Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43A, No.1,
January-June 2024: P.165-175

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: https://doi.org/10.52710/bpas-zoo.35

Original Research Article *Content Available online at: https://www.bpasjournals.com/zoology*

Analysis of Heavy Metal Contents in *Litopenaeus vannamei* and *Metapenaeus monoceros* Cultivated Within Selected Aquaculture establishments in Kerala

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ABSTRACT:
This study investigated the concentrations of heavy metals in two species of prawns (*Litopenaeus vannamei* and *Metapenaeus monoceros*) cultured in aquaculture farms in Kerala, India. A total of 22 samples were analyzed for the presence of lead (Pb), cadmium (Cd), and mercury (Hg). The findings showed that the hepatopancreas of prawns had different amounts of heavy metal pollution, with Cd and Hg being the most prominent. The quantities of Cd and Hg were higher than safety standards, indicating possible health concerns associated with eating contaminated prawns, even if Pb levels were within allowable limits. This study examines how these findings may affect environmental management and food safety in aquaculture environments. The statistical data assessments and the real data results were aligned, revealing exactly the same.

Keywords:
Aquaculture, Cadmium, Heavy metal pollution, Lead, *Litopenaeus vannamei, Metapenaeus monoceros*, Mercury.

Received on 16.01.2024
Revised on 26.04.2024
Accepted on 16.05.2024

How to cite this article: Jaya M., Aja M., Prasanth C.B., Vasudevan A. , Krishnendu V., Sasi S., Siya Chandran S., Ranjana P.B. and Adhithya T.M. (2024). Analysis of Heavy Metal Contents in *Litopenaeus vannamei* and *Metapenaeus monoceros* Cultivated Within Selected Aquaculture establishments in Kerala. *Bulletin of Pure and Applied Sciences-Zoology*, 43A (1), 165-175.

INTRODUCTION

Aquatic ecosystems harbor a significant portion of the planet's biological diversity. To protect this biodiversity, it is essential to implement conservation and management strategies such as establishing bio-reserve areas, employing bioregional management practices, and conducting global monitoring efforts (Irfan and Alatawi, 2019).

The release of heavy metals into marine habitats is especially concerning since it threatens human health and biodiversity. Many coastal communities depend on marine-based foods like fish, prawns, crabs, and mussels, which can accumulate these hazardous chemicals and pose serious health hazards to consumers. Persistent contaminants, including lead, cadmium, mercury, zinc, copper, manganese, iron, cobalt, chromium, nickel, and zinc, can bioaccumulate in marine creatures. Eating contaminated seafood can cause a number of health problems, such as cancer, heart disease, liver damage, hepatic dysfunction, dermatological disorders, and developmental anomalies. Given the potential health risks associated with heavy metal contamination in seafood, there is a pressing need for monitoring programs to assess the concentration of these contaminants in marine organisms.

The alarming frequency of heavy metal contamination in seafood has been brought to light by recent research, underscoring the urgent necessity of extensive regulatory measures and monitoring (Smith *et al*., 2023; Chen *et al*., 2022; Wang *et al*., 2019; Amiard *et al*., 2016; Almroth *et al*., 2015). The magnitude of environmental contamination and its effects on human health have been illuminated by the more precise measurement of heavy metal concentrations in aquatic creatures made possible by advancements in analytical techniques (Jones *et al*., 2021; Lin *et al*., 2018; Huang *et al*., 2017). Because heavy metals are bioaccumulative, consuming polluted seafood exposes humans at serious risk for health problems. Prolonged exposure to elevated levels of heavy metals found in marine products such as fish and prawns has been associated with a number of negative health impacts, including cancer, neurological disorders, liver damage, and renal malfunction. Thus, in order ensure food safety and safeguard the public's health, it is crucial to evaluate the levels of heavy metals in prawns from aquaculture farms (Chen *et al*., 2022; Wang *et al*., 2019). Rajaram *et al.* (2020) reported Cd, Pb, Cu, and Zn in *Stolephorus commersonii* and *Penaeus monodon*. Shalini *et al.* (2020) studied As, Cd, Hg, and Pb in flower shrimp (*Penaeus semisulcatus*).

Regular monitoring of the shrimp aquaculture system throughout the crop will provide evidence of heavy metals bioaccumulation in shrimps (Arisekar *et al.* 2022). Early detection can help prevent adverse health effects and ensure public safety. Aquaculture systems need to limit chemical inputs, maximize the use of renewable resources, and adjust to climate change in order to meet these challenges. Monitoring initiatives can also aid in identifying possible health hazards to the general public related to aquaculture products. Risks to human health from heavy metal contamination in aquaculture are significant. By comprehending the source and anticipation of these impurities in seafood, we can put policies in place that guarantees food safety and safeguard public health. To reduce these dangers and protect the health of coastal people, monitoring programs and sustainable aquaculture techniques are necessary.

In the light of this, this study aims to assess the accumulation of selected heavy metals in cultivated prawns sourced from local aquaculture farms. We aim to assess potential health concerns associated with consuming these seafood products and ascertain the levels of heavy metal contamination by evaluating the hepatopancreas of prawns.

MATERIALS AND METHODS

Study Site

Prawn samples were collected from two commercially operated aquaculture farms located in Thrissur District, Kerala, India, designated as Site 1 and Site 2, respectively. Each site was assigned a sample code (Table 1).

Collection of Sample Organisms

Live *Litopenaeus vannamei* (vannami) samples (sample code: KD12) were taken from Site 1 on January 12, 2023 (Fig. 1). A total of 11 random samples were obtained and transported to the laboratory for further analysis from each site. Samples of *Metapenaeus monoceros* (choodan chemmeen) samples (sample code: NP14) were taken on January 14, 2023, from Site 2, where shrimp post larvae and several fish species were being grown (Fig. 2).

Figure 1: ***Litopenaeus vannamei*** **collected from Site 1: KD 12**

Figure 2: ***Metapenaeus monoceros*** **Collected from Site 2: NP 14**

Sample Extrusion

Post larval samples were immediately dissected upon arrival at the laboratory. Gills, muscles, and hepatopancreas were extracted from each sample. The hepatopancreas, identified as a bilaterally bilobed brown-yellowish organ, was particularly targeted as the site of heavy metal accumulation. Hepatopancreas and other tissues had to be carefully removed once the carapace was split apart during dissection. After being cleaned with distilled water and allowed to air dry for two days, the isolated tissues were examined further (Fig. 3 A and 3B).

Figure 3 (A and B): Dissected and exposed hepatopancreas

Sample Analysis and Processing

Dried samples were weighed (Sample 1: 1.8 mg, Sample 2: 2.5 mg) and sent to the central laboratory of Veterinary Animal Sciences College, Mannuthy, Thrissur for analysis. In the laboratory, samples were placed in separate test tubes and treated with concentrated nitric acid. The mixture was homogenized and digested using a microwave high-pressure digester (model: TITAN MPS) for approximately 1 ½ hours. Upon digestion, samples emitted yellowish-brown fumes. A portion of the digested sample was diluted with distilled water to a final volume of 10 ml and thoroughly mixed for analysis of heavy metal accumulation. Blank samples were prepared alongside the experimental samples using the same method. The analysis focused on three heavy metals: lead (Pb), cadmium (Cd), and mercury (Hg) and results were analyzed statistically.

Statistical Analysis

Statistical data analysis especially to testing the equality of means at a level of significance (LS) $\alpha = 0.05$ is done by using Student t-test and independent sample t-test since here the sample size is less and the population standard deviation (sd) σ is unknown.

Table 1: showing the details of sample collection form two different localities

Sl. No.	Collection Site	Collection code	Species selected Scientific name and local name	Total number collected
1	Site 1	KD12	*Litopenaeus vannamei* (vannami)	11
2	Site 2	NP14	*Metapenaeus monoceros* (choodan chemmeen)	11

RESULTS

In this study, we examined the concentrations of heavy metals in two species of prawn from different aquaculture farms in Kerala, namely *Litopenaeus vannamei* and *Metapenaeus monoceros*. Out of the 22 samples analyzed, three heavy metals (Pb, Cd, and Hg) were detected. The findings revealed that Cd and Hg were the most abundant metals in the hepatopancreas of both prawn species, while Pb was the least abundant. Metal concentrations differed between the two sites, with samples from Site 2 showing no detectable Cd.

Lead (Pb) concentrations in *L. vannamei* from Site 1 and *M. monoceros* from Site 2 were found to be 0.14 mg/kg and 0.215 mg/kg, respectively. Although Pb levels were higher in river samples compared to those from Site 2, all samples remained below the permissible limit set by the EU at 0.5 mg/kg (Fatema *et al*., 2015a). The study suggested that the sources of Pb contamination could include air, food, and water, with food and water contributing the most to human intake. Fortunately, the Pb levels observed in the prawn samples were deemed non-toxic to consumers (Fig. 4).

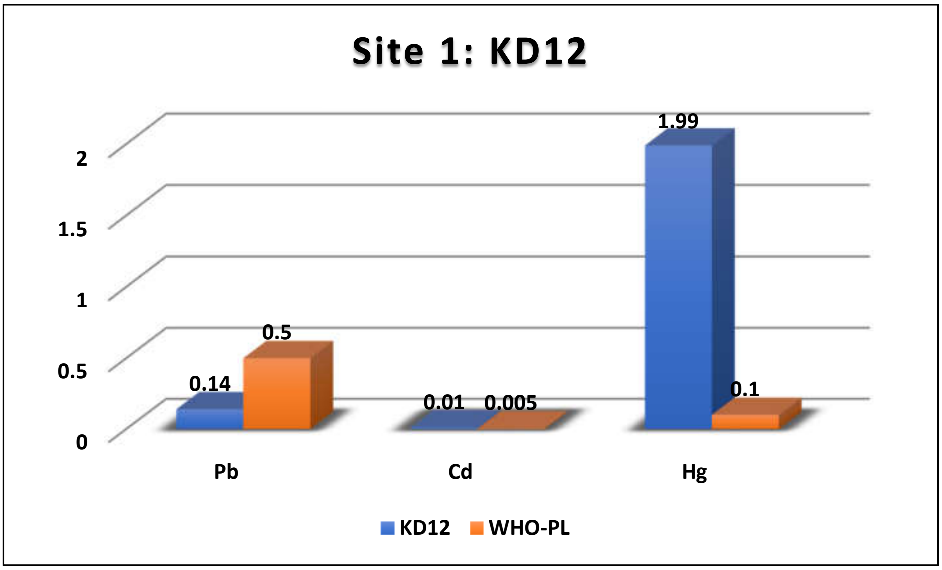

Figure 4: Comparison of different heavy metal concentrations of samples collected from Site 1 with that of WHO permissible levels

Cadmium (Cd) levels varied between the two sites, with Site 2 samples showing no detectable amounts, while Site 1 samples recorded a concentration of 0.010 mg/kg in *L. vannamei*. The data indicates that the samples from Site 2 have a lower concentration of cadmium than those from Site 1, making them safe for consuming and non-toxic to consumers.

Mercury (Hg) levels were significantly higher, with *L. vannamei* from Site 1 and *M. monoceros* from Site 2 recording concentrations of 1.990

mg/kg and 0.975 mg/kg, respectively, surpassing permissible limits (Fig. 5). This poses a serious health risk to consumers, as mercury, especially in its organic form, is highly toxic. According to the study, humans are not the only ones that consume fish as a significant cause of mercury exposure, since aquatic organisms also acquire mercury from natural sources and human activity.

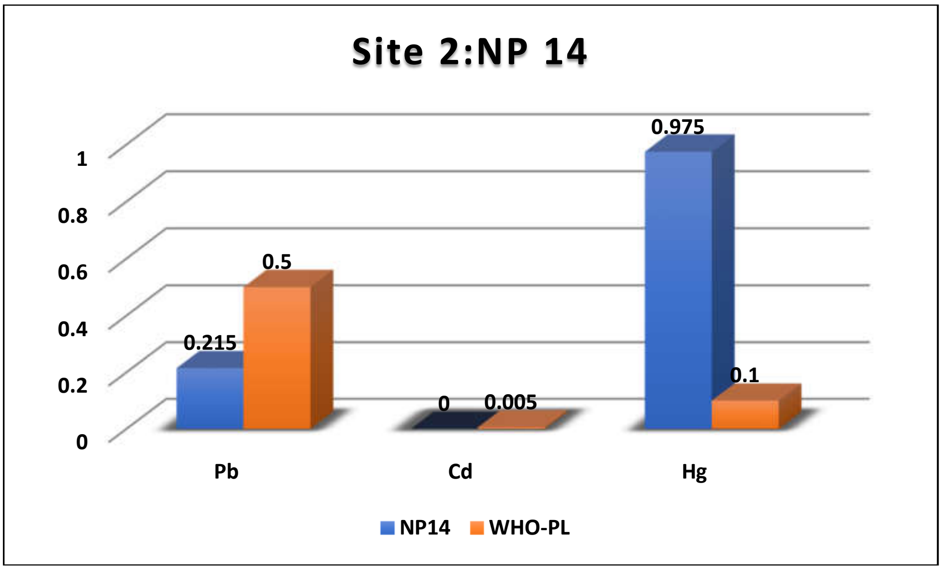

Figure 5: Comparison of different heavy metal concentrations of samples collected from Site 2 with that of WHO permissible levels

Table 2: showing analysis of different sample for detection of heavy metals

Sl. No.	Sample Code	Pb mg/kg	Cd mg/kg	Hg mg/kg
1	Site 1 -KD12	0.14	0.010	1.990
2	Site 2- NP14	0.215	0	0.975
3	WHO -EU max limit	0.5	0.005	0.1

Test (1) Let us consider the null hypothesis H_0: the mean Lead (Pb) concentrations in *L. vannamei* (μ_1) from Site 1 and *M. monoceros* (μ_2) from Site 2 are same. That is $\mu_1 - \mu_2 = 0$. Against the alternate hypothesis H_1: the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2 is greater than *L. vannamei* (μ_1) from Site 1. That is $\mu_2 - \mu_1 > 0$.Here the sample size n = 11, is small (less than 30) and population standard deviation (sd) σ is unknown. Estimate the sd (*Sp*) from sample data and apply t – test for checking the equality of means at level of significance (LS) α = 0.05. The test statistics t is, $t = \frac{XL - XM}{SP\ ROOT[(n1+n2)/n1n2]}$ follows t (n_1-1, n_2-1) at LS α, where X_L = the mean Lead (Pb) concentrations in *L. vannamei* (μ_1) from Site 1 and X_M = the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2. n_1 sample size from site 1 and n_2 sample size from site 2.The estimated pooled sd of the population *SP* = root $\{[(n_1-1)\, s_1^2 + (n_2-1) s_2^2] / (n_1 + n_2 - 2)\}$. If the p – value is greater than α then there is no reason to reject the null hypothesis. Here we get t = (-2.38), the p-value is .0136, the result is significant since p is less than α = 0.05. Hence the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2 is greater than *L. vannamei* (μ_1) from Site 1.

Test (2) Consider the null hypothesis H_0: the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2, $\mu_2 = 0.3$ Against the alternate hypothesis H_1:the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2, $\mu_2 < 0.3$ By using single population t-test for testing the value of mean, we get the value of the test statistic t is (-2.819). The value of p is 0.009. The result is significant since $p < \alpha = 0.05$. That is the mean Lead (Pb) concentrations in *M. monoceros* (μ_2) from Site 2, $\mu_2 < 0.3$. So from Test1 and Test 2, it can be established that the mean Lead (Pb) concentrations in *L. vannamei* (μ_1) from Site 1 and *M. monoceros* (μ_2) from Site 2 are under permissible limits, which is less than 0.5 mg/kg.

Test (3) Consider the null hypothesis H_0: the mean Cadmium (Cd) concentrations in *L. vannamei* (μ_1) from Site 1 is 0.005, Against the alternate hypothesis H_1: the mean Cadmium (Cd) concentrations in *L. vannamei* (μ_1) from Site 1 is greater than 0.005 by using single population t-test for testing the value of mean. The t-value is 16.58. The value of p is 0.00001. The result is significant since $p < \alpha = 0.05$. That is a Cadmium (Cd) concentration in *L. vannamei* (µ1) from Site 1 is greater than the permissible limit 0.005 mg/kg.

Test (4) Consider the null hypothesis H_0: the mean Mercury (Hg) concentrations in *L. vannamei* (μ_1) from Site 1 and *M. monoceros* (μ_2) from Site 2 are same. That is $\mu_1 - \mu_2 = 0$. Against the alternate hypothesis H_1: the mean Mercury (Hg) concentrations in *L. vannamei* (μ_1) from Site 1 is greater than *M. monoceros* (μ_2) from Site 2. That is $\mu_1 - \mu_2 > 0$. The t-value is 16.32. The value of p is 0.00001. The result is significant since $p < \alpha = 0.05$. The mean Mercury (Hg) concentrations in *L. vannamei* (μ_1)from Site 1 is greater than *M. monoceros* (μ_2) from Site 2.

Test (5) Consider the null hypothesis H_0: the mean Mercury (Hg) concentrations in *M. monoceros* (μ_2) from Site 2 = 0.1 That is $\mu_2 = 0.1$, against the alternate hypothesis H_1: the mean Mercury (Hg) concentrations in *M. monoceros* (μ_2) from Site 2 is greater than 0.1 Here from t-test, the value of p is 0.00001. The result is significant since $p < \alpha = 0.05$ and reject the Null Hypothesis. The mean Mercury (Hg) concentrations in *M. monoceros* (μ_2) from Site 2 is greater than the permissible limit 0.1 mg/kg.

From Test 4 and Test 5 it is clear that the mean Mercury (Hg) concentrations in *L. vannamei* (μ_1) from Site 1 and *M. monoceros* (μ_2) from Site 2 are greater than the permissible limit 0.1 mg/kg.

All these tests imply some significant results and should consider seriously clearing such important issues. Many cases show the concentrations of heavy metals lead (Pb), cadmium (Cd), and mercury (Hg) in two species of prawns (*Litopenaeus vannamei* and *Metapenaeus monoceros*) cultured in aquaculture farms in Kerala, India is more than the permissible limit prescribed by WHO -EU max limit.

DISCUSSION

The results of the study emphasize how crucial it is to keep an eye on the levels of heavy metals in aquatic species, especially those that are intended for human consumption. While some samples remained within permissible limits, others exceeded them, highlighting the need for stricter regulations and better waste management practices to prevent further contamination of aquatic environments. In order to pinpoint the precise causes of heavy metal contamination and reduce its negative effects on aquatic ecosystems and human health, more investigation is also necessary.

Sources of Cd contamination include runoff from agricultural lands, sewage sludge, industrial activities such as electroplating and mining, and the combustion of fossil fuels. Aquatic invertebrates, including prawns, absorb Cd from their surroundings and diets, with most unable to regulate its uptake and excretion. Our results showed that samples from site 1 had Cd contents above the WHO permitted levels (0.005 mg/Kg), which may indicate health hazards from consumption.

High concentrations of Cd and Hg accumulate within tissues causing death of organisms (Tavabe *et al*., 2019; Frias- Espericueta *et al*., 2001). When prawns were exposed to more than 60 µg/L of cadmium, their survival rates were reduced than those of the lower concentrations. Additionally, when exposed to different amounts of cadmium, they showed histological abnormalities in their muscles, gills, and hepatopancreas (Kaoud and Rezk, 2011). Sulthana *et al*. (2022) reported the concentrations of Cd, As and Hg were lower than the threshold limits whereas the concentrations of Pb and Cu levels in all shrimp samples were higher than the recommended limit. Nsofor *et al*. (2014) discussed the effects of lead pollution on the aquatic environment, emphasizing its toxicity and potential health risks. Our study found Pb levels below permissible limits but highlights the importance of continuous monitoring to prevent adverse effects on aquatic organisms and human health.

Heavy metal pollution in Sabah, Malaysia's coastal areas was documented by Hashmi *et al*. (2002), identifying possible sources of contamination from land-based operations. This is consistent with our findings, which indicate that interactions between inshore water and land-based activities might result in significant levels of heavy metals in the water in prawn ponds. According to Lee *et al.* (2017), heavy metal contamination in aquaculture settings is a common occurrence. They detected heavy metal concentrations in *Penaeus vannamei* from Korean aquaculture farms. By evaluating the level of heavy metal contamination in prawns from aquaculture farms in Kerala, India, our work adds even more to this body of knowledge. Satarug *et al.* (2010) shed light on the physiological and metabolic consequences of cadmium, providing insights into its toxicological effects. The finding of higher than average levels of Cd in prawns highlights the possible health hazards linked to consuming contaminated seafood.

Similar to our observation, Mostafiz *et al.* (2020) also concluded that the consumption of the studied prawn species contaminated with elevated levels of toxic metals is associated with higher degree of potential health risks.

Amaral *et al*. (2005) found evidence supporting the possibility of metal accumulation in aquaculture settings by analyzing the amounts of metals in *L. vannamei* shrimp from coast shrimp ponds in Peru. By evaluating the level of heavy metal contamination in prawns from aquaculture farms in Kerala, India, our study expands upon previous research. Prawns have the ability to ingest and collect metals from their surroundings, as demonstrated by Stewart *et al.'s* (2014) assessment on the cadmium accumulation in *Penaeus vannamei* exposed to dietary cadmium.

Our finding that prawns from contaminated aquaculture ponds had higher than average levels of Cd is corroborated by this. The significance of keeping an eye on the levels of heavy metals in seafood was highlighted by Fatema *et al.* (2015) when they evaluated the contamination of shrimp and prawn from Bangladesh. Islam *et al.* (2017) also evaluated the bioaccumulation of heavy metals in shrimp and fish from the Meghna River estuary in Bangladesh, as well as the contamination of sediment with these metals. Their research demonstrated the widespread impact of heavy metal pollution in aquatic ecosystems. Our research supports this by pointing to comparable issues in Kerala, India's aquaculture settings.

Cadmium was one of the heavy metals that Hashmi *et al.* (2002) found accumulating in prawn tissues that were taken from different farms; the main source of contamination was probably from the nearby river. According to Stewart *et al.* (2014), prawns have the capacity to efficiently absorb cadmium from their diet and may also be able to gradually eliminate it through the hepatopancreas' granules rich in cadmium. According to Heidarieh *et al.* (2013), metal accumulation was observed in the hepatopancreas of Persian Gulf prawns, underscoring the significance of this organ as a metal storage location in crustaceans. This is in line with the findings we observed which suggested that the hepatopancreas of prawns from Kerala had higher concentrations of Cd and Hg. These results are all consistent with what we have seen.

In the same way, increased mercury (Hg) levels in prawn samples from sites 1 and 2 raise questions concerning potential hazards to human health. One of the most significant aspects is that the EU only allows 0.1 mg/kg of mercury in feed. In contrast to certain earlier research that found lower mercury contents in shrimp, our results show levels that are significantly higher than allowable limits, threatening shrimp consumers.

The bioaccumulation of mercury and cadmium in prawn tissues highlights the urgent need for better aquaculture monitoring and management techniques. The primary source of mercury exposure for humans and animals is fish intake, although plants and livestock can also be exposed to mercury due to environmental concentrations in freshwater and saltwater, sediment, soil, and air. This happens because organisms in the food chain eat other organisms with mercury, making it build up in their bodies (Muzyed, 2011; EC, 2006; FAO, 1976). Our study adds to this body of research by providing insights into heavy metal contamination in prawns from aquaculture farms in Kerala, India.

The toxicity of heavy metals such as Cd and Hg isn't restricted to their direct physiological effects on living things; they can also cause acute and long-term health problems through bioaccumulation in organ tissues. The heavy metal prevalence order in our samples Hg being the most abundant, followed by Pb and Cd aligns with previous research findings. The findings underscore the significance of persistent investigation and regulatory actions aimed at reducing the hazards associated with heavy metal pollution in aquatic environments and safeguarding human well-being.

CONCLUSION

In this study, we investigated the levels of heavy metals in two popular prawn species from two aquaculture farms in Kerala: *Litopenaeus vannamei* and *Metapenaeus monoceros*. According to our research, lead (Pb) was the least prevalent element detected in the hepatopancreas of both prawn species, while cadmium (Cd) and mercury (Hg) were the most prominent metals. Notably, lead contents in all shrimp samples taken from Sites 1 and 2 were within permitted limits, proving the non-toxicity of the species. Nevertheless, the samples from Site 1 had much higher than recommended levels of cadmium (0.010 mg/kg) and mercury (1.9 and 0.97 mg/kg), making them unsuitable for consumption and presenting a major health risk to consumers. Here the statistical data analysis also synchronized with the real data results and emphasizing the same. These findings underscore the potential risks associated with prawn consumption, particularly in an area where prawn production and export are significant economic activities. While Pb levels were within acceptable limits, the presence of elevated mercury and cadmium levels highlights the urgent need for improved prawn farm management practices and further research into the interactions and synergies of these metals. Our study sheds light on the importance of monitoring heavy metal concentrations in prawn aquaculture, emphasizing the need for stringent regulations and proactive measures to safeguard both human health and the sustainability of the aquaculture industry in the region.

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