Assessment of Lead and Mercury Contamination in Amphidromous Goby Larvae (Nike), Water Quality, and Associated Human Health Risks in Bone Estuary, Indonesia

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ABSTRACT

The high market demand for Nike fish as a food source necessitates attention to the sustainability of natural resources and food safety. This study aimed to determine the concentrations of lead (Pb) and mercury (Hg) in Nike fish, assess the water quality of their habitat, and evaluate potential health risks to consumers. Sampling was conducted in September 2022 and July 2023 at three stations along the downstream and estuary of the Bone watershed. Atomic absorption spectrophotometry was utilized to measure the heavy metal concentrations in 250 fish samples. Water parameters were analyzed using principal component analysis (PCA) to explore their relationship with the heavy metals A Hazard Index analysis was employed to estimate potential health risks to humans consuming the fish. The results revealed that heavy metal concentrations in Nike fish were low: Pb ranged from 0.0019 to 0.0060 ppm and Hg ranged from 0.0013 to 0.0036 ppm. A significant difference ($p<0.05$) in Pb concentrations among the sampling sites was observed, whereas Hg concentrations showed no statistically significant difference ($p\geq0.05$) between site 1 and sites 2 and 3. Water quality assessments indicated pollution levels ranging from clean to moderately polluted. Human health risk evaluations through total hazard quotient (8.9×10^{-4} for Pb, 2.7×10^{-2} for Hg) and Hazard Index analyses (2.8×10^{-2} for total metals) confirmed that the levels of heavy metals in Nike fish from the Bone Estuary pose no significant risk to human health. These findings confirm the safety of consuming Nike fish, supporting its continued use as a valuable food resource in the Bone watershed.

Keywords: Heavy metals, Nike fish, Pollution Index, STORET, Tomini Bay

INTRODUCTION

Nike, a group of goby fish belonging to the Gobiidae and Eleotridae families, is an important aquatic resource in the Gorontalo region (Pasisingi and Abdullah, 2018; Sahami *et al*., 2020; Pasisingi and Olii, 2023). The fish schools comprises multiple species (Sahami *et al*., 2019; 2020). In 2020, the Ministry of Law and Human Rights of the Republic of Indonesia officially recognized Nike fish as a Communal Intellectual Property Right for the Gorontalo Province (Botutihe, 2020). This fish has a unique life cycle known as amphidromous, characterized by habitat transitions, inhabiting both marine and estuarine environments, with periodic migrations to freshwater for growth and spawning purposes (McDowall, 1988; McDowall, 1997). They migrate from Tomini Bay to the upper reaches of the Bone River across the Bone Estuary (Olii *et al*., 2017). Olii *et al*. (2019) and Sahami and Habibie (2020) documented that a significant portion of adult Nike fish inhabits the waters of the Bone River, which ultimately flows into the Bone Estuary. They only appear for a limited duration during the early phase of the moon in the estuary (Pasisingi *et al*., 2021a; 2021b; Olii and Pasisingi, 2022b).

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The unique ecological nature of the estuary, serving as a meeting area for freshwater and seawater, makes this ecosystem crucial and strategic for the Nike population. The estuary is pivotal in ensuring the sustainability of the Nike fish population. During their larval stage, Nike fish exhibit a high degree of vulnerability, making them particularly sensitive to fluctuations in water quality, highlighting the need to assess their habitat. Nike fish is also favored seafood choice among the local community, accompanied by concerns related to heavy metal contamination.

Heavy metals are a growing global issue due to their persistence in the environment, impact on biogeochemical cycles, and ecological risks (Gu *et al*., 2018). Previous studies, including those by Ruaeny *et al*. (2015), Ismarti *et al*. (2017), Amqam *et al*. (2020), and Lestari *et al*. (2021), have reported that several fish species consumed from Indonesian waters were contaminated with heavy metals such as lead (Pb), mercury (Hg), cadmium (Cd), copper (Cu), argon (Ar), and zinc (Zn). Studies by Doe *et al*. (2014) and Noor (2018) detected mercury (Hg), lead (Pb), and cadmium (Cd) in the Bone Estuary. Investigations by Lihawa and Mahmud (2012), Koniyo (2020), and Kadim *et al*. (2022a) have highlighted heavy metal contamination in the Bone River. Pb, Hg, and Cd are non-essential heavy metals, among the most toxic, and difficult to degrade naturally (Dewi *et al*., 2015; Erasmus, 2020). Consuming seafood contaminated with heavy metals can significantly impact human health.

Methylmercury is highly toxic due to its strong binding power and high solubility, especially in the bodies of aquatic organisms (Lihawa and Mahmud, 2012). Heavy metals enter the environment through various biogeochemical cycles, infiltrate the human food chain, and eventually undergo bioaccumulation and biomagnification (Doyi *et al*., 2018). Accumulated Pb and Hg in the human body can cause acute and chronic toxic effects, disrupting physiological processes (Amqam *et al*., 2020). Numerous studies have reported heavy metal contamination accumulating in organisms ranging from plankton and benthos to fish and humans. The case of Itai-itai disease in Toyama, Japan, was a significant event caused by cadmium exposure, leading to bone softening, muscle contraction, and severe pain (Kawano *et al*., 1986; Wang *et al*., 1994; Nogawa and Suwazono, 2011; Horiguchi, 2014). Minamata disease in Japan, related to industrial pollution, led to the release of methylmercury into waters, subsequently accumulating in seafood and causing serious neurological symptoms and even death (Takaoka *et al*., 2018).

There is an urgent need to investigate the potential health risks associated with consuming contaminated aquatic species. This research aims to quantify Pb and Hg concentrations in Nike fish, assess the water quality of their habitat, and evaluate the potential health risks to consumers in Bone watershed, Gorontalo Province, Indonesia.

MATERIALS AND METHODS

Sampling sites and collection methods

Nike fish in Gorontalo waters is a school of goby amphidromous larval and juvenile stages composed of the families Gobiidae and Eleotridae. Sahami *et al* (2024) identified the Nike fishes in Bone Estuary, consisting of 13 species: *Belobranchus belobarchus, B. elano, Bunaka gyrinoides, Eleotris elanosome, Eleotris fusca, Awaous ocellaris, Sicyopterus cynocephalus, S. lagocephalus, S. longifilis, S. microcephalus, S. parvei, Sicyopus zosterophorus,* and *Stiphodon semoni*. Fish samples were collected from three distinct locations within the Bone watershed, covering both downstream and estuarine regions (Figure 1). These areas were intentionally, selected to represent different stages of Nike fish development, transitioning from more saline to predominantly freshwater conditions, reflecting various larval growth phases. Site 3 was further divided into two areas: the eastern (Leato) and western (Pohe) seas, based on the natural emergence pattern of the Nike fish, which were never simultaneously present in both areas. Given that Nike fish appear seasonally in Gorontalo waters and are not always abundant, samples were randomly collected during periods of high abundance in September 2022 and July 2023. Sampling was conducted daily as long as Nike fish were present in the waters. The climatic conditions during the sampling period are presented in Table 1.

Measurement of water quality parameters as a description of the habitat of Nike fish was carried out. Temperature, pH, salinity, total dissolved solids (TDS), and conductivity parameters were assessed using a Water Quality Meter Model EZ 9909SP. Transparency was measured using a Secchi Disc, dissolved oxygen (DO) levels were monitored with a Dissolved Oxygen Meter Model AR8210, and turbidity was gauged using a Spectrophotometer. Depth and water current speed were determined using a scale stick and a current kite, respectively.

Heavy metal analysis of fish samples

Approximately 250 fish samples were gathered from each of the designated study locations. These samples were carefully placed within polyethylene bags, preserved in a coolbox to prevent contamination, and subsequently transported to the laboratory for heavy metal analysis. The preparation method followed the procedures outlined by Yu *et al*. (2020). To determine the heavy metal concentrations within the fish sample, the Standard Methods for the Examination of Water and Wastewater Ed. 23rd, part 3120 B (Rice *et al*., 2017) was employed. Fish samples were rinsed with distilled water, placed on a petri dish, and dried in an oven at 105 °C. A fish sample weighing approximately 5 g was then placed into a porcelain cup and heated at 500 °C for 2–3 h until the sample turned to white ash. Next, the ash sample was cooled, and 50 mL of distilled water was added. The mixture was filtered using polycarbonate filter paper (0.40–0.45 µm), stirred, and analyzed with an atomic absorption spectroscopy

Table 1. Climatic conditions during sampling in Bone Estuary area.

Year	Air temperature (°C)	Humidity (%)	Rainfall (mm)	Sunlight duration (h)	Wind speed (m·s^{-1})
2022	29.47±3.48	85±0.10	8.22±1.99	4.60±0.79	0.95±0.77
2023	32.63±1.57	73.71±0.15	2.23±2.39	6.32±1.39	0.877±0.68

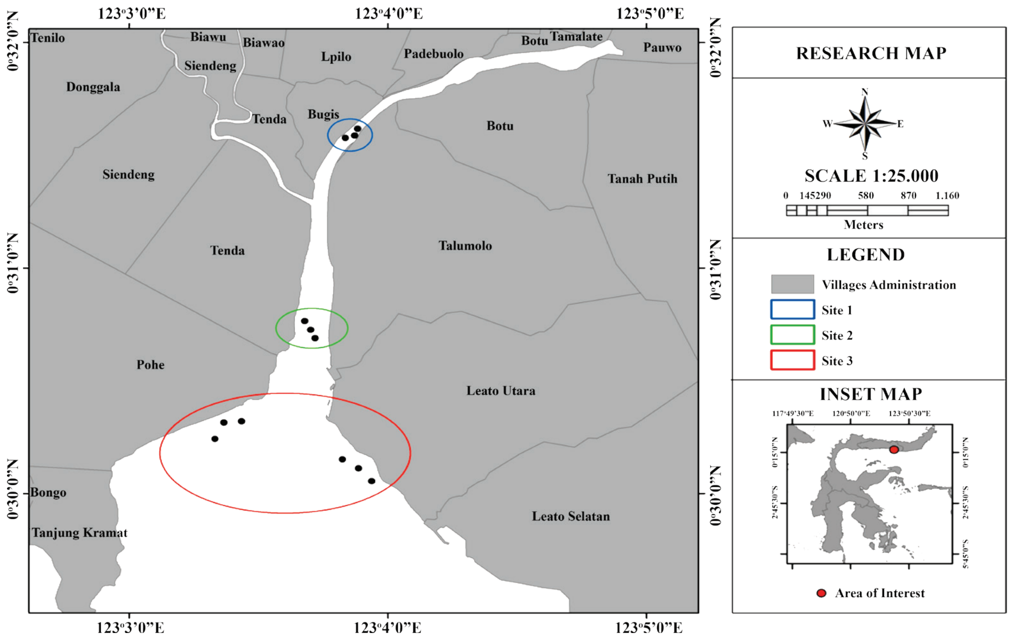

Figure 1. The research location and sampling sites in Bone Estuary area for fish sampling and water quality measurement in Indonesia.

(AAS)-Shimadzu 7000, with a detection limit of 0.001 $mg{\cdot}kg^{-1}$. The standard solution for further analysis was prepared by diluting 5 mL of a standard solution containing 100 $mg{\cdot}L^{-1}$ of Pb and Hg into a 10 mL measuring flask with distilled water. This solution was further diluted to concentrations of 0.1 $mg{\cdot}L^{-1}$, 0.2 $mg{\cdot}L^{-1}$, 0.3 $mg{\cdot}L^{-1}$, 0.4 $mg{\cdot}L^{-1}$, and 0.5 $mg{\cdot}L^{-1}$. The readings obtained from the spectrophotometer were recorded as numerical values corresponding to the heavy metal contents in the samples. These data were reported in parts per million (ppm).

Data analysis

The analysis of in-situ water quality parameters was conducted using the STORET method and the Pollution Index (PI) method, in accordance with the Decree of Environmental Ministry Regulation No. 115/2003 (Indonesian Ministry of Environment, 2003). The Water Quality Index (WQI) used in this study refers to Brown *et al*. (1970). The standards for water parameter quality in the STORET and PI analyses complied with the Government Regulation of the Republic of Indonesia Number 22 of 2021. These methods assessed the water pollution status at the time of fish samples collection. Water quality status based on STORET is classified into 4 classes: Class A (meets quality standards, score 0); Class B (lightly polluted, score -1 to -10); Class C (moderately polluted, score -11 to -30); Class D (heavily polluted, score ≤-31). Water quality, referred to as PI, is also divided into four categories: good condition ($0<PI<1$), lightly polluted ($1.1<PI<5.0$), moderately polluted ($5.1<PI<10$), and heavily polluted ($PI>10$). Assessing water quality using WQI is divided into six criteria: very clean ($WQI\leq0.30$), clean ($0.31\leq WQI\leq0.89$); lightly polluted ($0.90\leq WQI\leq2.49$); moderately polluted ($2.50\leq WQI\leq3.99$); heavily polluted ($4.00\leq WQI\leq5.99$); dirty ($WQI\geq6.00$).

The data generated from these analyses were visualized using appropriate graphs and figures, utilizing the GraphPad Prism 9 application. Principal Component Analysis (PCA) using Minitab 14.00 software was employed to unveil patterns of relationships among Pb and Hg concentrations in Nike fish samples and the measured water quality parameters. This multifaceted approach enhances our understanding of the interplay between heavy metal content in the fish and the associated water quality characteristics.

Statistical analysis

A two-way ANOVA (Analysis of Variance) with a 95% confidence level ($\alpha = 0.05$) was applied to determine significant differences in heavy metal concentrations in fish across the three sampling stations. This analysis also testes significant variations in water quality parameters measured at different times and stations in Bone Estuary. A Post Hoc t-test was carried out when the ANOVA p-value was statistically significant ($p<0.05$) to identify the different source groups of diversity with certainty.

Health potential risk assessment

The people of Gorontalo commonly consume Nike fish. Therefore, the health risk from consuming these fish was evaluated using Target Hazard Quotients (THQ) and the total Hazard Index (HI) (United States Environmental Protection Agency (USEPA), 2011; Yap *et al*., 2016; Yu *et al*., 2020). The THQ was employed to evaluate the potential dangers associated with the consumption of various aquatic creatures, following the formula outlined by USEPA (1989) using Equation 1:

$$THQ = \frac{E_D \times F_{IR} \times E_F \times C}{R_{FD} \times W_{AB} \times T_A} \times 10^{-3} \quad (1)$$

Where;

E_D: the exposure duration (70 years)

F_{IR}: daily ingestion rate in $g{\cdot}capita{\cdot}day^{-1}$ = 57.58 for fish (Food and Agriculture Organization (FAO), 2013).

E_F: the exposure frequency (365 $days{\cdot}year^{-1}$).

C: the concentration of heavy metals in aquatic samples ($mg{\cdot}kg^{-1}$).

R_{FD}: the reference oral dose in $mg \times kg_{bw}^{-1}d^{-1}$ (1×10^{-4} for Hg and 4×10^{-3} for Pb)

W_{AB}: the average body eight for an adult consumer (60) (Purbonegoro, 2020).

T_A: the average exposure time (365 $days{\cdot}year^{-1} \times E_D$).

A THQ value greater than 1 indicates that exposure exceeds the established safety limit, potentially endangering health (Copat *et al*., 2014). Daily exposure limits exceeding the safe reference can result in health risks (Copat *et al*., 2014; Fang *et al*., 2014). Moreover, Hallenbeck and Cunningham (1988) also noted that exposure to multiple heavy metals may produce an additive effect of potential risks. The Hazard Index (HI) was calculated as the sum of hazard quotients, using Equation 2 (USEPA, 1989).

$$HI = THQ_{Pb} + THQ_{Hg} \quad (2)$$

RESULTS AND DISCUSSION

Heavy metals concentration in Nike fish

The analysis of heavy metal concentrations, specifically Pb and Hg, within Nike fish tissues, revealed noteworthy findings across all sampling locations. As illustrated in Figure 2a, the results of the statistical tests indicate a statistically significant difference ($p<0.05$) in Pb concentrations among the samples obtained from the three distinct sampling sites. Conversely, the concentration of Hg in fish from Site 1 did not exhibit a statistically significant difference ($p>0.05$) compared to the samples collected from Site 2 and Site 3, as clearly depicted in Figure 2b.

In the year 2022, it is noteworthy that Hg concentrations in Nike fish samples from Site 1 and Site 2 displayed a similar trend, with an average concentration of 0.0033 ppm (Figure 2b). In contrast, samples gathered from Site 3 exhibited the Hg concentration, with an average of 0.0023 ppm. A contrasting pattern was observed concerning Pb concentrations, with the highest Pb concentration recorded in samples collected from Site 2, averaging at 0.0053 ppm. Samples from Site 1 displayed a slightly lower average Pb concentration of 0.0037 ppm, while the lowest Pb concentration was evident in samples originating from Site 3, registering at 0.0024 ppm. These findings underscore the variability in heavy metal concentrations across the different sampling sites and highlight the need for further investigation into the factors contributing to these variations.

In the year 2023, there appears to be a noticeable trend in the Hg concentrations within Nike fish tissues, indicating an decrease from Site 1 to Site 3. Specifically, the Hg content in fish samples collected from Site 1, Site 2, and Site 3 displayed average concentrations of 0.0031 ppm, 0.0026 ppm, and 0.0019 ppm, respectively. Simultaneously, about Pb content in Nike fish, the highest Pb concentration was observed in samples obtained from Site 2, with an average concentration of 0.0046 ppm. Samples from Site 1 exhibited an average Pb concentration

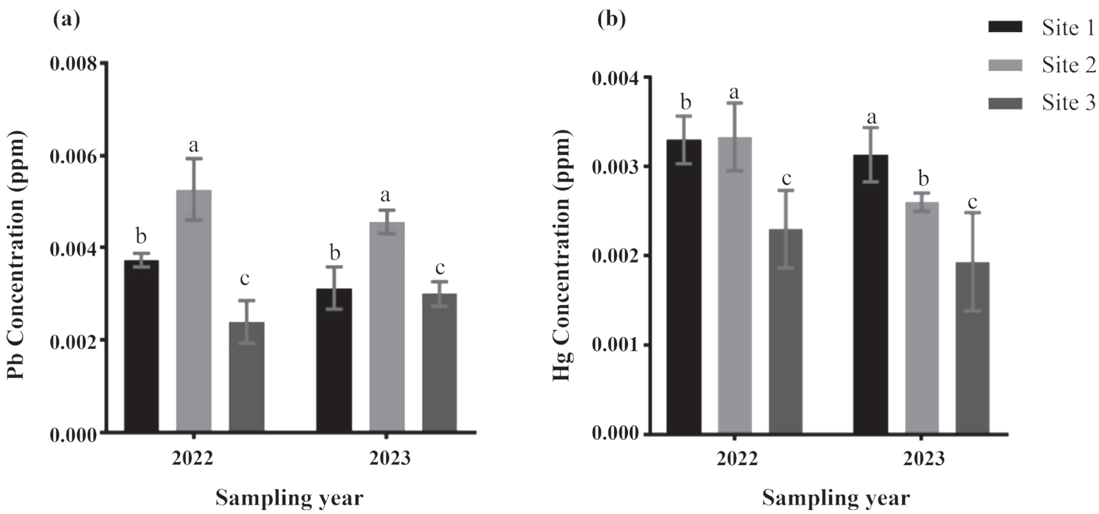

Figure 2. Heavy metal concentrations in nike fish at the three study sites in 2022 and 2023: (a) Pb; (b) Hg; Different lowercase letters above bars denote significant ($p<0.05$) difference within each year; error bars represent SD.

of 0.0031 ppm, while those from Site 3 had an average Pb concentration of 0.0030 ppm. These findings suggest a potential shift in the heavy metal composition within Nike fish tissues, particularly a rising trend in Hg concentrations from Site 3 to Site 1 in 2023. It is notable that Site 2 consistently exhibited the highest Pb concentration among the sampling locations. Further investigation and monitoring may be warranted to better understand the dynamics and factors contributing to these variations in heavy metal content. The study findings indicate a noteworthy trend: the heavy metal content,specifically Pb and Hg, in Nike fish bodies, tended to be highest in samples collected from the river and estuary areas (Site 1 and Site 2), in contrast to the sea (Site 3). This pattern suggests that heavy metals, possibly originating from sediment carried by the Bone River (Olii and Pasisingi, 2022a), may accumulate at the bottom of the estuary and subsequently be released into the surrounding waters. Nike fish are then exposed to these heavy metals through feeding and respiratory processes. The work of Kadim *et al*. (2022b) supports this hypothesis, linking anthropogenic activities in the Bone River to the presence of Pb, Hg, and Cd metals, primarily sourced from domestic and agricultural waste. These heavy metals are more concentrated in the downstream areas of the river due to the cumulative impact of activities in the upstream and middle sections of the river. Heavy rainfall and flooding events have likely contributed to the transportation of river sediment into the estuary and adjacent areas. It is essential to consider the developmental aspects of Nike fish larvae concerning their proximity to the river. As Nike fish move closer to the river, their age and the maturation of their respiratory and digestive systems increase. This progression aligns with the developmental phases of individual Nike within the population, further underscoring the intricate dynamics at play in this ecosystem.

Water quality status of Bone Estuary

The data of water quality parameters in the Bone Estuary, measured concurrently with fish sampling, have been succinctly presented in Table 2. Furthermore, the results of the statistical analysis test the various water quality parameters for comparing the differences among three sampling sites and two durations using the ANOVA test were presented in Table 3.

In simple terms, it can be seen that there are variations in the average values of the water quality parameter measurements in the Bone Estuary. A discernible observation from Table 2 is that the average water depth was deepest at Site 2, while it was shallower at Site 1. Notably, transparency and pH values at all sites in 2022 were relatively lower compared to those recorded in 2023. The salinity parameter at Site 1 registered at zero, attributed to its proximity to the lower reaches of the Bone River, whereas it increased progressively towards the sea, reaching a value of 23.67‰ in 2023. Water turbidity measurements for all sites in 2022 were notably higher than the data recorded in 2023. Total Dissolved Solids (TDS) and water conductivity values measured at Site 2 and Site 3 exceeded those measured at Site 1. Current speed, water surface temperature, and Dissolved Oxygen (DO) parameters exhibited minor variations among the three sites. However, there were no significant differences in all water quality parameters measured at two different times and in three different areas, as can be seen from all ANOVA analysis $p>0.05$, as shown in Table 3.

The analysis results employing the STORET, Pollution Index, and WQI methods collectively indicated that the waters of the Bone Estuary during the sampling period were categorized as ranging from clean to moderately polluted, as detailed in Table 4.

Based on 10 measured water quality parameters at Site 2 and Site 3, the water quality status based on STORET and PI is moderately polluted, while based on WQI those two sites are categorized as clean to lightly polluted. As for Site 1, the water quality status tends to be better with the categories clean, meets quality standard, and lightly polluted. The water quality parameters that are thought to be the cause of the low water quality status at several sites in this research are pH, transperancy, turbidity, and salinity whose concentration values do not meet quality standards. The possible cause of the poor water quality parameters is the input of

Table 2. Measurement of water quality parameters of Bone Estuary for 3 sampling sites during 2022 and 2023.

Sampling time	Area Site	Depth (m)	Current speed ($m \cdot s^{-1}$)	Transparency (m)	Temperature (°C)	pH	Salinity (‰)	DO ($mg \cdot L^{-1}$)	Turbidity (NTU)	TDS ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)
2022	1	1.15±0.13	0.61±0.09	0.28±0.04	29.09±0.23	6.94±1.29	0.00±0.00	7.63±0.07	57.29±29.63	80.03±11.63	154.61±16.87
	2	2.01±0.47	0.03±0.01	0.28±0.05	30.20±0.47	6.92±1.46	1.61±1.13	7.37±0.16	37.54±14.86	3,445.89±760.92	5,817.33±890.85
	3	1.56±0.20	0.19±0.14	0.60±0.45	30.22±2.13	6.80±1.23	7.83±4.17	7.48±0.29	28.49±33.25	7,346.67±1,114.52	14,690.00±2,229.62
2023	1	1.50±0.13	0.25±0.10	1.38±0.12	26.42±1.40	7.61±0.10	0.00±0.00	7.90±0.41	5.47±3.05	76.68±0.67	153.46±1.35
	2	1.60±0.36	0.24±0.14	1.17±0.27	27.49±2.19	7.36±0.34	1.33±2.31	7.91±0.19	8.09±0.35	1,699.06±2,396.41	774.33±241.79
	3	1.22±0.18	0.21±0.13	1.00±0.50	28.60±0.45	7.75±0.17	23.67±5.85	7.61±0.11	4.01±3.77	9,521.67±742.69	1,913.83±132.05

Table 3. p-value of ANOVA analysis result of water quality parameters of Bone Estuary among three sampling sites and between two years.

Source of Variance	Depth (m)	Current speed ($m \cdot s^{-1}$)	Transparency (m)	Temperature (°C)	pH	Salinity (‰)	DO ($mg \cdot L^{-1}$)	Turbidity (NTU)	TDS ($mg \cdot L^{-1}$)	Conductivity ($\mu S \cdot cm^{-1}$)
Time (Year)	0.061	0.437	0.229	0.157	0.229	0.511	0.346	0.058	0.931	0.261
Area (Site)	0.055	0.615	0.808	0.490	0.703	0.327	0.717	0.230	0.206	0.419

domestic waste&agricultural wastes and other sources of pollution into the Bone River. Apart from that, the river body modification factor that occurs results in erosion, which affects pH, turbidity, and transperancy due to the input of soil particles. Waste can affect the pH of river water through soil erosion. Agricultural waste or industrial waste that pollutes rivers can increase the amount of sediment that enters the river. These soil particles can react with water and produce acidic substances. This is thought to be the factor that makes the pH value of Bone Estuary water tend to be acidic. The flow of river water will affect the condition of the estuary.

Regarding the PCA, as depicted in Figure 3, it is evident that no individual water quality parameter displayed a clear association with heavy metals found in the body tissues of Nike fish throughout the study, except for water depth.

Specifically, the eigenvalues for the PCA accounted for 61.3% and 38.7% of the variance,

Table 4. Pollution status of Bone waters as Nike fish habitat in 3 sampling sites during 2022 and 2023.

Time	Methods	Sampling area		
		Site 1	Site 2	Site 3
2022	STORET	Meet quality standard	moderately polluted	moderately polluted
	PI	lightly polluted	moderately polluted	lightly polluted
	WQI	Clean	lightly polluted	lightly polluted
2023	STORET	Meet quality standard	moderately polluted	moderately polluted
	PI	lightly polluted	moderately polluted	moderately polluted
	WQI	Clean	Clean	Clean

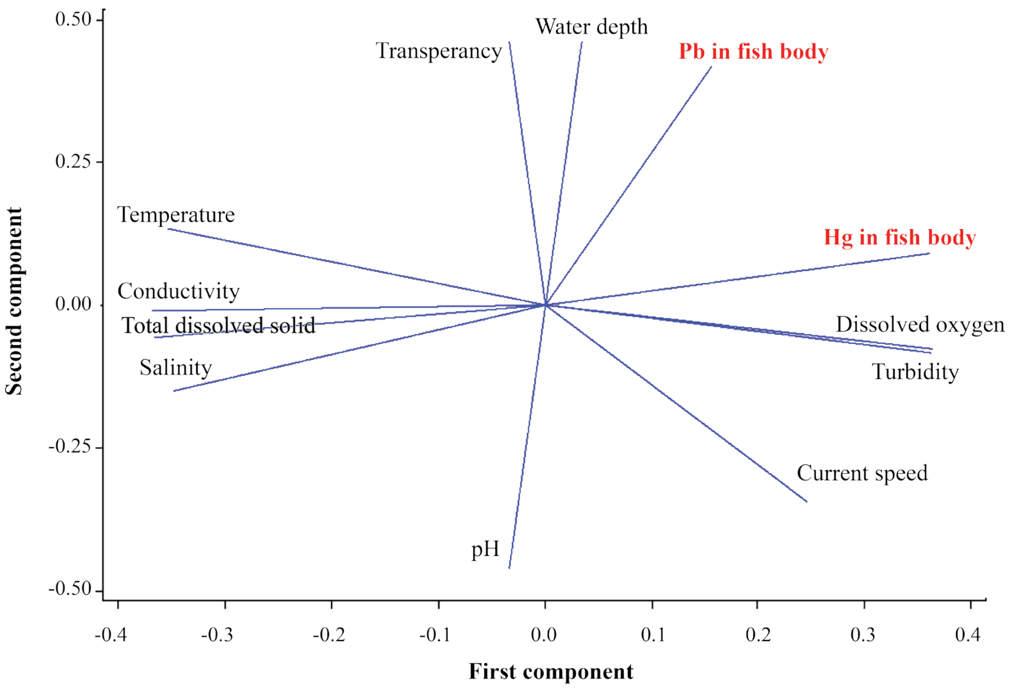

Figure 3. Correlation of heavy metals in Nike's body with water quality parameters displayed using Principal Component Analysis.

respectively, for the first principal component (PC1) and the second principal component (PC2). Variables that exhibited positive correlations with both PC1 and PC2 were Pb and Hg concentrations within the fish body tissues, along with water depth. Conversely, current speed, dissolved oxygen, and turbidity parameters demonstrated positive correlations solely with PC1 and not with PC2. The remaining variables displayed negative correlations with both PC1 and PC2. These findings collectively offer valuable insights into the complex interplay between water quality parameters and heavy metal concentrations in Nike fish tissues, with water depth being a notably influential factor.

The effect of water depth on Pb and Hg concentrations is directly related to sedimentation rate, water flow dynamics, and contaminant sources (Griscom and Fisher, 2004). In general, deeper waters may have lower Pb and Hg concentrations due to the effects of sediment dilution and settling (Mohiuddin *et al*., 2010). On the other hand, shallow waters plus areas with slow water flow can accumulate higher concentrations of these metals due to reduced dilution and increased sedimentation rates. In deep waters, Pb and Hg concentrations may be found to be lower than in shallow waters due to the relatively large water volume and dilution effects (Zhang *et al*., 2014). Additionally, deeper waters may have higher sedimentation rates, which can help trap and bury contaminants, resulting in lower concentrations of heavy metals in the water column (Chouvelon *et al*., 2019). This is what is thought to have caused the research results to show low levels of Pb and Hg in the Bone Estuary.

Potential risks to humans

The evaluation of potential human health risks associated with the consumption of Nike fish is conducted by calculating the THQ for individual heavy metals. In this context, the THQ_{Hg} value is the highest, with a calculated magnitude of 2.7×10^{-2}. On the other hand, the THQ_{Pb} value is notably lower, measuring 8.9×10^{-4}. When considering the cumulative risk from both heavy metals, the Total Hazard Index for these elements collectively amounts to 2.8×10^{-2}. This index indicates the overall potential health risk posed by the combined exposure to Hg and Pb through the consumption of Nike fish. These findings are essential for assessing and managing potential health concerns associated with the consumption of these fish and for making informed decisions regarding dietary choices. The acceptable guideline value for the THQ is set at 1, as per the guidelines from the United States Environmental Protection Agency (USEPA, 2011). In the context of this study, the THQ calculations for Pb and Hg resulting from Nike fish consumption were found to be below 1. This outcome indicates no apparent potential health risk associated with consuming Nike fish about these heavy metals. It is important to recognize that when humans are exposed to multiple heavy metals simultaneously, there is the potential for combined or interactive effects, as noted by Loaiza *et al*. (2018). This study delves into the cumulative impact of various heavy metals on the human body by calculating the HI. The findings from this study reveal that the combined effects of Pb and Hg, as indicated by the HI value, remain below the acceptable limit of 1. This underscores that Pb and Hg in Nike fish bodies within the Bone Estuary do not pose a substantial or serious risk to human health. As a comparison, in research conducted by Gu *et al*. (2018) the likelihood of carcinogenic risks from lead (Pb) exposure to both urban and rural residents was determined to be below the acceptable threshold ($<1\times10^{-4}$). The target hazard quotient (THQ) values for individual metals, as well as the total THQ values for all metals examined, suggested that there was no notable risk of non-carcinogenic effects to urban and rural residents from consuming marine organisms sourced from the South China Sea.

Several government agencies and international organizations have set standards and guidelines to determine the maximum levels of Pb and Hg contamination allowed in fishery products. These thresholds are designed to ensure public health and food safety. However, the specific thresholds in these regulations may vary depending on the type of fishery product, the intended consumer population (e.g. adults or children), and suitability for regional conditions. The United States Food and Drug Administration (FDA) has established action levels for Pb and Hg in certain types of fishery products. Pb levels in candy and other foods can be tolerated at a maximum of 0.1 ppm, while for certain types of fish and seafood, such as shark, swordfish, king mackerel and tilefish, the Hg level is 1.0 ppm. The European Union (EU) has also set maximum residue limits (MRL) for various contaminants in food, including fishery products. The MRL for Pb in fishery products in the EU is generally set at 0.1 ppm, while the MRL for Hg ranges from 0.3–1 ppm depending on the type of fish (EC, 2023). The World Health Organization (WHO) also guides food exposure to contaminants such as Pb and Hg to protect public health globally. Meanwhile, Indonesia, through the Food and Drug Supervisory Agency, has set a maximum limit for metal contamination in fish and fishery products, including mollusks, crustaceans, and echinoderms, for Pb and Hg at 0.2 ppm and 0.5 ppm, respectively (IFDA, 2018). Compliance with these maximum standards and applicable regulations helps ensure that fishery products are safe for consumption and minimizes the risk of adverse health impacts due to heavy metal contamination.

Assessing the metal concentrations in seafood and their adherence to quality standards is crucial in determining their safety for consumption. Seafood, encompassing a variety of products such as fish, shellfish, and shrimp, is renowned for its nutritional benefits. It can also pose health risks if contaminants within them surpass specific concentration limits (Purbonegoro, 2020). The accumulation of heavy metals in fish can have direct implications for the health of consumers, particularly those residing near fishing areas. Conducting health risk assessments is an essential step for individuals who consume contaminated fish, including Nike fish. As Yadav *et al*. (2015) noted, health risk assessments are typically grounded on the assumption that most chemicals exhibiting non-cancer effects demonstrate a threshold response. This approach helps gauge the potential risks associated with seafood consumption and provides valuable information for making informed dietary choices. By evaluating the concentrations of heavy metals and comparing them to established quality standards, health authorities can determine whether the seafood in question poses any health risks and, if necessary, issue guidelines or recommendations to mitigate these risks and safeguard public health.

Heavy metals are classified as potentially toxic, and can be bound in the bodies of organisms (Kadim and Risjani, 2022), no exception for non-essential metals such as Pb and Hg (Hertika *et al*., 2019). Aquatic organisms can absorb heavy metals either directly from their surrounding environment and sediments or through ingestion along the food chain (Jiang *et al*., 2018). The concentration of heavy metals in water and fish food plays a crucial role in determining the accumulation of these metals in fish (Maceda-Veiga *et al*., 2012). The contamination present in aquatic animals is a significant concern due to the threat it poses to the health of these animals, and subsequently, it creates hazards for humans who consume them (Varol and Sünbül, 2017). When present at low levels, toxic metals have the potential to endanger human health when consumed over prolonged periods (Yu *et al*., 2020).

With all the information in this work, the limitation of this research lies in the certainty of the source of heavy metals. This study did not examine what factors the heavy metals detected in the bodies of Nike fish samples came from. Aquatic animals accumulate metals may from the surrounding water, sediment, and their diet (Jayaprakash *et al*., 2015). Beside it, this research does not include Cd analysis and only focuses on the heavy metals Pb and Hg with the assumption that there is no potential source of Cd in the waters of the Gorontalo Bone Estuary then it can be ingnored. Gorontalo is not an industrial area like other cities whose industrial activities have the potential to contribute Cd to the waters. The potential sources for Hg and Pb enter the Gorontalo waters area because there are community mining activities, ship fuel, agriculture, domestic activities which in fact have the potential to produce these two heavy metals.

CONCLUSIONS

Pb and Hg concentrations within Nike fish tissues revealed noteworthy findings in all sampling stations along Estuary Bone, Gorontalo, Indonesia. This study revealed that Pb and Hg tended to be highest in fish body samples collected from the river and estuary stations (Site 1 and Site 2), compared with the sea area (Site 3). These heavy metals are more concentrated in the downstream areas of the river due to the cumulative impact of activities in the upstream and middle sections of the river. It is essential to consider the developmental aspects of Nike fish larvae concerning their proximity to the river. As Nike fish move closer to the river, their age and the maturation of their respiratory and digestive systems increase. Based on STORET, Pollution Index, and WQI methods involving other water quality parameters collectively indicated that the waters of the Bone Estuary during the sampling period were categorized as clean to moderately polluted. The novelty of this research is the tendency for Pb and Hg contaminants in the body of nike fish to be higher the closer they are to fresh water. Also, these metals detected in the bodies of Nike fish do not have the potential to harm consumers. This can be meaningful input data for relevant stakeholders to monitor and maintain the condition of Bone waters so that they remain safe from heavy metal pollution and control activities that have the potential to pollute the nike fish habitat.

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