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Microplastics with Heavy Metals Pollution in Water Supply of *Litopenaeus Vannamei* Aquaculture in Probolinggo, Indonesia

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ABSTRACT

The quality of the water supply for aquaculture determines the success of vanamei shrimp farming. The presence of heavy metals and microplastics in aquaculture waters are hazardous pollutants that pose a toxicological risk to aquatic organisms and the aquatic environment. The study aimed to determine the heavy metal content and abundance of microplastics in the water supply, as well as to determine the correlation between water quality factors with heavy metals and microplastics. Data collection was carried out through sampling at five locations determined based on close to Vannamei ponds and in the vicinity of settlements, tourism activities, and industrial activities criteria. The ICP-OES technique was used to measure heavy metal concentrations. NOAA standard techniques were used to identify and quantify the number of microplastics. One-way ANOVA and correlation tests were used for data analysis. The results of heavy metal measurements of Hg were 0.00001–0.0000133 mgL^{-1}, Pb ranged from 0.015–0.06 mgL^{-1} and Cd ranged from 0.003–0.008 mgL^{-1}. The abundance of fragment-type microplastics was dominant compared to other types of microplastics was 733.33 particles/m^3. Variance testing for microplastic fragment types differed significantly ($p<0.1$) between sampling points. Heavy metal concentrations between sampling points are not statistically different ($p>0.1$) and the results of correlation analysis show temperature correlates with heavy metal Cd. Temperature (30–32°C), pH (7.5–7.6), dissolved oxygen (5.53–7.23 mgL^{-1}), salinity (16.33–28.33 ppt), and total suspended solid (37–48.33 mgL^{-1}) are the physicochemical parameters that measure and has not exceeded the established quality standards.

Keywords: heavy metals, microplastic, shrimp, pollution, coastal.

INTRODUCTION

Shrimp farming is a viable business since shrimp is a high-demand export item with relatively constant prices. *Litopenaeus vannamei* is a major aquaculture commodity in Indonesia, accounting for the most foreign exchange earnings with oil and gas (Sitompul et al., 2018). The development of shrimp cultivation is in line with the high demand and price of shrimp in the international market so the intensification of the vanamei shrimp cultivation system is being carried out (Ahmed et al., 2018; Emerenciano et al., 2022). The success of aquaculture activities is greatly influenced by the water quality conditions used for these activities. Water is an unavoidable medium in aquaculture; hence water quality plays an important role in fish farming operations due to its various interacting effects on the productivity of farmed animals (Jana & Sarkar, 2005). The availability of water in the environment today has experienced pollution from organic matter, heavy metals, pesticides, and other pollution caused by anthropogenic activities in the aquatic environment such as microplastics so its quality has decreased for aquaculture activities (Wicaksono, 2022).

Heavy metals are substances that are difficult to eliminate, which is one of the causes of water pollution and accumulate for a long time in the bodies of organisms (Briffa et al., 2020; Lambert et al., 2000). Heavy metals are poisonous, difficult to decompose, and have a lengthy residual duration, making them a potential hazard to aquatic ecosystems (Zhang et al., 2023). Heavy metals that enter the water column over time will go down to the bottom of the water affecting organisms such as shellfish, crabs, and shrimp that look for food at the bottom of the water (Chan et al., 2021). This creates the opportunity for heavy metals to enter the organism's body. Many marine organisms (fish, shrimp, and crabs) are at the highest point of their natural food source and aggregate high amounts of metals from water and sediment (Yousif et al., 2021). Metal concentrations in aquatic organisms are several times greater than in water (Mitra et al., 2012). Crustaceans contaminated with heavy metals from the aquatic environment can be dangerous to human health. As a result, estimating heavy metal buildup is critical for this organism community sector.

Microplastics with a size of 1µm – 5 mm hurt aquatic organisms and ecosystems. Microplastics are fixed in surface water, beach sand, freshwater sediments, seawater, and the deep sea (Sandra & Radityaningrum, 2021). The presence of microplastics in water is a hazardous pollutant that poses a toxicological risk to aquatic organisms that consume these pollutants (W. Wang & Wang, 2018). Microplastics threaten the aquatic food chain by affecting organisms from lower trophic levels (Dewi et al., 2015). The presence of MP in the biota can also further have negative effects on humans and other biota in the food chain (Browne et al., 2011). Microplastics consumed by aquatic organisms can impair growth, and (Tongnunui et al., 2022)survival health caused by plasticizers, colorants, and chemical processes of microplastics that can accumulate hydrophobic toxins from the environment (Mani et al., 2015). Microplastic toxicity arises due to plastic additives such as phthalates and bisphenol A (BPA) that are applied to plastics during processing to improve plastic consistency (Zanolli, 2019). Additives in plastics can harm organisms by disrupting the body's metabolic and hormonal functions (Lee et al., 2019). Microplastics can affect shrimp, related to genetics, reproduction, populations, and abundance, and may pose risks to human health (Tongnunui et al., 2022).

The availability of water that can be used for shrimp farming activities is often found to be hampered by pollution due to industrial waste or increased plastic waste, settlements, and tourism. Source water quality standards for shrimp farming need to be examined, especially in coastal areas because in general, the raw water source used for ponds comes from coastal areas. Water quality used in ponds is correlated with vanname shrimp farming, because if the water quality in the pond is considered good then shrimp and aquatic biota ponds will develop well (Ariadi et al., 2020; Harlina et al., 2022). If the water quality is not maintained, the biota in the pond will be affected. For example, in shrimp ponds, unsuitable water quality will affect shrimp and cause shrimp stress, and reduced appetite, which prevents them from consuming food and turns into organic waste that can settle and damage the pond aquaculture ecosystem The reason is an important concern and needs to be researched and analyzed about the content of heavy metals and microplastics in water sources used to supply vaname shrimp ponds. In addition, it is important to determine the concentration of heavy metals and microplastics in commercial fish and shrimp to evaluate the possible risk of human consumption.

The study aimed to analyze the heavy metal content and the abundance of microplastics in water in coastal waters used as a water supply shrimp ponds. In addition, to determine the relationship of heavy metals and microplastics with supply water quality factors of shrimp ponds. It is reasonable to suspect the contamination of heavy metals and microplastic in the water supply of vannamei aquaculture so as not to exacerbate environmental events and potential risks.

MATERIAL AND METHODS

Study sites

The research was conducted on the coast of Probolinggo, East Java, which is an area dominated by vannamei shrimp ponds. The study was conducted between April and June of 2023. The study used sampling points consisting of 5 stations determined based on several criteria, namely close to vannamei ponds and in the vicinity of settlements, tourism activities, and industrial activities (Fig. 1) with descriptions of each respectively in Table 1. Sampling and determination of sampling points are done using the purposive sampling method in which 5 stations were selected to represent the area that became the point of water source for shrimp ponds with a sampling frequency of three times.

Measurement of heavy metals

Measurements were made to determine the levels of heavy metals Hg, Pb, and Cd in water using the ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) verification method. ICP-OES is a new excitation source atomic spectroscopy technique in the form of plasma that gives rise to alternative analytical techniques for determining heavy metal levels that are generally present in the aquatic environment (Rinawati et al., 2008). ICP-OES can perform simultaneous analyte measurements, with high sensitivity, with low analyte detection limits up to ppb units. In addition, measurements can be made quickly and easily (Pirdaus et al., 2018). Water samples were taken as much as 150 ml and given HNO_3 as much as 1 ml then the results can be read on the ICP-OES tool. Each water sample was measured using ICP with heavy metal levels determined by the linear regression equation of the standard solution calibration curve.

Sampling and microplastics identification

Microplastics were collected from water samples in the water supply of vaname shrimp farming pond. Microplastics were identified and quantified in water samples using the National Oceanic and Atmospheric Administration (NOAA) standard 2015 (Hidalgo-ruz et al., 2012). A Plankton net with a 25µm mesh size was used to collect microplastics from water samples. Water samples were then dried in an oven at 80°C for 24 hours (Hidalgo-ruz et al., 2012). The samples were dried and then placed in a solution of 2 ml of 30% H_2O_2 and 6 g NaCl/20 ml and allowed to stand for 24 hours and then incubated using a water bath at 80°C. Then the sample was filtered using Whatman paper number 42 assisted by a vacuum pump. Microplastic types were determined using a microscope with 100-fold magnification (Olympus CX-23 LED Lighting System tem, Light-Emitting Diode). The abundance of microplastic particles for water samples was calculated in particles per m^3 (Masura et al., 2015; Tongnunui et al., 2022).

Figure 1. Research location of heavy metal and microplastic samples in Probolinggo Coastal

Measurement of water quality parameters

Measurement of environmental parameters or water quality parameters was carried out in situ and ex-situ. The parameters measured were temperature (°C), pH, dissolved oxygen (DO) (mg L^{-1}), salinity (ppt), and total suspended solid (TSS) (mg L^{-1}). Temperature and DO measurements were carried out using a DO meter. pH was measured using a pH meter, for salinity using a refractometer while TSS with the gravimetric method. Water quality parameters are tested using the Indonesian National Standard (SNI) measurement method (Tanjung et al., 2019).

Statistical analysis

In this study, differences in heavy metal concentration and abundance of microplastics at each station were analyzed using One-Way ANOVA. Before ANOVA, homogeneity and normality tests were performed. In addition, correlation analysis between heavy metals and water quality and microplastics and water quality was also conducted to determine the closeness of the relationship between the variables. The complete data analysis using R software by R Core 2021 Team.

RESULT AND DISCUSSION

In this study, the concentrations of heavy metals Hg, Pb, and Cd in the water supply of vannamei ponds can be seen in Figure 2. Heavy metals Hg, Pb, and Cd are the focus of research in coastal environments including the Probolinggo region because of the source of heavy metal pollution from various sources such as industrial waste, agricultural activities, household waste, and shipping. Hg can come from mining or industrial activities, while Pb is often found in vehicle and industrial waste. In addition, because these heavy metals are highly toxic to living things, they are also harmful to humans. The values of heavy metal Hg ranged from 1.33333E-05–0.00001 mgL^{-1}, Pb (0.015–0.06 mgL^{-1}), and Cd (0.003–0.008 mgL^{-1}). Heavy

Tabel 1. Description of research location

Point	Sites	Location			Coordinate point	
		Village	Subdistrict	District/City	Longitude	Latitude
1	The coastal areas (Alim Muntasor Pond)	Bayeman	Tongas	Probolinggo District	113° 7’ 47.31”	8° 16’ 31.61”
2	The coastal areas (Sumber Lancar Pond)	Bayeman	Tongas	Probolinggo District	113° 7’ 57.78”	8° 16’ 28.12”
3	Residential areas (PT.TBAI Probolinggo Pond)	Banjarsari	Tongas	Probolinggo District	113° 8’ 29.28”	8° 16’ 21.46”
4	The Tourism of Beejay Bakau Resort (BJBR) East	Mangunharjo	Mayangan	Probolinggo City	113° 13’ 36.19”	8° 15’ 41.37”
5.	Mangrove (Experimental Laboratory FPIK Probolinggo)	Mangunharjo	Mayangan	Probolinggo City	113° 14’ 0.51”	8° 15’ 29.75”

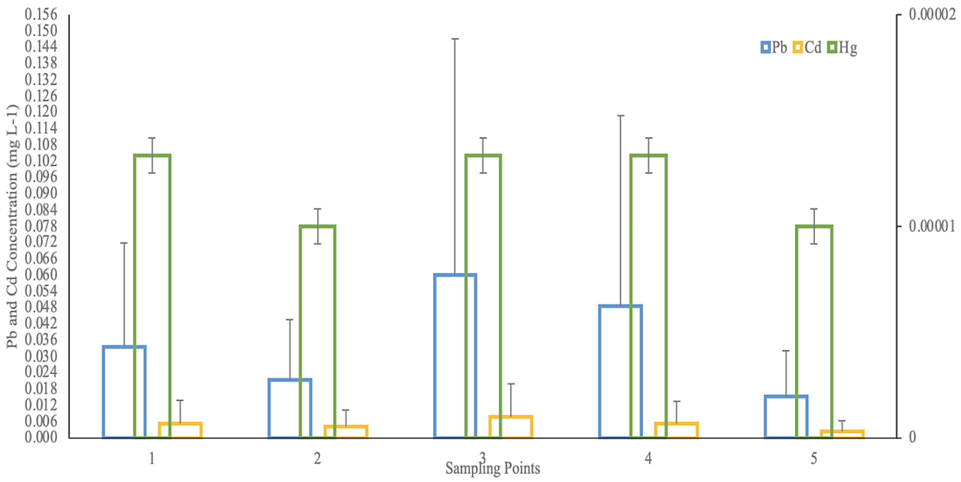

Figure 2. Metal concentration (Hg, Pb, and Cd) in water supply of vannamei ponds in Probolinggo

metal Hg had the lowest value of 1.33333E-05 mgL^{-1} at sampling locations 1, 3, and 4, while the highest value was 0.00001 mgL^{-1} at sampling points 2 and 5. The lowest Pb heavy metal content was 0.015 mgL^{-1} at sampling point 5, while the maximum was 0.06 mgL^{-1} at sampling point 3. Heavy metal Cd showed the lowest concentration distribution at sampling point 5 of 0.003 mgL^{-1} and the highest at point 3 of 0.008 mgL^{-1}.

Heavy metals Hg, Pb, and Cd have been identified in the water supply of Vannamei ponds. The measurement results of heavy metal Hg showed a small concentration compared to other heavy metals. Hg was dominate at sampling points 1, 3, and 4 compared to other sampling points but the concentration value was very small below the safe limit. The main source of heavy metal Hg is mining activities in addition to factory and domestic waste (Kumar et al., 2013).This is due to the condition of the sampling point area which is a residential area and there are no mining activities in the neighborhood. The highest concentration of Pb heavy metal was found at sampling point 3. The high Pb heavy metal is due to the amount of community waste that enters the waters such as fuel waste and agricultural waste from pesticides containing heavy metals. High Pb waters are caused by receiving a lot of input from agricultural waste that uses fertilizers or pesticides containing heavy metals that are carried along with anthropogenic runoff so there is an increase in waste in the water column (Rustiah et al., 2019). In addition, boats or ships that use fuel oil contribute heavy metals to the waters, because the fuel oil contains lead which is useful in improving the quality of the fuel used (Nurfadhilla et al., 2020).

Heavy metal Cd was also found to dominate at sampling point 3. Sampling point 3 is a denser residential area compared to other stations which allows higher input of waste into the waters. Heavy metal Cd is found in many locations due to its source, which mostly comes from environmental pollution and anthropogenic activities by humans (Tchounwou et al., 2012). Human activities such as industrial activities and the use of metals in daily activities will produce waste that can enter the water column. The results of variance analysis showed that heavy metal concentrations were not significantly different between stations. This may be related to the relatively similar characteristics of sampling points along the coast of Probolinggo. Based on the quality standards set in the Regulation of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 75 of 2016 concerning General Guidelines for the Cultivation of Wind Shrimp and Vaname Shrimp, namely for the maximum heavy metal content of Hg of 0.002 mg/L, it is still below the safe limit of Hg for aquaculture source water. The maximum Pb heavy metal content of 0.03 mg/L has exceeded the specified quality standards, and the maximum Cd heavy metal content of 0.01 mg/L has exceeded the quality standards for shrimp aquaculture source water.

Only three types of microplastics were found in the Vannamei ponds' water supply: fiber, fragments, and film. To determine the type of microplastics using visual characteristics of detected microplastic particles (Buwono et al., 2021). Figure 3. shows the identification results of microplastics found in the water supply of vannamei ponds. Fiber, film, and fragments are the three forms of microplastics discovered in water samples.

The abundance of microplastics by type found in Probolinggo coastal waters used to supply water for vannamei shrimp ponds is showed in Figure 4. The abundance of fragment type dominates compared to the abundance of fiber type followed by the lowest abundance of film type. The highest

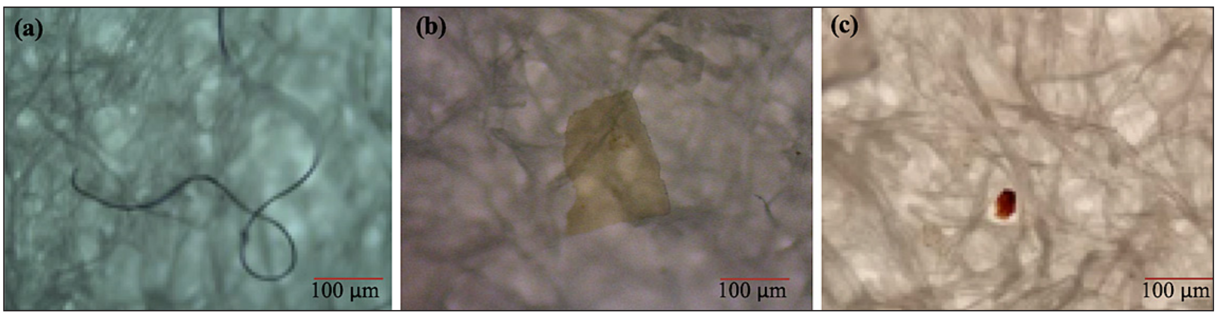

Figure 3. Microplastics type in water supply of vannamei ponds in Probolinggo: (a) fiber, (b) film and (c) fragments

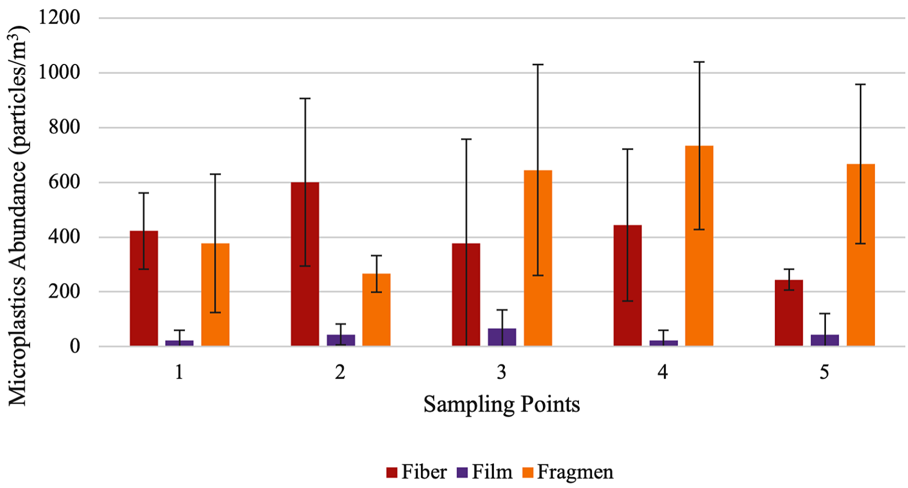

Figure 4. Microplastic abundance (fiber, film and fragments) in water supply of vannamei ponds in Probolinggo

fiber abundance value was 600 ± 305.51 particles/m^3 at sampling point 2 and the lowest was 244.44 ± 38.49 particles/m^3 at sampling point 5. The highest film abundance was 66.67 ± 66.67 particles/m^3 at sampling point 3 while the lowest was 22.22 ± 38.49 particles/m^3 at sampling points 1 and 4. The highest fragment abundance at sampling point 4 was 733.33 ± 305.51 particles/m^3 and the lowest at point 2 was 266.67 ± 66.67 particles/m^3.

The highest abundance of fragments was found at station 4. Station 4 is a tourism area utilizing mangrove ecosystems. Many microplastic fragments were found at the station because there was a lot of plastic waste trapped in mangrove roots. The garbage decomposes into microplastics and enters the water. Microplastic fragments at the station come from tourism sites and community activities and use plastic tools. It also includes plastic waste used in food and beverage packaging which is the waste of visitors to tourism sites. Microplastics tethered by mangrove roots make microplastics a lot on the surface of the waters (Y. Wang et al., 2023). Tourism activities are a contributor to microplastics at this station which comes from food packaging made of plastic by visitors to tourist attractions and recreational equipment that uses materials made of plastic.

The abundance of microplastics found at sampling point 2, namely the coastal waters of the Sumber Lancar pond, is dominated by fiber microplastics. This area is an area with dense and crowded anthropogenic activities and is close to the city center so it gets a lot of input of plastic waste and residual disposal from fishing boats. The source of fiber microplastics comes from leftover threads from washing clothes and degraded plastic ropes (Lo et al., 2018; Mauludy et al., 2019). The higher abundance of fibers is expected as research shows that fibers come from the production, washing, and natural aging of textiles, and cannot be completely removed through sewage treatment (Zhao et al., 2018). The distribution of fiber microplastics can come from fisheries activities or activities in the form of fishing that come from fishing gear such as fishing nets and degraded fishing rods (Katsanevakis & Katsarou, 2004). The film was found to have the lowest concentration compared to fragments and fiber. The highest abundance of the film was found at station 3. Station 3 is closer to residential areas, causing a lot of garbage or plastic waste to enter the waters. Plastic waste and waste from the community can be in the form of plastic bags, and household appliances made from plastic and plastic bottles. films are plastic particles that are thin, flexible, and generally transparent (Buwono et al., 2022). The fragmentation of large plastic-wrapped sand bags can result in microplastics of the film type (W. Wang et al., 2017).

Table 2 shows the findings of water quality parameters in the water supply of vannamei aquaculture in Probolinggo. The physical parameters measured were temperature ranging from 30 - 32°C. The chemical parameters measured are pH, DO, salinity, and TSS. The pH ranged from 7.5 to 7.6, while the dissolved oxygen level ranged from 5.53 to 7.23 mgL^{-1}. The salinity value is 16.33–28.33 ppt and the TSS is range 37–48.33 mgL^{-1}. The results of water quality measurements in this study show good results and are still by the established quality standards, namely the Regulation of the

Table 2. Water quality value of water supply of vannamei aquaculture in Probolinggo

Point	Temperature (∘C)		pH		DO (mgL^{-1})		Salinity (ppt)		TSS (mgL^{-1})	
	Value	SD (σ)	Value	SD (σ)	Value	SD (σ)	Value	SD (σ)	Value	SD (σ)
1	32	2.95	7.6	0.63	6.47	1.21	28.33	8.14	45.67	4.16
2	31	2.42	7.6	0.76	6.30	1.15	16.33	1.53	48.33	4.51
3	31	2.52	7.5	0.14	5.83	0.25	22.50	9.58	47.33	5.03
4	30	1.92	7.5	0.57	7.23	1.12	20.33	2.57	37.00	4.58
5	31	2.08	7.6	0.24	5.53	1.17	26.00	8.54	40.67	2.52

Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No. 75 of 2016 concerning General Guidelines for Growing Wind Shrimp and Vaname Shrimp. The range of temperature, pH, DO and TSS values in the coastal waters of Probolinggo is still relatively safe and has not exceeded the established quality standards.

The results of the analysis of variance comparing the concentrations of heavy metals between sampling points can be seen in Table 3. The results show that the amounts of heavy metals Hg, Pb, and Cd are not statistically different with a p-value greater than 0.1. The heavy metal content is relatively the same because the source of the entry of heavy metals and the activities found around the waters producing heavy metals are the same (Irawan et al., 2015) These results can occur due to the similarity of water characteristics and sources of heavy metal input at each research station. In addition, it can also be caused by conditions or seasons when taking the same sample so that there is no striking or dominant difference in heavy metal content. (Permana et al., 2022). The presence of heavy metals in the aquatic environment can come from natural or anthropogenic activities. Lead heavy metals in fuel can be a lead source in the aquatic environment; for example, it can come from power plants that use coal as fuel (Sudarmaji et al., 2006; Verma et al., 2013). Through anthropogenic activities, mercury heavy metals can come from the metal casting industry and pesticides, in addition to naturally coming from volcanic gases found at the bottom of the waters (Authman, 2015). Cadmium heavy metals can come from the use of cadmium as the main ingredient in the alloy industry, zinc (Zn) metal refining, and in pesticides in the aquatic environment can also come from the battery industry and the plastic industry (Iswantari et al., 2021)

Table 4 shows the results of ANOVA by comparing the abundance of microplastics of each type, namely fiber, film, and fragments at all

Table 3. One Way ANOVA result of heavy metal comparison between sampling points

Source	MS	F	*p*
Metal Hg	3.333e-12	0.183	0.676
Metal Pb	0.0000247	0.01	0.924
Metal Cd	5.550e-06	0.098	0.76

Table 4. One Way ANOVA result of microplastics type comparison between sampling points

Source	MS	F	*p*
Microplastic fiber	78370	1.293	0.276
Microplastic film	148.1	0.057	0.815
Microplastic fragments	327259	4.446	0.055
Microplastic total	85333	0.52	0.484

sampling locations. Microplastic fragment types show significant differences (p-value < 0.1) between sampling points. While the fiber and film types of microplastics are not significantly different between sampling points. ANOVA results showed that the abundance of fragments was significantly different at all stations. The abundance of fragments dominates compared to the abundance of fiber and film at all sampling points. Fragments are found in a location because it is close to the mainland, where the mainland is a place where people do activities and produce waste that is discharged into the river which is then carried towards the ocean (Hiwari et al., 2019). Microplastic types of fragments are irregularly shaped, clumps, or elongated and can come from mining ropes, plastic bottles, ship paint, or peeling fishing boats and epoxy (plastic-based polymers) which then enter the waters (Hiwari et al., 2019; Jaini & Namboothri, 2023). Fragments are found in a research location because the nature of this type of microplastic has a low density, making it easier to float on the surface of the waters (Hidalgo-ruz et al., 2012).

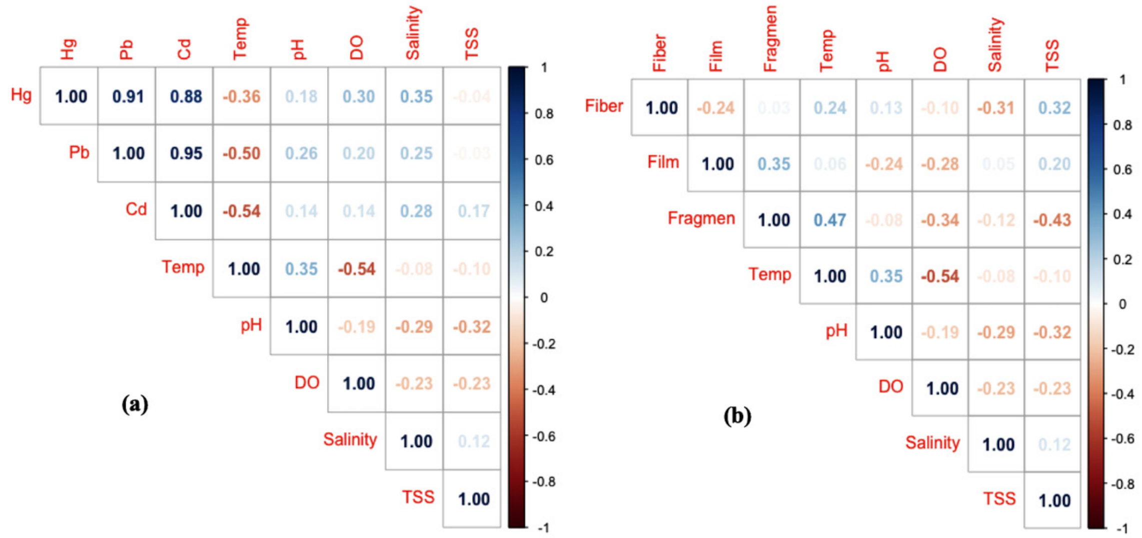

Figure 5. Correlation results of (a) water quality with heavy metals and (b) water quality with microplastics (coefficients in blue so significant correlation)

Correlation analysis in this study was used to determine the correlation between water quality parameters namely temperature, pH, DO, salinity, and TSS with heavy metal concentrations (Pb, Hg, and Cd). The correlation test was also used to determine the relationship between water quality parameters and the abundance of microplastics (fiber, film, and fragments). Figure 5a demonstrates that the correlation coefficient (r) between heavy metal Cd concentration and temperature is -0.54. From this result, it can be explained that there is a negative correlation between Cd and temperature. Figure 5b demonstrates that there is no association between the varied abundance of microplastic kinds and the assessed water quality parameters.

The negative correlation results show the relationship between temperature and the concentration of heavy metal Cd. Where Cd heavy metal concentrations increase in conditions of falling water temperature. When the water temperature decreases, it will cause heavy metals to easily settle in the sediment and will affect water quality (Sukoasih et al., 2016). The relationship between water quality parameters and microplastics still does not show a significant correlation value. This can occur due to the relatively short research time. Microplastics are formed through a degradation and fragmentation process that takes place over a very long period in the environment (Fachrul et al., 2021). So, in observing water quality parameters associated with the abundance of microplastics, it is not enough to only do it in a short period, it is necessary to monitor for a longer period. Heavy metals could be present in microplastics due to environmental absorption from water environment and additives that contain many toxic substances such as heavy metals, bisphenol A, and phthalates in the original plastic (Brennecke et al., 2016). Microplastics can also adsorb metals from the aquatic environment due to their surface modification through the erosion of charged materials and photooxidation (Turner and Holmes, 2015).

CONCLUSIONS

According to this research, the content of the heavy metal Hg is still considered safe because it is almost undetectable, while the content of the heavy metals Pb and Cd exceeds the safe limit for source water for shrimp cultivation. Microplastics in the form of fibers, fragments, and films have been found in the water supply of vannamei ponds. The distribution of microplastics in the water supply of vannamei ponds is the same between sampling points. Further research needs to be carried out regarding the toxic effects of heavy metals and microplastics on cultivated organisms that use contaminated water supplies.

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