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Research Article

Microplastic Abundance From Pig Farm Effluent and Surface Water In Sungai Tuang, Melaka, Malaysia

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ABSTRACT

Livestock is one of the country's important economic resources, nevertheless, an unsystematic livestock farm management system contributes to microplastic pollution. Microplastics (MPs) pollutant hurts the environment and human life, limited studies have been done in Malaysia's freshwater ecosystems. Therefore, this research was to determine the abundance of MPs in surface water and sediments from the nearby river and the last catchment pond of pig farm effluent in Paya Mengkuang and Sungai Tuang, Melaka. The concentration of MPs was compared with six water quality parameters (pH, biochemical oxygen demand (BOD), suspended solids (SS), dissolved oxygen (DO), total ammoniacal nitrogen (NH3-N), and chemical oxygen demand (COD). This study found that the average concentration of MPs was 487.38 particles/L and 50.96 particles/g for water and sediment samples consequently. This study showed a significant correlation between COD concentration and microplastic count in sediment samples. The source of microplastics in rivers is associated with anthropogenic activities such as unsystematic garbage disposal and poultry manure. The prevalence of microplastics in the environment of MPs could threaten the safety of resource utilization as MPs enter the food chain in aquatic ecology and pose a severe threat to aquatic organisms directly and subsequently to humans. Our study provides essential data on microplastic pollution in river estuaries and livestock farm areas.

Key words: Livestock, microplastic, pig farm, water quality

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INTRODUCTION

In 2020, the pig farming sector supplied 221 324.5 metric tonnes of pork, which is about 91% of the pork demand in Malaysia (Jabatan Perkhidmatan Veterinar, 2019). Economic demands such as ruminant farming, mainly pig farming, are often associated with the problem of environmental pollution, which usually gets public attention. The pollution is associated with water, soil, odor, and hygiene resulting from daily activities in the barn, such as waste and wastewater management and farm hygiene (Mateo-Sagasta *et al*. 2017; Steinfeld *et al*. 2006).

MPs in surface water can be classified as fibers, pellets, and fragments. However, this study required further analysis to identify the type of microplastic produced. For example, fiber particles may originate from conventional activities such as fishing (Castelvetro *et al*., 2021) and laundry (Napper & Thompson 2016; De Falco *et al*., 2019). The most apprehension about MPs was physically resembling the size of plankton and potentially ingested by other organisms (de Souza Machado *et al*., 2018) then conceivably entering the human food chain.

Sewage water from livestock farms, especially livestock farms that still practice the traditional farming concept, will release the wastewater into inland waters such as drains, waterways, and rivers. The release of untreated sewage wastewater will cause the deterioration of river water quality and, at the same time, harm our ecosystem. Based on the Guidelines on Pollution Control from Pig Breeding Activities issued by the Jabatan Alam Sekitar & Jabatan Perkhidmatan Veterinar (2014), livestock activities have been identified as one of the contributors to river pollution in Malaysia. The Environmental Quality Report in 2020 (Jabatan Alam Sekitar, 2020) showed the pollution load from pig farming activities was 463.32 tons/day. The Environmental Quality Report showed a rise in polluted rivers from 50 rivers in 2018 to 59 rivers in 2019 (Jabatan Alam Sekitar 2019) and a reduction in 2020 of 34 rivers (Jabatan Alam Sekitar 2020) in due to the Mobility Control Order enforced by the Government of Malaysia to prevent and control the spread of Covid19.

In Melaka, Malaysia one of the rivers associated with pollution from pig farming is Sungai Tuang. Sungai Tuang is located within a 1 km radius of the Pengkalan Balak Beach resort, which houses 35 pig farmers. Sungai Tuang recorded a water quality index at class III, polluted status in 2018, 2019, and 2020 with index values of 70 for 2018, 57 for 2019 to 59 for 2020. The water quality of this river declined from 2017 to 2019 and its polluted status is allegedly due to pig farming activities (Jabatan Alam Sekitar, 2020). However, efforts were made to improve the quality of effluent discharged from the wastewater pond outlet to the river area. This sewage waste not only contains dirt from pig manure but also there is garbage waste from farm management.

Among the pollutants studied in the effluent of pig wastewater treatment ponds are MPs, which are pollutants that are beginning to gain attention. MPs are plastic particles with a diameter size of less than 5 mm, resembling the size of a sesame seed (Galvão *et al*., 2020). Microfibers shed from clothing and other fabrics, such as fishing nets, as well as microscopic particles made for commercial use, such as those found in cosmetics, are the two main types of primary MPs. Particles known as secondary MPs are produced when bigger plastic objects, such as water bottles, break down. The sun's radiation is the key environmental variable that contributes to this degradation in the freshwater ecosystem. Many studies are conducted abroad to confirm the presence of microplastics in livestock sewage effluents. However, primary microplastic research is still poorly executed and considered insignificant (Cheung & Fok, 2016), especially in Malaysia. Therefore, this study aims to determine the rate of microplastic discharge from effluent discharged by pig farm sewage ponds, which has the potential to pollute Sungai Tuang, Melaka. This result can determine the relationship between the concentration of MPs in water and Sungai Tuang water quality parameters. The findings of this study can provide an initial overview to assist the country's management in developing action plans and plans to address the problems caused by MPs pollution.

MATERIALS AND METHODS

Sampling location

Sampling activities for this study were conducted in Masjid Tanah district, Melaka state, in September and December 2021. A total of seven sampling locations were selected around Paya Mengkuang and involved pig farms located in the Sungai Tuang area, which crosses the Paya Mengkuang area and flows to Gong Bay Beach (Figure 1 & Table 1). This study consists of two categories: samples from pig farm effluents around the Paya Mengkuang area and surface water samples of Sungai Tuang, Melaka.

Sampling and analysis

All equipment used to conduct sampling at designated locations was pre-washed and soaked with nitric acid, and rinsed several times with distilled water. Containers used for water and sediment sampling are pre-labeled before emptying. Containers are labeled with station number and replication number.

The glass bottle for water sample collection was rinsed first with the river water. Then, a water sample was taken using a bottle by inserting the bottle into the water 30 cm from the water's surface. Samples were taken using a pair of clean rubber gloves to avoid cross-contamination during sample collection. The mouth of the sample bottle for MPs analysis should be covered with aluminum foil to prevent MPs contamination from the air and bottle caps. The bottle was tightly closed for water quality analysis samples especially for BOD analysis, to ensure the absence of oxygen to dissolve the water sample. Water samples were stored in containers cooled to 4 °C with three replications and maintained at that temperature until brought to the laboratory for analysis of ex-situ parameters such as COD, BOD, TSS, and NH_3-N. River bed sediment samples were taken using a stainless-steel scoop. Triplicate sediment samples that have been taken were placed in glass containers and labeled. All sample containers were covered with aluminum foil to prevent MPs contamination.

The samples went through two processes, the MPs detection process through the red nile staining process adopted by Prata *et al*. (2021) and Maes *et al*. (2017). Then, the process of calculating the number of MP particles present using ImageJ software. Quality control measures were highly emphasized during these tests conducted in the laboratory to prevent MP contamination from the air and laboratory surfaces. All laboratory table surfaces used should be cleaned with Mili-Q water before use. All glassware was washed with nitric

Fig. 1. Map of the sampling station at Sungai Tuang and the pig farm in Paya Mengkuang Melaka.

Table 1. Sampling location

Sampling station	Label	Latitude	Longitude	Description
Sungai Tuang 1	ST01	2.352105	102.060200	Upstream, nearby solid waste dump site
Sungai Tuang 2	ST02	2.347577	102.061481	Nearby pig farm
Sungai Tuang 3	ST03	2.341922	102.060533	Near village residential area
Sungai Tuang 4	ST04	2.337832	102.060319	At the river mouth
Pig farm effluent M014	PF01	2.348000	102.061870	Last wastewater pond in the pig farm A
Pig farm effluent M018	PF02	2.347700	102.061030	Last wastewater pond in the pig farm B
Pig farm effluent M017	PF03	2.345760	102.061070	Last wastewater pond in the pig farm C

acid and rinsed with Milli-Q water before and between sample analyses. All processed samples were stored in a closed glass petri dish to prevent airborne particles with minimal movement to reduce the particles released from the filter disc.

Red nile powder was mixed with methanol to obtain a 1 mg/mL concentration for the stock solution. For the staining process, a 20 µm stock of Red Nile solution was dissolved in 200 mL of Mili-Q water. The MP samples present in the sample will absorb the red nile lipophilic dye and give fluorescent light to the microplastic during the imaging process under a blue light and orange lens microscope (Prata *et al.*, 2021). In the wet peroxide oxidation (WPO) process, iron sulfate powder was mixed with distilled water to obtain a concentration of 0.05 M. Then, the solution was dissolved with a concentrated solution of sulfuric acid to ensure complete dissolution and stored at room temperature. Both iron solution and 30% hydrogen peroxide were used during the WPO process. Then, for density separation, zinc chloride 1.5 g/cm^3 was used.

A total of 1 L of water was collected for each sampling point with three replications for MPs analysis. A homogeneous water sample was digested by the WPO method and filtered with a 10 µm polycarbonate membrane filter. Then, 20 mL zinc chloride was added for the density separation process. Only floated particles were filtered and stained using a red nile working solution. Samples were stained in red nile solution for at least 15 min before being removed by using a vacuum pump. Filtered membrane filters were removed and stored in a glass petri dish before being analyzed with an imaging device (Canon EOS 600 or EOS 1200 digital SLR camera) under a macro-orange filter lens (Kobo, 529 nm) camera with a powerful blue LED light source (Crimelite 450–510 nm, Foster & Freeman, U.K.). To block out incident blue light, fluorescent pictures were captured using an orange filter. For sediment samples, the samples were dried using an oven at a temperature of 70 °C. Dry samples were sieved using a 5 mm sieve and only 20 g of sample were processed. For sediment samples, the density separation process was first, then the floating particles were filtered using a 10 µm polycarbonate membrane filter. Subsequently, the filtered particles were digested with the WPO method and again filtered. The remaining particles were processed with density separation to ensure a minimized disturbance from organic materials, filtered, and stained with red nile solution. A similar method with MPs in water was used to analyze the MPs from sediment below the imaging device.

Statistical analysis

In this study, a one-way ANOVA test was used to determine the concentration of microplastics in water and sediment between the sampling stations. In addition, a correlation was also used to identify the relationship between the concentration of microplastics in water with the water quality of Sungai Tuang.

RESULTS AND DISCUSSIONS

Microplastic abundance in water and sediment

Based on observations made during the sampling process and visits to these pig farms, MPs contamination can be caused by many factors, including feed containers made of plastic (troughs or self-feeders), animal feed packaging, medical equipment containers, garbage waste, and probably pig manure too. However, it is estimated that the MPs released by livestock farms are mostly secondary. Secondary MPs are larger plastics and are broken down into smaller fragments through the natural effects of nature, such as UV light, wind, and waves (Eerkes-Medrano & Thompson, 2018; Priestnall *et al*., 2020) and plastics fragment from listed plastic sources. Plastics are not easily biodegradable materials and will break down into small particles and decompose when exposed to ultraviolet radiation, oxygen, high temperatures, and the activity of microorganisms (Carson *et al*., 2011). Therefore, this breakdown process in our environment does not cause the plastic to disappear but only makes it smaller and becomes a source of dangerous silent pollution because it cannot be seen with the naked eye.

The subsequent MP will enter the land drainage system through groundwater runoff, discharge in drains, and even sewage effluent. Based on the report from the pig waste management guidelines issued by the Jabatan Perkhidmatan Veterinar (2020), the average wastewater production for a 70 kg pig (finisher), which is a pig that is mature enough and ready for slaughter, is approximately 40 liters/day including 2.1-2.5 liters/day of urine. Most pig farmers clean their livestock about two times a day. Yet to this day, there are not many studies related to MPs concerning effluents from ruminant farms such as pig farms (to pens).

The distribution of MPs concentrations was evaluated from seven sampling points around Sungai Tuang which are surrounded by pig farms, farming areas, village residential areas, forests, and mangroves. MPs were present in surface water (Figure 2) and sediment samples (Figure 3) at all sampling points. Screening of MPs in water and sediment samples for each sampling station were categorized according to particle sizes of 10–100 μm, 101–300 μm, 301–500 μm, and 501–1000 μm which is similar to Masura *et al*. (2015) and Zaki *et al*. (2021). For this study, the particle size range obtained did not exceed 1 mm (Tables 2 & 3). According to Rozaimi *et al*. (2021), most MPs in surface water are between 300–1000 μm in size. Because larger particles tend to float on the water's surface, the sampling container did not capture MP particles larger than 1000 m in size.

The numbers of MPs in the water were high for all pig farm final water treatment ponds, which could be from the pig manure and waste management. The ST01 sampling area showed the lowest numbers of MPs which were located far from the anthropogenic activity and received relatively low amounts of pollutants (Yonkos *et al*., 2014). For the first sampling activity, station PF02 had the most MPs in a water sample, which was 1315.7 particles/L, while station ST01 had the least number of MPs which was 177 particles/L.

In the sediment samples, station PF02 had the highest number of MPs, while ST03 had the least number, 67.1 particles/g, and 25.9 particles/g respectively. For the second sampling, station ST02 had the highest number of MPs, 100.4 particles/g, while station ST03 had the least number of MPs, which is 20.2 particles/g. Station ST02 was the nearest sampling location with the pig farms for the river. So, this could contribute to the highest number of MPs due to inefficient wastewater treatment (Mason *et al*., 2016).

Overall, it was found that the average concentration of MPs for water samples was 121.9 particles/g and 12.74 particles/L for sediment samples (Table 2 & Table 3). The concentration varied for each sampling point, day of samples taken and type of sample analyzed. Inefficient wastewater systems from pig farms and anthropogenic activities along the river contributed to MP pollution in the river. Based on one-way ANOVA statistical analysis conducted for all sampling stations, it was found that there was no significant difference ($p \leq 0.05$) between each particle size range and each sampling station. The ineffective wastewater treatment was rich in fibers from the garbage (Galvão *et al*., 2020) and vary according to the type of plastic used (Carson *et al*., 2011), which instigated the MPs in sediment because between 80 to 90% of the microplastics that passed in the drainage system would remain in the sediment (Tsang *et al*., 2017).

Water quality index

The analysis results for a sampling conducted at all sampling stations around Sungai Tuang and pig farms in Paya Mengkuang, Melaka, are shown in

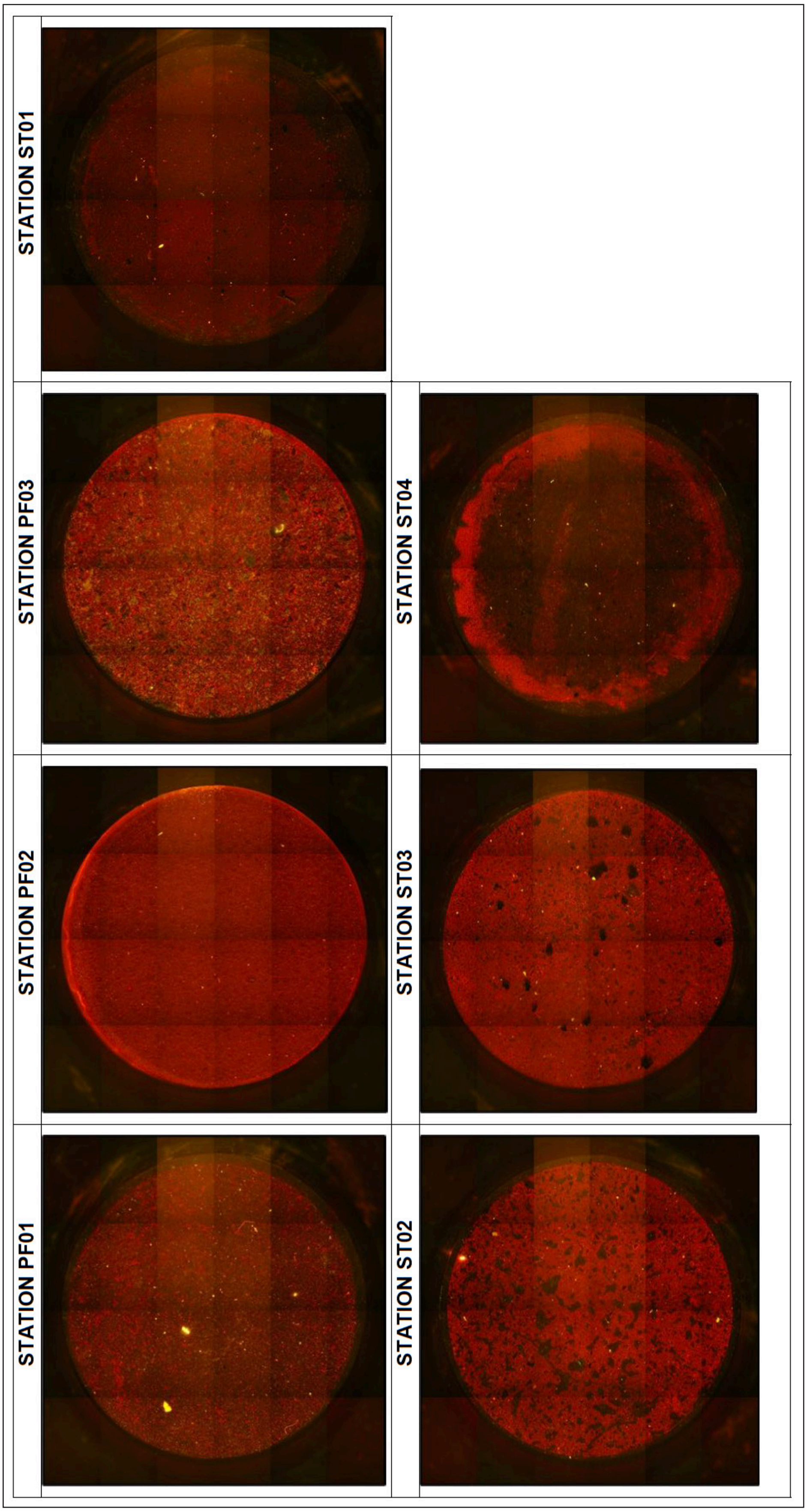

Fig. 3. MPs found in water samples for each sampling station.

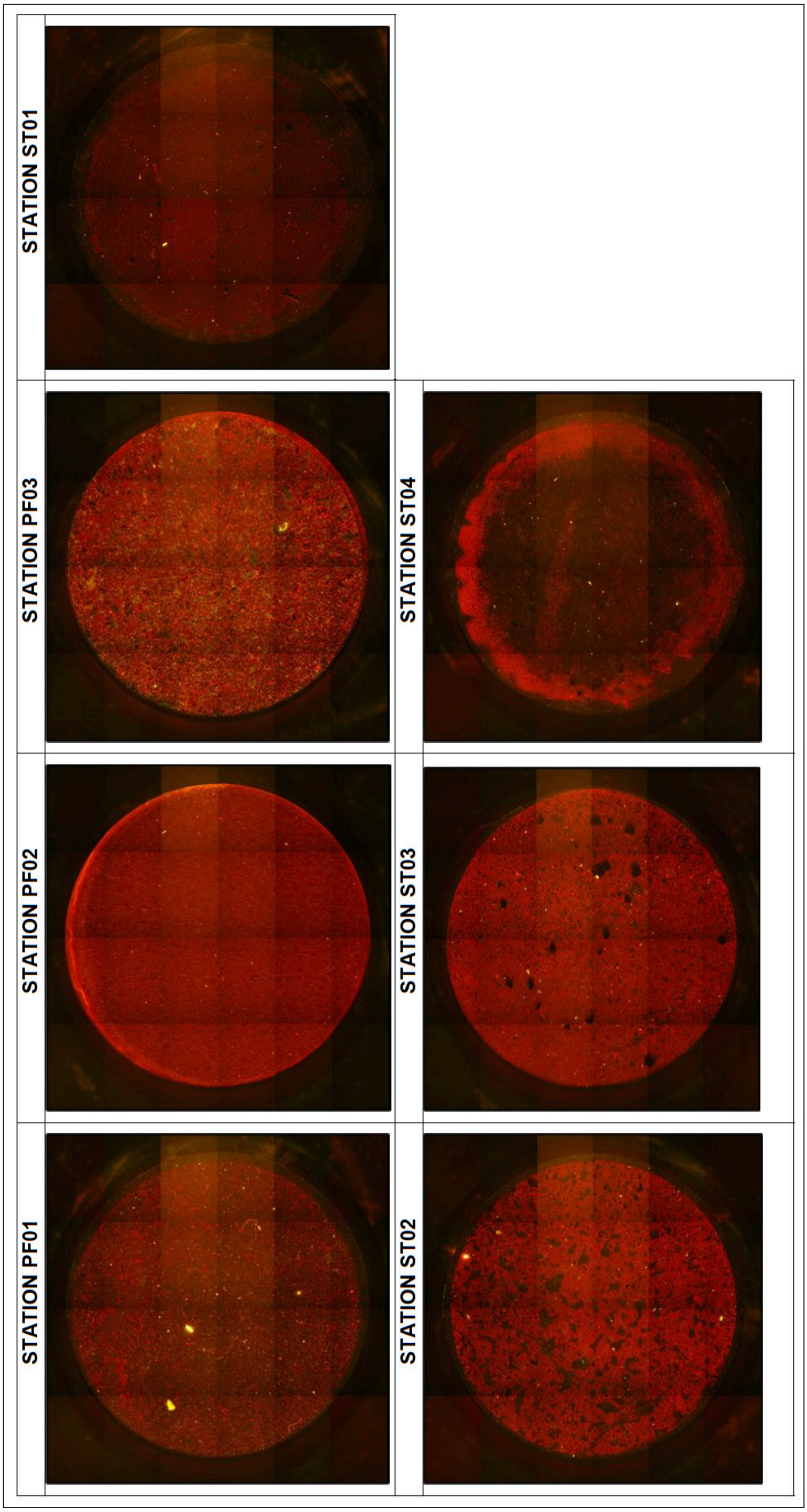

Fig. 3. MPs found in sediment samples for each sampling station

Table 2. Number of MPs in water for each sampling station by size

Particle Size (µm)	Sampling station (water)													
	First sampling							Second sampling						
	PF01	PF02	PF03	ST01	STO2	ST03	ST04	PF01	PF02	PF03	ST01	STO2	ST03	ST04
10-100	590.67± 845.34a	1289.3± 969.42a	552.33± 148.67a	169.67± 60.71a	233.33± 89.39a	267.33± 81.82a	379.33± 39.83a	330.33± 235.6a	619.33± 376.47a	615.67± 230.85a	370.33± 67.16a	428.00 ± 79.73a	310.67± 200.63a	398.67± 53.41a
101-300	33.67± 33.8a	24.67± 24.68a	9.33± 6.51a	7.00± 1.00a	4.33± 1.53a	9.67± 3.51a	8.67± 8.96a	7.67± 5.51a	27.00± 21.79a	30.00± 30.27a	13.67± 10.07a	18.67 ± 4.16a	23.67± 8.14a	38.33± 20.13a
301-500	0.33± 0.58a	1.67± 2.08a	0.33± 0.58a	0.33± 0.58a	0	0.33± 0.58a	0	0	1.33± 0.58a	1.00± 0.00a	0.33 ± 0.58a	0.67 ± 0.58a	1.67± 0.58a	2.00± 1.73a
501-1000	0.67± 1.15a	0	0.33± 0.58a	0	0	0	0	0	0.33± 0.5a	0.33± 0.58a	0	0.33 ± 0.58a	0	0

Table 3. Number of MPs in sediment for each sampling station by size

Particle Size (µm)	Sampling station (sediment)													
	First Sampling (particles/g)							Second Sampling (particles/g)						
	PF01	PF02	PF03	ST01	STO2	ST03	ST04	PF01	PF02	PF03	ST01	STO2	ST03	ST04
10-100	24.55± 6.21a	57.88± 206.9a	59.00± 25.23a	35.09± 23.25a	57.93 ± 9.46 a	23.41 ± 3.1 a	58.85 ± 26.46a	76.52 ± 84.86a	50.27 ± 7.85a	43.67 ± 32.74a	23.45± 4.28a	96.60± 21.91a	18.84 ± 11.16a	42.37 ± 29.46a
101-300	8.53± 11.73a	7.67 ± 10.51a	1.77± 1.22a	4.13± 3.25a	1.31± 0.62 a	2.24± 0.56a	2.36± 0.87a	3.08± 2.16a	1.32± 0.72a	2.19± 1.10a	1.43± 0.76a	3.53± 0.33a	1.25± 0.58a	1.40± 0.85a
301-500	0.47± 0.39a	0.87 ± 1.22a	0.29± 0.22a	0.87± 0.16a	0.08± 0.08 a	0.19± 0.09a	0.20± 0.11a	0.29± 0.34a	0.20± 0.24a	0.23± 0.06a	0.09± 0.06a	0.13± 0.14a	0.08± 0.04a	0.16± 0.08a
501-1000	0.23± 0.20a	0.27 ± 0.26a	0.12± 0.17a	0.24± 0.18a	0.03± 0.02a	0.08± 0.0a	0.09± 0.06a	0.29± 0.30a	0.13± 0.13a	0.07± 0.02a	0.03± 0.02a	0.12± 0.08a	0.07± 0.02a	0.11± 0.10a

Table 4. Analysis results for seven sampling areas around Sungai Tuang and pig farms in Paya Mengkuang, Melaka

SAMPLING	STATION NO.	DO %	BOD (mg/L)	COD (mg/L)	NH3-N (mg/L)	TSS (mg/L)	PH	DO index	BOD I index	COD index	NH_3-N index	SS index	pH index	WQI	Class	Water Quality Index (WQI)
1	ST01	114.17	3	53	1.14	0	6.08	100	87	42	45	97	92	78.7	II	SP
2	ST01	81.72	2.9	23	0	0.38	4	91	88	71	70	97	35	78.4	II	SP
1	STO2	50.40	4	166	89.00	4	7.91	50	84	1	0	95	92	53.5	III	P
2	STO2	12.39	1.0	220	1	0.94	7	4	96	-6	51	97	98	53.1	III	P
1	ST03	46.14	4	216	133.67	4	7.34	44	85	-5	0	95	98	52.0	III	P
2	ST03	30.80	0.6	449	1	0.94	7	22	98	-18	50	97	99	55.5	III	P
1	ST04	55.32	4	199	20.17	0	7.13	58	82	-3	0	97	99	55.1	III	P
2	ST04	85.07	3.4	1183	0	0.46	8	94	86	-47	87	97	96	69.5	III	SP
1	PF01	13.48	3	347	207.00	0	7.71	5	89	-13	0	97	95	42.7	IV	P
2	PF01	13.48	1.0	339	2	1.96	7	5	96	-13	22	96	100	47.9	IV	P
1	PF02	7.64	1	1474	34.67	0	7.40	0	98	-59	0	97	97	36.5	IV	P
2	PF02	5.54	0.4	585	3	9.08	8	0	99	-23	6	92	96	42.1	IV	P
1	PF03	103.71	9	239	18.93	0	8.24	100	67	-7	0	97	87	59.6	III	P
2	PF03	64.58	3.6	54	1	0.85	8	71	85	42	60	97	95	74.4	III	SP

Table 4. In this study, the water quality index value of station ST01 was in class IIB, where the water can be used for recreation. The location of station ST01, which was far from the anthropogenic activity without seawater encroachment, contributed to the value of the water quality index for this station (Dris *et al*., 2016).

The water quality index for stations PF03, ST02, ST03, and ST04 were in class III, where the water requires intensive treatment. According to the report from the Jabatan Alam Sekitar Melaka (2020), severe pollution in Sungai Tuang was due to the release of pig waste around Paya Mengkuang, this made the water no longer suitable for any purpose related to the lives of the surrounding residents and the water quality once declined to Class V in 2014. The water quality index values for stations PF01 and PF02 were in class IV, where the water was only suitable for irrigation purposes. Improper maintenance and inefficient treated pig farm wastewater systems could contribute to poor water quality.

Relationship between water quality and MPs

To explain the general features of the parameters, different data sets were examined based on the sample periods (sampling 1 & sampling 2). The calculated correlation values between the water quality index parameters and microplastics are shown in Table 5. In sediment samples, the correlation test showed that microplastic concentration had a significant positive relationship with COD (r^2=0.96, p<0.05) for the first sampling and did not show any significant relationship with other parameters. However, there was no significant relationship between the water quality parameter and MPs concentration in sediment for the second sampling. According to Kataoka *et al*. (2019), MPs concentration had a positive relationship with COD which was an indicator of river pollution. While in water samples, for both sampling periods, there was no significant relationship that could be proven between water quality parameters and MPs. Nevertheless, only for COD parameter for the first sampling showed a moderate relationship (r^2=0.42, p<0.05) with MPs. The abundance of rain in December diluted the COD concentration, which is why there is variation in these relationships. In particular, MPs' pollution of the freshwater ecosystem had grown most rapidly in polluted rivers with poor water quality status (Kataoka *et al*., 2019).

CONCLUSION

This study found MPs found in all sampling points. The concentration of MPs in pig farms may reflect the unsystematic waste management that contributed to the pollution of MPs. There was a significant correlation between COD and MP concentrations of the sediment samples in this study. Consequently, the data obtained from this study has enough evidence that the pig farms around Paya Mengkuang should be relocated to a new systematic site that provides modern and environmentally friendly facilities. Pig farming requires modern indoor farming and provides a clean water discharge system. Apart from that, it is also a step to respond to the government's aspirations towards achieving the Sustainable Development Goals (SDGs), especially the third goal (health and well-being) and the sixth (clean water and sanitation) by the United Nations, the 2030 agenda.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table 5. Correlation between Number of MPs in Water and Sediment with Water Quality Parameters

Sediment	Sampling 1	Sampling 2	Water	Sampling 1	Sampling 2
		MPs			
MPs	1	1		1	1
DO (mg/L)	-0.62	-0.04		-0.08	-0.58
pH	0.26	0.44		0.27	0.37
BOD5(mg/L)	-0.41	0.19		0.16	-0.33
COD (mg/L)	0.96	-0.05		0.42	-0.09
TSS (mg/L)	-0.44	0.57		-0.28	0.08
NH3-N (mg/L)	-0.04	0.30		-0.67	0.30

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