33	0.53	0.00	1.73	0.00	7.60	12.27	6.87	4.80
	blue	1.13	0.00	0.00	0.00	0.00	0.00	0.07	0.00	1.13	0.20	0.07	0.07
	other	9.40	2.93	1.00	0.73	0.00	0.00	0.27	0.00	5.73	2.93	1.93	0.73
Post sediment	green	0.00	0.00	0.47	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.67
	purple	2.20	0.33	0.00	2.27	0.00	0.00	0.00	0.00	2.20	0.33	0.00	2.27
	black	0.00	0.73	1.60	1.40	0.40	0.00	0.00	0.93	0.40	1.40	1.67	2.40
	red	0.33	0.27	0.13	0.87	0.27	0.00	0.00	0.33	0.60	0.27	0.20	1.20
	yellow	0.13	0.07	0.13	0.33	0.13	0.00	0.00	0.00	0.27	0.07	0.33	0.33
	pink	0.07	0.07	0.27	0.07	0.00	0.00	0.00	0.00	0.07	0.07	0.47	0.07
	white	2.80	2.87	3.00	3.60	0.33	0.00	0.00	0.80	3.13	3.53	3.33	4.40
	blue	0.07	0.40	0.00	0.07	0.07	0.00	0.00	0.00	0.13	0.60	0.13	0.07
	other	0.33	0.13	0.53	0.87	0.00	0.00	0.00	0.00	0.33	0.13	0.73	0.87

Table A6 Shape of microplastics in wastewater from WWTP B.

	Influent			Grit			Aeration			Sediment		
fiber	15.53	15.60	15.47	15.67	15.60	15.73	21.53	21.60	15.47	7.93	8.00	7.87
pellet	63.93	63.87	63.80	65.73	65.80	65.67	21.07	21.00	21.13	9.87	9.93	9.80
fragment	18.20	18.27	18.33	16.47	16.40	16.53	27.73	27.67	27.80	8.60	8.53	8.67
film	22.80	22.87	22.73	11.47	11.40	11.53	16.53	16.47	16.60	4.80	4.73	4.87

Table A7 The size distribution and abundance of microplastics in sludge from both study sites.

Size	WWTP A			avr	WWTP B			avr
>500	368.00	368.00	370.00	368.67	312.00	314.00	316.00	314.00
500 - 212	130.00	130.00	132.00	130.67	1566.00	1556.00	1544.00	1555.33
212 - 100	1266.00	1254.00	1258.00	1259.33	120.00	122.00	120.00	120.67
100 - 20	172.00	170.00	174.00	172.00	410.00	410.00	406.00	408.67
Total	1936.00	1922.00	1934.00	1930.67	2408.00	2402.00	2386.00	2398.67

Table A8 The colors of microplastics in sludge from both study sites.

Site	Colors	NaCl				NaI				total
		>500	212-500	100-212	20-100	>500	212-500	100-212	20-100	
WWTP A	green	0	0	5	2	1	0	1	0	9
	purple	2	0	0	0	0	0	0	0	2
	black	46	11	110	30	6	5	24	2	234
	red	0	1	9	0	0	1	1	0	12
	yellow	0	1	24	1	6	3	8	0	43
	pink	5	0	0	1	0	0	0	0	6
	white	110	34	411	37	7	8	13	10	630
	blue	0	0	6	0	0	1	0	0	7
	other	1	0	20	3	0	0	1	0	25
WWTP B	green	6	25	3	31	0	5	2	18	90
	purple	0	3	0	0	0	0	0	0	3
	black	28	220	9	44	21	5	12	66	405
	red	13	36	4	3	0	1	1	0	58
	yellow	10	120	5	41	18	9	4	0	207
	pink	0	24	0	18	0	1	0	6	49

	white	84	209	23	21	102	13	26	56	534
	blue	1	0	1	1	0	1	0	2	6
	other	1	106	0	23	2	5	0	33	170

Table A9 Shape of microplastics in sludge from both sites.

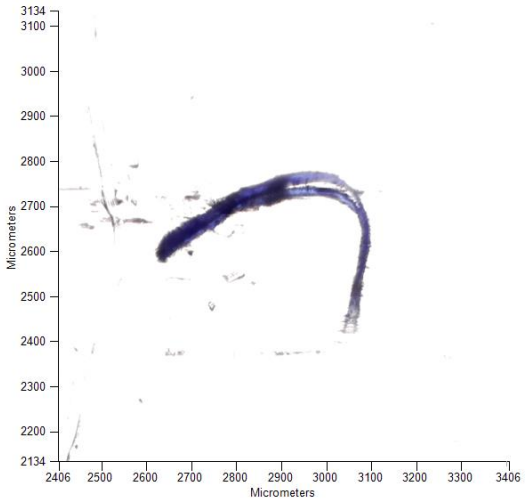
	Size	WWTP A				WWTP B			
		fiber	pellet	fragment	film	fiber	pellet	fragment	film
NaCl	>500	42	69	0	54	50	83	0	9
	500 - 212	7	29	0	11	60	587	4	92
	212 - 100	181	341	0	62	18	27	0	0
	100 - 20	6	65	0	4	27	151	0	3
NaI	>500	3	16	0	1	1	12	0	1
	500 - 212	7	9	0	3	9	27	1	3
	212 - 100	6	39	0	3	2	13	0	1
	100 - 20	2	10	0	0	3	17	1	2
Total		254	578	0	138	170	917	6	111

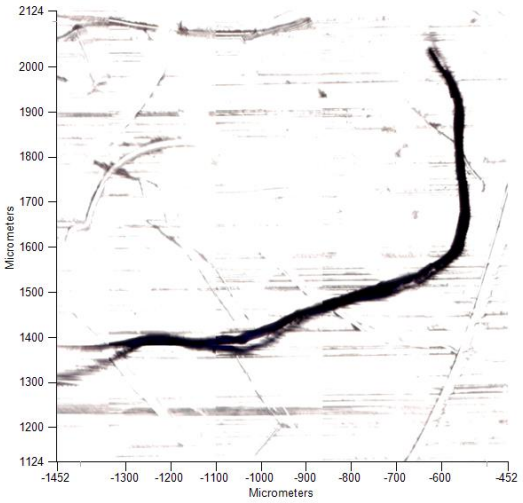
Table A10 Polymer of microplastics from both sites.

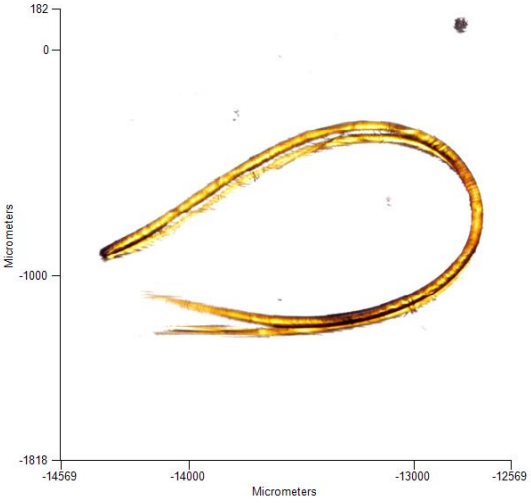
Polymer	Wastewater		Sludge	
	WWTP A	WWTP B	WWTP A	WWTP B
PE	2	5	4	1
PVC	0	0	1	0
PP	4	8	2	7
PS	2	0	1	2
PC	0	0	0	0
PET	2	1	9	2
PL	0	0	1	0
PUR	0	0	1	0
PA	2	0	0	0
PTFE	0	0	1	0
PCL	0	0	1	0
PBD	0	0	0	12
PDMS	0	1	0	0
Rayon	1	0	13	12
Nylon	1	1	0	0
cellulose	2	7	12	8
other	10	26	14	11

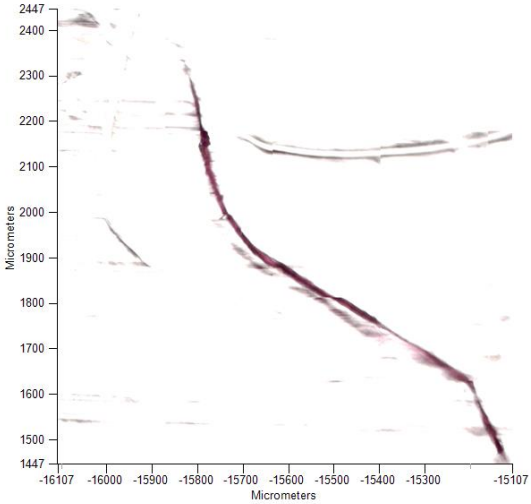
APPENDIX B

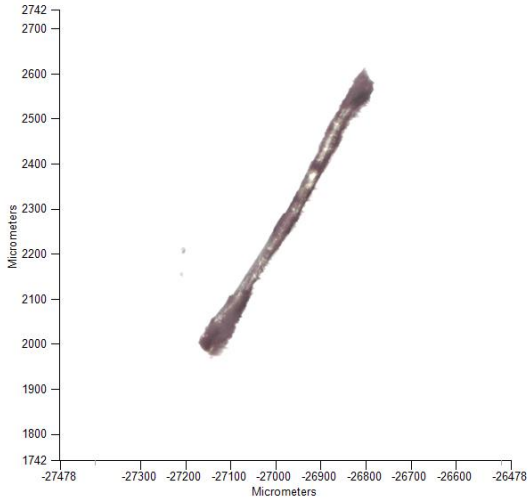
B1 Microplastic in fiber shape

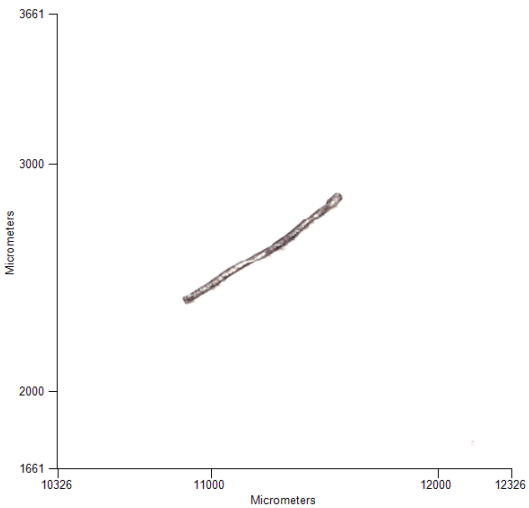




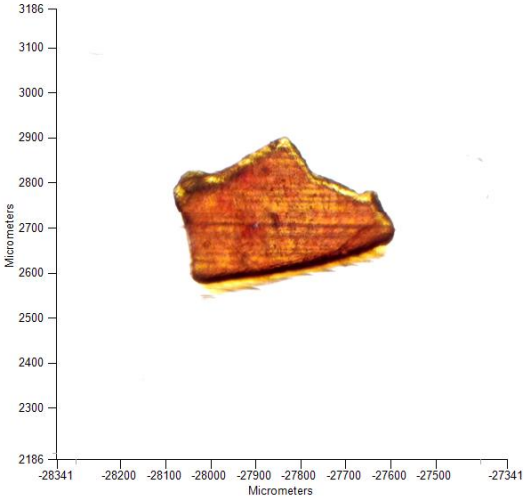


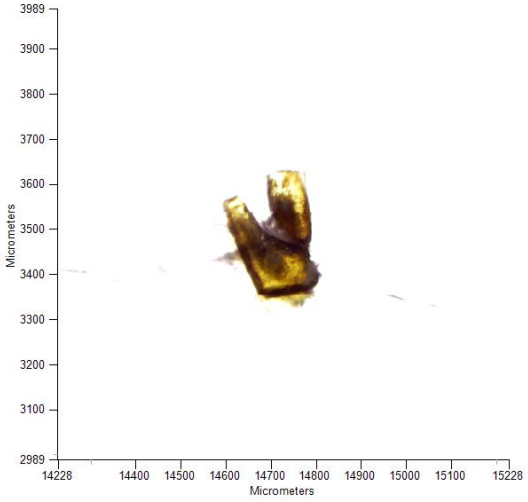


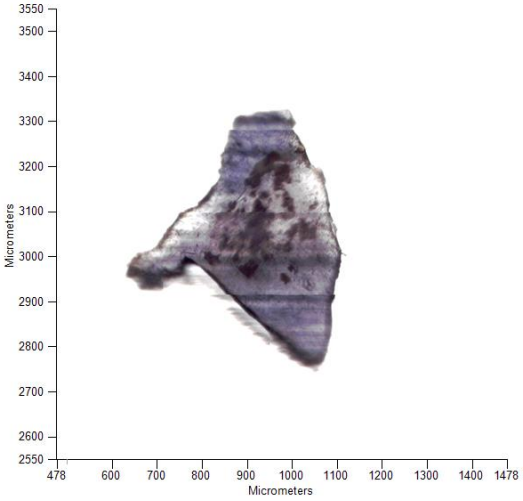


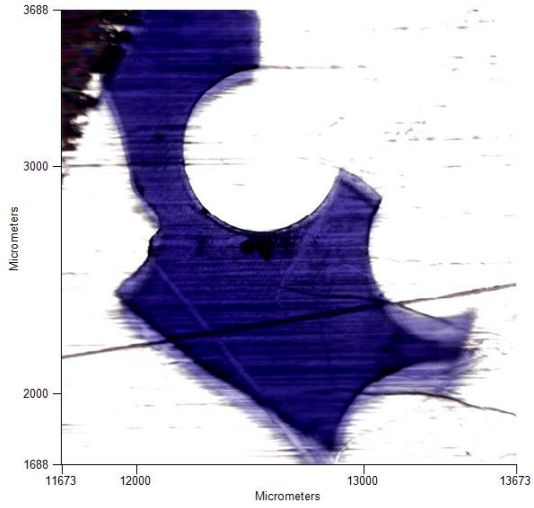


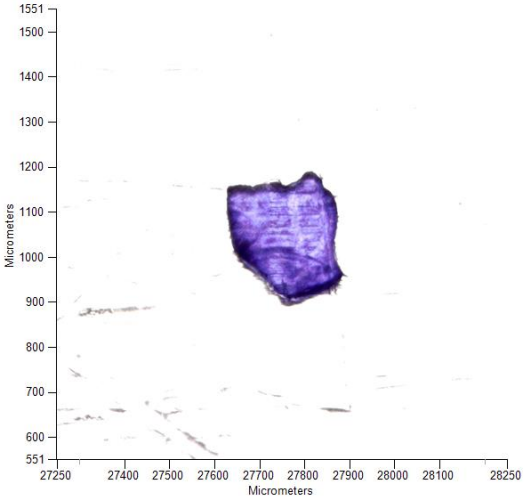
B2 Microplastic in pellet shape

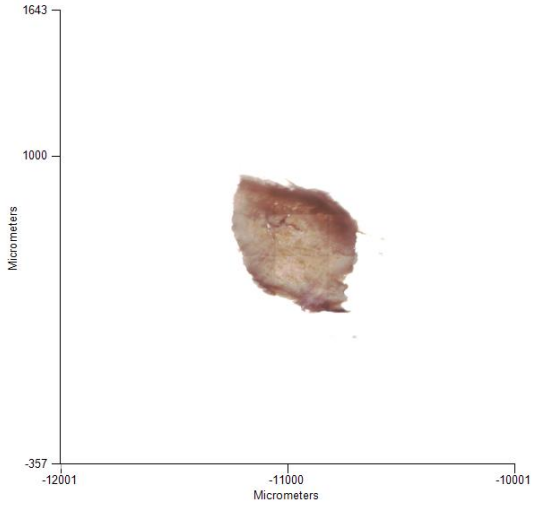




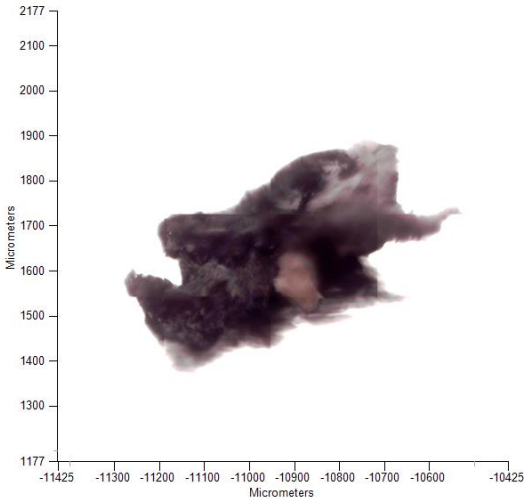


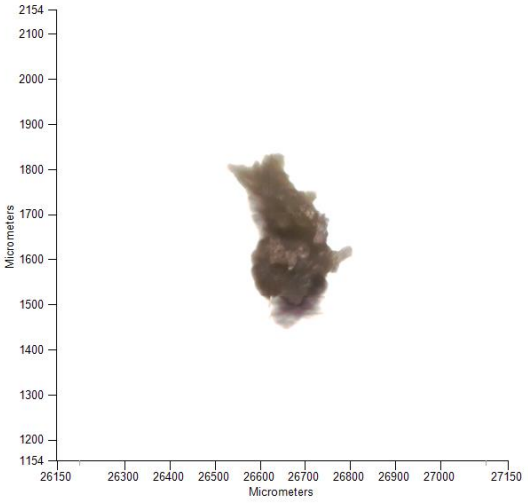


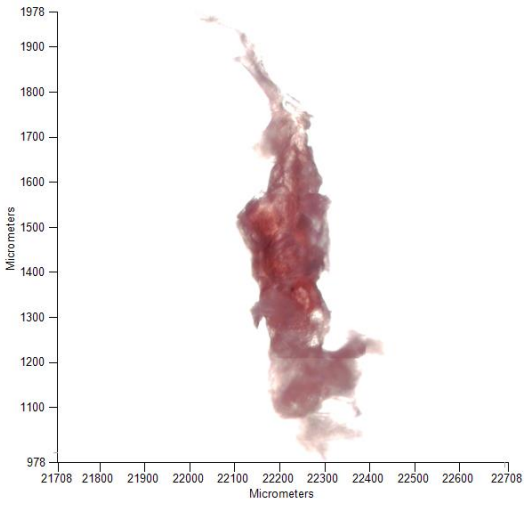


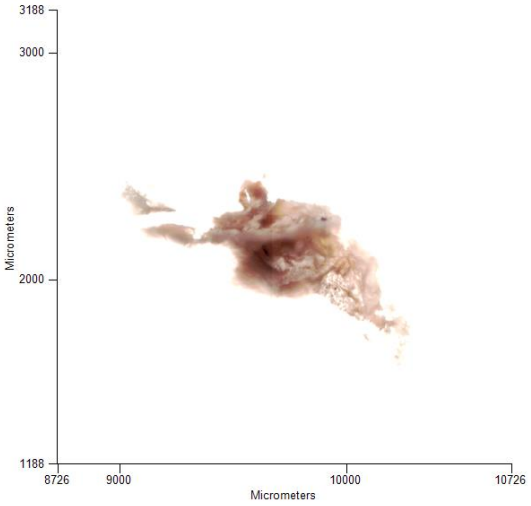


B3 Microplastic in fragment shape

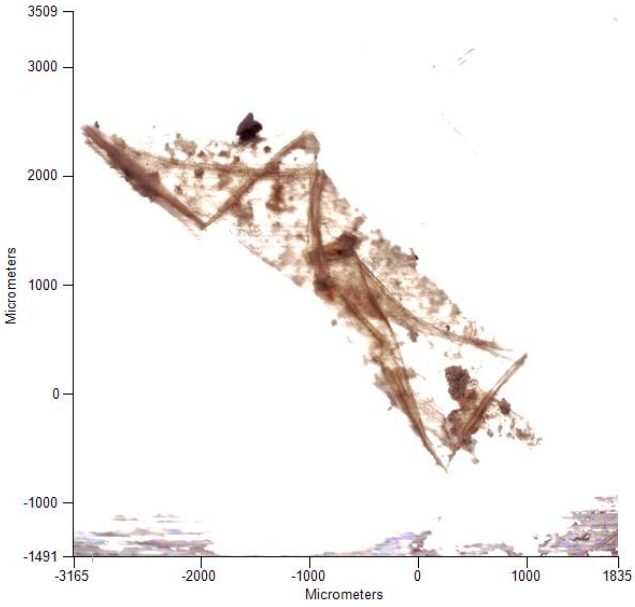


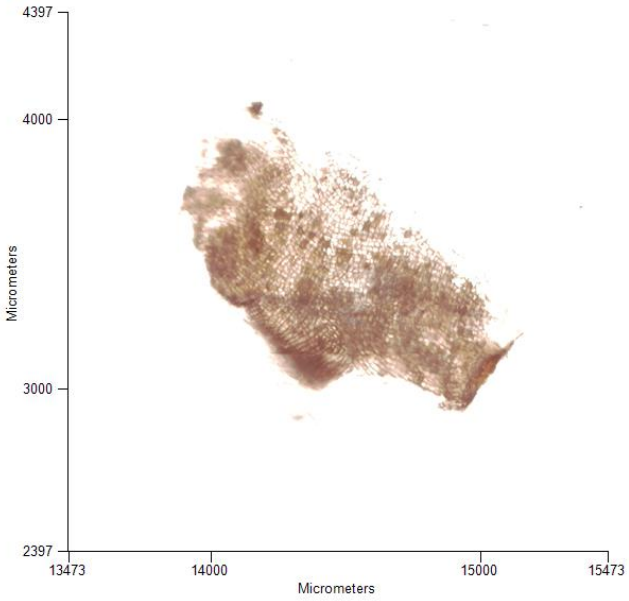


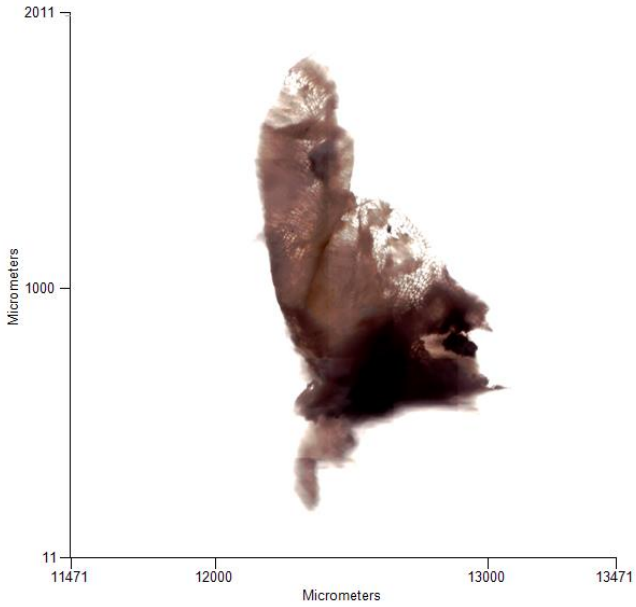




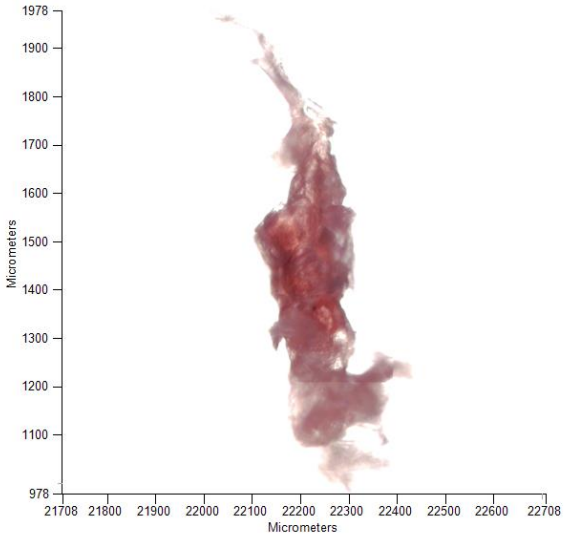
B4 Microplastic in film shape

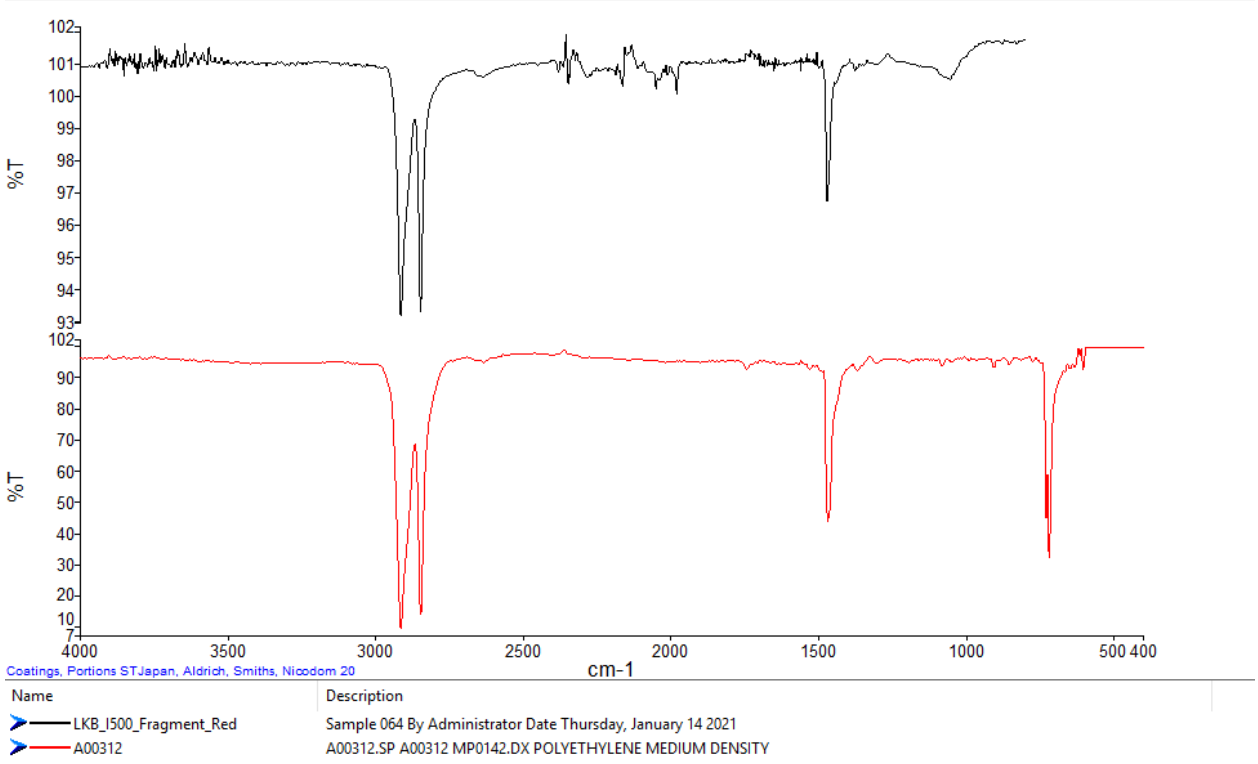




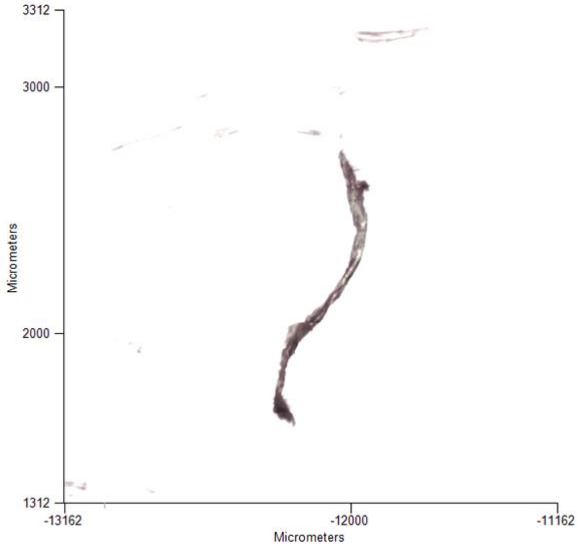


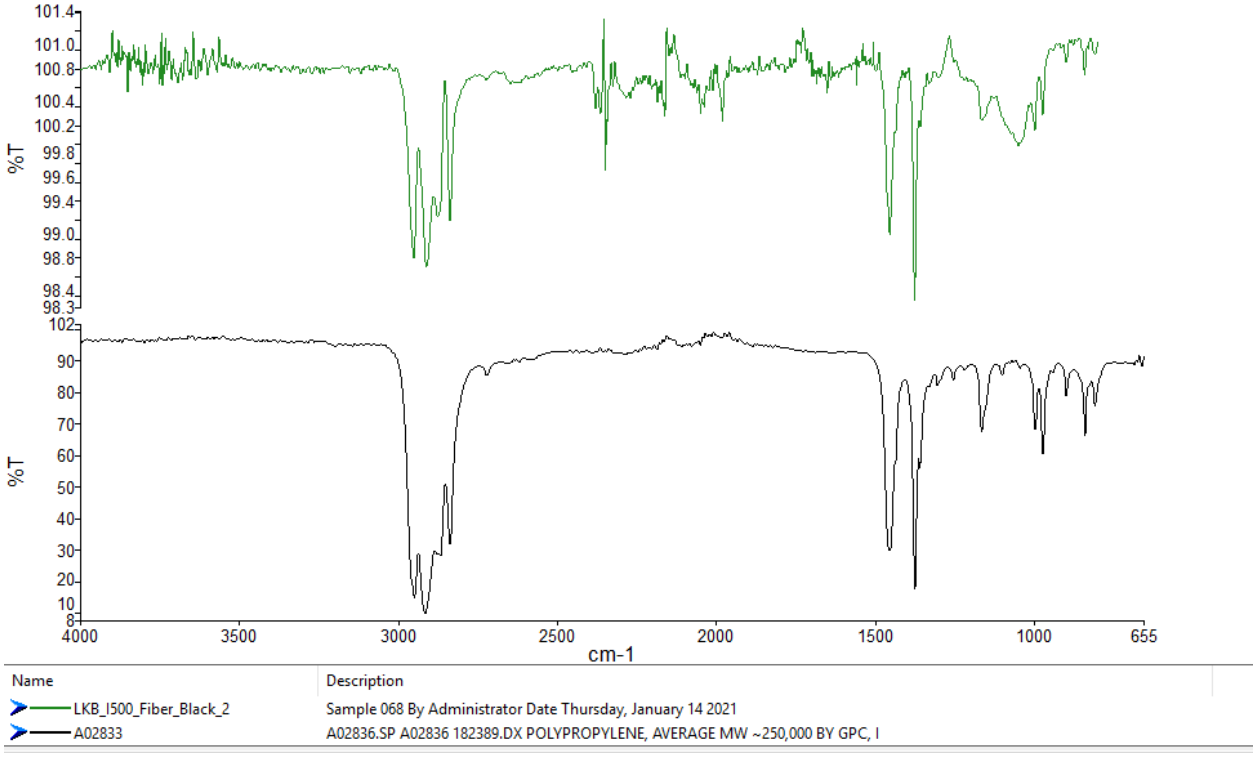
B5 Polyethylene



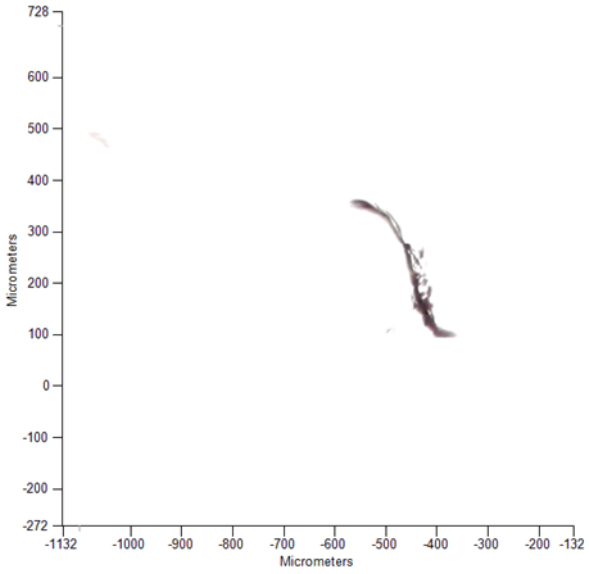


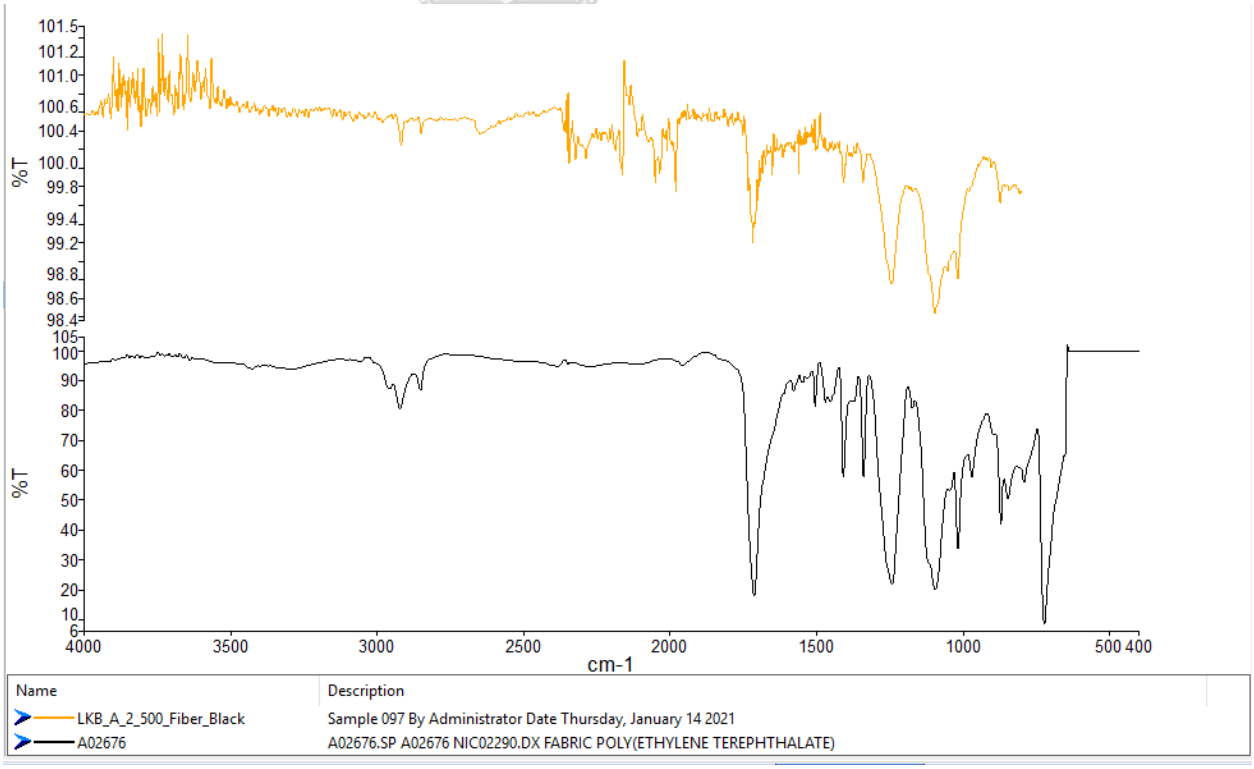
B6 Polypropylene



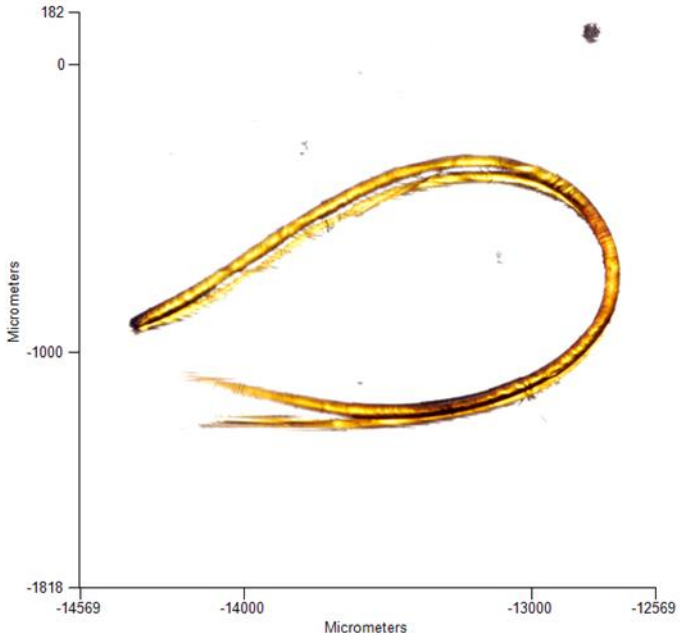


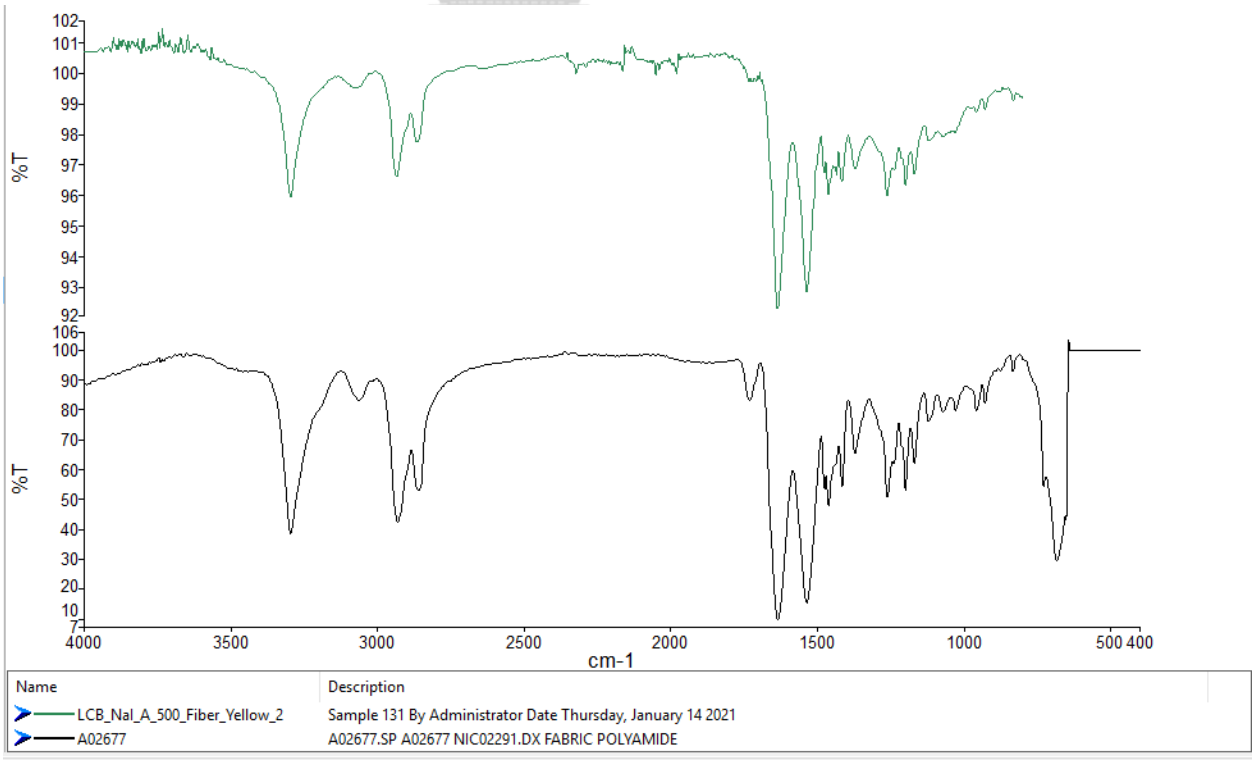
B7 Polyethylene terephthalate



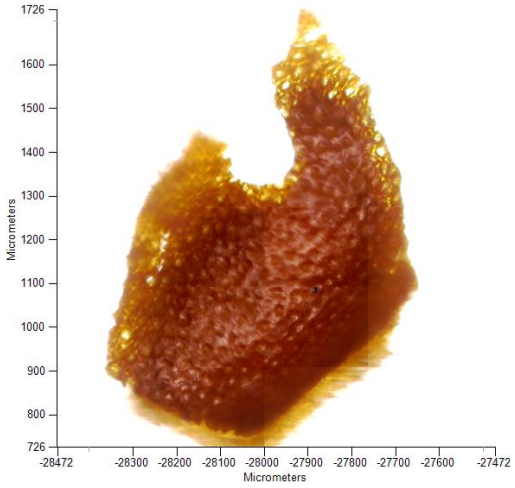


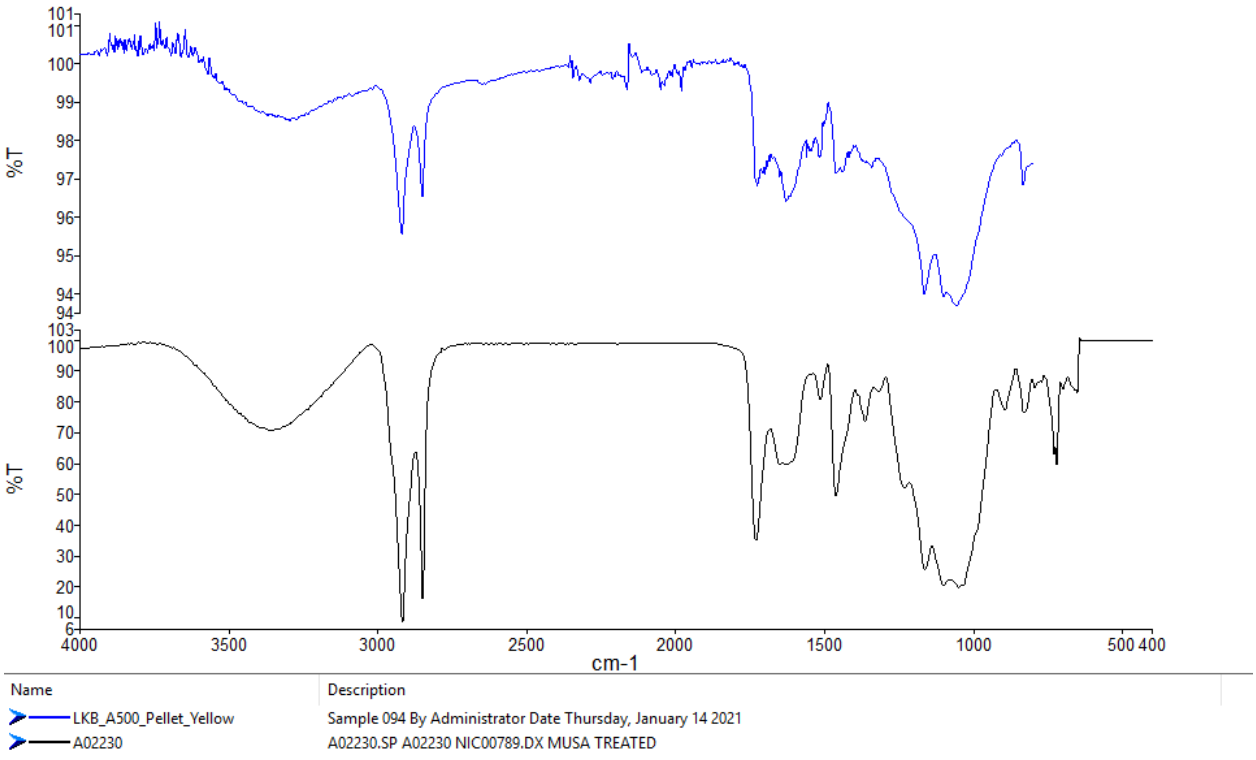
B8 Polyamide



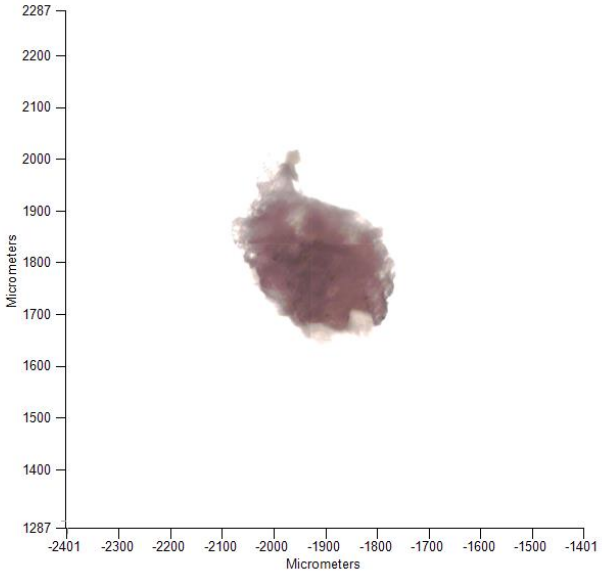


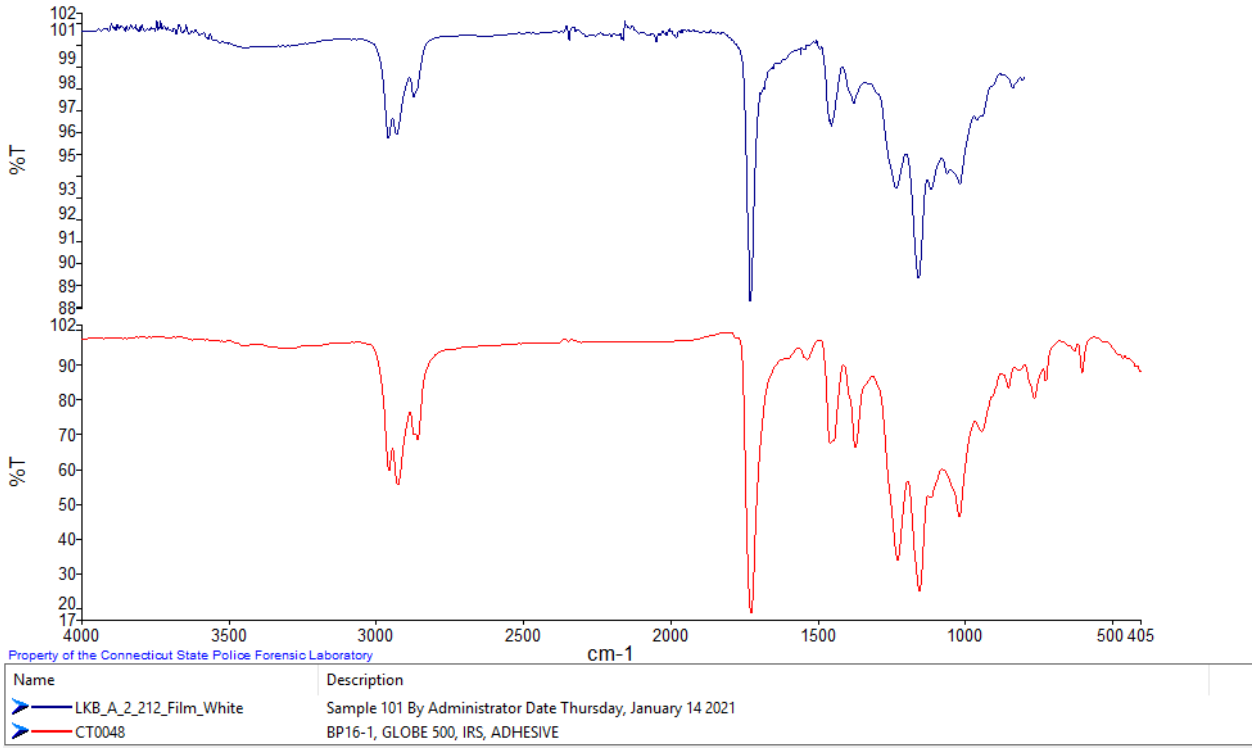
B9 Cork


Micrometers
Micrometers


%T
%T
cm-1
Name
Description
LKB_A500_Pellet_Yellow
Sample 094 By Administrator Date Thursday, January 14 2021
A02230
A02230.SP A02230 NIC00789.DX MUSA TREATED

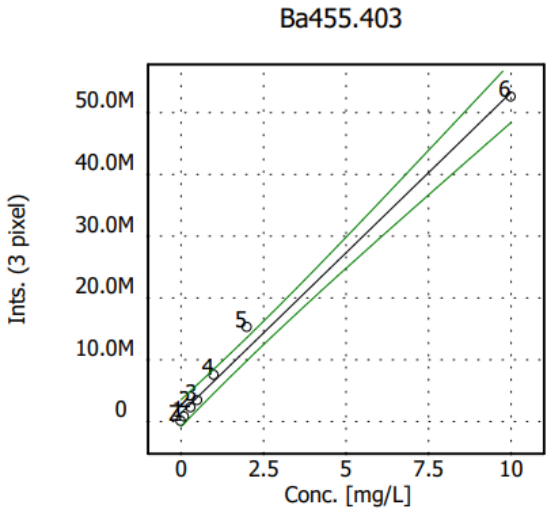
B10 Adhesive





APPENDIX C

C1 Graph standard concentration of Ba.



R^2(adj.): 0.987571174 Slope:5190940.6 Ints./(mg/L)

Method SD: 0.36044 mg/L

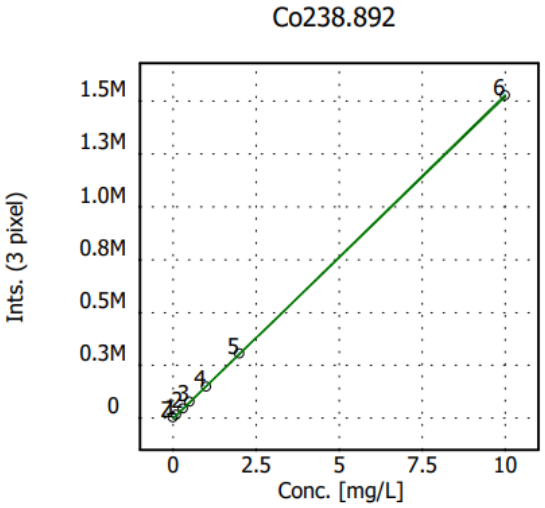
y=a+bx a=1364018.5 b=5190940.6

SD Blank (DL):
705.84792794

Table Ba455.403

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	32515	738	2.27		
Cal-Std1	0.1	750850	8221	1.09		
Cal-Std2	0.3	2230800	32208	1.44		
Cal-Std3	0.5	3406516	93252	2.74		
Cal-Std4	1	7498274	130916	1.75		
Cal-Std5	2	15244110	415649	2.73		
Cal-Std6	10	52539137	391935	0.75		

C2 Graph standard concentration of Co.



R^2(adj.): 0.999978071 Slope:152627.89 Ints./(mg/L)

Method SD: 0.01508 mg/L

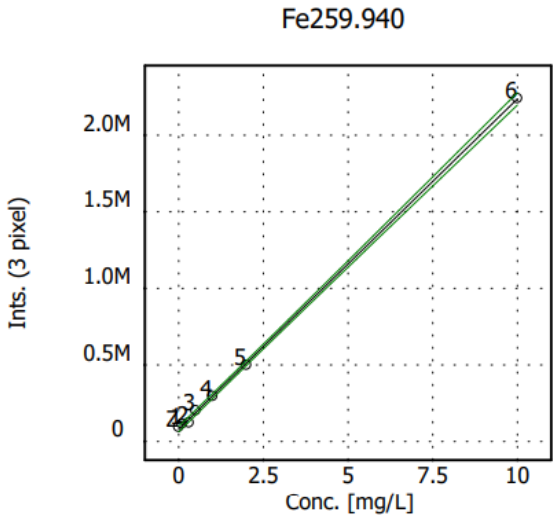
y=a+bx a=-1212.815 b=152627.89

SD Blank (DL):
55.10962614

Table Co238.892

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	1935	17	0.87		
Cal-Std1	0.1	14517	100	0.69		
Cal-Std2	0.3	44060	373	0.85		
Cal-Std3	0.5	75613	690	0.91		
Cal-Std4	1	147457	2987	2.03		
Cal-Std5	2	304001	1447	0.48		
Cal-Std6	10	1525456	6747	0.44		

C3 Graph standard concentration of Fe.



R²(adj.): 0.999499625 Slope:215587.72 Ints./(mg/L)

Method SD: 0.07203 mg/L

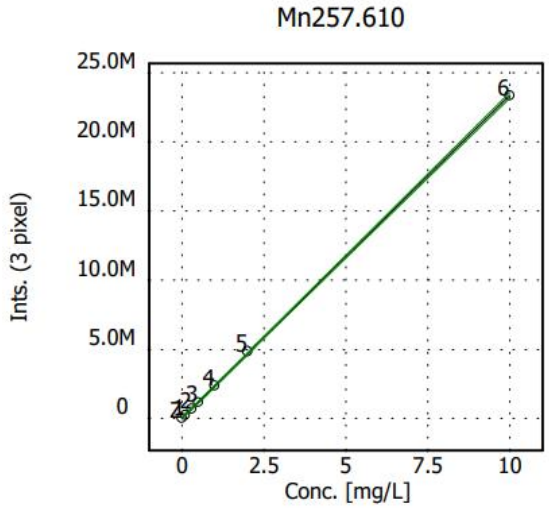
y=a+bx a=81503.694 b=215587.72

SD Blank (DL):
1776.86690857

Table Fe259.940

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	93492	814	0.87		
Cal-Std1	0.1	114469	1555	1.36		
Cal-Std2	0.3	122410	20537	16.78		
Cal-Std3	0.5	202251	5641	2.79		
Cal-Std4	1	295357	6476	2.19		
Cal-Std5	2	498968	8690	1.74		
Cal-Std6	10	2240248	14905	0.67		

C4 Graph standard concentration of Mn.



R²(adj.): 0.999892869 Slope:2333166.5 Ints./(mg/L)

Method SD: 0.03333 mg/L

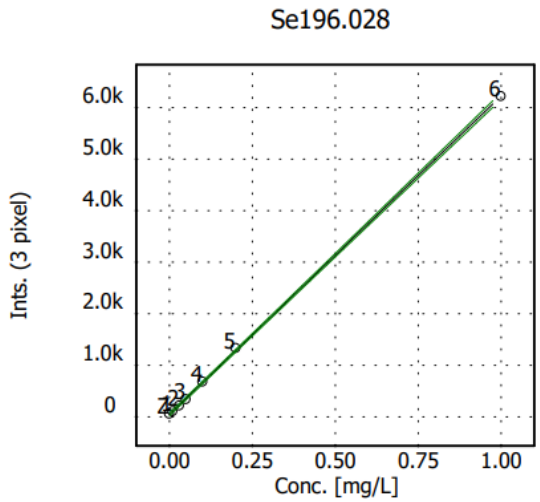
y=a+bx a=32571.516 b=2333166.5

SD Blank (DL):
486.72595938

Table Mn257.610

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	10212	29	0.29		
Cal-Std1	0.1	222819	1793	0.80		
Cal-Std2	0.3	684405	7529	1.10		
Cal-Std3	0.5	1166960	11971	1.03		
Cal-Std4	1	2388560	23511	0.98		
Cal-Std5	2	4851051	21917	0.45		
Cal-Std6	10	23335009	77314	0.33		

C5 Graph standard concentration of Se.



R²(adj.): 0.999840992 Slope:6174.7785 Ints./(mg/L)

Method SD: 0.00406 mg/L

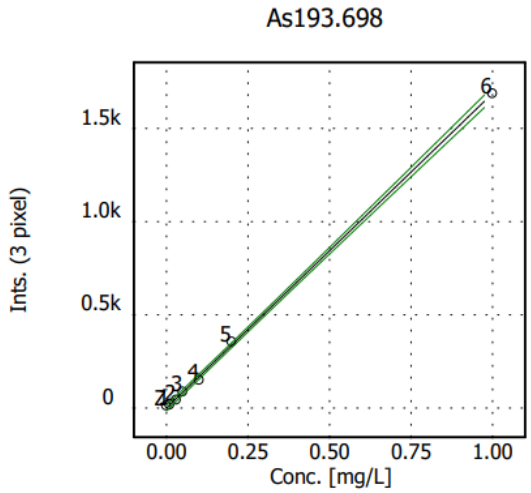
y=a+bx a=48.437974 b=6174.7785

SD Blank (DL):
6.74795682

Table Se196.028

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	53	6	10.97		
Cal-Std1	0.01	102	7	7.23		
Cal-Std2	0.03	211	5	2.37		
Cal-Std3	0.05	337	6	1.74		
Cal-Std4	0.1	675	9	1.39		
Cal-Std5	0.2	1328	2	0.12		
Cal-Std6	1	6215	56	0.90		

C6 Graph standard concentration of As.



R²(adj.): 0.999408593 Slope:1687.3093 Ints./(mg/L)

Method SD: 0.00783 mg/L

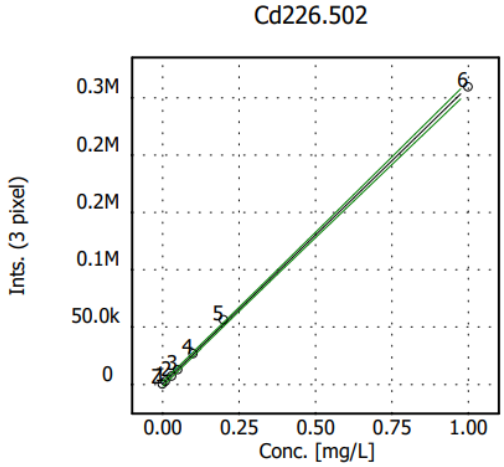
y=a+bx a=0.5443634 b=1687.3093

SD Blank (DL):
8.19913610

Table As193.698

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	12	9	74.59		
Cal-Std1	0.01	16	12	76.21		
Cal-Std2	0.03	43	16	37.21		
Cal-Std3	0.05	87	8	8.81		
Cal-Std4	0.1	150	8	5.46		
Cal-Std5	0.2	355	18	5.15		
Cal-Std6	1	1687	31	1.81		

C7 Graph standard concentration of Cd.



R²(adj.): 0.999562086 Slope:259449.89 Ints./(mg/L)

Method SD: 0.00674 mg/L

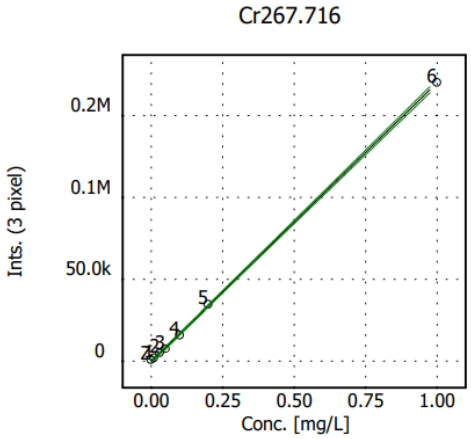
y=a+bx a=521.66944 b=259449.89

SD Blank (DL):
12.16944972

Table Cd226.502

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	153	8	5.34		
Cal-Std1	0.01	2531	9	0.37		
Cal-Std2	0.03	7047	769	10.91		
Cal-Std3	0.05	12694	136	1.07		
Cal-Std4	0.1	26620	465	1.75		
Cal-Std5	0.2	55899	632	1.13		
Cal-Std6	1	259342	5157	1.99		

C8 Graph standard concentration of Cr.



R^2(adj.): 0.999811715 Slope:170216.92 Ints./(mg/L)

Method SD: 0.00442 mg/L

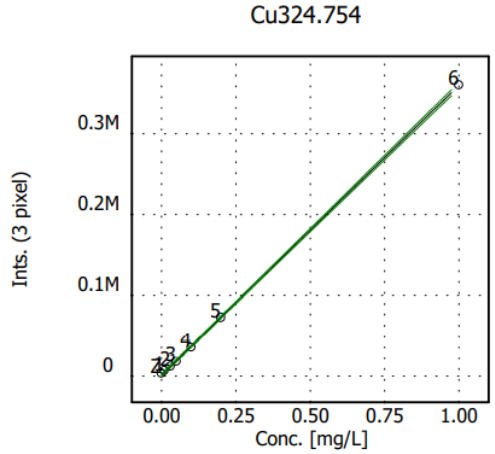
y=a+bx a=-160.4755 b=170216.92

SD Blank (DL):
37.33680431

Table Cr267.716

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	689	17	2.51		
Cal-Std1	0.01	1814	52	2.86		
Cal-Std2	0.03	4977	16	0.31		
Cal-Std3	0.05	7538	317	4.21		
Cal-Std4	0.1	15878	21	0.13		
Cal-Std5	0.2	34517	1020	2.96		
Cal-Std6	1	170065	2213	1.30		

C9 Graph standard concentration of Cu.



R²(adj.): 0.999837324 Slope:358698.64 Ints./(mg/L)

Method SD: 0.00411 mg/L

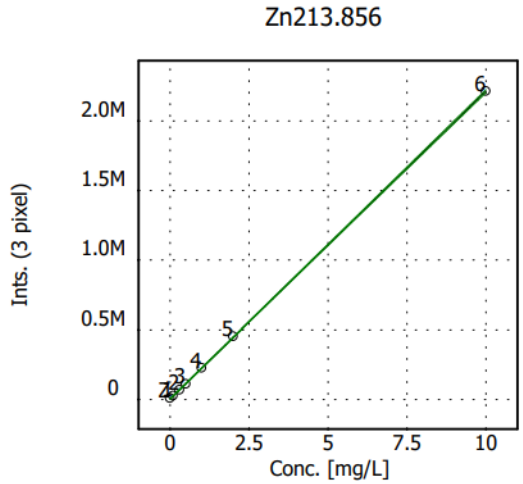
y=a+bx a=1461.9102 b=358698.64

SD Blank (DL):
21.00037436

Table Cu324.754

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	3376	27	0.80		
Cal-Std1	0.01	6572	39	0.60		
Cal-Std2	0.03	12020	177	1.48		
Cal-Std3	0.05	18094	25	0.14		
Cal-Std4	0.1	35748	37	0.10		
Cal-Std5	0.2	72498	399	0.55		
Cal-Std6	1	360516	1041	0.29		

C10 Graph standard concentration of Zn.



R²(adj.): 0.999967089 Slope:220928.19 Ints./(mg/L)

Method SD: 0.01847 mg/L

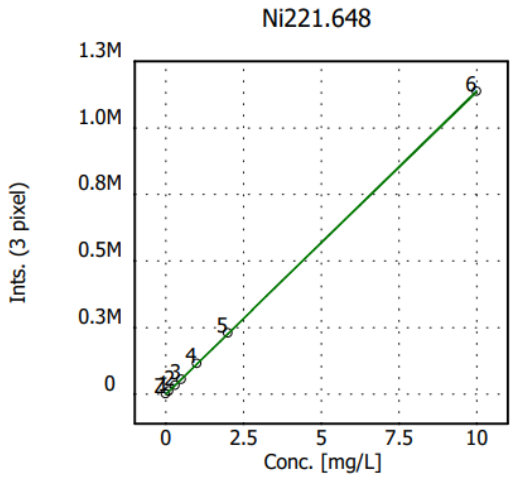
y=a+bx a=4584.7000 b=220928.19

SD Blank (DL):
90.26106596

Table Zn213.856

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	8343	132	1.58		
Cal-Std1	0.1	25328	77	0.30		
Cal-Std2	0.3	67637	146	0.22		
Cal-Std3	0.5	110694	1399	1.26		
Cal-Std4	1	225476	2939	1.30		
Cal-Std5	2	452537	3067	0.68		
Cal-Std6	10	2212979	14259	0.64		

C11 Graph standard concentration of Ni.



R²(adj.): 0.999982111 Slope:113832.17 Ints./(mg/L)

Method SD: 0.01362 mg/L

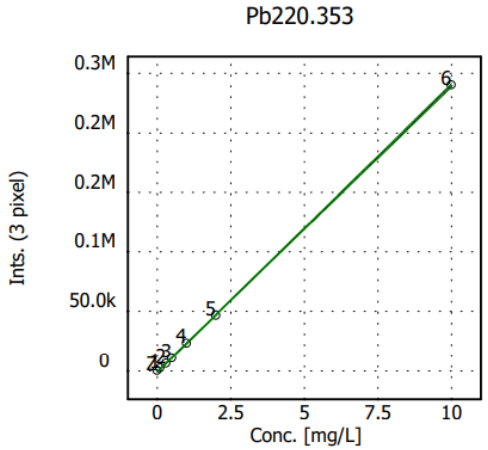
y=a+bx a=-518.2631 b=113832.17

SD Blank (DL):
10.32506738

Table Ni221.648

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	573	10	1.66		
Cal-Std1	0.1	10520	99	0.95		
Cal-Std2	0.3	32059	103	0.32		
Cal-Std3	0.5	54594	352	0.64		
Cal-Std4	1	114352	792	0.69		
Cal-Std5	2	229089	1241	0.54		
Cal-Std6	10	1137452	4935	0.43		

C12 Graph standard concentration of Pb.



R²(adj.): 0.999944513 Slope:24057.282 Ints./(mg/L)

Method SD: 0.02398 mg/L

y=a+bx a=-690.8547 b=24057.282

SD Blank (DL):
10.61467945

Table Pb220.353

Type	*Conc. [mg/L]*	*Ints.*	*SD*	*RSD/%*	*Ints. Recal.*	*Rem.*
Cal-Zero1	0	127	1	0.98		
Cal-Std1	0.1	2202	24	1.07		
Cal-Std2	0.3	6299	30	0.48		
Cal-Std3	0.5	10894	128	1.17		
Cal-Std4	1	23242	30	0.13		
Cal-Std5	2	46742	436	0.93		
Cal-Std6	10	240055	4105	1.71		

APPENDIX D

Table D1 Concentration of heavy metals in wastewater from WWTP A.

Sites	Ba	As	Co	Cd	Cr	Se	Ni	Pb	Zn	Mn	Cu	Fe
Influent	0.03	0.00	0.00	0.00	0.00	0.00	0.16	0.01	0.03	0.27	0.65	0.03
Grit chamber	0.17	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.60	0.54	0.20	0.43
Aeration tank	0.24	0.00	0.00	0.00	0.00	0.00	0.19	0.01	8.42	3.14	1.75	0.10
Effluent	0.07	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.97	0.67	0.07	0.22
RO	0.03	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.05	0.05	0.00	0.00

Table D2 Concentration of heavy metals in wastewater from WWTP B.

Sites	Ba	As	Co	Cd	Cr	Se	Ni	Pb	Zn	Fe	Mn	Cu
Influent	0.16	0.00	0.00	0.00	0.17	0.00	0.06	0.00	0.30	1.98	0.33	0.03
Grit chamber	0.15	0.01	0.00	0.00	0.07	0.00	0.07	0.00	0.47	2.02	0.28	0.02
Aeration tank	0.32	0.03	0.06	0.02	1.03	0.00	0.84	0.02	8.10	0.20	2.21	1.42
Effluent	0.05	0.00	0.00	0.00	0.02	0.00	0.05	0.00	0.20	0.34	0.21	0.01

TableD3 Concentration of heavy metals in sludge from both WWTPs.

Sites	Ba	As	Co	Cd	Fe	Cr	Mn	Cu	Se	Zn	Ni	Pb
WWTP A	7.87	0.16	0.53	0.48	336.46	24.52	8.83	10.46	0.15	110.69	7.63	0.89
WWTP B	6.85	0.04	0.13	0.01	111.53	1.30	2.81	23.02	0.03	76.75	0.76	0.52

TableD4 Concentration of heavy metals on microplastic in wastewater from both WWTPs.

Location	site	Size fraction	As	Co	Cd	Mn	Se	Ni	Pb	Fe	Ba	Cu	Zn	Cr
WWTP A	Inf	LCB I 20	0.00	0.01	0.00	0.00	0.00	0.01	0.00	19.50	0.00	0.00	0.04	0.04
		LCB I 100	0.04	0.28	0.03	0.29	0.03	0.29	0.26	1.26	0.59	0.03	0.33	0.04
		LCB I 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.34	0.10	0.00	0.02	0.01
		LCB I 500	0.04	0.28	0.03	0.29	0.03	0.28	0.26	1.31	0.58	0.03	0.34	0.05
	Grit	LCB G 20	0.03	0.30	0.04	0.30	0.03	0.29	0.26	51.04	0.52	0.03	0.35	0.06
		LCB G 100	0.00	0.00	0.00	0.00	0.00	0.00	0.01	2.00	0.33	0.04	0.04	0.02
		LCB G 200	0.04	0.28	0.03	0.30	0.03	0.29	0.26	3.91	0.27	0.03	0.33	0.05

		LCB G 500	0.00	0.00	0.00	0.02	0.02	0.01	0.00	12.04	0.01	0.01	0.04	0.07
	Aeration	LCB A 20	0.00	0.01	0.00	0.52	0.00	0.04	0.05	29.30	0.46	3.89	3.97	0.10
		LCB A 100	0.04	0.28	0.03	0.40	0.03	0.31	0.27	4.55	0.33	0.90	1.30	0.10
		LCB A 200	0.01	0.01	0.00	0.19	0.00	0.02	0.03	38.19	0.29	2.81	2.30	0.05
		LCB A 500	0.00	0.00	0.00	0.00	0.00	0.01	0.00	6.46	0.02	0.01	0.07	0.03
	Sediment	LCB S 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.04	0.00	0.00	0.00	0.11
		LCB S 100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.62	0.03	0.01	0.02	0.02
		LCB S 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.78	0.00	0.00	0.00	0.01
		LCB S 500	0.00	0.00	0.00	0.01	0.00	0.01	0.00	5.19	0.14	0.00	0.02	0.06
	RO	LCB RO 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.90	2.07	0.00	0.01	0.06
		LCB RO 100	0.01	0.00	0.00	0.00	0.00	0.02	0.00	2.77	0.00	0.00	0.00	0.01
		LCB RO 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.72	2.37	0.00	0.01	0.07
		LCB RO 500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.73	0.83	0.00	0.01	0.04

WWTP B	Inf	LKB i 20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.45	0.00	0.00	0.00	0.02
		LKB i 100	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.00	0.00	0.01	0.01
		LKB i 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.00	0.00
		LKB i 500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84	0.00	0.00	0.02	0.00
	Grit	LKB g 20	0.00	0.01	0.00	0.01	0.00	0.03	0.01	11.68	0.15	0.01	0.07	0.10
		LKB g 100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.37	0.00	0.00	0.00	0.09
		LKB g 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.05	0.00	0.00	0.00	0.07
		LKB g 500	0.00	0.01	0.00	0.01	0.01	0.04	0.01	15.86	0.16	0.00	0.04	0.24
	Aeration	LKB a 20	0.04	0.10	0.01	0.07	0.01	0.33	0.06	158.40	1.65	0.20	0.44	2.44
		LKB a 100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.35	0.09	0.59	0.30	0.36
		LKB a 200	0.00	0.00	0.00	0.00	0.00	0.00	0.00	34.22	0.01	0.00	0.00	0.21
		LKB a 500	0.00	0.02	0.00	0.05	0.00	0.05	0.01	31.54	0.45	0.02	0.06	0.37
	Sediment	LKB s 20	0.00	0.00	0.00	0.01	0.01	0.02	0.00	6.82	0.14	0.01	0.04	0.08
		LKB s 100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.41	0.00	0.00	0.00	0.04
		LKB s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.40	0.00	0.00	0.00	0.01

		200												
		LKB s 500	0.00	0.00	0.00	0.01	0.00	0.01	0.00	5.84	0.75	0.00	0.03	0.07

TableD5 Concentration of heavy metals on microplastic in sludge from both WWTPs.

Location	Size fraction	As	Co	Cd	Cr	Mn	Se	Ni	Pb	Ba	Fe	Cu	Zn
WWTP A	HM MP LKB 20	0.000	0.009	0.002	0.026	0.007	0.001	0.008	0.052	0.125	12.326	0.069	0.117
	HM MP LKB 100	0.005	0.001	0.000	0.015	0.004	0.000	0.002	0.013	0.031	0.986	0.009	0.018
	HM MP LKB 200	0.002	0.000	0.001	0.029	0.017	0.000	0.004	0.038	15.473	0.787	0.075	42.660
	HM MP LKB 500	0.003	0.013	0.000	0.015	0.000	0.001	0.012	1.498	0.000	3.540	2.244	0.057
WWTP B	HM MP LCB 20	0.000	0.039	0.003	0.553	0.155	0.002	0.139	0.066	0.285	50.110	0.046	1.940
	HM MP LCB 100	0.000	0.012	0.000	0.040	0.000	0.002	0.019	0.040	0.000	0.695	0.002	0.033
	HM MP LCB 200	0.000	0.010	0.000	0.032	0.009	0.002	0.010	0.045	15.140	0.337	0.011	42.000
	HM MP LCB 500	0.000	0.011	0.000	0.034	0.042	0.003	0.015	0.099	0.000	1.005	0.023	0.022

APPENDIX E

Figure E1 Influent at WWTP A.

Figure E2 Grit chamber at WWTP A.

Figure E3 Aeration tank at WWTP A.

Figure E4 Sedimentation tank at WWTP A.

Figure E5 RO process at WWTP A.

Figure E6 Influent at WWTP B.

Figure E7 Sedimentation tank at WWTP B.

Figure E8 Sludge storage at WWTP B.

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