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Distribution of Heavy Metals in Aquaculture Ponds and Bioaccumulation in Vannamei Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

This study evaluated the amounts of heavy metals (Cd, Cu, Cr, and Pb) in water, sediment, and the vannamei shrimp (*Litopenaeus vannamei*) from three typical ponds in Gresik, Indonesia. Heavy metal analysis was performed with Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES). The research examined the concentration of heavy metals in the vannamei shrimp aquaculture ecosystem and assessed the associated human health concerns. The findings indicated a greater concentration of heavy metals in sediment than in water, particularly for cadmium and lead. The maximum concentration in water was Cd (2.94 ppm) in Pond 1, whereas the greatest concentration in sediment was Pb (1.73 ppm) in Pond 6. Copper was the predominant metal in prawn tissue, with a maximum value of 1.76ppm in Pond 6. Copper is vital for the metabolism and excretion of the vannamei prawns. Notwithstanding these findings, the human health risk assessment (HRA), predicated on Lifetime Cancer Risk (LCR), Target Hazard Quotient (THQ), and Total THQ (TTHQ), suggested that the levels remained within acceptable consumption limits. This research is significant since it offers critical data on environmental contamination levels in aquaculture environments, assisting stakeholders, including farmers and policymakers, in making educated decisions to ensure food safety. Nonetheless, consistent monitoring and sustainable agricultural techniques are crucial to avert heavy metal buildup in the vannamei shrimp and to guarantee the safety of aquaculture products.

INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is a principal brackish water fishery product of significant economic importance. Due to its rapid growth, excellent feed conversion rate, and high market value, vannamei prawns are the preferred option for farmers globally. Southeast Asia, including China, Indonesia, and Vietnam, accounted for

81.7% of total shrimp production from 2000 to 2017 **(Tacon, 2020)**. In 2018, worldwide shrimp production reached around 3.8 million tonnes **(Kilawati *et al.*, 2022)**.

Shrimp is a crucial source of protein, providing all essential amino acids. While nutritionally valuable, shrimp can accumulate toxins and environmental pollutants **(Pandion *et al.*, 2022)**. Water contamination is a significant issue in the vannamei shrimp aquaculture **(Kumar *et al.*, 2022)**. Numerous industrial, pesticide, and domestic pollutants can contaminate the environment **(Ditia, 2024)**. Pesticides are extensively utilized globally in agriculture to safeguard crops and in public health to manage illnesses. Although a pesticide is designed to target specific groups or species, it is inherently toxic to all organisms, including both target and non-target animals such as fish **(Islamy *et al.*, 2017)**. Heavy metals, which are poisonous and detrimental to living organisms, are one of the hazardous components in these wastes **(Mitra *et al.*, 2022)**. Heavy metals that serve as contaminants in the vannamei shrimp aquaculture include Cu, Cr, Cd, and Pb **(Wang *et al.*, 2023)**. These metals are typically associated with proteins in the organism's body, such as metallothionein, complicating their natural elimination **(Balali-Mood *et al.*, 2021)**.

The quality of the vannamei shrimp may be compromised by deleterious elements in the aquaculture pond environment, including heavy metals that can elevate the risk of health issues **(Pandion *et al.*, 2022)**. Heavy metals may infiltrate aquaculture ponds through many routes, including food sources, water runoff, and the internal transference of contaminants from sediments to culture water **(Dietrich & Ayers, 2021)**. Heavy metals can accumulate in the tissues of shrimp, which are often located at the highest trophic levels of aquatic food chains and may negatively impact human health upon consumption, although having little effect on the biota **(Rainbow, 2018)**. Consequently, it is essential to quantify heavy metal concentrations in several media that may influence the heavy metal content in the vannamei shrimp. Heavy metal pollution in shrimp can negatively impact human health due to the accumulation of these metals in the body, leading to potential long-term health issues **(Wang *et al.*, 2023)**. Heavy metals that infiltrate the human body are challenging to metabolize normally and can lead to many ailments, including diabetes, Alzheimer's disease, and multiple forms of cancer **(Jara-Marini *et al.*, 2020)**.

Evaluating the environmental quality of the vannamei shrimp aquaculture (*Litopenaeus vannamei*) is crucial because of the potential for heavy metal contamination from anthropogenic activities in the vicinity, including agricultural, animal, and residential waste. Heavy metals, such as cadmium (Cd), copper (Cu), chromium (Cr), and lead (Pb), are acknowledged for their buildup in pond waters, adverse effects on the health of cultivated organisms, and decrease in productivity. Gresik, an industrial and agricultural region, possesses significant potential for accumulating these metals in ponds **(Marchellina *et al.*, 2024)**. The environment of aquatic organisms, encompassing cultured water and sediment, markedly affects the bioaccumulation of heavy metals

(Wang *et al.*, 2023). The vannamei shrimp is an indicator organism due to its direct contact with the culture medium and its ability to absorb heavy metals from the environment. An examination of heavy metal levels in sediments was performed to identify possible enduring pollution that might affect the long-term health of the aquaculture ecosystem. This study examined the heavy metal concentration in the vannamei shrimp aquaculture ecosystem and assessed the possible health concerns for humans.

MATERIALS AND METHODS

Samples collection

Water, vannamei shrimp, and sediment samples were collected from an agricultural pond in the Gresik District. All samples were collected from three ponds with three replicates. The ponds utilized for sampling are situated near agricultural, aquacultural, and residential zones. Observations were conducted thrice at 20-day intervals. Water samples were collected using a water sampler, silt with a grab tool, and vannamei shrimp with a net tool. Upon arrival at the laboratory, vannamei shrimp samples (DOC 30 - 70) were frozen at -20 °C for further analysis. The pond is situated in the geographical coordinates 6°54'23.040" N, 112°30'58.320" E to 6°54'25.920" N, 112°31'4.800" E. Fig. (1) illustrates the sample location map.

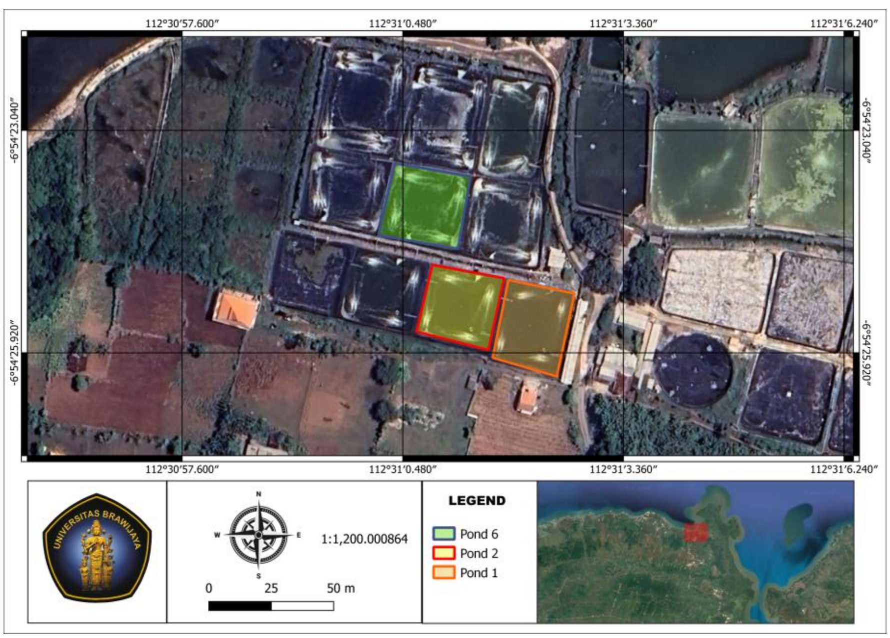

Fig. 1. Map of sampling points

Analysis of heavy metals

Heavy metal analyses were conducted on water, sediment, and vannamei shrimp samples for Cd, Cu, Cr, and Pb, which were first subjected to digestion using an acid solution. The vannamei shrimp samples, 250±5mg, were crushed using a mortar, and

100±5mg of sediment was placed into a microwave vessel (Multiwave Go Plus, Anton Paar, LRT UB). The samples were then added to 5ml of 65% HNO_3 (Merck, Sigma-Aldrich) and 2ml of 30% H_2O_2 (Merck, Sigma-Aldrich), following a gradient temperature program with temperature varying from ambient to 160°C for 10min, while in the case of sediment samples it increased to 180°C for 10min. For the water samples, 100ml of pond water was put into a glass jar and was filtered using Whatman No.41 filter paper to remove dust particles. From the filtrate obtained, 9ml was taken and added to 1ml of 65% HNO_3; then, the digestion process was carried out. The microwave digestion system was set with a temperature program that reached 160°C for 20 minutes. Then, the results were analyzed using ICP AES wavelengths of 226.502 (Cd), 267.716 (Cr), 327.396 (Cu), and 220.353 (Pb).

The analysis of heavy metal contents in all samples was conducted using ICP-AES (ICPE-9820; Shimadzu, LRT UB), with findings interpreted based on six calibration curve points: 0, 0.1, 0.5, 1, 2, and 5ppm **(Arisekar *et al.*, 2022)**. The limit of detection (LOD) and limit of quantification (LOQ) of the technique are determined by the standard deviation of the response and the slope, namely 3.3 and 10, respectively.

A recovery study was conducted using certified reference material, specifically a multi-elemental standard mix (CRM, periodic table mix 1 for ICP, Merck, Sigma-Aldrich), to evaluate the instrument's accuracy and the efficiency of the digestion process. This material was spiked into pond water, sediment, and vannamei shrimp as Quality Control samples. The concentration was measured before and after spiking, allowing for the determination of the recovery ratio. The average recovery ratio of the examined heavy metals varied from 81.00 to 90.20%. By EU regulations (EC/SANTE/11813/2017, 2017), the recovery of the analyzed materials fell within the allowed range of 80–120 %, with a standard deviation (SD) of less than 10%.

Human health risk assessment (HRA) through the consumption of shrimps

Human Health Risk Assessment was conducted based on heavy metal concentrations accumulated in shrimp tissue. The evaluation was conducted by calculating the target hazard quotient (THQ), total THQ (TTHQ), and lifetime cancer risk (LCR). THQ and TTHQ assess non-carcinogenic health risks due to ingesting heavy metals through food. A THQ value of <1 indicates no adverse health risk, while a THQ >1 indicates the potential for long-term health effects **(Balamanikandan *et al.*, 2024)**. THQ and TTHQ are using equation 1.

In addition, carcinogenic risk is calculated using the Lifetime Cancer Risk (LCR) parameter, specifically for metals classified as carcinogenic by the USEPA. LCR values $< 10^{-6}$ are considered to pose no cancer risk, values between 10^{-6} to 10^{-4} are within the acceptable risk range, while LCR values $> 10^{-4}$ are categorized as unacceptable risk **(EPA, 2011)**. LCR using equation 2.

$$THQ = \frac{Cs \times EFr \times ED \times FIR}{RfD \times Bw \times AT} \times CF \qquad (1)$$

$$LCR = \frac{Cs \times EFr \times ED \times CSF}{Bw \times AT} \times 10^{-3} \quad \text{..} (2)$$

Where, Cs is the heavy metal concentration in shrimps; EFr (Exposure frequency); ED (Exposure duration); FIR (Food Ingestion rate (crustaceans)); RfD (Recommended daily reference dose); Bw (Body weight); AT (Average lifespan); CSF (Cancer slope factor); CF (Conversion factor).

Data analysis

Data analysis was conducted using the SPSS application **(Feng *et al.*, 2023)**. To identify significant relationships in heavy metal content in water and sediment samples with the vannamei shrimp, linear regression tests were used with a significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Heavy metals in water

The results of the analysis of heavy metal content in the vannamei shrimp culture water in three ponds are presented in Fig. (2). This study found the presence of Cd, Cr, Cu, and Pb metals with varying concentrations. Cd content showed the highest value among other metals, with concentrations reaching 2.94ppm in Pond 1, followed by Pond 2 at 2.49ppm and Pond 6 at 2.47ppm. Pb metal was also detected at high levels at 2.35ppm in Pond 1, 1.81 ppm in Pond 2, and 1.79 ppm in Pond 6. Meanwhile, Cu and Cr contents were relatively lower, with Cu contents ranging from 0.47-0.75ppm and Cr between 0.08 & 0.21ppm.

These heavy metal concentrations indicate a potential risk to water quality and shrimp health, as some heavy metals, such as Cd and Pb, are toxic even at low concentrations. The consistently high levels of Cd in all three ponds indicate a uniform source of pollution, such as from biogeochemical processes and anthropogenic activities **(Xing *et al.*, 2017)**. In addition, the source of Pb heavy metal content in aquaculture water can come from industrial waste, settlements, agriculture, aquaculture, combustion products of motor vehicle fuels, and plastic waste containing Pb **(Komala *et al.*, 2022)**.

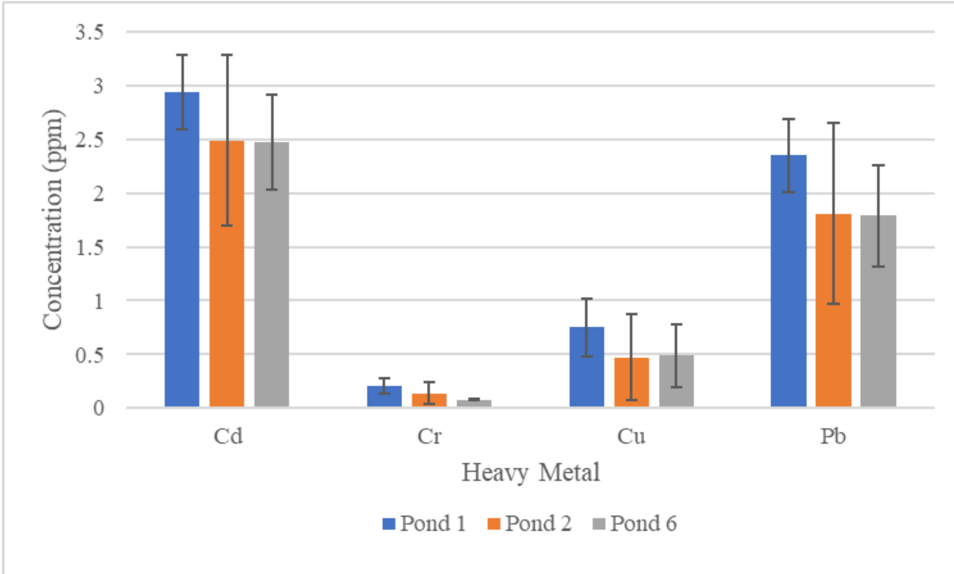

Fig. 2. Heavy metals in water

There are various pathways for heavy metal pollutants to enter the vannamei shrimp culture water. Surface water seepage that carries pollutants into groundwater or through the atmosphere due to rain is one source of heavy metal entry into aquaculture water **(Singh *et al.*, 2022)**. The increase in heavy metals accumulated in aquaculture water over a long time can lead to the accumulation of heavy metals into the body of the vannamei shrimp, which can then damage the organs of the vannamei shrimp if it lasts a long time because it has exceeded the tolerance limit of heavy metals in the body and can cause death in the vannamei shrimp **(Apresia *et al.*, 2023)**.

Heavy metals in sediment

The results of the analysis of heavy metal content in sediments from the three vannamei shrimp farming ponds are presented in Fig. (3). Sediment samples showed the presence of heavy metals Cd, Cu, and Pb, while Cr was not detected in the three ponds. Cd content was recorded between 0.77 and 1.01 ppm, with the highest value in Pond 2. Cu metal showed greater variation, with the highest concentration in Pond 2 at 1.18ppm and the lowest in Pond 1 at 0.59ppm. Pb was detected at relatively high levels and increased gradually from Pond 1 at 1.45ppm to Pond 6 at 1.73ppm.

The absence of heavy metal chromium levels in all sediment tests indicates that this metal is not substantially deposited at the bottom of the culture ponds. The presence of Cd, Cu, and Pb signifies the buildup of heavy metals, likely sourced from feed, aquaculture chemicals, or environmental contamination **(Wang *et al.*, 2023)**. The occurrence of these metals in sediments warrants particular consideration, as they may act as a secondary source of exposure for benthic shrimp **(Zhang *et al.*, 2016)**. Consistent oversight and adopting sustainable aquaculture techniques are crucial to avert the buildup of heavy metals in pond ecosystems and guarantee the safety of aquaculture products.

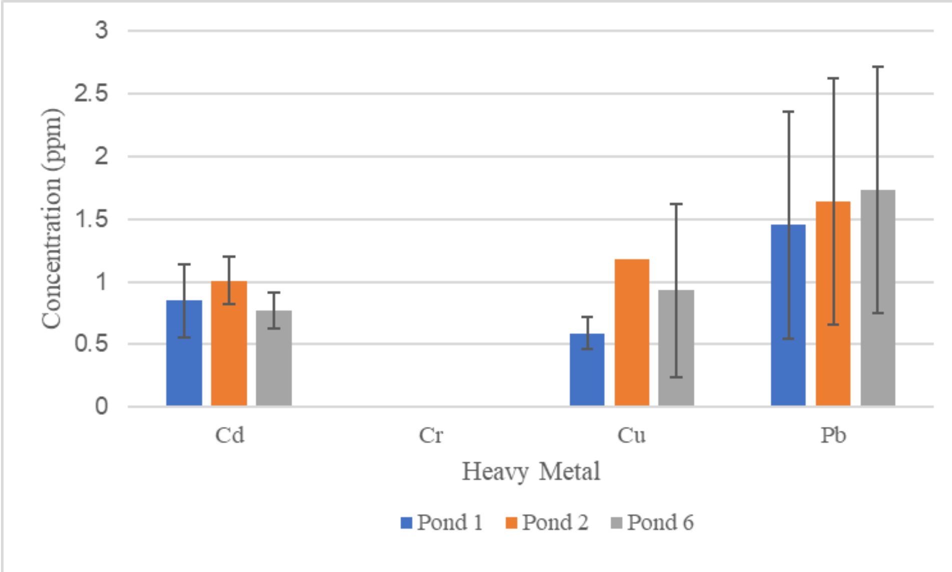

Fig. 3. Heavy metals in sediment

In shrimp aquaculture, the syphon technique is employed to mitigate silt accumulation. Syphons facilitate the extraction of accumulated metals from sediments and water that settle at the pond's bottom. This technique is sometimes referred to as an 'aquaculture lavatory' due to its function of collecting and disposing of undesirable contaminants **(Newton *et al.*, 2019)**.

Heavy metal content in vannamei shrimp

The analysis of heavy metal content in the vannamei shrimp cultured in the three ponds is presented in Fig. (4). The test results on the vannamei shrimp showed the accumulation of heavy metals Cd, Cr, Cu, and Pb with varying concentrations. Cu showed the highest concentration among the other metals, with values ranging from 1.41 ppm in Pond 1 to 1.76ppm in Pond 6. Cd content was relatively low, ranging from 0.06 to 0.10 ppm, while Pb was detected between 0.25 and 0.47ppm. Cr was only detected in very low amounts (0.01-0.02ppm) and not in Pond 2. Cd and Pb concentrations in vannamei shrimp did not exceed the limits set by the FAO (Cd:0.5; Pb:2 ppm) **(Aytekin *et al.*, 2019; Arisekar *et al.*, 2022)**.

Despite the comparatively low heavy metal levels in shrimp compared to water and sediment, the presence of these metals is a significant concern for consumer safety. The buildup of heavy metals in shrimp may arise from the culture environment via bioaccumulation **(Ramos-Miras *et al.*, 2023)**. Linear regression analyses indicated a substantial correlation ($P < 0.05$) between culture water and silt. Thus, cultural water and sediment substantially affected heavy metal bioaccumulation in the vannamei shrimp. Consequently, our findings underscore the necessity of overseeing the quality of the cultural environment and adopting sustainable agricultural methods to mitigate heavy metal pollution in fishing products.

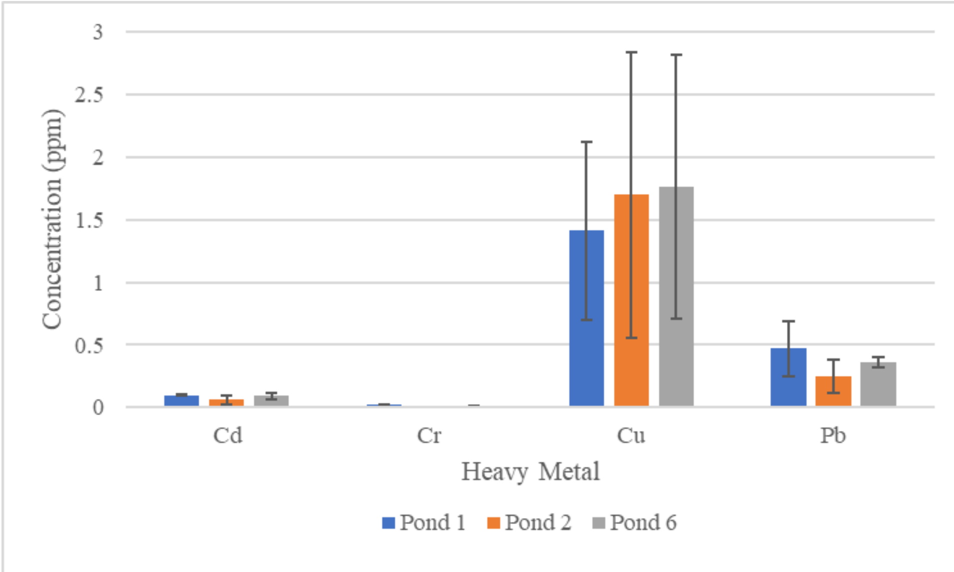

Fig. 4. Heavy metals in the vannamei shrimp

Vannamei shrimp acquire higher concentrations of heavy metals than fish, particularly in the head region, encompassing the gills, antennae, and hepatopancreas **(Albuquerque *et al.*, 2020)**. Heavy metals may accumulate in shrimp tissues, which typically occupy the terminal position in the aquatic food chain. While these elements may not consistently impact biota directly, the buildup of heavy metals can detrimentally influence human health **(Rainbow, 2018)**.

A significant buildup of heavy metals in the vannamei shrimp, particularly copper and lead, may result from several causes, including feed utilization, water quality, and chemicals in the culture system **(León-Cañedo *et al.*, 2017)**. Elevated copper contents in vannamei shrimp suggest an increased vulnerability to heavy metal bioaccumulation **(Gulmez *et al.*, 2021)**. Furthermore, a specific concentration of the heavy metal copper (Cu) is necessary for the vannamei shrimp since it plays a crucial role in metabolic and excretory functions **(Ortiz-Moriano *et al.*, 2024)**.

Human health risk assessment (HRA) through the consumption of shrimps

Table (1) shows the Lifetime Cancer Risk (LCR) values of heavy metals Cr, Cd, and Pb detected in the vannamei shrimp samples. Heavy metal Cu was not detected (nd) in all samples. Cancer risk assessment based on LCR values refers to three categories **(EPA, 2011)**.

Table 1. Lifetime cancer risk (LCR)

Sample	Cu	Cr	Cd	Pb
Pond 1	nd	1.57×10^{-7}	6.68×10^{-6}	4.30×10^{-8}
Pond 2	nd	nd	3.80×10^{-6}	1.80×10^{-8}
Pond 3	nd	9.50×10^{-8}	8.20×10^{-6}	2.60×10^{-8}

The LCR value of heavy metal Cr was also within the safe category, with the highest value recorded in Pond 1 at 1.57×10^{-7}. However, heavy metal Cd showed higher LCR values, ranging from 3.80×10^{-6} to 8.20×10^{-6}. These values are within the acceptable risk category, but close to the upper limit of the threshold (10^{-6} to 10^{-4}). Overall, the analysis results show that heavy metal Cd is the element that contributes the most to cancer risk compared to other components. If the LCR value increases and is consumed on a large scale, it can cause symptoms of disruption in the human internal organ system **(Pragnya *et al.*, 2021)**.

Table (2) presents the results of calculating heavy metals' Target Hazard Quotient (THQ) and Total THQ (TTHQ) values in the vannamei shrimp. THQ values are used to evaluate the potential non-carcinogenic risk to human health from ingestion of heavy metals through food.

Table 2. Target hazard quotient (THQ) and total THQ (TTHQ)

***Total Hazard Quotient* (THQ**					
Sample	**Cu**	**Cr**	**Cd**	**Pb**	**TTHQ**
Pond 1	0.4×10^{-4}	0.1×10^{-4}	1.5×10^{-4}	18.1×10^{-4}	5.1×10^{-4}
Pond 2	0.4×10^{-4}	0	0.8×10^{-4}	7.6×10^{-4}	2.2×10^{-4}
Pond 3	0.4×10^{-4}	0.1×10^{-4}	1.9×10^{-4}	11.1×10^{-4}	3.4×10^{-4}

TTHQ values tend to be higher, with a range of 2.2×10^{-4} to 5.1×10^{-4}. Although well below the risk threshold (TTHQ < 1), this increase indicates that intensive systems have a higher potential for heavy metal exposure. Pb metal contributed the most to the TTHQ value, especially in Pond 1, which showed a THQ value for Pb of 18.1×10^{-4}, much higher than other heavy metals. This suggests that, although still within the safe category, special attention needs to be paid to Pb contamination. Overall, the THQ and TTHQ values were below the recommended limit, and shrimps cultured in this study region are safe for human consumption.

Future research should incorporate physiological indicators, including histopathological examination of reproductive organs, molecular diagnostics, and hemocyanin (HMC) gene expression profiles **(Islamy *et al.*, 2025; Kilawati *et al.*, 2025)**, alongside human impact analysis, to enhance the understanding of the internal physiological conditions and reproductive capabilities of aquatic organisms subjected to heavy metal exposure in aquaculture environments.

CONCLUSION

These findings indicate that the vannamei shrimp farms in Gresik are exposed to varying levels of heavy metals, with Cd in water and Pb in sediment showing the highest concentrations. Although Cu was the predominant metal detected in shrimp tissue, all metal concentrations, including those related to human health risk indicators (LCR, THQ, and TTHQ), remained within acceptable safety thresholds. These results underscore the importance of continuous monitoring and adopting sustainable aquaculture practices, including carefully evaluating inputs such as feed, fertilizers, and chemicals, to mitigate potential long-term risks of heavy metal accumulation in the production environment.

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