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Physiological impacts of microplastics, heavy metals, and metallothionein in milkfish (*Chanos chanos*) in Jakarta Bay, Indonesia

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Abstract Several anthropogenic activities are renowned for causing microplastic (MP) pollution and heavy metal accumulation. Therefore, this study aimed to analyze the concentrations of MPs, metallothionein (MT) and heavy metals found in milkfish (*Chanos chanos*) in Jakarta Bay. The samples were taken from five locations, namely, Marunda in North Jakarta, Muara Gembong in Bekasi, Teluk Naga and Pandeglang in Banten and East Lampung in Lampung. Separation and destruction of the gills and the digestive tract of milkfish were carried out via nitric acid (HNO3). The samples were subsequently analyzed to assess the abundance of MPs via light microscopy, and the MT concentration was measured via the MT ELISA Kit (enzyme-linked immunosorbent assay kit). MT analysis was then complemented by an evaluation based on literature studies and assessment of protein interactions via STRING (string-db.org). Heavy metal analysis in milkfish was performed via the inductively coupled plasma (ICP) method. The results revealed that the average number of MP fibers across the five locations was 11.67. In terms of MT, Marunda had the highest protein expression in the gills and digestive tract, with values of 72.56 pg/mg and 245.44 pg/mg, respectively. The observation of lead (Pb) heavy metals suggested that the highest level was found in East Lampung (0.11 mm/kg), whereas Marunda (0.07 mm/kg) had the lowest level. The MT protein is involved in the mechanism of cell stress pathways, indicating the occurrence of severe pollution in Jakarta Bay. Thus, this study provides evidence of pollution in Jakarta Bay by evaluating the MP and heavy metal statuses and offering information regarding the physiological impacts on aquatic organisms. This valuable information is essential for future regulations.

Keywords: heavy metals, microplastics (mps), metallothionein (mt), pb, protein interactions

1. Introduction

Environmental pollution is a global issue caused by various household and industrial waste activities (Lau et al., 2020; Schmaltz et al., 2020). Various types of pollution on land and sea always harm environmental organisms (Hu et al., 2019). In this context, bioaccumulation and biomagnification of pollution significantly occur in aquatic ecosystems. Several dangerous micro- and macropollutants easily settle on water bodies and can have long-lasting impacts on aquatic life (Worm et al., 2017; Germanov et al., 2019). Examples of pollutants in waters resulting from anthropogenic activities include heavy metals from ships in harbors, agricultural fertilizers and plastics (Karapanagioti and Kalavrouziotis 2019).

Plastic has been used for water in daily life for the past 100 years (Cordova et al., 2021). The amount of plastic in the marine environment is estimated to be > 5 trillion or more than 250,000 tons of plastic particles floating on the ocean (Thiele et al.,2021). It can take approximately 20 to 50 years for plastic to be completely decomposed, depending on the type and material that makes it up (Wright et al., 2013). Microplastics (MPs) are the products of plastic decomposition in the form of microsized flakes (<5 mm) (Barboza et al., 2018; Mahamud et al., 2022). They may appear in various forms, including pellets, fibers, fragments, and films, which facilitate their strong endurance so that they may penetrate into the smallest tissues of organisms (Cordova et al., 2021).

Several studies indicate that MPs can be very dangerous when they accumulate in the bodies of organisms, including the gills and intestines of Skipjack tuna fish (Andreas et al., 2021), the distribution of MPs to the blood circulation of zebrafish

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(Qiao et al., 2019), and the transfer of chemicals contained in the MPs from the original organism through predators (Barboza et al., 2018).

MPs can be easily found in various species of marine life; for example, milkfish (*Chanos chanos*) can be exposed to relatively high levels of MPs. Milkfish can be processed into different favorite dishes (Luqman et al., 2021). The digestive tract is a part of the milkfish body that is highly likely to be exposed to MPs. Although Indonesians rarely consume the digestive tract of milkfish, such exposure can affect fish metabolism. MPs usually enter the fish body through two routes, both passively through filters on the gills and actively when the fish digests and consumes prey (Alberghini et al., 2023). These particles are dangerous when consumed, and they may also damage milkfish DNA (Luqman et al., 2021).

In addition to MPs that harm aquatic life, heavy metals also pollute water (Cheung and Cheung 1995). Arsenic (As), cadmium (Cd), lead (Pb), mercury/hydrargyrum (Hg), nickel (Ni) and zinc (Zn) are harmful substances that can accumulate in the bodies of aquatic organisms. These heavy metals have toxic characteristics, can trigger cancer (carcinogenic) and have a biomagnification effect (Yap et al.,2004). Pollution by heavy metals such as Hg, Pb, and Cd potentially causes poisoning when consumed and has neurotoxic characteristics. Pb also influences all organ systems in an organism (Filipović Marijić et al.,2023), such as nerves, blood circulation and the heart. Exposure to heavy metals, such as Pb, can significantly affect child growth and development. The consumption of marine biota, such as fish, shrimp, shellfish, sea cucumber and other animals, can be dangerous when the coastal sea in an area is polluted by heavy metals (Scutarașu and Trincă 2023).

A prominent natural biomarker that has been used to detect the presence of heavy metals in marine biota is the metallothionein (MT) protein (Strogyloudi et al., 2021), which is a short-chain polypeptide with 61–68 amino acids. MT proteins were discovered in 1960 and are effective biomarkers (Hertika et al., 2021). These proteins serve as a defense mechanism in several organisms and are encoded by MT genes that bind heavy metals to peptides. According to various studies, MT is present in almost all marine biota, specifically in several cells and tissues, such as the intestinal or digestive tissue and the gills of fish, which are the first locations exposed to heavy metals.

The impacts of MP and heavy metal exposure on organisms can be dangerous at certain levels and times. Therefore, this study aimed to analyze the characteristics of MPs, Pb levels, and MT contents in milkfish. This type of fish was selected because of its widespread consumption among Indonesians. This study focused on data limitations regarding MP and Pb detection in milkfish from Jakarta Bay.

2. Materials and Methods

2.1. Collecting and preparation

Milkfish (*Chanos chanos*) with the age criterion of 5 to 6 months were collected from five farms in northern Jakarta and Lampung. The detailed locations include Marunda in North Jakarta, Muara Gembong in Bekasi, Teluk Naga in Tangerang, Labuan Pandeglang in Banten and Labuhan Maringgai in East Lampung, Sumatera. The fish gills and digestive tracts were then stored in a -20°C freezer, and the average weight of the five samples from the five locations was 248.32 grams. The average length of each sample was 31.84 cm. The sample was subsequently prepared and analyzed according to the procedures presented in Figure 1. The sample was collected in several locations that are shown in Figure 2.

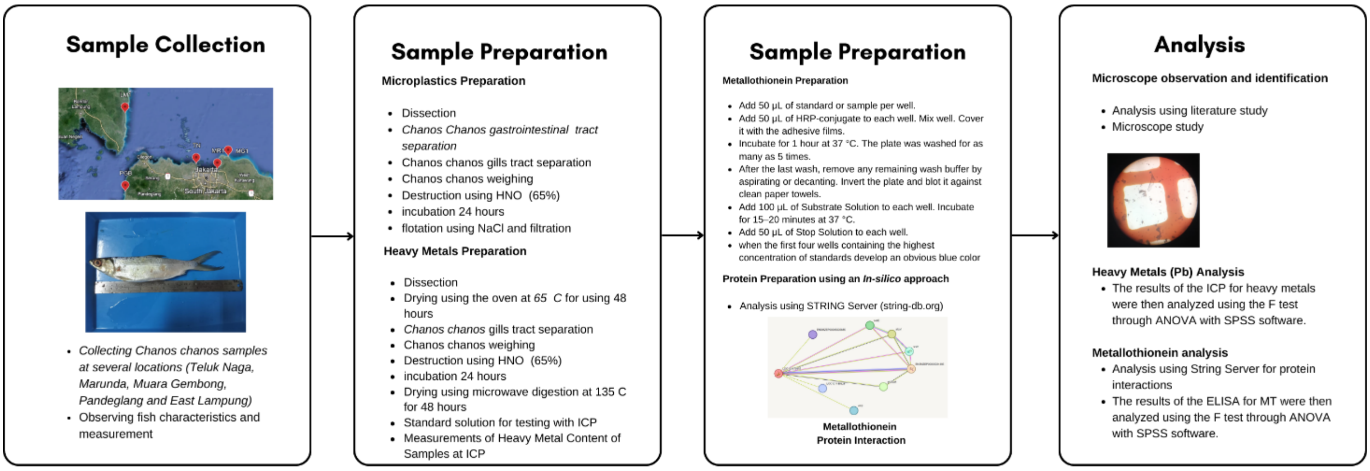

Figure 1 Research flow of milkfish microplastic sample preparation and analysis.

2.2. Extraction and observation of MPs

Gills and digestive tracts were extracted from each milkfish sample. The samples were then destroyed with 65% HNO3 at a ratio of 1:10 for the gross weight of the gills and digestive tract. The mixture was left for 72 hours, and concentrated NaCl solution was added at a ratio of 1:4. The concentrated salt solution was allowed for the flotation of MPs via density separation.

The water samples were filtered a second time with a sieve net of 5 mm mesh size, and the sediment was dried in an oven until all the water content was lost. A concentrated NaCl solution was used to achieve MP flotation on the filtered water sample at a ratio of 1:3 water volume (mL) against the volume of NaCl solution (mL) (Priscilla and Patria 2019).

Figure 2 Location of sample collection. Marunda (MR1) in North Jakarta, Muara Gembong (MG) in Bekasi, Teluk Naga (TN) in Tangerang, Labuan Pandeglang (PGB) in Banten and Labuhan Maringgai (LM) in East Lampung, Sumatera.

The samples from the gills and digestive tract were left for 24 hours to allow for density separation, and then 20 mL was removed from the surface of each sample and transferred to Erlenmeyer flasks for homogenization. After homogenization, a representative 1 mL sample was taken from each Erlenmeyer flask and transferred to a Sedgwick Rafter Chamber to count the MPs. The samples were observed with a light microscope. The visualization of MPs via dark color in gill tissues was performed. The color was visualized via a staining microscope using Harris hematoxylin and then blocked with paraffin.

2.3. Heavy metal (Pb) preparation

The samples were cleaned with distilled water and dried in an oven at 65°C for 48 hours; then, they were crushed using a mortar and pestle. The samples were placed into clean vessels for metal destruction, and 2.5 ml of 65% nitric acid (HNO3) and 5 ml of distilled water were added. Subsequently, microwave digestion was carried out for 40 minutes at 135°C, followed by cooling. In preparation for testing, the samples were dissolved in distilled water to a final volume of 50 mL in test tubes. To prepare standard solutions for inductively coupled plasma (ICP) testing, 0.5 mL of a 1000 ppm stock solution was diluted to 50 mL with 2% HNO3 and mixed until homogeneous.

Calibration curve solutions were prepared by pipetting 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the standard solution into 50 mL volumetric flasks and diluting to the mark with 2% HNO3. The samples were then made into standard solutions with concentrations of 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 ppm. For the ICP–MS measurements, the ICP was turned on for at least 30 minutes before use, and argon gas was introduced. The computer was turned on, and the TEVA software was opened. A calibration curve was generated using the standard solutions. After verifying the calibration curve, sample measurements were performed. A method for measuring Pb levels was created on a computer for heavy metal analysis via ICP (Alina et al.,2012).

2.4. MT analysis

The fish MT ELISA Kit (enzyme-linked immunosorbent assay kit) was used according to the instructions in the product package Abebio No. AE32620FI. Initially, the reagent, sample, and standard solutions were prepared. Approximately 50 µL of standard or sample was added to each well, along with 50 µL of HRP conjugate. The mixture was homogenized using adhesive films and incubated for 1 hour at 37°C. The plate was washed 5 times, and after the last wash, any remaining wash buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels, and 100 µL of substrate solution was added to each well, followed by incubation for 15–20 minutes at 37°C. Approximately 50 µL of Stop Solution was then added to each well. A positive result was indicated when the first four wells containing the highest concentration of standards developed an obvious blue color (Adam et al., 2019).

2.5. MT protein interactions

The MT protein was analyzed via an in-silico approach via the String server (https://string-db.org/). STRING analysis was performed to examine the interaction of the MT protein with other proteins in fish cells. Protein interaction analysis using strings includes full-string network type, evidence of network edges, a medium confidence level for interaction scores and active interaction sources with text mining, neighborhoods, experiments, gene fusion, databases, coexpression, and co-occurrence. The maximum number of interactors was then displayed with no more than ten interactions.

2.6. Statistical analysis

The data on microplastics (MPs) and lead (Pb) in the digestive tract and gills of milkfish (*Chanos chanos*) were analyzed via one-way analysis of variance (ANOVA) with a significance level of 5%. The between-group variables were the variations or differences in the mean MP, Pb, and MT levels among the different groups (in this case, 5 different locations), and the within-group variables were the variations or differences in the MP, Pb, and MT levels within the same group. Metallothionein (MT) levels in the gills and digestive tract were also subjected to ANOVA to identify significant differences between these tissues. Additionally, Spearman's rank correlation was employed to evaluate the relationships among Pb, MT, and MPs.

3. Results and Discussion

3.1. Extraction and observation of MPs

MPs were detected in milkfish across five locations: Marunda, Muara Gembong, Teluk Naga, East Lampung, and Pandeglang. The types of MPs found included fibers, fragments and films, which were found in each sample obtained from the gills and digestive tract of milkfish can be found in Figure 3.

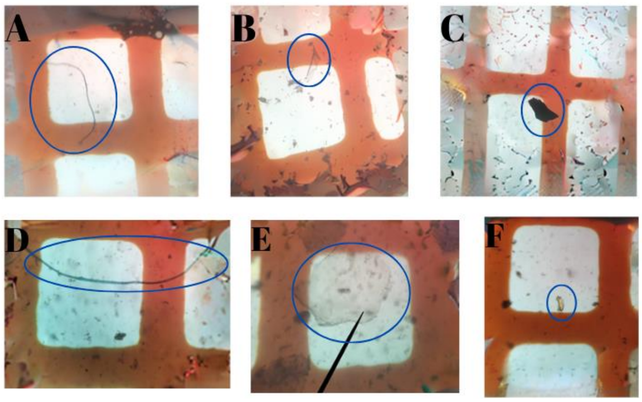

Figure 3 Types of MPs in the gill and digestive tract of milkfish obtained from all locations. A: Fiber in gills, B: film in gills, C: fragment in gills, D: fiber in digestive tracts, E: film in digestive tracts, F: fragment in digestive tracts.

The results of observations via a Nikon light microscope revealed many MP types, including fibers, films and fragments. Fiber was the dominant type, with an average of 11.67, followed by film and fragments, with averages of 9.71 and 1.71, respectively. However, the pellet type was not found in this study.

Figure 4 shows that fiber was the most common MP type found in Jakarta Bay, amounting to 954. This mixture was followed by fragments and films with amounts of 1001 and 162, respectively. The results for the control area were also similar to those for the samples from Jakarta Bay, in which the most common form of MPs was fiber, totaling 748, followed by fragments and films of 497 and 92, respectively.

The contents of MPs measured across the five locations were analyzed, and the results are presented in Figure 5. The number of MPs found in the gills of milkfish obtained from Marunda, Muara Gembong, Teluk Naga, East Lampung, and Pandeglang was 27.67, 23.00, 14.87, 8.67 and 21.20, respectively. Moreover, 22.60, 21.66, 21.33, 28.07, and 21.20 MPs were detected in the digestive tract, respectively. The highest MP level was found in March, with an average value of 50.26; the lowest was recorded in Pandeglang, with an average value of 42.40.

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ANOVA (analysis of variance) of the MP content in gills in Table 1, Table 2, Table 3, Table 4, Table 5 and Table 6 revealed p values of 0.849, 0.114, and 0.203 for fiber, film, and fragment types, respectively. However, there were no significant differences in the types of MPs found in the gills across the five locations. ANOVA of MP levels in the digestive tract revealed p values of 0.000, 0.7344, and 0.0038 for fibers, films, and fragments, respectively. The results suggested significant differences in the number of fibers and fragments, whereas the number of fibers and fragments in the film did not significantly differ.

The results of the ANOVA test on the MP level in the intestine generated a p value. The fiber type was 0.000, the film type was 0.7344, and the fragment type was 0.0038. The results suggested a significant difference in the number of MPs in milkfish intestines between fiber and fragment types across the five locations. The MP content in the intestine was not significantly different among the film types.

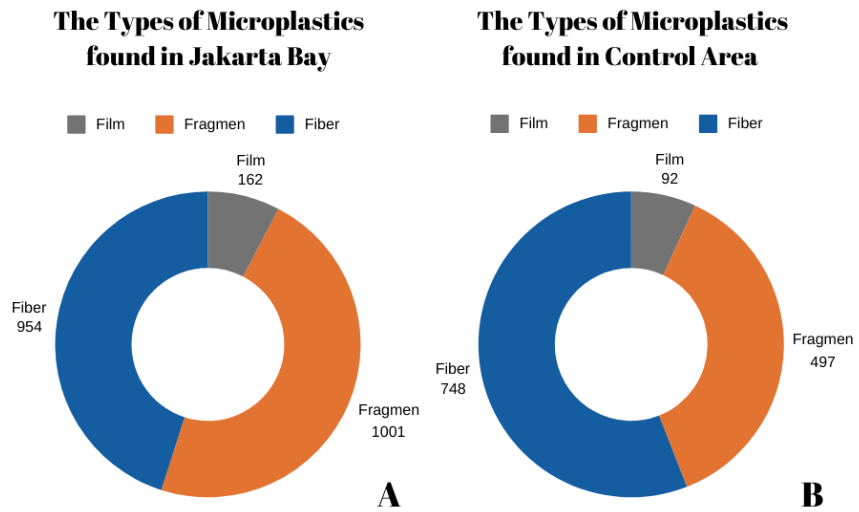

Figure 4 The MP types found in jakarta bay and the control area. A. Jakarta bay area (Teluk Naga, Marunda, Muara Gembong). B. Control area (East Lampung & Pandeglang).

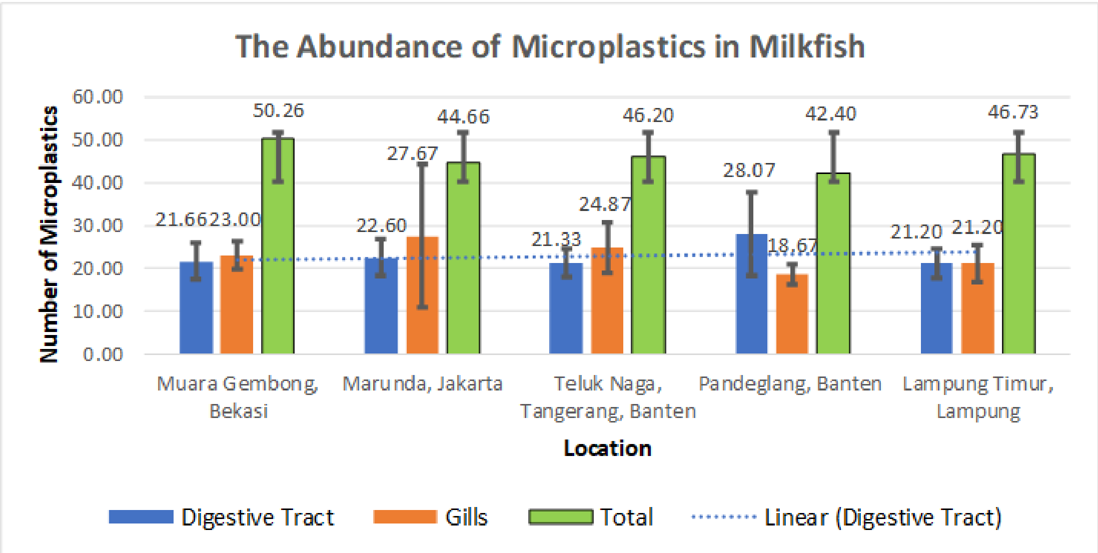

Figure 5 The number of MPs in milkfish from several locations.

Table 1 Analysis of variance (ANOVA) of MP abundance with fiber type in milkfish gills.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.290	4	6.322	.338	.849
Within Groups	374.475	20	18.724		
Total	399.765	24			

Table 2 Analysis of variance (ANOVA) of MP abundance with film type in milkfish gills.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	267.524	4	66.881	2.136	.114
Within Groups	626.328	20	31.316		
Total	893.853	24			

Image analysis via hematoxylin staining was used to identify the presence of MPs inside and around the intestinal epithelial tissue. The staining revealed the histological structure of the intestinal tissue in fairly straightforward detail, including the lumen and the surrounding epithelial layer. MPs were visualized as small, slightly light-colored particles spread in various locations in the tissue. Figure 6 shows several MPs detected around the intestinal epithelium and lumen. The particles have different forms and sizes, indicating that milkfish consume multiple types of MPs from the environment.

Table 3 Analysis of variance (ANOVA) of MP abundance with film type in milkfish gills.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.407	4	1.602	1.642	.203
Within Groups	19.509	20	.975		
Total	25.917	24			

Table 4 Analysis of variance (ANOVA) of MP abundance with fiber type in milkfish digestive tracts.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	489.260	4	122.315	13.140	.000
Within Groups	186.165	20	9.308		
Total	675.426	24			

Table 5 Analysis of variance (ANOVA) of MP abundance with film type in milkfish digestive tracts.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	28.976	4	7.244	.503	.734
Within Groups	287.803	20	14.390		
Total	316.779	24			

Table 6 Analysis of variance (ANOVA) of MP abundance with fragment type in milkfish digestive tracts.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	8.627	4	2.157	3.121	.038
Within Groups	13.822	20	.691		
Total	22.450	24			

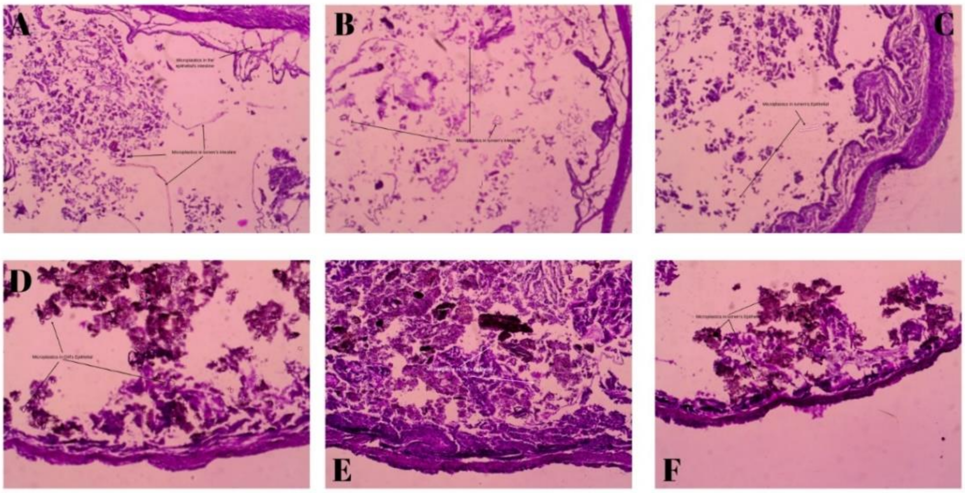

Figure 6 The staining of MPs in the digestive lumen. Preparations were stained with Harris hematoxylin. There are various types, numbers, and sizes of MPs in each preparation. 6A-6C show MPs in digestive lumen tissue, and there was a film and fragment of MPs. 6D-6F show MPs in gill tract epithelial tissues. Fragments, films, and fibers are found in gill and digestive tract tissues.

3.2. Heavy metals (Pb)

The level of Pb was observed in milkfish collected from the five locations. The average Pb concentrations in Marunda, Muara Gembong, Teluk Naga, East Lampung, and Pandeglang were 0.07 mg/kg, 0.80 mg/kg, 0.09 mg/kg, 0.11 mg/kg, and 0.09 mg/kg, respectively. As shown in Table 7, the highest Pb concentration was found in East Lampung, at 0.11 mg/kg, whereas the lowest was recorded in Marunda, at 0.07 mg/kg. Furthermore, the one-way ANOVA test generated a p value of 0.64, indicating that the Pb content in milkfish was not significantly different. The Pb concentrations obtained from the five study locations were still below the threshold.

Table 7 indicates that the highest lead (Pb) concentration, 0.114 mg/kg, was detected in Lampung Timur (East Lampung), Lampung. The lowest lead concentration, 0.072 mg/kg, was found in Marunda Jakarta. The one-way ANOVA test results. In Table 8 it generated a P value of 0.645, which indicates that the metal content in the milkfish meat from the five locations was not significantly different. The Pb concentration in the milkfish from the five research locations was still below the threshold.

Table 7 Heavy metal (Pb) concentrations in milkfish.

No	Location	Pb Mean (mg/kg)	Size Mean (length: cm)
	Marunda, Jakarta	0.07	33.4
	Muara Gembong, Bekasi, West Java	0.08	31.6
	Teluk Naga, Tanggerang, Banten	0.09	31.6
	Lampung Timur, Lampung	0.11	34.4
	Pandeglang, Banten	0.09	28.2
	Maximum Threshold (BPOM, 2022)	0.30	

Table 8 Analysis of the variance in heavy metal (Pb) content in milkfish digestive tracts.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.006	4	.002	.632	.645
Within Groups	.048	20	.002		
Total	.054	24			

3.3. MT Protein Interactions

Figure 7 (LOC101479115) shows that some MT interacts with several proteins via the STRING database. These interactions are related to the mechanism of heavy metal binding and detoxification. Moreover, the interaction of MT with superoxide dismutases (Sod1 and Sod3) indicated protection against oxidative stress.

The antioxidant 1 copper chaperone (Atox1) protein with a solute carrier (Slc30a4) was also identified. Both proteins play crucial roles in the regulation of metal ions. Other proteins were VRK3 (LOC101486928) and melatonin receptor (ENSMZEP00005005885), which suggested the interaction of signaling pathways and cell regulatory functions.

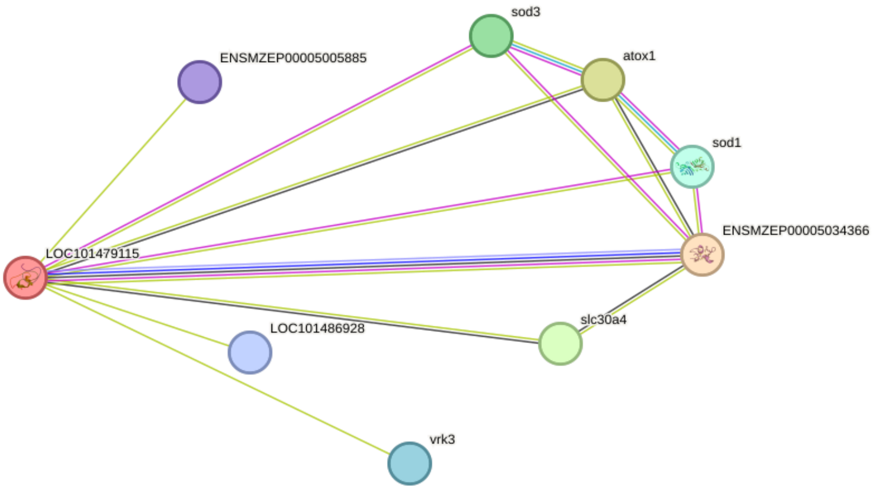

Figure 7 Interactions between MT and protein in fish species.

3.4. MT analysis

MT analysis was conducted via the KIT MT, and the results for the five locations are presented in Figure 8. The levels of MT in the gills and digestive tract of milkfish samples obtained from Marunda, Muara Gembong, Teluk Naga, East Lampung, and Pandeglang were 72.56 pg/mg and 245.44 pg/mg protein, 80.24 pg/mg and 153.55 pg/mg protein, 92.94 pg/mg and 202.804 pg/mg protein, 58.85 pg/mg and 130.69 pg/mg protein, and 100.20 pg/mg and 224.091 pg/mg protein, respectively.

The highest MT level was found in the gills of milkfish from Marunda, with an average of 245.44 pg/mg protein, whereas the lowest was recorded in East Lampung at 58.85 pg/mg protein. One-way ANOVA in Table 9 – Table 10 generated a p value of 0.77 for the gills and 0.41 for the digestive tract. These results indicate that the MT levels in milkfish gills and digestive tracts from the five locations were not significantly different.

The Spearman Rho correlation test results in Table 11 reveal a weak and insignificant correlation between MP levels and MT concentrations in milkfish. Table 12 & Table 13 show a negligible correlation between the levels of MTs-MPs and the concentration of Pb. The results of the analysis revealed a significant interaction between MPs in the gills and those in the digestive tract. However, there was no significant correlation with lead or lead (Pb) levels.

4. Discussion

In general, MPs can be found in various types of marine biota, especially those close to human anthropogenic activities (Rubio-Armendáriz et al.,2022). This phenomenon is particularly true on the northern coast of Jakarta, which is recognized as an industrial area. One of the marine biota that is commonly consumed by people is milkfish. In general, milkfish inhabiting coastal regions in Jakarta are at greater risk of MP exposure than those inhabiting other areas are (Yona et al., 2019). In terms of form, MPs can be differentiated into sheets, films, line/fiber pellets/granules, fragments and foams (Utami et al., 2021). As indicated in Figure 3, this study revealed only three types of MP particles in milkfish: fibers, films, and fragments. The three types were found in the gills and digestive tract. Fibers were the most dominant type, followed by films and fragments (Buwono et al., 2021).

The MP fiber type was successfully observed via a light microscope at a magnification of 10x10. Figures 3A and 3D clearly show fiber-type MPs in milkfish gills and digestive tract cross-sections with long, thread-like lines (Hariharan et al., 2021). The fiber/line type is generally found from degradation in the textile industry. Several studies, such as De Falco, have suggested that 5 kg of clothing made from polyester fibers could emit 6 million microfiber particles (De Falco et al., 2018). Another possible origin is damaged and degraded fishing nets in the sea (Liu et al., 2022).

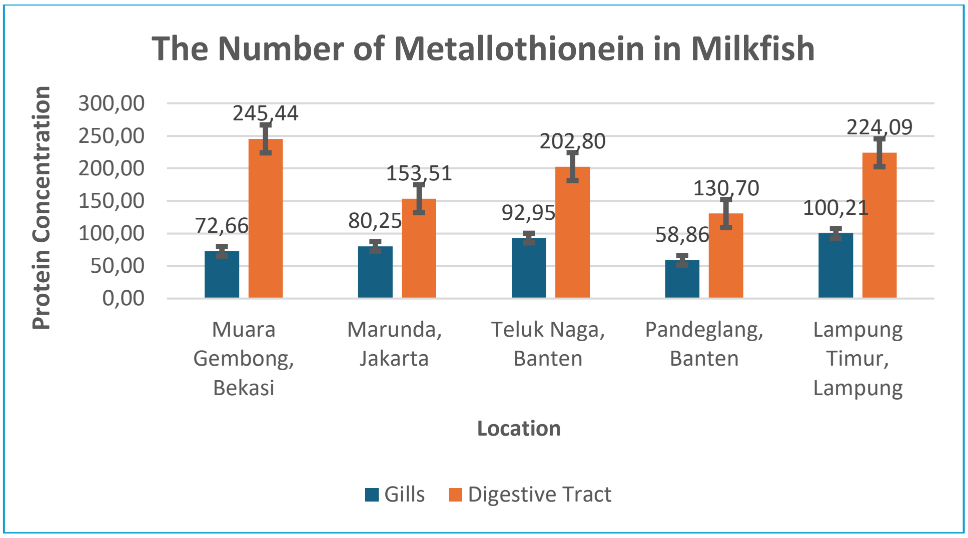

Figure 8 The number of MTs found in milkfish species obtained from five locations.

Table 9 Analysis of variance in mt in milkfish gills.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5360.898	4	1340.225	.441	.777
Within Groups	60772.113	20	3038.606		
Total	66133.011	24			

Table 10 Analysis of variance in MT in milkfish digestive tracts.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46197.813	4	11549.453	1.033	.414
Within Groups	223520.658	20	11176.033		
Total	269718.471	24			

Table 11 Spearman rho analysis of MPs with different MT concentrations.

		MP Gills	MP Digest	MT Gills	MT Digest
MP Gills	Correlation coefficient	1.000	-0.436	0.226	-0.045
	Sig. (2 tailed)	.000	.0290	0.277	0.829
MP digest	Correlation coefficient	-0.436	1.000	-0.113	0.232
	Sig. (2 tailed)	.0290	.000	0.591	0.265
MT Gills	Correlation coefficient	-.045	0.232	0.255	1.000
	Sig. (2 tailed)	0.829	0.265	0.218	0.000
MT digest	Correlation coefficient	0.226	-.113	1.000	0.255
	Sig. (2 tailed)	0.277	0.591	0.000	0.218

The MP fiber type was successfully observed via a light microscope at a magnification of 10x10. Figures 3A and 3D clearly show fiber-type MPs in milkfish gills and digestive tract cross-sections with long, thread-like lines (Hariharan et al., 2021). The fiber/line type is generally found from degradation in the textile industry. Several studies, such as De Falco, have suggested that 5 kg of clothing made from polyester fibers could emit 6 million microfiber particles (De Falco et al., 2018). Another possible origin is damaged and degraded fishing nets in the sea (Liu et al., 2022).

Table 12 Spearman rho analysis of MT digest and MT gills with lead (Pb).

		MT Gills	MT Digest	Plumbum (Pb)
MT Gills	Correlation coefficient	1.000	0.255	-0.176
	Sig. (2 tailed)	.000	0.218	0.399
MT digest	Correlation coefficient	0.255	1.000	0.041
	Sig. (2 tailed)	0.218	.000	0.847
Plumbum	Correlation coefficient	-0.176	0.041	1.000
	Sig. (2 tailed)	0.399	0.847	.000

Table 13 Spearman rho analysis of MT digest and MT gills with lead (Pb).

Spearman rho			MPs Gills	MPs Digest	Plumbum
	MPs Gills	Correlation coefficient	1.000	-.436*	0.043
		Sig. (2 tailed)	0.000	0.029	0.840
	MPs digest	Correlation coefficient	-.436*	1.000	0.202
		Sig. (2 tailed)	0.029	0.000	0.332
	Plumbum	Correlation coefficient	0.043	0.202	1.000
		Sig. (2 tailed)	0.840	0.332	0.000

Figures 3B and 3E show the results of MP film-type analysis of the gills and digestive tract preparations. The film type is thin and transparent (Gan et al., 2023), which is usually produced from plastic bag degradation (Wright et al., 2013). Each day, plastic bags naturally degrade in the environment and form film-type MPs. Figures 3C and 3F show fragment-type MPs in gills and digestive tract preparations. As shown in Figure 4, the fragment type was the least common type of MP found in the study locations. This result is attributed to polymer degradation, which is quite dense and takes a longer time than other polymers do. Fiber- and film-type MPs have a long degradation rate, and they have a reasonably high density, thereby allowing exposure to marine biota.

The histological results of the milkfish preparation images revealed that MPs are present in the digestive tract of milkfish. Figures 6A-6E present strong evidence that MPs were found in the digestive tract lumen. This figure indicates accumulation that reaches the digestive muscle tissue of the milkfish (Deng et al., 2017; Espinosa et al., 2018). The findings of MPs around the intestinal epithelium suggest that these particles can directly interact with epithelial cells and potentially cause cellular stress or tissue damage. Furthermore, the presence of MPs in the intestinal lumen indicates that these particles can pass through the digestive tract without becoming trapped (Deng et al., 2017). The presence of MPs revealed that milkfish in the studied locations were significantly exposed to contamination.

In addition to MP exposure, heavy metal exposure is a threat to marine biota. Figure 8 shows the MT concentration in Marunda, which was found to be the highest compared with the other locations. Moreover, the MT level in East Lampung was lower than those in the other regions. These results align with other studies suggesting that heavy metals have polluted Jakarta water (Cordova et al., 2016; Putri et al., 2018; Suratno et al., 2020; Rusdi et al., 2021). Lead (Pb) has also contaminated several locations in Jakarta Bay, such as Kepulauan Seribu; however, the concentration is still below the standard threshold.

Table 7 shows the lead (Pb) levels found in milkfish across several locations. The highest level was found in samples from East Lampung, at 0.114 mg/kg. However, this level is still below the standard threshold set by the National Agency of Drug and Food Control of Indonesia, 0.3 mg/kg. The lowest Pb level was found in Marunda, at 0.072 mg/kg, primarily because of several factors, such as milkfish being obtained from managed ponds instead of sea ponds. Despite the low Pb level in Marunda, the MT level at this location was the highest, as indicated in Figure 8. Other studies have also shown that Jakarta Bay is exposed to Pb pollutants and other heavy metals, such as Cd, Hg, Ni, and Zn (Yap et al., 2004; Aifin et al., 2012; Priscilla and Patria, 2019). MT is expressed in response to Pb and other heavy metals on the basis of interactions with STRING (string-db.org).

According to a previous report, MT proteins can function as biomarkers of heavy metal binding (Kadim and Risjani, 2022). ELISAs revealed that the MT protein was expressed in the gills since they are the first organ implicated in fish respiration and osmoregulation. Several studies have also shown that the accumulation of Pb in gills indicates MT expression (Richardson et al.,2008). In this case, gills play a role in the first homeostatic pathway in milkfish. Figure 7 also shows that MT expression in the digestive tract is greater than that in the gills. A previous study (Sujitha et al., 2017) described heavy metals that enter the body through fish food (Sujitha et al., 2017). Heavy metals accumulate in the gills, digestive tract and kidneys. This finding also indicates that heavy metals, namely, Pb, accumulate in the digestive tract. This study revealed that MT acts as a self-defense mechanism in milkfish.

Table 11 shows the results of the interaction between MPs in the gills and digestive tract compared with those in the MT, whereas Table 12 shows a weak correlation with the Pb level. As shown in Table 13, there was a significant correlation between MPs in the gills and those in the digestive tract. However, there was no strong correlation between Pb and MPs in either organ. These results indicate that the Pb concentrations in milkfish from Jakarta Bay are within safe limits. The results also revealed that MPs had no significant effect on MT proteins, suggesting that milkfish are safe for consumption.

Interaction analysis via the STRING server (string-db.org) suggested that MT proteins contain cysteine residues. Proteins are important for heavy metal homeostasis and detoxification, and they interact directly with melatonin receptors (Zalewska et al., 2014). For example, melatonin receptors such as ENSMZEP00005005885 and LOC101486928 are included in the G-protein coupled receptor 1 (GPCR-1) family category. These receptors play a role in the central nervous system and the regulation of heavy metals (Kalyan et al., 2022).

The interaction between MT and superoxide disismutase (SOD1 and SOD3) converts superoxide radical catalysts into oxygen and hydrogen peroxides and protects against oxidative damage. In this case, the MT interaction can regulate the availability of copper and zinc ions in cells. MT and solute carrier family 30 member 4 (SLC30A44) interact in Zn regulation. Another interaction involves VRK serine/threonine kinase 3 (VRK3). This interaction could protect cells from free-radical-related damage (Zalewska et al., 2014). Therefore, the expression of MT indicates protection for cells in milkfish gills and the digestive tract.

5. Conclusion

In conclusion, MPs and heavy metals, such as Pb, threaten marine biota, including milkfish, in Jakarta Bay. The results revealed that MP types of fibers, films and fragments were found in milkfish gills and digestive tracts, with fibers being more dominant than the other MP types. Moreover, the highest MT concentration was found in Marunda, indicating a high level of heavy metal pollution at this location; however, the Pb levels remained within the set threshold. The interaction between MT proteins and other molecules suggests a role for heavy metal homeostasis and protection against oxidative damage. These results underscore the need for action to reduce MP and heavy metal pollution to protect aquatic ecosystems and marine biota in these areas.

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Ethical Considerations

All process during this research were carried out according to ethical approval from Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah, Jakarta, No: B-046/F12/KEPK/TL.00/9/2022.

Conflicts of Interest

All of the authors declare that there are no conflicts of interest associated with this research.

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