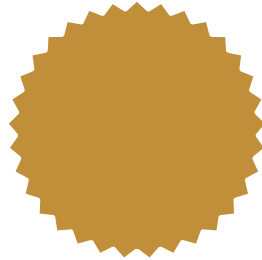


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## Manuscript Title

Iron Bioaccumulation and Ecological Implications in the Estuarine Ecosystem of South Kalimantan: Insights from Giant Mudskipper Fish as Bioindicators

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# Iron Bioaccumulation and Ecological Implications in the Estuarine Ecosystem of South Kalimantan: Insights from Giant Mudskipper Fish as Bioindicators

*by Author Author*

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*Research article*

## **Iron Bioaccumulation and Ecological Implications in the Estuarine Ecosystem of South Kalimantan: Insights from Giant Mudskipper Fish as Bioindicators**

**Abstract:** This study investigated iron (Fe) accumulation in South Kalimantan's estuarine ecosystem, utilizing giant mudskipper fish as indicators of heavy metal pollution. By analyzing Fe levels in water, sediment, and fish organs over time, insights into its effects on the environment and human health were gained. Furthermore, through Atomic Absorption Spectrometry, Fe concentrations in Kuala Lupak's coastal wetland were measured, revealing significant correlations between Fe levels in water, sediment, and fish tissues. These results enhance our understanding and inform better management strategies.. Anthropogenic and natural sources contribute to the accumulation of heavy metals, particularly Fe, with anthropogenic pollution being the most dominant. This study presented the escalating concentrations of Fe within the Kuala Lupak estuary and raised concerns regarding the ecological and human health implications. Continuous monitoring, source identification, public awareness, regulations, remediation, and long-term exploration were essential for addressing heavy metal pollution and its ecological impact. Therefore, valuable insights were provided for environmental management and conservation efforts.

**Keywords:**

Iron Accumulation Dynamics; Estuarine Fish Contamination; Environmental Heavy Metal Impact; Aquatic Ecosystem Health.

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### **1. Introduction**

Heavy metals are a significant threat to the health of estuarine ecosystems, affecting the biodiversity of aquatic organisms at both individual and community levels [1-3]. This negative impact is attributed to the bioaccumulation of heavy metals in aquatic biota, resulting from the discharge of industrial and domestic waste into the estuarine environment. Yogeshwaran et al. [4] reported that the bioaccumulation of the metals in estuarine aquatic biota is directly linked to water contamination. The presence of heavy metals in aquatic biota was due to their availability in water bodies and sediment,

as well as the food consumed by the fish [5]. These trace elements exist in sediments in various forms, including dissolved, bound to suspended particulate matter, or accumulated, enhancing their incorporation into biological processes [6].

Iron, as the fourth most dominant metal on Earth, possesses the capacity to change valence and form complexes with oxygen. Furthermore, it plays a crucial role in respiration in nearly all aerobic organisms. However, unless suitably sequestered, Fe can catalyze the formation of radicals capable of damaging biological molecules, cells, tissues, and entire organisms (7). Certain trace metals such as Fe, Cu, Mn, and Zn are essential to biological systems but can become toxic at high concentrations. Metals including Zn, Fe, Cu, and Mo are integral components of metal-protein complexes in enzymes, contributing to enzymatic activities. Fe is a constituent of oxygen transport within living organisms, while it also plays a vital role in oxidative metabolism, serving as a cofactor for proteins and enzymes, DNA synthesis, and electron transport (8).

Other heavy metals such as Cd, Cr, Pb, and Hg have adverse effects even at deficient concentrations (9). Despite its physiological functions in fish, environmental pollution can render Fe toxic due to excessive accumulation (10). The continuous and substantial discharge of trace metal pollutants into the sea poses a significant threat to estuarine and coastal ecosystems due to their chronic toxicity, non-biodegradability, and bioaccumulation in biota (11). The metals can be transferred and biomagnified through food chains, presenting significant risks to human health. Metal contamination in estuarine ecosystems can induce toxic effects in fish and humans, primarily by consuming contaminated food (12) (13). Therefore, evaluating the occurrence of Fe content in the estuarine ecosystem of South Kalimantan is essential.

Analyzing the levels of Fe in the water body, sediments, and its bioaccumulation in wild fish, particularly those that inhabit estuaries for a significant portion of their life, such as the giant mudskipper fish (*Periophthalmodon schlosseri*), is of great importance. This fish species holds potential as a bioindicator for monitoring heavy metal pollution in estuarine waters, aiming to mitigate the adverse impacts of Fe on biodiversity conservation and environmental health. Evaluating the presence of Fe in the ecosystem serves as a valuable environmental indicator to understand anthropogenic influences and associated risks to the estuary environment.

Fe is an abundant transition metal in Earth's crust (14), and it can exist in aquatic environments in dissolved or suspended forms in water or sediment. Investigating Fe in water, sediment, and fish tissues aids in determining contamination status and loads. Therefore, quantifying the presence of this metal is pivotal in analyzing the health of aquatic ecosystems. Valuable information about the impacts of environmental changes and anthropogenic activities over time are obtained through long-term bioaccumulation of heavy metals. As a result, biomonitoring studies are essential in tracking trends in the concentrations of these metals, enabling scientists to identify changes in estuarine health (15). Contamination of aquatic ecosystems by trace metals has garnered global attention from environmental and biological experts, leading to numerous studies on their accumulation in estuarine and marine biota (16)(12). Bioaccumulation of Fe in commercially important fish species in estuarine ecosystems has been extensively investigated in various countries. For instance, studies have focused on the bioaccumulation of Fe in fish species, including *Solea solea*, *Sparus aurata*, and *Penaeus semiculatus*



in Turkey (17), *Rastrelliger kanagurta* in India (18), *Penyatrems luteus*, *Macrodon ancylodon*, *Sciades herzbergii*, and *Mugil curema* in Brazil (19), *Sciades herzbergii*, *Cetengraulis edentulus*, *Macrodon ancylodon*, *Pseudauchenipterus nodosus*, and *Stellifer rastrifer* in the Amazon region of Brazil (20), 13 potential heavy metal bioindicator fish species in the Persian and Arabian Gulfs (21), *Sciades herzbergii* in the Amazon region of Brazil (22), Sillaginid species in Iran (23); *Chrysiichthys nigrodigitatus*, *Sarotherodon melanotheron*, *Scomber scombrus*, *Sardinella maderensis*, *Dentex canariensis*, and *Pseudotolithus typus* in Nigeria (24), as well as *Brevoortia aurea*, *Cynoscion guatucupa*, *Micropogonias furnieri*, *Mustelus schmitti*, *Odontesthes argentinensis*, and *Paralichthys orbignyanus* in Argentina (16). Trace metals enter fish bodies through various routes, namely direct food consumption, absorption through gills, and dermal absorption. After absorption, they circulate through the bloodstream and transported to various organs such as the kidneys, gills, liver, and muscles before being excreted. However, the concentrations of heavy metals absorbed/accumulated in fish muscle tissue remain relatively constant (15). There is a pressing need for comprehensive information on the evaluation of Fe presence, toxicity, and effects on giant mudskipper fish in the South Kalimantan estuary. Therefore, effective monitoring and evaluation of the availability of this metal in the estuarine environment (water, sediment, biota) are crucial (25).

The giant mudskipper fish has potential as a bioindicator species for heavy metal pollution biomonitoring programs in the Barito River estuary because it meets the requirements, including natural abundance (26), easy to obtain in large quantities, wide tolerance to polluted environmental conditions, benthic living habits, high trophic level in the aquatic food chain, and capability to accumulate heavy metals (27)(28)(29). Fish, renowned for their capacity to accumulate heavy metals, are well-suited to serve as bioindicator species in pollution biomonitoring, enabling the assessment of estuarine ecological health (30)(31)(32). The giant mudskipper fish, in particular, possesses unique biological characteristics, namely having amphibian-like characters, which distinguish them from other species. Additionally, they are often called "fish walking on land" or "fishes out of water" (33)

The habitat of giant mudskipper is mostly mudflats in mangrove forests and is widely distributed across Africa, Madagascar, India, Southeast Asia, northern Australia, southern China, southern Japan, Samoa, Tonga Islands, Saudi Arabia, Gulf of Kuwait, Polynesia, and Indonesia (34). The highest species diversity was observed in coastal areas of Southeast Asia, Australia, and Papua (35). Furthermore, giant mudskipper fish excel at making burrows in the mud of coastal swamps, estuaries, and intertidal zones. Their diet primarily consists of algae, detritus, diatoms, nematodes, polychaetes, and fish eggs, alongside mud and sand particles (33).

Kuala Lupak in the Barito River estuary is a coastal swamp wetland area characterized by extensive transportation activities, loading and unloading of coal, and coal mining waste disposal. The continuous input of waste from the coal mining process and coal-related activities into the water body has the potential to cause estuary pollution when prolonged and accumulated. These waste materials contain heavy metals such as Fe, Mn, Hg, Cd, Cr, Pb, and Cu (36). Furthermore, Kuala Lupak has witnessed the conversion of mangrove land to ponds, a place for ship transportation traffic and settlements. The several industries along the riverbank such as wood/plywood processing factories, rubber, fisheries, and oil palm plantations, pollute estuary waters through waste discharge (37). It is hypothesized that the unchecked release of these wastes into water bodies poses a significant risk to

both aquatic ecosystems and human health. Therefore, urgent biomonitoring efforts were warranted for early detection of the negative impacts of pollution. In light of these considerations, it is important to investigate the presence and occurrence of Fe in the Kuala Lupak estuary ecosystem during the period from 2020 to 2022. The objectives of the study were to evaluate the presence of Fe levels in water bodies, sediments, and giant mudskipper fish, assess the bioaccumulation of Fe in various tissues of giant mudskipper fish (liver, kidney, muscle, skin, and gills), evaluate the relationship or correlation between Fe content in water bodies and sediments as well as the concentrations in the liver, kidney, skin, muscle, and gill tissues of giant mudskipper fish, and validate the potential of giant mudskipper fish as a bioindicator of Fe contamination in the Kuala Lupak estuary. The trends and intricacies of iron (Fe) contamination are investigated into by this research, especially within estuarine environments. The accessibility of this metal was primarily evaluated, and its annual fluctuations were also monitored. The knowledge acquired from this study provides significant insights, especially for comprehending the possible consequences of future environmental changes. The results of this multi-year trace metal monitoring are of paramount importance in understanding the bioaccumulation potential of trace metals and constitute a crucial step in the management of estuarine and coastal ecosystems. These outcomes serve as a foundational framework for stakeholders to assess and address anthropogenic effects in the future.

## 2. Materials and methods

### 2.1. Study Area

The study was conducted in the coastal wetland of Kuala Lupak, situated in the Barito Kuala Regency. This region is approximately 8 km west of the mouth of the Barito River, the largest and longest river in South Kalimantan Province, Indonesia. The coastal wetland area of Kuala Lupak directly merges with the Java Sea coastline to the south. A comprehensive review of each study station/site is presented in Table 1, while the precise sampling locations are visually shown in Figure 1.

**Table 1.** Study station description

Location	Stations/sites	Description
Kuala Lupak	Estuarine	The estuarine waters of Kuala Lupak were a coastal wetland area with the status of Margasatwa Wildlife Sanctuary, specifically a mangrove swamp ecosystem. The mouth of the Kuala Lupak river was surrounded by human settlements and it flows into the Java Sea. The water samples were collected from the coordinates 3°27'28.223"S 114°22'04.780" E in the estuarine waters.
	Coastal	The coastal waters of Kuala Lupak were located approximately 8 km west of the mouth of the Barito River and border the Java Sea to the south, with a coastline of 30 km. Furthermore, they were



characterized by mangrove swamps. At the coordinates  $3^{\circ}28'02.383''\text{S}$   $114^{\circ}21'31.282''\text{E}$  water was collected in the coastal waters where the Kuala Lupak River flows into the sea.

## 2.2. Sample collections and analysis

The process of collecting water, sediment, and white snapper fish samples was conducted during August in 2020, 2021, and 2022. This was carried out using the purposive sampling method, where sample selection is based on specific criteria, such as the potential sources of pollution. To accurately identify these sources, various activities that contribute to contamination have been taken into consideration. Figure 1 shows the collection of fish, water, and sediment samples at the study location.

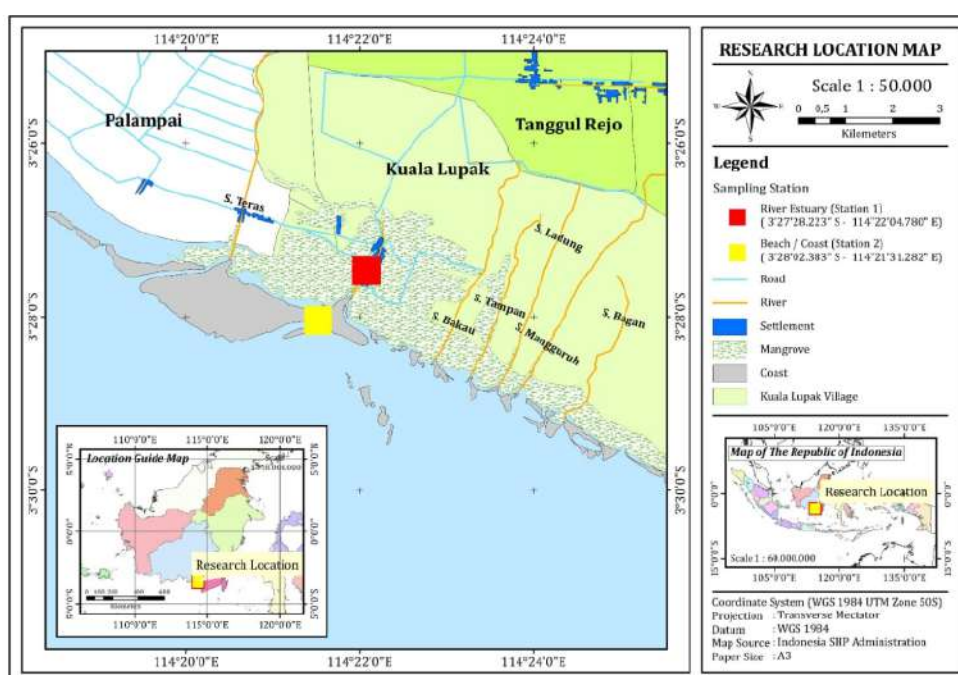


Figure 1. Study area map and sampling locations in the estuary Kuala Lupak South Kalimantan.

### 2.2.1. Water

Using Aqua trap water sampler, surface water samples were collected from 2 stations scattered throughout the study region. In preparation for further analysis, approximately 2 ml of concentrated  $\text{HNO}_3$  was added to each 1 sample after filtration. The study adopted the liquid-liquid extraction technique (38) to determine the concentration level of dissolved Fe. A 100 ml unfiltered sample was placed in a separating funnel and mixed with 2 ml of 2% ammonium pyrrolidine dithiocarbamate

(APDC). Subsequently, 10 ml of iso-butyl methyl ketone (IBMK) was used for extraction after were agitation. The extracted aqueous phase was subjected to further extraction using concentrated  $\text{HNO}_3$  and high-purity water. Any remaining traces of organic solvents in the solutes were evaporated on a low-temperature hot plate, and the final solution was analyzed using Atomic Absorption Spectrometry (AAS Thermo Scientific ICE 3500 series Germany). This instrument was used to measure the concentration level of Fe in water, sediments, and fish samples. For water, the concentration was expressed in mg/L. Finally, samples for metal determination were conditioned following Jayaprakash et al. (38).

#### 2.2.2. Sediment.

Sediment samples were procured from 2 estuary stations using a Van Veen grab sampler. Furthermore, they were dried at  $40^\circ\text{C}$  and powdered in an agate mortar for the digestion procedures. Approximately 1 g of the dried sediment sample was digested with an acid mixture containing  $\text{HClO}_4 + \text{HF} + \text{HCl}$ , and the residue was dissolved with concentrated HCl, then diluted to 25 ml. The solution was analyzed using flame AAS to estimate the Fe concentrations which were expressed in mg/Kg for sediment.

#### 2.2.3. Fish Sampling and Analysis

Fish samples were obtained from the estuarine area by local fishermen. A total of 10 giant mudskipper fish with identical dimensions, ranging between 19 to 20 cm in length and weighing from 130 to 170 g were collected from each sampling station to ensure representativeness. The obtained samples were transported to the laboratory in ice boxes to maintain their preservation and appropriate conditions for further analysis. Furthermore, the dissection of the fish was conducted using surgical stainless-steel instruments, facilitating the extraction of organs, including the liver, kidneys, gills, muscles, and skin.

A digestion tube, pre-loaded with 5 ml of  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$  was utilized for each organ sample to initiate the reaction. The samples were subjected to digestion using a hot block digestion apparatus, maintained at  $60^\circ\text{C}$  for 30 minutes. Following the cooling of the samples, 10 ml of  $\text{HNO}_3$  was added, and the mixture was further heated at temperatures ranging from 120 to  $150^\circ\text{C}$  until a discernibly dark colouration of the solution was achieved. For facilitating the subsequent filtration process, 1 ml of  $\text{H}_2\text{O}_2$  was introduced to yield a clear solution. Those filtered samples were then analyzed using Atomic Absorption Spectrometry (AAS) to quantify the concentration of Fe in the fish tissues, expressed mg/Kg.

### 2.3. Data Analysis

Data processing and analysis were executed employing GraphPad Prism software, specifically the 5.9.0 series from Dotmatics. The statistical evaluations comprised the determination of mean, standard deviations (SD), minimum and maximum values, the creation of graphical representations showing the interrelationship among variables, and the calculation of Pearson correlations. The analyses were conducted using GraphPad Prism 7.02, developed by the company of GraphPad

Software Inc., San Diego.

### 3. Results

#### 3.1. Occurrence of Fe Concentrations in Water and Sediment

The average concentrations of Fe in the water column from 2020 to 2022 ranged from 2.55 to 11.61 mg/L. This showed a significant trend of increase over each successive year. The highest average concentration of Fe was recorded in 2022 to be 11.61 mg/L, as presented in Table 1. The Maximum Permissible Limits (MPLs) for heavy metals in estuary water were not regulated by International Guideline values. The Fe in Kuala Lupak estuary water exceeded the threshold value set by Government Regulation of Indonesia number 22 of 2021 concerning the Implementation of Environmental Protection and Management. Furthermore, the seawater quality standard for marine biota was set at 0.30 mgL<sup>-1</sup>. In parallel, the average concentrations of Fe within the sediment, serving as the habitat of the giant mudskipper fish, was in the range of 1245.92 to 48911.78 mg/Kg.

Analogous to the pattern observed in the water column, the concentrations of Fe in the sediment also displayed a significant upward trend over each year, as shown in Figure 2. The highest average level of Fe within the sediment was reported in 2022, to be 48911.78 mg/Kg, as presented in Table 1. The concentration of this metal in Kuala Lupak estuary sediments exceeded the maximum limit of 20000 mgKg<sup>-1</sup> set by the International Guideline value, CBSQG 2003: Consensus-Based Sediment Quality Guidelines (Wisconsin Department of Natural Resources). The mean plot of Fe level in water and sediment from 2020 to 2022 demonstrated a significant interrelationship between variables, with a confidence level of 95%, as indicated by the statistical analysis ( $P < 0.05$ ,  $R^2 = 0.649$ ).

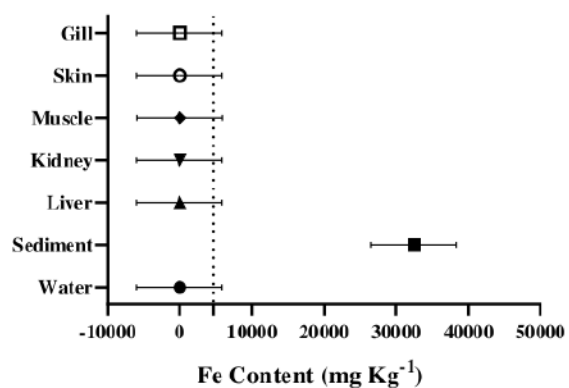
#### 3.2. Temporal Variations in Fe Concentrations in Giant Mudskipper Fish

The mean Fe concentrations in the liver, kidney, muscle, skin, and gills of giant mudskipper for the years 2020-2022 ranged from 5.52 to 12.84 mg/kg, 7.98 to 15.44 mg/kg, 40.44 to 69.74 mg/kg, 4.42 to 8.80 mg/kg, and 1.23 to 6.22 mg/kg, as presented in Table 1. The average level of this metal in several fish tissues exhibited a significant upward trend annually, with the highest value being recorded in 2022, as specified in Figure 2. Based on the results in Table 1, muscle had the highest accumulation among the tested fish tissues, followed by kidney, liver, skin, and gills. The Maximum Permissible Limit (MPL) for Fe in fish meat was not regulated by national but not international guideline values. The Fe in giant mudskipper fish organs exceeds the threshold value of 1 mgKg<sup>-1</sup>, as regulated by the Indonesian National Standard (SNI 7387:2009) regarding the maximum limit of heavy metal contamination. When comparing the content of this metal among the tested samples in the Kuala Lupak estuarine ecosystem, the average concentration in sediment was the highest.

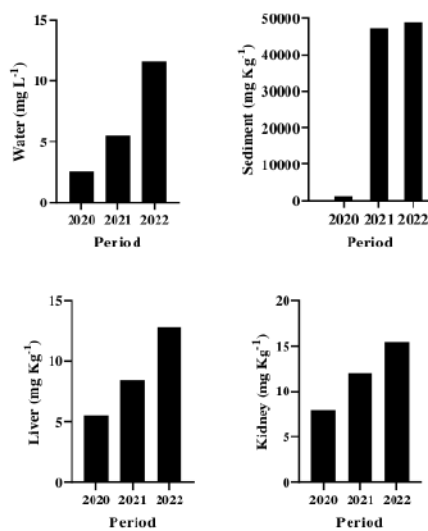
**Table 1.** Mean values of Fe concentration in water, sediment, and some tissues of giant mudskipper fish from the Kuala Lupak estuary between the period of 2020 to 2022.

Sampling Period	Water (mg L <sup>-1</sup> )	Sediment (mg kg <sup>-1</sup> )	Liver (mg kg <sup>-1</sup> )	Kidney (mg kg <sup>-1</sup> )	Muscle (mg kg <sup>-1</sup> )	Skin (mg kg <sup>-1</sup> )	Gill (mg kg <sup>-1</sup> )
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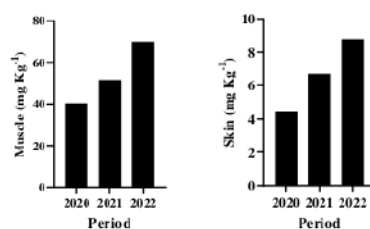
2020	2.55	1245.92	5.52	7.98	40.44	4.42	1.23
2021	5.54	47348.42	8.43	11.99	51.56	6.70	3.32
2022	11.61	48911.78	12.84	15.44	69.74	8.80	6.22



**Figure 1.** The mean plot of Fe concentration in water, sediment, and some tissue of giant mudskipper fish from 2020 to 2022 ( $P < 0,05$   $R^2 = 0,649$ ).







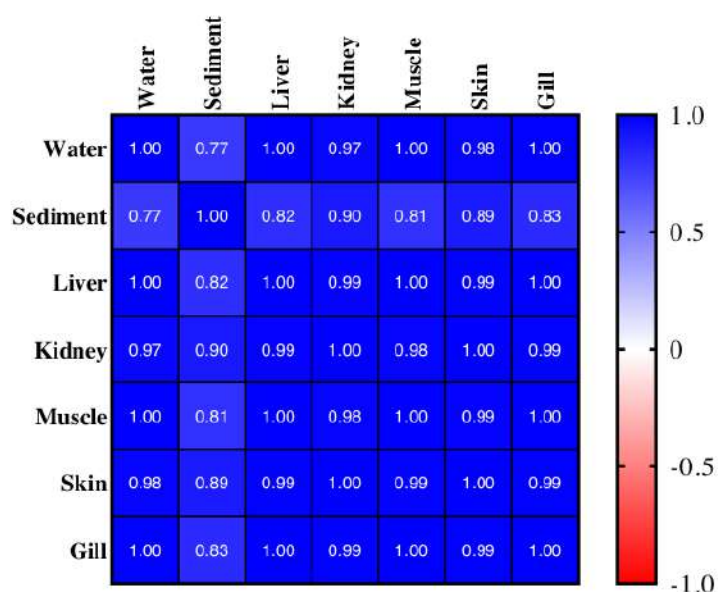
**Figure 2.** Fe content trend in some tissue of giant mudskipper fish period 2020-2022

### 3.3. The relationship between Fe contents in water, sediment, and giant mudskipper fish tissues

The interrelationships among heavy metal concentrations in water, sediment, and the tissues of giant mudskipper fish from 2020 to 2022 were evaluated and presented in Figure 3. The graphical representation showed a substantial positive correlation between the concentration of Fe in the water and the levels identified in the liver, muscle, and gills, with a correlation coefficient of 1. This implied that elevated levels of this metal in the water correspond with its heightened concentrations in the liver, muscles, and gills. Furthermore, a robust positive correlation was observed between waterborne Fe concentrations and the levels of the metal in the kidney and skin, with correlation coefficients of 0.97 and 0.98, respectively. The correlation coefficient between Fe levels in the water and sediment was 0.77. A highly significant positive correlation among the concentrations present in the water, sediment, and tissues of the giant mudskipper fish, as indicated by the outcomes of this investigation. Based on these observations, the accumulation in water and sediment has augmented Fe levels within the tissues of the fish. This correlation was in line with the consistently high concentrations in water and sediment.

Figure 3 further showed a robust positive correlation between sediment-bound Fe concentrations and those in the kidney, skin, gills, liver, and muscle tissues, with correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81, respectively. These results underscored the effect of sediment-bound Fe accumulation on the corresponding values within the tissues of the fish. This correlation was in line with the consistent elevation of sediment-bound Fe concentrations over 3 years, exhibiting an annual increment, as presented in Figure 1. The study also underscored a significant positive correlation across various organs of the giant mudskipper fish. This indicated a uniform distribution across diverse organs after Fe uptake into the body of the fish. In Figure 3, a close positive correlation was evident between the Fe concentration in the skin, serving as an entry point for Fe uptake. In the kidney, gills, muscle, and liver, the level of this metal exhibited correlation coefficients of 1.00, 0.99, 0.99, and 0.99, respectively. Similarly, a positive, strong correlation existed among the concentration in the gills, another site of Fe entry, and the Fe levels in the liver, muscle, skin, as well as kidney. These featured coefficients of 1.00, 1.00, 0.99, and 0.99, respectively. The results showed that elevated Fe concentrations in the skin and gills precipitate were directly proportional to the liver, kidney, and muscle tissues.





**Figure 3.** Pearson correlation of Fe content in some tissue of mudskipper fish period 2020-2022

#### 4. Discussion

##### 4.1. Fe Contamination in Estuarine Waters: Sources and Implications

The heightened prevalence of Fe within the Kuala Lupak estuary in 2022 was attributed to the effects of extreme rainfall and substantial flooding in the region of Kalimantan Selatan from December 2020 to February 2021. This event led to the dissolution of heavy metal deposits from upstream areas, resulting in their transport into the estuarine environment and eventual deposition within the estuary. This result <sup>17</sup> is in line with the study conducted by Čmelík et al. (39), which identified a substantial increase in heavy metal concentrations in both water and sediment along the course of the Bílina River in the Czech Republic, directly attributed to extreme rainfall events. However, it is important to note that floods had a comparatively shorter-lived influence on the quality of water and sediment compared to the cumulative impacts of anthropogenic activities. A study by Supriyantini and Endrawati (40) also reported elevated concentrations within water bodies, sediment, and tissues of the green mussel (*Perna viridis*) in the coastal rivers of Tanjung Emas Semarang. In this investigation, the elevated Fe content in the estuary stemmed from various sources, including soil and anthropogenic activities occurring on land. These activities comprise household waste containing Fe, water reservoirs sourced from Fe, industrial waste deposits, and corrosion from Fe-containing pipes transported into the estuary by river flow. According to Haeruddin et al. (41), the highest heavy metal concentrations within sediment were proximal to contamination sources.

Fe contamination within estuarine waters emanates from natural processes, such as geological weathering, rock decomposition, and anthropogenic activities. These anthropogenic sources include Fe release from ore materials and volcanic eruptions that discharge Fe-rich materials into water bodies through runoff, erosion, and flooding. Human activities such as coal transportation and mining operations contribute significantly to this pollution (42). Anthropogenic Fe sources also include land-based activities, such as the discharge of household waste containing Fe, Fe-laden water reservoirs, industrial waste deposits, and the corrosion of Fe-containing pipes, releasing Fe<sup>2+</sup> and sulfuric acid due to the oxidation of Fe pyrite (FeS<sub>2</sub>) present in coal layers (14).

The escalating temporal increase in Fe concentrations in both water bodies and sediments, as observed in this study, was supported by an investigation conducted by Weber et al. (43) concerning the contamination in Brazil's Piranga and Doce rivers. Both rivers were contaminated with Fe due to the collapse of the Samarco mine tailings dam in Brazil in 2015. Environmental catastrophes such as dam failures that release tailings profoundly impacted the landscape and aquatic environments, progressively deteriorating the water quality of the Doce and Piranga rivers over time. Another source of this contamination stems from mineral weathering/erosion and elevated Fe content in soil. The contamination of these rivers has led to the bioaccumulation of Fe in *Hoplias intermedius* and *Hypostomus affini* fish. This caused histopathological lesions in the liver, including vacuolization, hepatocyte hypertrophy, and elevated necrotic areas compared to fish from non-tailings-contaminated rivers. This study strengthens the notion that fish living in heavy metal-contaminated waters experience declining aquatic health quality, leading to heavy metal bioaccumulation and histopathological lesions.

#### 4.2. Accumulation of Metals in Sediments: Multifaceted Sources and Ecological Implications

In this study, sediments in the Kuala Lupak estuary accumulate higher levels of heavy metals than the water column. This has rendered the sediments a significant potential source and a primary repository for all pollutants (44). These particles can store diverse heavy metals in substantial and consistent quantities, hence, they become valuable indicators for assessing water pollution status (41). Liline et al. (45) reported that sediments in the Rutong, Waai, and Poka mangrove areas in Maluku function as a medium for transferring heavy metals such as chromium (Cr) and Fe to mudskipper fish. The heavy metals in estuarine sediments result from sedimentation processes that retain significant amounts of adsorbed metal particles, leading to their deposition at the bottom. Wastes containing these metallic elements were deposited in sediments upon entering estuaries. This was facilitated by reduced environmental conditions, low dissolved oxygen, and elevated pH, causing metal concentration or enrichment (46). The result was in line with the study by Dalu et al. (42), stating that heavy metal content in sediment was significantly higher than in the water column, as it was being absorbed by suspended particles. High Fe content in sediment can lead to contamination of water bodies, which can have negative impacts on the health of aquatic biota and the overall ecological balance of aquatic ecosystems.

Sediments serve as indicators of land-based pollution by acting as "sinks" for pollutants. The rise in heavy metal levels in sediments suggests that heavy metals in the water column are deposited along with suspended solids, posing a potential threat to estuarine and coastal ecosystems. These sediments, serving as sinks for various human activities, may also act as sources of heavy metals, underscoring

the ongoing necessity for monitoring to evaluate their role as pollution sources. Heavy metals are prone to accumulating in sediments, resulting in consistently higher concentrations than those in the water column. Suspended sediment particles quickly transport these metals into water through the movement of water masses (44). The high volume of merchant and coal transport vessels contributes to water mass movement in the Barito River estuary.

1 The accumulation of heavy metal in sediments was attributed to a variety of sources, including leaded gasoline emissions, chemical manufacturing industries, motorized transportation, underground pipe corrosion, coal-based thermal power plants, and urban waste (38). The results of this study were in line with the report of Jayaprakash et al. (38), indicating that heavy metal concentrations in water were consistently lower than in sediment within contaminated water bodies. This is because sediment particles tend to absorb metals from the water. In sediment-rich environments, organisms such as the giant mudskipper fish accumulated heavy metals from their food and water through gills, mouth, and skin mucus, further underscoring the potential for metal bioaccumulation.

The intricate interplay between suspended sediment particles and their propensity to absorb heavy metals from the aqueous phase was a crucial mechanism underlying the contrasting concentrations of these metals in water and sediment. This phenomenon accentuated the role of sediments as repositories for heavy metals, specifically in aquatic environments subject to anthropogenic influences and diverse pollutant sources.

A previous study by Jayaprakash et al. (38) described the multiple pathways through which heavy metals infiltrate sediment systems, originating from various anthropogenic activities. The disposal of leaded gasoline, effluents from chemical industries and motorized transport, underground pipe corrosion, emissions from coal-based thermal power plants, and the discharge of urban waste all contribute to metal accumulation within sedimentary matrices.

The congruence between the report of Jayaprakash et al. (38) and this study 7 underscores the broader ecological implications of metal accumulation in sediments. The distinct levels of heavy metals within water and sediment reflect the differential interactions between these metals and particulate matter suspended in water. Consequently, sedimentary environments act as crucial hubs for storing heavy metals, providing a pertinent context for comprehending the potential ecological 17 ramifications of anthropogenic pollution in aquatic ecosystems. High Fe content in sediment can alter the physical and chemical properties, such as color, texture, and pH. This could also affect the habitat of benthic organisms and the overall ecological balance of aquatic ecosystems (47). These results elaborate on the necessity for robust monitoring practices to assess the extent and impacts of contamination within sediment systems.

During this study, direct observations showed that the deteriorating water quality in the Kuala Lupak Estuary stems from anthropogenic activities. The sources of contamination affecting water, sediment, and the organs of the giant mudskipper fish were believed to originate from domestic and non-domestic waste. Domestic waste originates from residential areas, while non-domestic waste sources comprise industrial, agricultural, livestock, fisheries, mining, and other non-residential activities. The fact was in line with the study of Čmelík et al. (39), who stated that anthropogenic



pollution, as opposed to short-term flood effects, was the dominant contributor to a significant increase in heavy metal concentrations in both water and sediment along the Bilina River in the Czech Republic. Additionally, anthropogenic pollution exhibited a more pronounced temporal impact on the water quality of the Bilina River.

Direct observations during this study, as well as interviews with residents, led to the identification of sources of coastal swamp water pollution in Kuala Lupak. These sources include residential waste and the waste products of factories situated along the banks of the Barito River estuary. Factories, such as plywood processing plants perform wood washing activities as well as generate solid waste such as wood fragments, bark, sawdust, and sanding residues. Other sources of contamination were believed to arise from gold mining activities in the Barito River basin, the use of paint in shipyards, agricultural activities including the application of pesticides and fungicides, converting mangrove areas to fish ponds, and the application of explosives for fishing. Fuel companies discharging waste were also suspected sources of pollution. These companies distributed diesel fuel through water transport routes that traverse the Barito River estuary. Additionally, the high density of commercial and coal transport ships, alongside coal spillage and debris entering the river, contributed to the degraded water quality of the river. According to the National Transportation Safety Committee, approximately 7.101 units of coal barges navigate the Barito River [64].

In addition to anthropogenic activities, Fe is sourced from natural processes comprising regular biogeochemical cycles, volcanic activities, soil erosion, continental runoff, shelf inputs, as well as atmospheric deposition from volcanic events and wildfires (6). Furthermore, as the most dominant transition metal in the Earth's crust, it undergoes multiple natural cycles (14). Anthropogenic pollution of trace metals, including Fe, in estuarine waters has deteriorated water quality and negatively impacted fish health (9)(48)(49).

#### 4.3. Bioaccumulation Potential and Ecological Impact of Heavy Metals in Giant Mudskipper Fish

Many studies have documented that indicator species accumulate contaminants in their tissues at higher levels than the surrounding habitat, such as water and sediment. This characteristic makes them valuable as potential bioindicators within ecosystem health biomonitoring programs. According to Table 1, the concentrations of Fe in the muscle, kidney, liver, and skin of giant mudskipper fish exceeded those in the surrounding aquatic environment. The elevated concentrations within fish tissues were attributed to the increased levels present both sediment and water. These results corroborate with those of Abdel Gawad (50), who posited that high concentrations of heavy metals in sediment and water allow aquatic organisms to accumulate metals biologically and transport them through the food chain. The ability of giant mudskipper fish to accumulate heavy metals in tissues stems from their position at the pinnacle of the food chain. These organisms reside benthically in mud within the intertidal coastal zone and mangrove forest floor, hence, they are susceptible to direct exposure to pollutants within the estuarine environment. According to Ferreira et al. (51), estuaries represent habitats with continuous and direct exposure to heavy metal pollutants.

Estuaries have gained wide recognition as net sinks for trace metals, including Fe, which precipitate rapidly within the environment (52). While rivers transport substantial amounts of dissolved

and particulate Fe into the sea, a significant portion was retained and accumulated within sediments (53)(54). This phenomenon was attributed to the exceptional capacity of the sediments as a final trap and adsorptive reservoir for heavy metals in aquatic environments (55). Fe colloids were immobilized within surface estuarine sediments and can become mobilized by organic matter and reduction agents (53). When these values exceed a certain threshold, the mobility and deposition of Fe lead to pollution, thereby posing toxicity risks to fish. This risk arose primarily because of the persistent, non-biodegradable, accumulative, and diverse sources of trace metals (55).

Continued and significant discharges of trace metal pollutants into the sea can damage estuarine and coastal ecosystems, given their chronic toxicity, non-biodegradability, and bioaccumulation in biota, including fish (11). Trace metals can transfer and biomagnify through food chains, posing severe threats to ecosystem health and human well-being. Furthermore, the contamination of metal within estuarine ecosystems can engender toxic effects in fish and humans, primarily by consuming contaminated food sources (13).

Several studies have noted that indicator species accumulated contaminants in their tissues higher than in the environment of habitat, such as water and sediment. This phenomenon holds potential for the utilization of these species as bioindicator or tools within aquatic ecosystem biomonitoring programs. Table 1 presents the Fe concentrations in the muscle, kidney, liver, and skin tissues of the giant mudskipper fish, which were observed to be higher than in the water column. The elevated concentration in the fish tissues was attributed to the high levels of this metal in both sediment and water. This result was in line with the study conducted by Abdel Gawad (50), who postulated that the high amount of heavy metals in the water and sediments provided opportunities for aquatic organisms to accumulate these metals biologically and transport them through the food chain. The capacity of the giant mudskipper fish to accumulate heavy metals within its tissues was attributed to its position at the top of the food chain. This species thrived benthically in mud within the intertidal coastal zone and mangrove forest floor, experiencing direct exposure to pollutants in estuarine environments. Estuaries, as described by Ferreira et al. (56), represented habitats continually exposed to heavy metal pollutants.

People have widely recognized estuaries as net sinks for trace metals, including Fe. This was because of the rapid deposition of trace metals/Fe ions within estuaries (52). Despite that rivers transported substantial amounts of dissolved and particulate Fe into the ocean, a significant portion became entrapped and accumulated within estuarine sediments (53)(54). Sediment was well-established as a proficient final trap and adsorptive sink for heavy metals in aquatic environments (55). Fe colloids became immobilized within estuarine surface sediments and were mobilized with organic matter and reduction media (53). When Fe mobilization and sedimentation surpass certain thresholds, it leads to pollution and becomes toxic to fish. This toxicity arises primarily from the persistent, non-biodegradable, accumulative, and diverse nature of trace metal sourcing (55).

Continuous and substantial discharges of trace metal pollutants into the sea damage estuarine and coastal ecosystems. The chronic toxicity and non-biodegradability of these pollutants, combined with their bioaccumulation within biota, including fish, posed a severe threat to human health (11). Transfer and bio-magnification of trace metals along food chains severely jeopardized human health. Additionally, metal contamination within estuarine ecosystems yielded deleterious effects on both fish



and humans, stemming from the consumption of tainted food sources (13).

The investigation into the toxic effects of Fe on fish inhabiting estuarine ecosystems proved a compelling inquiry for biologists in South Kalimantan, attributable to the robust positive correlation between Fe levels in the water, sediment, and fish organs. The heightened concentrations of this metal were suspected to be a consequence of its extensive usage (57). Furthermore, Fe was a prevalent component of industrial and mining waste, frequently discharged into aquatic environments, hence, exhibiting a pronounced correlation with aquatic biota, including fish. Its elevated contents in water and sediment facilitated a more pronounced accumulation within fish organs. Fe in its ferrous form ( $\text{Fe}^{2+}$ ) had heightened toxicity towards fish compared to when it was ferric ( $\text{Fe}^{3+}$ ).  $\text{Fe}^{2+}$  exhibited toxic effects by binding to gill surfaces and undergoing oxidation to form insoluble  $\text{Fe}^{3+}$ , thereby inciting cellular damage, ultimately culminating in respiratory dysfunction (58). The bioconcentration of Fe within fish tissues attained its zenith within the liver and gonads, subsequently diminishing in the brain, muscles, and heart (59). The study conducted by Omar et al. (60) substantiated the liver as a prime target organ for Fe-induced toxicity in fish. Furthermore, the toxicity of this metal within gills disrupts respiration due to the physical obstruction of the surfaces (61). The alkaline nature of the gill surfaces promoted the facile oxidation of soluble Fe, forming insoluble Fe compounds that cloak gill lamellae, impeding respiratory function. Excessive Fe absorption led to poisoning in *Labeo rohita*, catalyzing the generation of ROS via Fenton reactions, which damaged biomolecules, cells, and tissues through oxidative stress mechanisms (58). This metal also potentiates the toxicity of other chemical agents, such as paraquat or 2,3,7,8-tetrachlorodibenzo-p-dioxin (62). In a study by Kaloyianni et al. (63), the toxicity of magnetite nanoparticles ( $\text{Fe}_3\text{O}_4$ ) was reported in *Danio rerio* and *Carassius gibelio* fish, evoking oxidative, proteolytic, genotoxic, and apoptotic effects, accompanied by elevated malondialdehyde and carbonyl protein levels.

## 5. Conclusions

In conclusion, the study elucidated substantial positive associations in Fe levels across water, sediment, and tissues of the giant mudskipper fish (*Periophthalmodon schlosseri*) during the 2020-2022 period. These associations underscored the dynamic interplay between environmental Fe buildup and its assimilation within fish tissues. The graphical representation accentuated a strong positive correlation (correlation coefficient 1) between waterborne concentrations and those in the liver, muscle, and gills. This indicated that higher Fe levels in water correspond to elevated levels in these fish tissues. Similarly, noteworthy positive correlations were observed between Fe levels in water as well as kidney and skin tissues, with correlation coefficients of 0.97 and 0.98, respectively. Sediment-bound Fe also correlated positively with kidney, skin, gills, liver, and muscle tissues, with sequential correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81.

The strong positive correlation within the various fish organs implied uniform Fe dispersion post-uptake. The skin and gills, primary entry points for this metal, exhibited robust positive correlations with kidney, gills, muscle, liver, and skin tissues. This inquiry into the detrimental impacts of Fe on estuarine fish in South Kalimantan assumed significance due to the strong positive correlations between the concentrations of this metal in water, sediment, and fish organs. The widespread dispersion of Fe from industrial and mining activities underscored its prevalence in aquatic ecosystems, often resulting from industrial and mining byproducts infiltrating water bodies.

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The intricate associations between Fe concentrations in water, sediment, and fish tissues illuminated the intricate relationship between environmental Fe accumulation and biological assimilation in estuarine ecosystems. The study underscored the importance of comprehending the impact of heavy metal contaminants, such as Fe, on aquatic organisms and habitats, with potential implications for ecosystem health and human well-being. Therefore, future studies should comprise a multifaceted approach including scientific investigations, policy formulation, public awareness, and collaborative endeavors among stakeholders to safeguard the well-being of aquatic ecosystems and human populations.

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**Use of AI tools declaration**

The authors declared that Artificial Intelligence (AI) tools were not used in the creation of this article.

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*Research article*

## **Iron Bioaccumulation and Ecological Implications in the Estuarine Ecosystem of South Kalimantan: Insights from Giant Mudskipper Fish as Bioindicators**

**Abstract:** This study investigated iron (Fe) accumulation in South Kalimantan's estuarine ecosystem, utilizing giant mudskipper fish as indicators of heavy metal pollution. By analyzing Fe levels in water, sediment, and fish organs over time, insights into its effects on the environment and human health were gained. Furthermore, through Atomic Absorption Spectrometry, Fe concentrations in Kuala Lupak's coastal wetland were measured, revealing significant correlations between Fe levels in water, sediment, and fish tissues. These results enhance our understanding and inform better management strategies.. Anthropogenic and natural sources contribute to the accumulation of heavy metals, particularly Fe, with anthropogenic pollution being the most dominant. This study presented the escalating concentrations of Fe within the Kuala Lupak estuary and raised concerns regarding the ecological and human health implications. Continuous monitoring, source identification, public awareness, regulations, remediation, and long-term exploration were essential for addressing heavy metal pollution and its ecological impact. Therefore, valuable insights were provided for environmental management and conservation efforts.

**Keywords:**

Iron Accumulation Dynamics; Estuarine Fish Contamination; Environmental Heavy Metal Impact; Aquatic Ecosystem Health.

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### **1. Introduction**

Heavy metals are a significant threat to the health of estuarine ecosystems, affecting the biodiversity of aquatic organisms at both individual and community levels [1-3]. This negative impact is attributed to the bioaccumulation of heavy metals in aquatic biota, resulting from the discharge of industrial and domestic waste into the estuarine environment. Yogeshwaran et al. [4] reported that the bioaccumulation of the metals in estuarine aquatic biota is directly linked to water contamination. The presence of heavy metals in aquatic biota was due to their availability in water bodies and sediment,

as well as the food consumed by the fish [5]. These trace elements exist in sediments in various forms, including dissolved, bound to suspended particulate matter, or accumulated, enhancing their incorporation into biological processes [6].

Iron, as the fourth most dominant metal on Earth, possesses the capacity to change valence and form complexes with oxygen. Furthermore, it plays a crucial role in respiration in nearly all aerobic organisms. However, unless suitably sequestered, Fe can catalyze the formation of radicals capable of damaging biological molecules, cells, tissues, and entire organisms (7). Certain trace metals such as Fe, Cu, Mn, and Zn are essential to biological systems but can become toxic at high concentrations. Metals including Zn, Fe, Cu, and Mo are integral components of metal-protein complexes in enzymes, contributing to enzymatic activities. Fe is a constituent of oxygen transport within living organisms, while it also plays a vital role in oxidative metabolism, serving as a cofactor for proteins and enzymes, DNA synthesis, and electron transport (8).

Other heavy metals such as Cd, Cr, Pb, and Hg have adverse effects even at deficient concentrations (9). Despite its physiological functions in fish, environmental pollution can render Fe toxic due to excessive accumulation (10). The continuous and substantial discharge of trace metal pollutants into the sea poses a significant threat to estuarine and coastal ecosystems due to their chronic toxicity, non-biodegradability, and bioaccumulation in biota (11). The metals can be transferred and biomagnified through food chains, presenting significant risks to human health. Metal contamination in estuarine ecosystems can induce toxic effects in fish and humans, primarily by consuming contaminated food (12) (13). Therefore, evaluating the occurrence of Fe content in the estuarine ecosystem of South Kalimantan is essential.

Analyzing the levels of Fe in the water body, sediments, and its bioaccumulation in wild fish, particularly those that inhabit estuaries for a significant portion of their life, such as the giant mudskipper fish (*Periophthalmodon schlosseri*), is of great importance. This fish species holds potential as a bioindicator for monitoring heavy metal pollution in estuarine waters, aiming to mitigate the adverse impacts of Fe on biodiversity conservation and environmental health. Evaluating the presence of Fe in the ecosystem serves as a valuable environmental indicator to understand anthropogenic influences and associated risks to the estuary environment.

Fe is an abundant transition metal in Earth's crust (14), and it can exist in aquatic environments in dissolved or suspended forms in water or sediment. Investigating Fe in water, sediment, and fish tissues aids in determining contamination status and loads. Therefore, quantifying the presence of this metal is pivotal in analyzing the health of aquatic ecosystems. Valuable information about the impacts of environmental changes and anthropogenic activities over time are obtained through long-term bioaccumulation of heavy metals. As a result, biomonitoring studies are essential in tracking trends in the concentrations of these metals, enabling scientists to identify changes in estuarine health (15). Contamination of aquatic ecosystems by trace metals has garnered global attention from environmental and biological experts, leading to numerous studies on their accumulation in estuarine and marine biota (16)(12). Bioaccumulation of Fe in commercially important fish species in estuarine ecosystems has been extensively investigated in various countries. For instance, studies have focused on the bioaccumulation of Fe in fish species, including *Solea solea*, *Sparus aurata*, and *Penaeus semiculatus*

in Turkey (17), *Rastrelliger kanagurta* in India (18), *Genyatremus luteus*, *Macrodon ancylodon*, *Sciades herzbergii*, and *Mugil curema* in Brazil (19), *Sciades herzbergii*, *Cetengraulis edentulus*, *Macrodon ancylodon*, *Pseudauchenipterus nodosus*, and *Stellifer rastrifer* in the Amazon region of Brazil (20), 13 potential heavy metal bioindicator fish species in the Persian and Arabian Gulfs (21), *Sciades herzbergii* in the Amazon region of Brazil (22), Sillaginid species in Iran (23); *Chrysichthys nigrodigitatus*, *Sarotherodon melanotheron*, *Scomber scombrus*, *Sardinella maderensis*, *Dentex canariensis*, and *Pseudotolithus typus* in Nigeria (24), as well as *Brevoortia aurea*, *Cynoscion guatucupa*, *Micropogonias furnieri*, *Mustelus schmitti*, *Odontesthes argentinensis*, and *Paralichthys orbignyanus* in Argentina (16). Trace metals enter fish bodies through various routes, namely direct food consumption, absorption through gills, and dermal absorption. After absorption, they circulate through the bloodstream and transported to various organs such as the kidneys, gills, liver, and muscles before being excreted. However, the concentrations of heavy metals absorbed/accumulated in fish muscle tissue remain relatively constant (15). There is a pressing need for comprehensive information on the evaluation of Fe presence, toxicity, and effects on giant mudskipper fish in the South Kalimantan estuary. Therefore, effective monitoring and evaluation of the availability of this metal in the estuarine environment (water, sediment, biota) are crucial (25).

The giant mudskipper fish has potential as a bioindicator species for heavy metal pollution biomonitoring programs in the Barito River estuary because it meets the requirements, including natural abundance (26), easy to obtain in large quantities, wide tolerance to polluted environmental conditions, benthic living habits, high trophic level in the aquatic food chain, and capability to accumulate heavy metals (27)(28)(29). Fish, renowned for their capacity to accumulate heavy metals, are well-suited to serve as bioindicator species in pollution biomonitoring, enabling the assessment of estuarine ecological health (30)(31)(32). The giant mudskipper fish, in particular, possesses unique biological characteristics, namely having amphibian-like characters, which distinguish them from other species. Additionally, they are often called "fish walking on land" or "fishes out of water" (33)

The habitat of giant mudskipper is mostly mudflats in mangrove forests and is widely distributed across Africa, Madagascar, India, Southeast Asia, northern Australia, southern China, southern Japan, Samoa, Tonga Islands, Saudi Arabia, Gulf of Kuwait, Polynesia, and Indonesia (34). The highest species diversity was observed in coastal areas of Southeast Asia, Australia, and Papua (35). Furthermore, giant mudskipper fish excel at making burrows in the mud of coastal swamps, estuaries, and intertidal zones. Their diet primarily consists of algae, detritus, diatoms, nematodes, polychaetes, and fish eggs, alongside mud and sand particles (33).

Kuala Lupak in the Barito River estuary is a coastal swamp wetland area characterized by extensive transportation activities, loading and unloading of coal, and coal mining waste disposal. The continuous input of waste from the coal mining process and coal-related activities into the water body has the potential to cause estuary pollution when prolonged and accumulated. These waste materials contain heavy metals such as Fe, Mn, Hg, Cd, Cr, Pb, and Cu (36). Furthermore, Kuala Lupak has witnessed the conversion of mangrove land to ponds, a place for ship transportation traffic and settlements. The several industries along the riverbank such as wood/plywood processing factories, rubber, fisheries, and oil palm plantations, pollute estuary waters through waste discharge (37). It is hypothesized that the unchecked release of these wastes into water bodies poses a significant risk to

both aquatic ecosystems and human health. Therefore, urgent biomonitoring efforts were warranted for early detection of the negative impacts of pollution. In light of these considerations, it is important to investigate the presence and occurrence of Fe in the Kuala Lupak estuary ecosystem during the period from 2020 to 2022. The objectives of the study were to evaluate the presence of Fe levels in water bodies, sediments, and giant mudskipper fish, assess the bioaccumulation of Fe in various tissues of giant mudskipper fish (liver, kidney, muscle, skin, and gills), evaluate the relationship or correlation between Fe content in water bodies and sediments as well as the concentrations in the liver, kidney, skin, muscle, and gill tissues of giant mudskipper fish, and validate the potential of giant mudskipper fish as a bioindicator of Fe contamination in the Kuala Lupak estuary. **The trends and intricacies of iron (Fe) contamination are investigated into by this research, especially within estuarine environments. The accessibility of this metal was primarily evaluated, and its annual fluctuations were also monitored.** The knowledge acquired from this study provides significant insights, especially for comprehending the possible consequences of future environmental changes. The results of this multi-year trace metal monitoring are of paramount importance in understanding the bioaccumulation potential of trace metals and constitute a crucial step in the management of estuarine and coastal ecosystems. These outcomes serve as a foundational framework for stakeholders to assess and address anthropogenic effects in the future.

## 2. Materials and methods

### 2.1. Study Area

The study was conducted in the coastal wetland of Kuala Lupak, situated in the Barito Kuala Regency. This region is approximately 8 km west of the mouth of the Barito River, the largest and longest river in South Kalimantan Province, Indonesia. The coastal wetland area of Kuala Lupak directly merges with the Java Sea coastline to the south. A comprehensive review of each study station/site is presented in Table 1, while the precise sampling locations are visually shown in Figure 1.

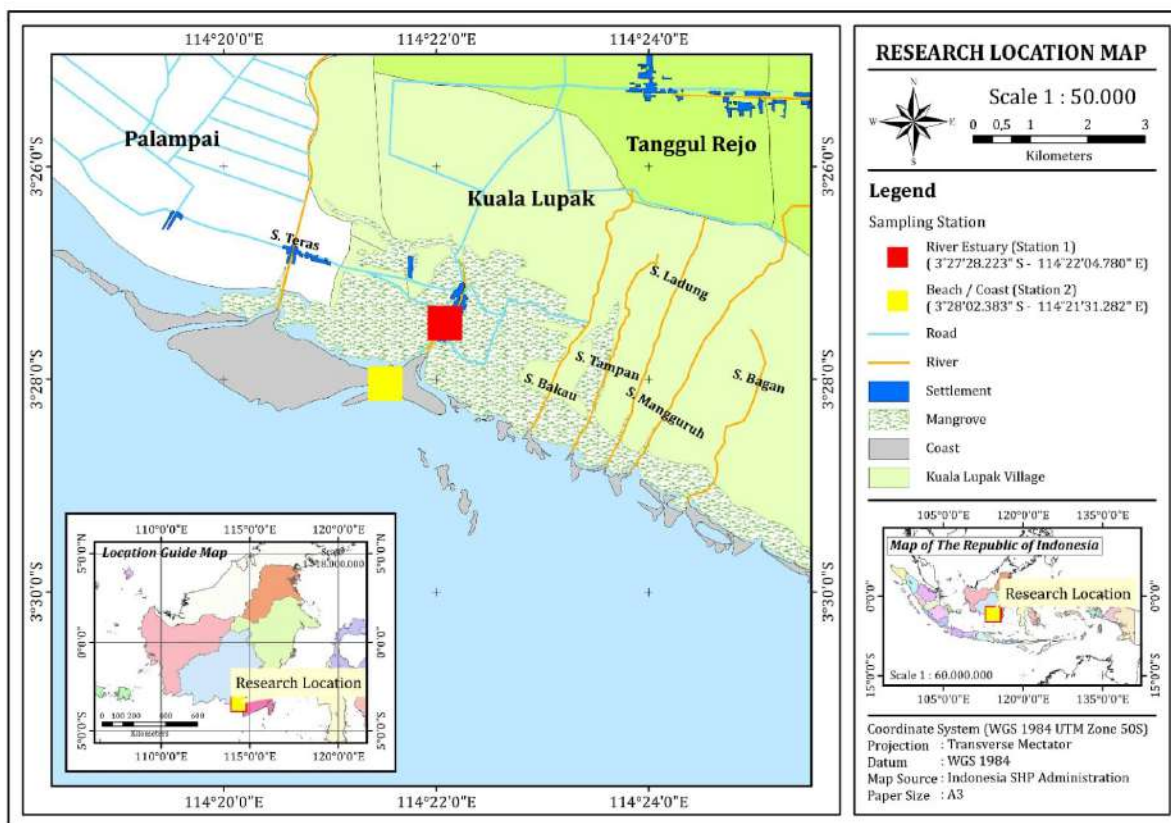
**Table 1.** Study station description

Location	Stations/sites	Description
Kuala Lupak	Estuarine	The estuarine waters of Kuala Lupak were a coastal wetland area with the status of Margasatwa Wildlife Sanctuary, specifically a mangrove swamp ecosystem. The mouth of the Kuala Lupak river was surrounded by human settlements and it flows into the Java Sea.
	Coastal	The water samples were collected from the coordinates 3°27'28.223"S 114°22'04.780" E in the estuarine waters. The coastal waters of Kuala Lupak were located approximately 8 km west of the mouth of the Barito River and border the Java Sea to the south, with a coastline of 30 km. Furthermore, they were

characterized by mangrove swamps. At the coordinates  $3^{\circ}28'02.383''\text{S}$   $114^{\circ}21'31.282''\text{E}$  water was collected in the coastal waters where the Kuala Lupak River flows into the sea.

## 2.2. Sample collections and analysis

The process of collecting water, sediment, and white snapper fish samples was conducted during August in 2020, 2021, and 2022. This was carried out using the purposive sampling method, where sample selection is based on specific criteria, such as the potential sources of pollution. To accurately identify these sources, various activities that contribute to contamination have been taken into consideration. Figure 1 shows the collection of fish, water, and sediment samples at the study location.



**Figure 1.** Study area map and sampling locations in the estuary Kuala Lupak South Kalimantan.

### 2.2.1. Water

Using Aqua trap water sampler, surface water samples were collected from 2 stations scattered throughout the study region. In preparation for further analysis, approximately 2 ml of concentrated  $\text{HNO}_3$  was added to each 1 L sample after filtration. The study adopted the liquid-liquid extraction technique (38) to determine the concentration level of dissolved Fe. A 100 ml unfiltered sample was placed in a separating funnel and mixed with 2 ml of 2% ammonium pyrrolidine dithiocarbamate



(APDC). Subsequently, 10 ml of iso-butyl methyl ketone (IBMK) was used for extraction after severe agitation. The extracted aqueous phase was subjected to further extraction using concentrated HNO<sub>3</sub> and high-purity water. Any remaining traces of organic solvents in the solutes were evaporated on a low-temperature hot plate, and the final solution was analyzed using Atomic Absorption Spectrometry (AAS Thermo Scientific ICE 3500 series Germany). This instrument was used to measure the concentration level of Fe in water, sediments, and fish samples. For water, the concentration was expressed in mg/L. Finally, samples for metal determination were conditioned following Jayaprakash et al. (38).

### 2.2.2. Sediment.

Sediment samples were procured from 2 estuary stations using a Van Veen grab sampler. Furthermore, they were dried at 40°C and powdered in an agate mortar for the digestion procedures. Approximately 1 g of the dried sediment sample was digested with an acid mixture containing HClO<sub>4</sub>+HF+HCl, and the residue was dissolved with concentrated HCl, then diluted to 25 ml. The solution was analyzed using flame AAS to estimate the Fe concentrations which were expressed in mg/Kg for sediment.

### 2.2.3. Fish Sampling and Analysis

Fish samples were obtained from the estuarine area by local fishermen. A total of 10 giant mudskipper fish with identical dimensions, ranging between 19 to 20 cm in length and weighing from 160 to 170 g were collected from each sampling station to ensure representativeness. The obtained samples were transported to the laboratory in ice boxes to maintain their preservation and appropriate conditions for further analysis. Furthermore, the dissection of the fish was conducted using surgical stainless-steel instruments, facilitating the extraction of organs, including the liver, kidneys, gills, muscles, and skin.

A digestion tube, pre-loaded with 5 ml of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, was utilized for each organ sample to initiate the reaction. The samples were subjected to digestion using a hot block digestion apparatus, maintained at 60°C for 30 minutes. Following the cooling of the samples, 10 ml of HNO<sub>3</sub> was added, and the mixture was further heated at temperatures ranging from 120 to 150 °C until a discernibly dark colouration of the solution was achieved. For facilitating the subsequent filtration process, 1 ml of H<sub>2</sub>O<sub>2</sub> was introduced to yield a clear solution. Those filtered samples were then analyzed using Atomic Absorption Spectrometry (AAS) to quantify the concentration of Fe in the fish tissues, expressed mg/Kg.

## 2.3. Data Analysis

Data processing and analysis were executed employing GraphPad Prism software, specifically the 5.9.0 series from Dotmatics. The statistical evaluations comprised the determination of mean, standard deviations (SD), minimum and maximum values, the creation of graphical representations showing the interrelationship among variables, and the calculation of Pearson correlations. The analyses were conducted using GraphPad Prism 7.02, developed by the company of GraphPad

Software Inc., San Diego.

### 3. Results

#### 3.1. Occurrence of Fe Concentrations in Water and Sediment

The average concentrations of Fe in the water column from 2020 to 2022 ranged from 2.55 to 11.61 mg/L. This showed a significant trend of increase over each successive year. The highest average concentration of Fe was recorded in 2022 to be 11.61 mg/L, as presented in Table 1. The Maximum Permissible Limits (MPLs) for heavy metals in estuary water were not regulated by International Guideline values. The Fe in Kuala Lupak estuary water exceeded the threshold value set by Government Regulation of Indonesia number 22 of 2021 concerning the Implementation of Environmental Protection and Management: Furthermore, the seawater quality standard for marine biota was set at 0.30 mgL<sup>-1</sup>. In parallel, the average concentrations of Fe within the sediment, serving as the habitat of the giant mudskipper fish, was in the range of 1245.92 to 48911.78 mg/Kg.

Analogous to the pattern observed in the water column, the concentrations of Fe in the sediment also displayed a significant upward trend over each year, as shown in Figure 2. The highest average level of Fe within the sediment was reported in 2022, to be 48911.78 mg/Kg, as presented in Table 1. The concentration of this metal in Kuala Lupak estuary sediments exceeded the maximum limit of 20000 mgKg<sup>-1</sup> set by the International Guideline value, CBSQG 2003: Consensus-Based Sediment Quality Guidelines (Wisconsin Department of Natural Resources). The mean plot of Fe level in water and sediment from 2020 to 2022 demonstrated a significant interrelationship between variables, with a confidence level of 95%, as indicated by the statistical analysis ( $P < 0.05$ ,  $R^2 = 0.649$ ).

#### 3.2. Temporal Variations in Fe Concentrations in Giant Mudskipper Fish

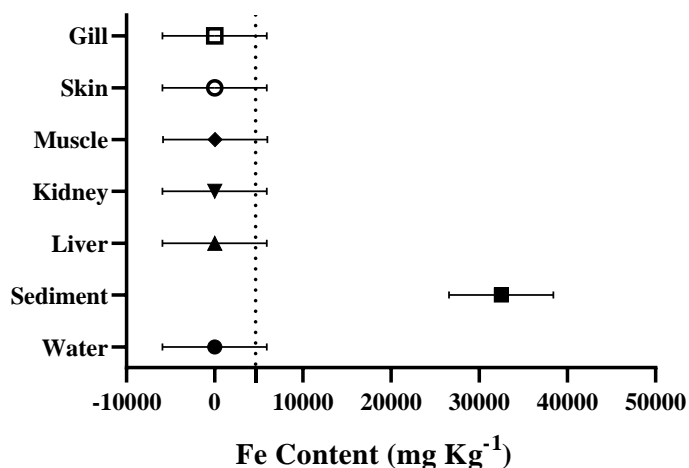
The mean Fe concentrations in the liver, kidney, muscle, skin, and gills of giant mudskipper for the years 2020-2022 ranged from 5.52 to 12.84 mg/kg, 7.98 to 15.44 mg/kg, 40.44 to 69.74 mg/kg, 4.42 to 8.80 mg/kg, and 1.23 to 6.22 mg/kg, as presented in Table 1. The average level of this metal in several fish tissues exhibited a significant upward trend annually, with the highest value being recorded in 2022, as specified in Figure 2. Based on the results in Table 1, muscle had the highest accumulation among the tested fish tissues, followed by kidney, liver, skin, and gills. The Maximum Permissible Limit (MPL) for Fe in fish meat was not regulated by national but not international guideline values. The Fe in giant mudskipper fish organs exceeds the threshold value of 1 mgKg<sup>-1</sup>, as regulated by the Indonesian National Standard (SNI 7387:2009) regarding the maximum limit of heavy metal contamination. When comparing the content of this metal among the tested samples in the Kuala Lupak estuarine ecosystem, the average concentration in sediment was the highest.

**Table 1.** Mean values of Fe concentration in water, sediment, and some tissues of giant mudskipper fish from the Kuala Lupak estuary between the period of 2020 to 2022.

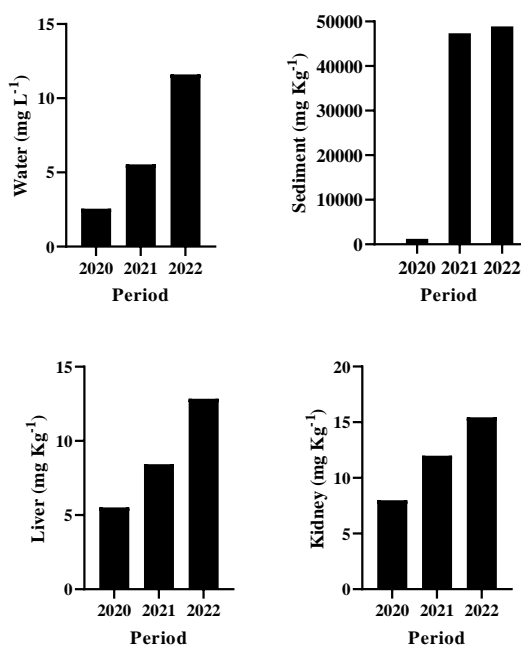
Sampling Period	Water (mg L <sup>-1</sup> )	Sediment (mg kg <sup>-1</sup> )	Liver (mg kg <sup>-1</sup> )	Kidney (mg kg <sup>-1</sup> )	Muscle (mg kg <sup>-1</sup> )	Skin (mg kg <sup>-1</sup> )	Gill (mg kg <sup>-1</sup> )

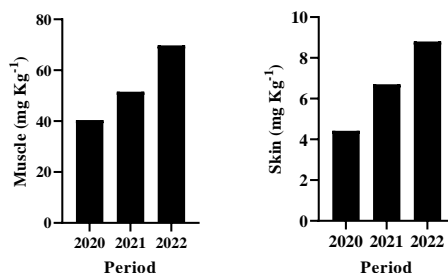


2020	2.55	1245.92	5.52	7.98	40.44	4.42	1.23
2021	5.54	47348.42	8.43	11.99	51.56	6.70	3.32
2022	11.61	48911.78	12.84	15.44	69.74	8.80	6.22



**Figure 1.** The mean plot of Fe concentration in water, sediment, and some tissue of giant mudskipper fish from 2020 to 2022 ( $P < 0,05$   $R^2 = 0,649$ ).



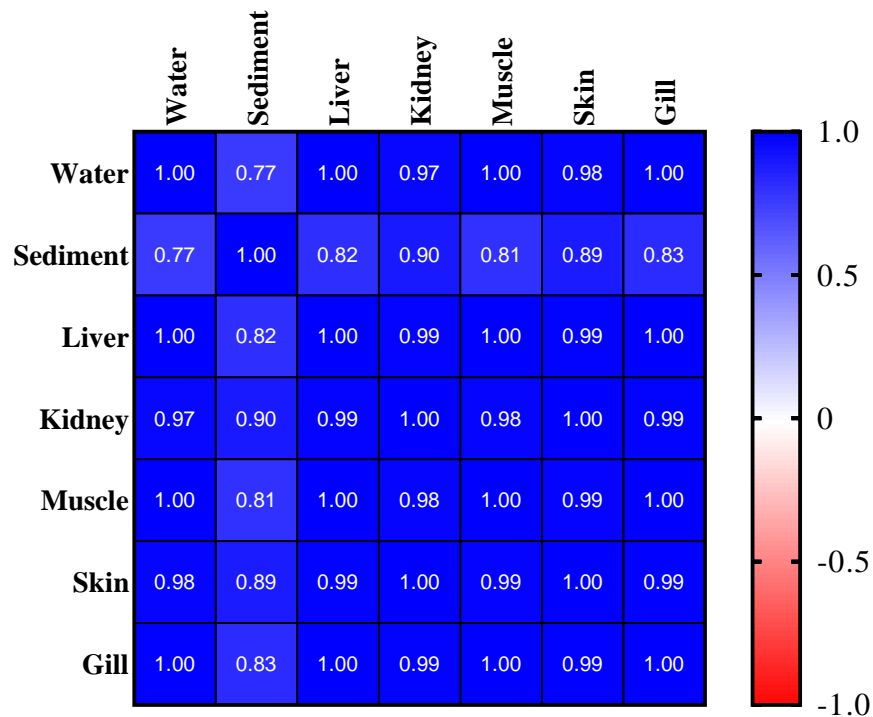


**Figure 2.** Fe content trend in some tissue of giant mudskipper fish period 2020-2022

### 3.3. The relationship between Fe contents in water, sediment, and giant mudskipper fish tissues

The interrelationships among heavy metal concentrations in water, sediment, and the tissues of giant mudskipper fish from 2020 to 2022 were evaluated and presented in Figure 3. The graphical representation showed a substantial positive correlation between the concentration of Fe in the water and the levels identified in the liver, muscle, and gills, with a correlation coefficient of 1. This implied that elevated levels of this metal in the water correspond with its heightened concentrations in the liver, muscles, and gills. Furthermore, a robust positive correlation was observed between waterborne Fe concentrations and the levels of the metal in the kidney and skin, with correlation coefficients of 0.97 and 0.98, respectively. The correlation coefficient between Fe levels in the water and sediment was 0.77. A highly significant positive correlation among the concentrations present in the water, sediment, and tissues of the giant mudskipper fish, as indicated by the outcomes of this investigation. Based on these observations, the accumulation in water and sediment has augmented Fe levels within the tissues of the fish. This correlation was in line with the consistently high concentrations in water and sediment.

Figure 3 further showed a robust positive correlation between sediment-bound Fe concentrations and those in the kidney, skin, gills, liver, and muscle tissues, with correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81, respectively. These results underscored the effect of sediment-bound Fe accumulation on the corresponding values within the tissues of the fish. This correlation was in line with the consistent elevation of sediment-bound Fe concentrations over 3 years, exhibiting an annual increment, as presented in Figure 1. The study also underscored a significant positive correlation across various organs of the giant mudskipper fish. This indicated a uniform distribution across diverse organs after Fe uptake into the body of the fish. In Figure 3, a close positive correlation was evident between the Fe concentration in the skin, serving as an entry point for Fe uptake. In the kidney, gills, muscle, and liver, the level of this metal exhibited correlation coefficients of 1.00, 0.99, 0.99, and 0.99, respectively. Similarly, a positive, strong correlation existed among the concentration in the gills, another site of Fe entry, and the Fe levels in the liver, muscle, skin, as well as kidney. These featured coefficients of 1.00, 1.00, 0.99, and 0.99, respectively. The results showed that elevated Fe concentrations in the skin and gills precipitate were directly proportional to the liver, kidney, and muscle tissues.



**Figure 3.** Pearson correlation of Fe content in some tissue of mudskipper fish period 2020-2022

#### 4. Discussion

##### 4.1. Fe Contamination in Estuarine Waters: Sources and Implications

The heightened prevalence of Fe within the Kuala Lupak estuary in 2022 was attributed to the effects of extreme rainfall and substantial flooding in the region of Kalimantan Selatan from December 2020 to February 2021. This event led to the dissolution of heavy metal deposits from upstream areas, resulting in their transport into the estuarine environment and eventual deposition within the estuary. This result was in line with the study conducted by Čmelík et al. (39), which identified a substantial increase in heavy metal concentrations in both water and sediment along the course of the Bílina River in the Czech Republic, directly attributed to extreme rainfall events. However, it is important to note that floods had a comparatively shorter-lived influence on the quality of water and sediment compared to the cumulative impacts of anthropogenic activities. A study by Supriyantini and Endrawati (40) also reported elevated concentrations within water bodies, sediment, and tissues of the green mussel (*Perna viridis*) in the coastal rivers of Tanjung Emas Semarang. In this investigation, the elevated Fe content in the estuary stemmed from various sources, including soil and anthropogenic activities occurring on land. These activities comprise household waste containing Fe, water reservoirs sourced from Fe, industrial waste deposits, and corrosion from Fe-containing pipes transported into the estuary by river flow. According to Haeruddin et al. (41), the highest heavy metal concentrations within sediment were proximal to contamination sources.

Fe contamination within estuarine waters emanates from natural processes, such as geological weathering, rock decomposition, and anthropogenic activities. These anthropogenic sources include Fe release from ore materials and volcanic eruptions that discharge Fe-rich materials into water bodies through runoff, erosion, and flooding. Human activities such as coal transportation and mining operations contribute significantly to this pollution (42). Anthropogenic Fe sources also include land-based activities, such as the discharge of household waste containing Fe, Fe-laden water reservoirs, industrial waste deposits, and the corrosion of Fe-containing pipes, releasing  $\text{Fe}^{2+}$  and sulfuric acid due to the oxidation of Fe pyrite ( $\text{FeS}_2$ ) present in coal layers (14).

The escalating temporal increase in Fe concentrations in both water bodies and sediments, as observed in this study, was supported by an investigation conducted by Weber et al. (43) concerning the contamination in Brazil's Piranga and Doce rivers. Both rivers were contaminated with Fe due to the collapse of the Samarco mine tailings dam in Brazil in 2015. Environmental catastrophes such as dam failures that release tailings profoundly impacted the landscape and aquatic environments, progressively deteriorating the water quality of the Doce and Piranga rivers over time. Another source of this contamination stems from mineral weathering/erosion and elevated Fe content in soil. The contamination of these rivers has led to the bioaccumulation of Fe in *Hoplias intermedius* and *Hypostomus affini* fish. This caused histopathological lesions in the liver, including vacuolization, hepatocyte hypertrophy, and elevated necrotic areas compared to fish from non-tailings-contaminated rivers. This study strengthens the notion that fish living in heavy metal-contaminated waters experience declining aquatic health quality, leading to heavy metal bioaccumulation and histopathological lesions.

#### 4.2. Accumulation of Metals in Sediments: Multifaceted Sources and Ecological Implications

In this study, sediments in the Kuala Lupak estuary accumulate higher levels of heavy metals than the water column. This has rendered the sediments a significant potential source and a primary repository for all pollutants (44). These particles can store diverse heavy metals in substantial and consistent quantities, hence, they become valuable indicators for assessing water pollution status (41). Liline et al. (45) reported that sediments in the Rutong, Waai, and Poka mangrove areas in Maluku function as a medium for transferring heavy metals such as chromium (Cr) and Fe to mudskipper fish. The heavy metals in estuarine sediments result from sedimentation processes that retain significant amounts of adsorbed metal particles, leading to their deposition at the bottom. Wastes containing these metallic elements were deposited in sediments upon entering estuaries. This was facilitated by reduced environmental conditions, low dissolved oxygen, and elevated pH, causing metal concentration or enrichment (46). The result was in line with the study by Dalu et al. (42), stating that heavy metal content in sediment was significantly higher than in the water column, as it was being absorbed by suspended particles. High Fe content in sediment can lead to contamination of water bodies, which can have negative impacts on the health of aquatic biota and the overall ecological balance of aquatic ecosystems.

Sediments serve as indicators of land-based pollution by acting as "sinks" for pollutants. The rise in heavy metal levels in sediments suggests that heavy metals in the water column are deposited along with suspended solids, posing a potential threat to estuarine and coastal ecosystems. These sediments, serving as sinks for various human activities, may also act as sources of heavy metals, underscoring

the ongoing necessity for monitoring to evaluate their role as pollution sources. Heavy metals are prone to accumulating in sediments, resulting in consistently higher concentrations than those in the water column. Suspended sediment particles quickly transport these metals into water through the movement of water masses (44). The high volume of merchant and coal transport vessels contributes to water mass movement in the Barito River estuary.

The accumulation of heavy metal in sediments was attributed to a variety of sources, including leaded gasoline emissions, chemical manufacturing industries, motorized transportation, underground pipe corrosion, coal-based thermal power plants, and urban waste (38). The results of this study were in line with the report of Jayaprakash et al. (38), indicating that heavy metal concentrations in water were consistently lower than in sediment within contaminated water bodies. This is because sediment particles tend to absorb metals from the water. In sediment-rich environments, organisms such as the giant mudskipper fish accumulated heavy metals from their food and water through gills, mouth, and skin mucus, further underscoring the potential for metal bioaccumulation.

The intricate interplay between suspended sediment particles and their propensity to absorb heavy metals from the aqueous phase was a crucial mechanism underlying the contrasting concentrations of these metals in water and sediment. This phenomenon accentuated the role of sediments as repositories for heavy metals, specifically in aquatic environments subject to anthropogenic influences and diverse pollutant sources.

A previous study by Jayaprakash et al. (38) described the multiple pathways through which heavy metals infiltrate sediment systems, originating from various anthropogenic activities. The disposal of leaded gasoline, effluents from chemical industries and motorized transport, underground pipe corrosion, emissions from coal-based thermal power plants, and the discharge of urban waste all contribute to metal accumulation within sedimentary matrices.

The congruence between the report of Jayaprakash et al. (38) and this study underscores the broader ecological implications of metal accumulation in sediments. The distinct levels of heavy metals within water and sediment reflect the differential interactions between these metals and particulate matter suspended in water. Consequently, sedimentary environments act as crucial hubs for storing heavy metals, providing a pertinent context for comprehending the potential ecological ramifications of anthropogenic pollution in aquatic ecosystems. High Fe content in sediment can alter the physical and chemical properties, such as color, texture, and pH. This could also affect the habitat of benthic organisms and the overall ecological balance of aquatic ecosystems (47). These results elaborate on the necessity for robust monitoring practices to assess the extent and impacts of contamination within sediment systems.

During this study, direct observations showed that the deteriorating water quality in the Kuala Lupak Estuary stems from anthropogenic activities. The sources of contamination affecting water, sediment, and the organs of the giant mudskipper fish were believed to originate from domestic and non-domestic waste. Domestic waste originates from residential areas, while non-domestic waste sources comprise industrial, agricultural, livestock, fisheries, mining, and other non-residential activities. The fact was in line with the study of Čmelík et al. (39), who stated that anthropogenic

pollution, as opposed to short-term flood effects, was the dominant contributor to a significant increase in heavy metal concentrations in both water and sediment along the Bílina River in the Czech Republic. Additionally, anthropogenic pollution exhibited a more pronounced temporal impact on the water quality of the Bílina River.

Direct observations during this study, as well as interviews with residents, led to the identification of sources of coastal swamp water pollution in Kuala Lupak. These sources include residential waste and the waste products of factories situated along the banks of the Barito River estuary. Factories, such as plywood processing plants perform wood washing activities as well as generate solid waste such as wood fragments, bark, sawdust, and sanding residues. Other sources of contamination were believed to arise from gold mining activities in the Barito River basin, the use of paint in shipyards, agricultural activities including the application of pesticides and fungicides, converting mangrove areas to fish ponds, and the application of explosives for fishing. Fuel companies discharging waste were also suspected sources of pollution. These companies distributed diesel fuel through water transport routes that traverse the Barito River estuary. Additionally, the high density of commercial and coal transport ships, alongside coal spillage and debris entering the river, contributed to the degraded water quality of the river. According to the National Transportation Safety Committee, approximately 7.101 units of coal barges navigate the Barito River [64].

In addition to anthropogenic activities, Fe is sourced from natural processes comprising regular biogeochemical cycles, volcanic activities, soil erosion, continental runoff, shelf inputs, as well as atmospheric deposition from volcanic events and wildfires (6). Furthermore, as the most dominant transition metal in the Earth's crust, it undergoes multiple natural cycles (14). Anthropogenic pollution of trace metals, including Fe, in estuarine waters has deteriorated water quality and negatively impacted fish health (9)(48)(49).

#### *4.3. Bioaccumulation Potential and Ecological Impact of Heavy Metals in Giant Mudskipper Fish*

Many studies have documented that indicator species accumulate contaminants in their tissues at higher levels than the surrounding habitat, such as water and sediment. This characteristic makes them valuable as potential bioindicators within ecosystem health biomonitoring programs. According to Table 1, the concentrations of Fe in the muscle, kidney, liver, and skin of giant mudskipper fish exceeded those in the surrounding aquatic environment. The elevated concentrations within fish tissues were attributed to the increased levels present in both sediment and water. These results corroborate with those of Abdel Gawad (50), who posited that high concentrations of heavy metals in sediment and water allow aquatic organisms to accumulate metals biologically and transport them through the food chain. The ability of giant mudskipper fish to accumulate heavy metals in tissues stems from their position at the pinnacle of the food chain. These organisms reside benthically in mud within the intertidal coastal zone and mangrove forest floor, hence, they are susceptible to direct exposure to pollutants within the estuarine environment. According to Ferreira et al. (51), estuaries represent habitats with continuous and direct exposure to heavy metal pollutants.

Estuaries have gained wide recognition as net sinks for trace metals, including Fe, which precipitate rapidly within the environment (52). While rivers transport substantial amounts of dissolved



and particulate Fe into the sea, a significant portion was retained and accumulated within sediments (53)(54). This phenomenon was attributed to the exceptional capacity of the sediments as a final trap and adsorptive reservoir for heavy metals in aquatic environments (55). Fe colloids were immobilized within surface estuarine sediments and can become mobilized by organic matter and reduction agents (53). When these values exceed a certain threshold, the mobility and deposition of Fe lead to pollution, thereby posing toxicity risks to fish. This risk arose primarily because of the persistent, non-biodegradable, accumulative, and diverse sources of trace metals (55).

Continued and significant discharges of trace metal pollutants into the sea can damage estuarine and coastal ecosystems, given their chronic toxicity, non-biodegradability, and bioaccumulation in biota, including fish (11). Trace metals can transfer and biomagnify through food chains, posing severe threats to ecosystem health and human well-being. Furthermore, the contamination of metal within estuarine ecosystems can engender toxic effects in fish and humans, primarily by consuming contaminated food sources (13).

Several studies have noted that indicator species accumulated contaminants in their tissues higher than in the environment of habitat, such as water and sediment. This phenomenon holds potential for the utilization of these species as bioindicator tools within aquatic ecosystem biomonitoring programs. Table 1 presents the Fe concentrations in the muscle, kidney, liver, and skin tissues of the giant mudskipper fish, which were observed to be higher than in the water column. The elevated concentration in the fish tissues was attributed to the high levels of this metal in both sediment and water. This result was in line with the study conducted by Abdel Gawad (50), who postulated that the high amount of heavy metals in the water and sediments provided opportunities for aquatic organisms to accumulate these metals biologically and transport them through the food chain. The capacity of the giant mudskipper fish to accumulate heavy metals within its tissues was attributed to its position at the top of the food chain. This species thrived benthically in mud within the intertidal coastal zone and mangrove forest floor, experiencing direct exposure to pollutants in estuarine environments. Estuaries, as described by Ferreira et al. (56), represented habitats continually exposed to heavy metal pollutants.

People have widely recognized estuaries as net sinks for trace metals, including Fe. This was because of the rapid deposition of trace metals/Fe ions within estuaries (52). Despite that rivers transported substantial amounts of dissolved and particulate Fe into the ocean, a significant portion became entrapped and accumulated within estuarine sediments (53)(54). Sediment was well-established as a proficient final trap and adsorptive sink for heavy metals in aquatic environments (55). Fe colloids became immobilized within estuarine surface sediments and were mobilized with organic matter and reduction media (53). When Fe mobilization and sedimentation surpass certain thresholds, it leads to pollution and becomes toxic to fish. This toxicity arises primarily from the persistent, non-biodegradable, accumulative, and diverse nature of trace metal sourcing (55).

Continuous and substantial discharges of trace metal pollutants into the sea damage estuarine and coastal ecosystems. The chronic toxicity and non-biodegradability of these pollutants, combined with their bioaccumulation within biota, including fish, posed a severe threat to human health (11). Transfer and bio-magnification of trace metals along food chains severely jeopardized human health. Additionally, metal contamination within estuarine ecosystems yielded deleterious effects on both fish



and humans, stemming from the consumption of tainted food sources (13).

The investigation into the toxic effects of Fe on fish inhabiting estuarine ecosystems proved a compelling inquiry for biologists in South Kalimantan, attributable to the robust positive correlation between Fe levels in the water, sediment, and fish organs. The heightened concentrations of this metal were suspected to be a consequence of its extensive usage (57). Furthermore, Fe was a prevalent component of industrial and mining waste, frequently discharged into aquatic environments, hence, exhibiting a pronounced correlation with aquatic biota, including fish. Its elevated contents in water and sediment facilitated a more pronounced accumulation within fish organs. Fe in its ferrous form ( $\text{Fe}^{2+}$ ) had heightened toxicity towards fish compared to when it was ferric ( $\text{Fe}^{3+}$ ).  $\text{Fe}^{2+}$  exhibited toxic effects by binding to gill surfaces and undergoing oxidation to form insoluble  $\text{Fe}^{3+}$ , thereby inciting cellular damage, ultimately culminating in respiratory dysfunction (58). The bioconcentration of Fe within fish tissues attained its zenith within the liver and gonads, subsequently diminishing in the brain, muscles, and heart (59). The study conducted by Omar et al. (60) substantiated the liver as a prime target organ for Fe-induced toxicity in fish. Furthermore, the toxicity of this metal within gills disrupts respiration due to the physical obstruction of the surfaces (61). The alkaline nature of the gill surfaces promoted the facile oxidation of soluble Fe, forming insoluble Fe compounds that cloak gill lamellae, impeding respiratory function. Excessive Fe absorption led to poisoning in *Labeo rohita*, catalyzing the generation of ROS via Fenton reactions, which damaged biomolecules, cells, and tissues through oxidative stress mechanisms (58). This metal also potentiates the toxicity of other chemical agents, such as paraquat or 2,3,7,8-tetrachlorodibenzo-p-dioxin (62). In a study by Kaloyianni et al. (63), the toxicity of magnetite nanoparticles ( $\text{Fe}_3\text{O}_4$ ) was reported in *Danio rerio* and *Carassius gibelio* fish, evoking oxidative, proteolytic, genotoxic, and apoptotic effects, accompanied by elevated malondialdehyde and carbonyl protein levels.

## 5. Conclusions

In conclusion, the study elucidated substantial positive associations in Fe levels across water, sediment, and tissues of the giant mudskipper fish (*Periophthalmodon schlosseri*) during the 2020-2022 period. These associations underscored the dynamic interplay between environmental Fe buildup and its assimilation within fish tissues. The graphical representation accentuated a strong positive correlation (correlation coefficient 1) between waterborne concentrations and those in the liver, muscle, and gills. This indicated that higher Fe levels in water correspond to elevated levels in these fish tissues. Similarly, noteworthy positive correlations were observed between Fe levels in water as well as kidney and skin tissues, with correlation coefficients of 0.97 and 0.98, respectively. Sediment-bound Fe also correlated positively with kidney, skin, gills, liver, and muscle tissues, with sequential correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81.

The strong positive correlation within the various fish organs implied uniform Fe dispersion post-uptake. The skin and gills, primary entry points for this metal, exhibited robust positive correlations with kidney, gills, muscle, liver, and skin tissues. This inquiry into the detrimental impacts of Fe on estuarine fish in South Kalimantan assumed significance due to the strong positive correlations between the concentrations of this metal in water, sediment, and fish organs. The widespread dispersion of Fe from industrial and mining activities underscored its prevalence in aquatic ecosystems, often resulting from industrial and mining byproducts infiltrating water bodies.

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The intricate associations between Fe concentrations in water, sediment, and fish tissues illuminated the intricate relationship between environmental Fe accumulation and biological assimilation in estuarine ecosystems. The study underscored the importance of comprehending the impact of heavy metal contaminants, such as Fe, on aquatic organisms and habitats, with potential implications for ecosystem health and human well-being. Therefore, future studies should comprise a multifaceted approach including scientific investigations, policy formulation, public awareness, and collaborative endeavors among stakeholders to safeguard the well-being of aquatic ecosystems and human populations.

### **Use of AI tools declaration**

The authors declared that Artificial Intelligence (AI) tools were not used in the creation of this article.

Submission & Acknowledgement to JDMLM  
**Journal of Degraded and Mining Lands  
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## [JDMLM] Submission Acknowledgement

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Editorial Team <editor.jdmlm@ub.ac.id>

Wed, Sep 27, 2023 at 7:15 AM

To: Mr HERI BUDI SANTOSO <heribudisantoso@ulm.ac.id>

Mr HERI BUDI SANTOSO:

Thank you for submitting the manuscript, "Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators" to Journal of Degraded and Mining Lands Management. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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Reviewer's comments (4 Nopember 2023)



Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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**[JDMLM] Reviewer's comments**

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JDMLM Editorial Office &lt;editor.jdmlm@ub.ac.id&gt;

Sat, Nov 4, 2023 at 4:51 PM

To: heribudisantoso@ulm.ac.id, krisdianto@ulm.ac.id, rizmiyunita@ulm.ac.id

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Your manuscript entitled "**Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**" has been reviewed by the Journal of Degraded and Mining Lands Management reviewer. Based on the reviewer's comments (attached), **your manuscript might be accepted for publication in the Journal of Degraded and Mining Lands Management, pending some minor corrections suggested by the reviewer.**

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Submitted the revised (9 Nopember 2023)



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## [JDMLM] Reviewer's comments

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**Heri Budi Santoso** <heribudisantoso@ulm.ac.id>  
To: JDMLM Editorial Office <editor.jdmlm@ub.ac.id>

Thu, Nov 9, 2023 at 2:10 PM

Dear Editor in Chief  
Prof Eko Handayanto PhD

I have submitted the revised result as suggested by the editor team. Manuscript attached.

Best regards  
Heri Budi Santoso

[Quoted text hidden]

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Regards,  
Heri Budi Santoso  
Faculty of Mathematics & Natural Science  
Lambung Mangkurat University  
Jl. A. Yani Km 36, Banjarbaru, 70714, Indonesia



**15953-SANTOSO et al-reviewed CWA\_revisi1.docx**  
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Reviewer's comments (11 Nopember 2023)



Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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## [JDMLM] Reviewer's comments

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JDMLM Editorial Office <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>

Sat, Nov 11, 2023 at 3:34 PM

Dear Authors

(Comments of 2<sup>nd</sup> reviewer)

Herewith, we are forwarding **the comments of the second reviewer** on your manuscript entitled "**Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**". The comments are as follows:

(1) *This manuscript was neatly prepared. However, the methods presented in the Materials and Methods section seem to be only the procedures Fe analysis in water, sediments, and fish tissue, NOT research methods designed to solve the negative effects of Fe in water and sediment. So, this manuscript only presents the results of a problem-identification study, NOT a problem-solving study. The results of this problem-identification study should have been further followed by research work(s) directed to the management of the degraded environments (Fe-contaminated water and soils/sediment) to fit within the scope of this journal. The correlation analysis made between Fe concentration in sediment and Fe concentration in fish kidneys, skin, gills, liver, and muscle tissues seems meaningless, as fish definitely interact with water, the medium used for their lives.*

(2) *The Results and Discussion section only presents comparative statements with others rather than presenting the mechanism of Fe accumulation. I understand it is difficult for the authors to present a good discussion as the results presented in this manuscript are not results of experimental research works; they are just the results of laboratory analysis of water and sediment samples.*

**Recommendation: Revisions.** *The authors should include the results of research work(s) based on this problem-identification study (Fe content in water and sediment), for example, bioremediation or phytoremediation of Fe-contaminated water or sediment.*

If you wish to revise your manuscript, please make sure you have followed and accommodated all the reviewer's comments before submitting the revised version.

All the best

Eko Handayanto  
Editor in Chief

[Quoted text hidden]

## Response to reviewer 2 (16 Nopember 2023)





Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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## [JDMLM] Reviewer's comments

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Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
To: JDMLM Editorial Office <editor.jdmlm@ub.ac.id>

Thu, Nov 16, 2023 at 9:10 PM

Dear

*Editor in Chief*

Prof. Eko Handayanto

We would like to thank you for your kind response to the article "**Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**". Could you please give us an opportunity to depend on our research article as follows:

Regarding your comment, "NOT research methods designed to solve the negative effects of Fe in water and sediment", we have implicatively answered by comparing the result to the safety standards for human consumption as recommended by national or international organizations. Thus, the intelligent solution is clear: to avoid utilizing the contaminated tissue of that fish. By the higher value of heavy metal concentrations than international and national permissible limits (MPLs), it is implicitly concluded that further management is needed, and not necessarily in the cases of environmental degradation. Implementing regulations to prevent people from further Fe contamination. We appreciate suggestions about other research solutions for the degraded environment, but it takes time, funding and many different approaches and designs. We thank you for your wise suggestion about bioremediation or phytoremediation; we will plan for the following research in the next Fiscal Year, 2024 since this research requires new design and acclimation to the field conditions.

Our results present not just laboratory analyses but also Fe contaminants in different tissues, such as fish kidneys, skin, gills, liver, and muscle tissues. Since the metabolism of metallothionein and metal enzymes play different activities in different tissues and organs of species, it is essential to clearly understand the accumulation of Fe under other species prior to determining the mechanism of accumulation of Fe in certain fish. We also present our research publication by considering other International Journal articles as baseline, such as: Zaynab, M., Al-yahyai, R., Ameen, A., Sharif, Y., Ali, L., Fatima, M., Ali, K., and Li, S. (2022). Health and environmental effects of heavy metals. Journal of King Saud University-Science, 34(1): 101653. doi:10.1016/j.jksus.2021.101653.

We chose to submit an article on the adverse effects of iron pollution to JDMLM because we wanted to inform the public that the coastal swamp environment at the estuarine of the Barito River has been degraded by heavy metals. In the last two decades, there is no information on the health of wetland ecosystems in the coastal swamps of South Kalimantan. We conducted a three-year research to confirm that the wetland was polluted with iron (2020-2022). The wetland ecosystem that has been studied includes water, sediment and fish. We hope that the results of this research in the form of problem identification can be followed up by the authorized institutions with degraded land management studies. We are ready to work together for collaborative research to address the degraded wetland environment.

We would thank you very much if you could consider publishing our research article in your journal.

Sincerely yours,  
Heri Budi Santoso

[Quoted text hidden]

Editor decision (12 Desember 2023) 9.34 AM



Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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**[JDMLM] Editor Decision**

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**JDMLM Editorial Office** <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
Cc: krisdianto@ulm.ac.id, rizmiyunita@ulm.ac.id

Tue, Dec 12, 2023 at 9:34 AM

**Dear Authors** (*Heri Budi Santoso, Krisdianto, Rizmi Yunita*),

After correcting a few typos and some other details, we are pleased to inform you that your revised manuscript entitled "**Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**" can be **ACCEPTED for publication in the Journal of Degraded and Mining Lands Management (JDMLM)**.

Before we further process your manuscript, we need to inform you that your article will likely come in JDMLM Vol. 11, No. 3 (1 April 2024) because JDMLM Vol. 11, No. 2 (1 January 2024) is already full.

If you agree your article will be published in JDMLM Vol 11 No 3 (1 April 2024), we will send the Galley Proof of your ready-to-publish article for proofreading. However, if you disagree, please ignore this email, and you may request a withdrawal of the submission.

We are waiting for your confirmation, and we apologize for this inconvenience.

Sincerely yours

Prof Eko Handayanto PhD  
Editor in Chief

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Response author to editor  
(12 Desember 2023) 9.45 AM



Heri Budi Santoso <heribudisantoso@ulm.ac.id>

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## [JDMLM] Editor Decision

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**Heri Budi Santoso** <heribudisantoso@ulm.ac.id>  
To: JDMLM Editorial Office <editor.jdmlm@ub.ac.id>

Tue, Dec 12, 2023 at 9:45 AM

Dear Editor

Yes, We agree our article will be published in JDMLM Vol 11 No 3 (1 April 2024)

[Quoted text hidden]

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Regards,  
Heri Budi Santoso  
Faculty of Mathematics & Natural Science  
Lambung Mangkurat University  
Jl. A. Yani Km 36, Banjarbaru, 70714, Indonesia

Response editor to author  
(12 Desember 2023) 6.11 PM





Heri Budi Santoso <heribudisantoso@ulm.ac.id>

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## [JDMLM] Editor Decision

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**JDMLM Editorial Office** <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>

Tue, Dec 12, 2023 at 6:11 PM

Thank you for your prompt response, and we will soon send the Acceptance Letter and Galley proof for proofreading.

JDMLM Editorial Team

[Quoted text hidden]

Galley Proof of MS 15953  
(30 Desember 2023)



Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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**[JDMLM] Galley Proof of MS 15953**

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**JDMLM Editorial Office** <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
Cc: krisdianto@ulm.ac.id, rizmiyunita@ulm.ac.id

Sat, Dec 30, 2023 at 2:45 AM

**Dear Authors**

[Heri Budi Santoso, Krisdianto, Rizmi Yunita]

We are sending the Galley Proof of your ready-to-publish article entitled "**Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**" for proofreading.

You can make corrections by marking yellow on the attached file, not on another new one, and send the corrections back to us through this email address soon. Your article will likely come in Vol. 11, No. 3 (1 April 2024). However, the article pages have not been set up yet; we await your confirmation.

Thank you for considering this journal as a venue for your work.

Sincerely yours

Prof Eko Handayanto PhD

*Editor in Chief*

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491K

Submitted the corrected MS (2 Januari 2024)



Heri Budi Santoso <heribudisantoso@ulm.ac.id>

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## [JDMLM] Galley Proof of MS 15953

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Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
To: JDMLM Editorial Office <editor.jdmlm@ub.ac.id>

Tue, Jan 2, 2024 at 11:58 AM

Dear Editor.

I am sending the corrected manuscript. Thank you for publishing our article.

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Regards,  
Heri Budi Santoso  
Faculty of Mathematics & Natural Science  
Lambung Mangkurat University  
Jl. A. Yani Km 36, Banjarbaru, 70714, Indonesia

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Article, APC 15953-SM, and LoA  
(3 Januari 2024)



Heri Budi Santoso &lt;heribudisantoso@ulm.ac.id&gt;

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**[JDMLM] Article and APC: 15953-SM**

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**JDMLM Editorial Office** <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
Cc: krisdianto@ulm.ac.id, rizmiyunita@ulm.ac.id

Wed, Jan 3, 2024 at 3:09 AM

**Dear Authors,**

We are sending herewith the **final version of your ready-to-publish article** entitled **""Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators"** (attached). If you still find mistakes or typos in your ready-to-publish article, please do not hesitate to contact us to make further corrections.

Journal of Degraded and Mining Lands Management applies APC (article processing charge) of US\$ 100 for an article of **no more than 12 pages** (10-point font, including figures, tables, and references). An additional APC of \$20 per page will be charged to ready-to-publish articles exceeding 12 pages <https://jdmlm.ub.ac.id/index.php/jdmlm/about#APC.> Regarding this, you are kindly requested to deposit **US\$ 100 (IDR 1,550,000)** to the following Bank Account. APC is used for maintaining international indexation memberships, reviewer incentives, and editorial staff allowances.

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Name of A/c holder : Eko Handayanto

\*) Please kindly notify us when you have made the payment.

Thank you for considering this journal as a venue for your work.

Kindest regards

Dr Reni Ustiatik

*JDMLM AEiC*

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[https://www.scimagojr.com/journalrank.php?area=2300&country=Asiatic%20Region&page=3&total\\_size=216](https://www.scimagojr.com/journalrank.php?area=2300&country=Asiatic%20Region&page=3&total_size=216) (no 110)


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**2 attachments**

 **5539-5550, 15953-SANTOSO et al.pdf**  
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Heri Budi Santoso <heribudisantoso@ulm.ac.id>

**[JDMLM] Article and APC: 15953-SM**

Heri Budi Santoso <heribudisantoso@ulm.ac.id>  
To: JDMLM Editorial Office <editor.jdmlm@ub.ac.id>

Wed, Jan 3, 2024 at 6:05 PM

Dear Dr Reni Ustiatik  
*JDMLM AEiC*  
Here I attach proof of payment or APC transfer  
Thank you for the opportunity to be published on JDMLM  
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Regards,  
Heri Budi Santoso  
Faculty of Mathematics & Natural Science  
Lambung Mangkurat University  
Jl. A. Yani Km 36, Banjarbaru, 70714, Indonesia



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Heri Budi Santoso <heribudisantoso@ulm.ac.id>

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## [JDMLM] Article and APC: 15953-SM

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**JDMLM Editorial Office** <editor.jdmlm@ub.ac.id>  
To: Heri Budi Santoso <heribudisantoso@ulm.ac.id>

Wed, Jan 3, 2024 at 6:24 PM

Dear Authors

The APC payment has been received by the JDMLM Editorial Office.

Thank you for supporting this journal.

JDMLM Editorial Team

[Quoted text hidden]

Article published (1 April 2024) **Vol. 11 No. 3**  
**(2024)**

**Research Article**

## **Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators**

**Heri Budi Santoso<sup>1\*</sup>, Krisdianto<sup>1</sup>, Rizmi Yunita<sup>2</sup>**

<sup>1</sup> Department of Biology, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

<sup>2</sup> Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

\*corresponding author: heribudisantoso@ulm.ac.id

### **Abstract**

#### *Article history:*

Received 27 September 2023

Revised 15 November 2023

Accepted 3 January 2024

#### *Keywords:*

aquatic ecosystem  
environmental impact  
estuarine fish  
iron accumulation

This study investigated iron (Fe) accumulation in South Kalimantan's coastal swamp wetlands ecosystem, utilizing giant mudskipper fish as indicators of heavy metal pollution. By analyzing Fe levels in water, sediment, and fish organs over time, insights into its effects on the environment and human health were gained. Furthermore, through Atomic Absorption Spectrometry, Fe concentrations in Kuala Lupak's coastal wetland were measured, revealing significant correlations between Fe levels in water, sediment, and fish tissues. These results enhance our understanding and inform better management strategies. Anthropogenic and natural sources contribute to the accumulation of heavy metals, particularly Fe, with anthropogenic pollution being the most dominant. This study presented the escalating concentrations of Fe within the Kuala Lupak estuary and raised concerns regarding the ecological and human health implications. Continuous monitoring, source identification, public awareness, regulations, remediation, and long-term exploration are essential for addressing heavy metal pollution and its ecological impact. Therefore, valuable insights are provided for environmental management and conservation efforts.

**To cite this article:** Santoso, H.B., Krisdianto, and Yunita, R. 2024. Iron bioaccumulation and ecological implications in the coastal swamp wetlands ecosystem of South Kalimantan: Insights from giant mudskipper fish as bioindicators. *Journal of Degraded and Mining Lands Management* 11(3):5539-5550, doi:10.15243/jdmlm.2024.113.5539.

### **Introduction**

Heavy metals are a significant threat to the health of estuarine ecosystems, affecting the biodiversity of aquatic organisms at both individual and community levels (Sarah et al., 2019; Tabrez et al., 2021; Zaoui et al., 2023). This negative impact is attributed to the bioaccumulation of heavy metals in aquatic biota, resulting from the discharge of industrial and domestic waste into the estuarine environment. Yogeshwaran et al. (2020) reported that the bioaccumulation of the metals in estuarine aquatic biota is directly linked to water contamination. The presence of heavy metals in aquatic biota is due to their availability in water bodies

and sediment, as well as the food consumed by the fish (Moniruzzaman and Saha, 2020). These trace elements exist in sediments in various forms, including dissolved, bound to suspended particulate matter, or accumulated, enhancing their incorporation into biological processes (Soltani et al., 2021).

Iron, as the fourth most dominant metal on Earth, possesses the capacity to change valence and form complexes with oxygen. Furthermore, it plays a crucial role in respiration in nearly all aerobic organisms. However, unless suitably sequestered, Fe can catalyze the formation of radicals capable of damaging biological molecules, cells, tissues, and entire organisms (Tenenbein and Huang, 2022). Certain trace



metals such as Fe, Cu, Mn, and Zn are essential to biological systems but can become toxic at high concentrations. Metals, including Zn, Fe, Cu, and Mo, are integral components of metal-protein complexes in enzymes, contributing to enzymatic activities. Fe is a constituent of oxygen transport within living organisms, while it also plays a vital role in oxidative metabolism, serving as a cofactor for proteins and enzymes, DNA synthesis, and electron transport (Zafar and Khan, 2020).

Other heavy metals such as Cd, Cr, Pb, and Hg have adverse effects even at deficient concentrations (Akinsanya et al., 2020). Despite its physiological functions in fish, environmental pollution can render Fe toxic due to excessive accumulation (Garg and Gauns, 2023). The continuous and substantial discharge of trace metal pollutants into the sea poses a significant threat to estuarine and coastal ecosystems due to their chronic toxicity, non-biodegradability, and bioaccumulation in biota (Zaynab et al., 2022). The metals can be transferred and biomagnified through food chains, presenting significant risks to human health. Metal contamination in estuarine ecosystems can induce toxic effects in fish and humans, primarily by consuming contaminated food (Franco-Fuentes et al., 2023; Prasad et al., 2023). Therefore, evaluating the occurrence of Fe content in the estuarine ecosystem of South Kalimantan is essential.

Analyzing the levels of Fe in the water body, sediments, and its bioaccumulation in wild fish, particularly those that inhabit estuaries for a significant portion of their life, such as the giant mudskipper fish (*Periophthalmodon schlosseri*), is of great importance. This fish species holds potential as a bioindicator for monitoring heavy metal pollution in estuarine waters, aiming to mitigate the adverse impacts of Fe on biodiversity conservation and environmental health. Evaluating the presence of Fe in the ecosystem serves as a valuable environmental indicator to understand anthropogenic influences and associated risks to the estuary environment.

Iron is an abundant transition metal in Earth's crust (Jaishankar et al., 2014), and it can exist in aquatic environments in dissolved or suspended forms in water or sediment. Investigating Fe in water, sediment, and fish tissues aids in determining contamination status and loads. Therefore, quantifying the presence of this metal is pivotal in analyzing the health of aquatic ecosystems. Valuable information about the impacts of environmental changes and anthropogenic activities over time is obtained through long-term bioaccumulation of heavy metals. As a result, biomonitoring studies are essential in tracking trends in the concentrations of these metals, enabling scientists to identify changes in estuarine health (Muhammad and Ahmad, 2020). Contamination of aquatic ecosystems by trace metals has garnered global attention from environmental and biological experts, leading to numerous studies on their accumulation in estuarine and marine biota (La Colla et al., 2017).

There is a pressing need for comprehensive information on the evaluation of Fe presence, toxicity, and effects on giant mudskipper fish in the South Kalimantan estuary. Therefore, effective monitoring and evaluation of the availability of this metal in the estuarine environment (water, sediment, biota) are crucial. The giant mudskipper fish has potential as a bioindicator species for heavy metal pollution biomonitoring programs in the estuarine ecosystem of South Kalimantan because it meets the requirements, including natural abundance (Hidayaturrahmah et al., 2019), easy to obtain in large quantities, wide tolerance to polluted environmental conditions, benthic living habits, high trophic level in the aquatic food chain, and capability to accumulate heavy metals (Santoso et al., 2020). Fish, renowned for their capacity to accumulate heavy metals, are well-suited to serve as bioindicator species in pollution biomonitoring, enabling the assessment of estuarine ecological health (Alizada et al., 2020). The giant mudskipper fish, in particular, possesses unique biological characteristics, namely having amphibian-like characters, which distinguish them from other species. Additionally, they are often called "fish walking on land" or "fishes out of water" (Kumaraguru et al., 2020).

Kuala Lupak in the Barito River estuary is a coastal swamp wetland area characterized by extensive transportation activities, loading and unloading of coal, and coal mining waste disposal. The continuous input of waste from the coal mining process and coal-related activities into the water body has the potential to cause estuary pollution when prolonged and accumulated. These waste materials contain heavy metals such as Mn, Fe, Hg, Cd, Cr, Pb, and Cu (Kasmiarti et al., 2021). Furthermore, Kuala Lupak has witnessed the conversion of mangrove land to ponds, a place for ship transportation traffic and settlements. Several industries along the riverbank, such as wood/plywood processing factories, rubber, fisheries, and oil palm plantations, pollute estuary waters through waste discharge (Sofarini et al., 2012). It is hypothesized that the unchecked release of these wastes into water bodies poses a significant risk to both aquatic ecosystems and human health. Therefore, urgent biomonitoring efforts are warranted for early detection of the negative impacts of pollution. In light of these considerations, it is important to investigate the presence and occurrence of Fe in the Kuala Lupak estuary ecosystem during the period from 2020 to 2022.

The objectives of this study were to evaluate the presence of Fe levels in water bodies, sediments, and giant mudskipper fish, assess the bioaccumulation of Fe in various tissues of giant mudskipper fish (liver, kidney, muscle, skin, and gills), evaluate the relationship or correlation between Fe content in water bodies and sediments as well as the concentrations in the liver, kidney, skin, muscle, and gill tissues of giant mudskipper fish, and validate the potential of giant mudskipper fish as a bioindicator of Fe contamination

in the Kuala Lupak estuary. The trends and intricacies of iron (Fe) contamination are investigated into by this research, especially within estuarine environments. The accessibility of this metal was primarily evaluated, and its annual fluctuations were also monitored. The knowledge acquired from this study provides significant insights, especially for comprehending the possible consequences of future environmental changes. The results of this multi-year trace metal monitoring are of paramount importance in understanding the bioaccumulation potential of trace metals and constitute a crucial step in the management of estuarine and coastal ecosystems. These outcomes serve as a foundational framework for stakeholders to assess and address anthropogenic effects in the future.

## Materials and Methods

### *Study area*

The study was conducted in the coastal wetland of Kuala Lupak, situated in the Barito Kuala Regency. This region is approximately 8 km west of the mouth of the Barito River, the largest and longest river in South Kalimantan Province, Indonesia. The coastal wetland area of Kuala Lupak directly merges with the Java Sea coastline to the south. A comprehensive review of each study station/site is presented in Table 1, while the precise sampling locations are visually shown in Figure 1.

### *Sample collections and analysis*

The process of collecting water, sediment, and giant mudskipper fish samples was conducted during August in 2020, 2021, and 2022. The sampling was carried out using the purposive sampling method, where sample selection was based on specific criteria, such as the potential sources of pollution. To accurately identify these sources, various activities that contribute to contamination were taken into consideration. Figure 1 shows the collection of fish, water, and sediment samples at the study location.

### *Water*

Using an Aqua trap water sampler, surface water samples were collected from two stations scattered throughout the study region. In preparation for further analysis, approximately 2 mL of concentrated HNO<sub>3</sub> was added to each 1 L sample after filtration. The study adopted the liquid-liquid extraction technique (Jayaprakash et al., 2015) to determine the concentration level of dissolved Fe. A 100 mL unfiltered sample was placed in a separating funnel and mixed with 2 mL of 2% ammonium pyrrolidine dithiocarbamate (APDC). Subsequently, 10 mL of iso-butyl methyl ketone (IBMK) was used for extraction after severe agitation. The extracted aqueous phase was subjected to further extraction using concentrated HNO<sub>3</sub> and high-purity water. Any remaining traces of organic solvents in the solutes were evaporated on a

low-temperature hot plate, and the final solution was analyzed using Atomic Absorption Spectrometry (AAS Thermo Scientific ICE 3500 series Germany). This instrument was used to measure the concentration level of Fe in water, sediments, and fish samples. For water, the concentration was expressed in mg L<sup>-1</sup>. Finally, samples for metal determination were conditioned following Jayaprakash et al. (2015).

### *Sediment*

Sediment samples were procured from two estuary stations using a Van Veen grab sampler. Furthermore, they were dried at 40°C and powdered in an agate mortar for the digestion procedures. Approximately 1 g of the dried sediment sample was digested with an acid mixture containing HClO<sub>4</sub>+HF+HCl, and the residue was dissolved with concentrated HCl, then diluted to 25 mL. The solution was analyzed using flame AAS to estimate the Fe concentrations which were expressed in mg kg<sup>-1</sup> for sediment.

### *Fish sampling and analysis*

Fish samples were obtained from the estuarine area by local fishermen. A total of 15 giant mudskipper fish with identical dimensions, ranging between 19 and 20 cm in length and weighing from 160 to 170 g, were collected from each sampling station to ensure representativeness. The obtained samples were transported to the laboratory in ice boxes to maintain their preservation and appropriate conditions for further analysis. Furthermore, the dissection of the fish was conducted using surgical stainless-steel instruments, facilitating the extraction of organs, including the liver, kidneys, gills, muscles, and skin. A digestion tube, pre-loaded with 5 mL of HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>, was utilized for each organ sample to initiate the reaction. The samples were subjected to digestion using a hot block digestion apparatus, maintained at 60°C for 30 minutes. Following the cooling of the samples, 10 mL of HNO<sub>3</sub> was added, and the mixture was further heated at temperatures ranging from 120 to 150 °C until a discernibly dark coloration of the solution was achieved. For facilitating the subsequent filtration process, 1 mL of H<sub>2</sub>O<sub>2</sub> was introduced to yield a clear solution. Those filtered samples were then analyzed using Atomic Absorption Spectrometry (AAS) to quantify the concentration of Fe in the fish tissues, expressed mg kg<sup>-1</sup>.

### *Statistical analysis*

Data processing and analysis were executed employing GraphPad Prism software, specifically the 5.9.0 series from Dotmatics. The statistical evaluations comprised the determination of mean, standard deviations (SD), minimum and maximum values, the creation of graphical representations showing the interrelationship among variables, and the calculation of Pearson correlations. The analyses were conducted using GraphPad Prism 7.02, developed by the company of GraphPad Software Inc., San Diego.

Table 1. Study station description.

Location	Stations/sites	Description
Kuala Lupak	Estuarine	The estuarine water of Kuala Lupak is a coastal wetland area with the status of Margasatwa Wildlife Sanctuary, specifically a mangrove swamp ecosystem. The mouth of the Kuala Lupak River is surrounded by human settlements, and it flows into the Java Sea. The samples were collected from the coordinates 3°27'28.223"S 114°22'04.780" E in the estuarine waters.
	Coastal	The coastal waters of Kuala Lupak are located approximately 8 km west of the mouth of the Barito River and border the Java Sea to the south, with a coastline of 30 km. Furthermore, they are characterized by mangrove swamps. At the coordinates 3°28'02.383"S 114°21'31.282" E samples were collected in the coastal waters where the Kuala Lupak River flows into the sea.

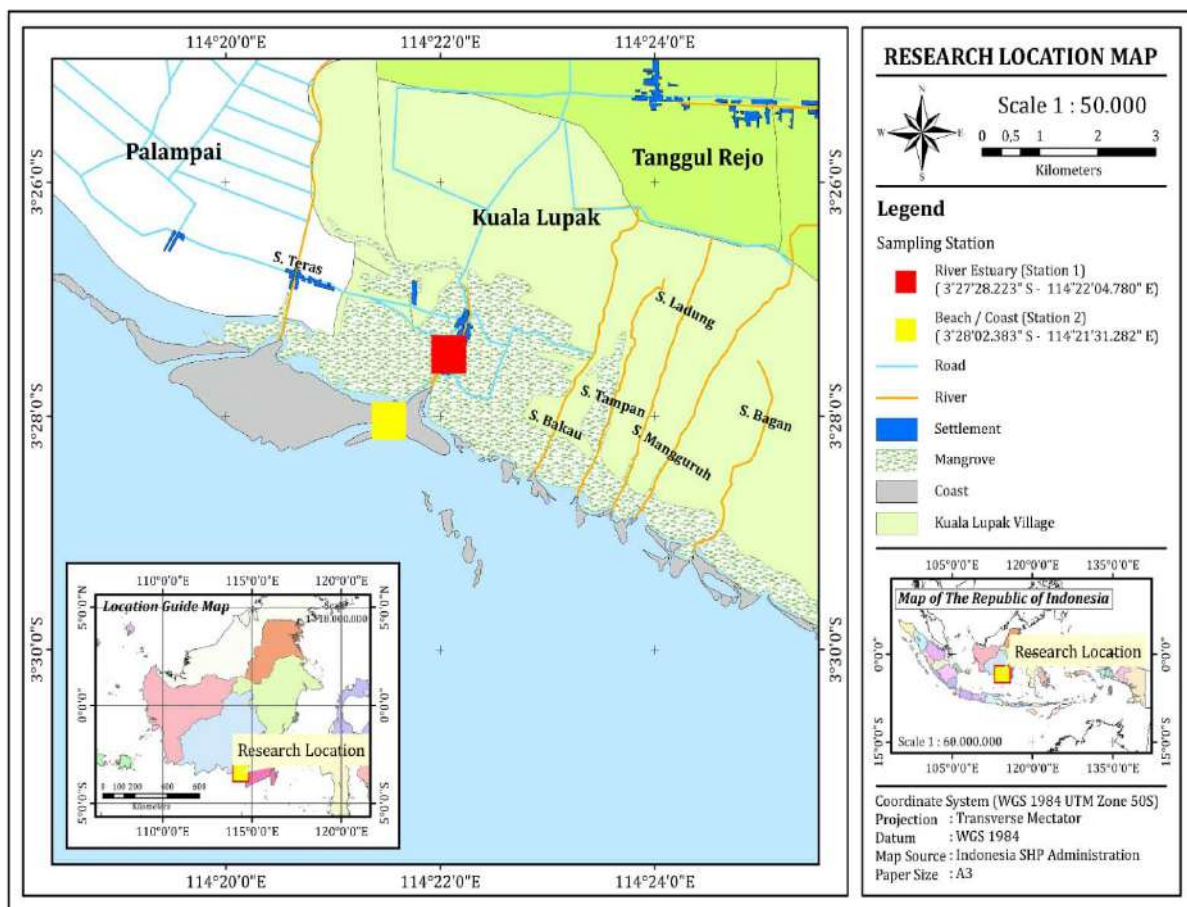


Figure 1. Study area map and sampling locations in the estuary Kuala Lupak South Kalimantan.

## Results

### Occurrence of Fe concentrations in water and sediment

The average concentrations of Fe in the water column from 2020 to 2022 ranged from 2.55 to 11.61 mg L<sup>-1</sup>. This showed a significant trend of increase over each successive year. The highest average concentration of Fe was recorded in 2022 to be 11.61 mg L<sup>-1</sup>, as presented in Table 2. The Maximum Permissible Limits (MPLs) for heavy metals in estuary water were

not regulated by International Guideline values. The Fe in Kuala Lupak estuary water exceeded the threshold value set by Government Regulation of Indonesia number 22 of 2021 concerning the Implementation of Environmental Protection and Management. Furthermore, the seawater quality standard for marine biota was set at 0.30 mg L<sup>-1</sup>. In parallel, the average concentrations of Fe within the sediment, serving as the habitat of the giant mudskipper fish, was in the range of 1,245.92 to 48,911.78 mg kg<sup>-1</sup>.

Analogous to the pattern observed in the water column, the concentrations of Fe in the sediment also displayed a significant upward trend over each year, as shown in Figure 2. The highest average level of Fe (48,911.78 mg kg<sup>-1</sup>) in the sediment was observed in 2022 (Table 2). The concentration of this metal in Kuala Lupak estuary sediments exceeded the maximum limit of 20,000 mg kg<sup>-1</sup> set by the

International Guideline value, CBSQG 2003: Consensus-Based Sediment Quality Guidelines (Wisconsin Department of Natural Resources). The mean plot of Fe level in water and sediment from 2020 to 2022 demonstrated a significant interrelationship between variables, with a confidence level of 95%, as indicated by the statistical analysis ( $p < 0.05$ ,  $R^2 = 0.649$ ).

Table 2. Mean values of Fe concentration in water, sediment, and some tissues of giant mudskipper fish from the Kuala Lupak estuary between the period of 2020 to 2022.

Sampling Period	Water (mg L <sup>-1</sup> )	Sediment (mg kg <sup>-1</sup> )	Liver (mg kg <sup>-1</sup> )	Kidney (mg kg <sup>-1</sup> )	Muscle (mg kg <sup>-1</sup> )	Skin (mg kg <sup>-1</sup> )	Gill (mg kg <sup>-1</sup> )
2020	2.55	1,245.92	5.52	7.98	40.44	4.42	1.23
2021	5.54	47,348.42	8.43	11.99	51.56	6.70	3.32
2022	11.61	48,911.78	12.84	15.44	69.74	8.80	6.22

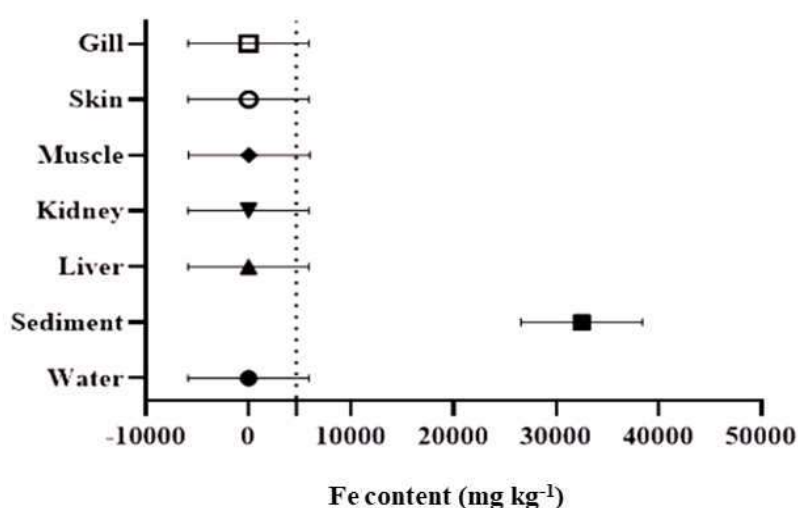


Figure 2. The mean plot of Fe concentration in water, sediment, and some tissue of giant mudskipper fish from 2020 to 2022.

#### Temporal variations in Fe concentrations in giant mudskipper fish

The mean Fe concentrations in the liver, kidney, muscle, skin, and gills of giant mudskipper for the years 2020-2022 ranged from 5.52 to 12.84 mg kg<sup>-1</sup>, 7.98 to 15.44 mg kg<sup>-1</sup>, 40.44 to 69.74 mg kg<sup>-1</sup>, 4.42 to 8.80 mg kg<sup>-1</sup>, and 1.23 to 6.22 mg kg<sup>-1</sup>, as presented in Table 2. The average level of this metal in several fish tissues exhibited a significant upward trend annually, with the highest value being recorded in 2022, as specified in Figures 2 and 3. Based on the results in Table 2, muscle had the highest accumulation among the tested fish tissues, followed by kidney, liver, skin, and gills. The Maximum Permissible Limit (MPL) for Fe in fish meat was not regulated by international guideline values but was regulated by national guideline values. The Fe in giant mudskipper fish organs exceeds the threshold value of 1 mg kg<sup>-1</sup>, as regulated by the Indonesian National Standard (SNI 7387:2009) regarding the maximum limit of

heavy metal contamination. When comparing the content of this metal among the tested samples in the Kuala Lupak estuarine ecosystem, the average concentration in sediment was the highest.

#### The relationship between Fe contents in water, sediment, and giant mudskipper fish tissues

The interrelationships among heavy metal concentrations in water, sediment, and the tissues of giant mudskipper fish from 2020 to 2022 were evaluated and presented in Figure 4. The graphical representation shows a substantial positive correlation between the concentration of Fe in the water and the levels identified in the liver, muscle, and gills, with a correlation coefficient of 1. This implied that elevated levels of this metal in the water correspond with its heightened concentrations in the liver, muscles, and gills. Furthermore, a robust positive correlation was observed between waterborne Fe concentrations and the levels of the metal in the kidney and skin, with correlation coefficients of 0.97 and 0.98, respectively.



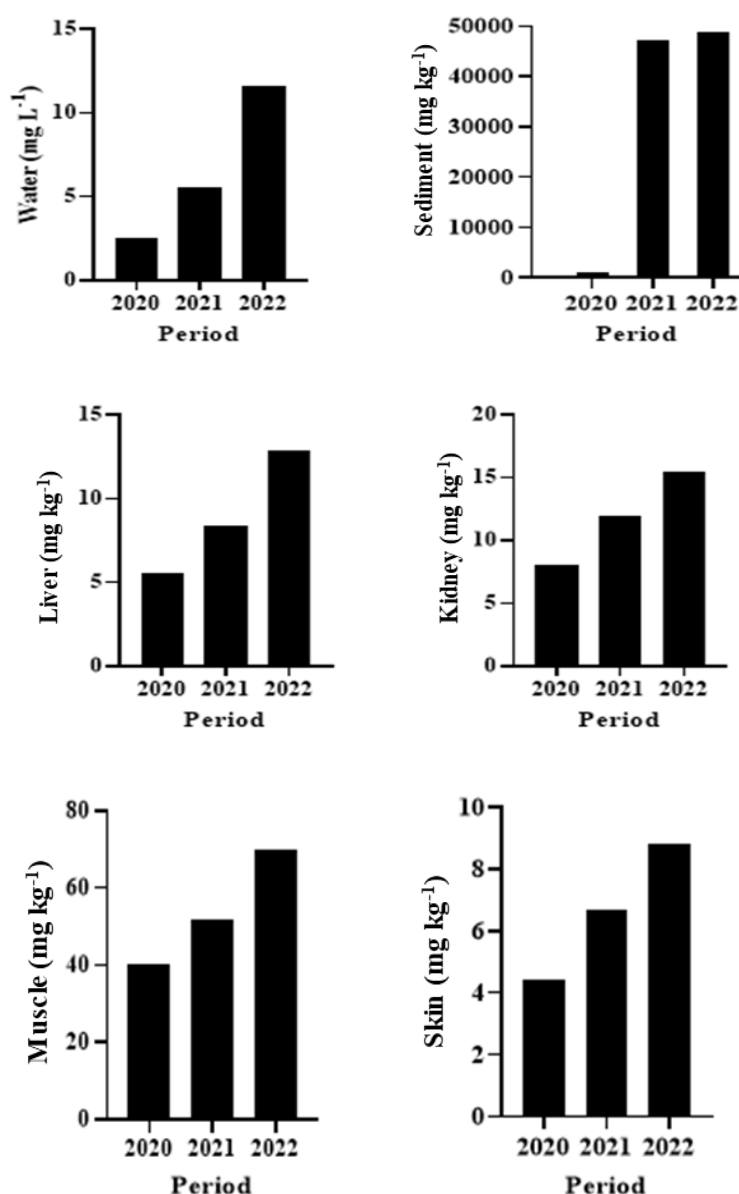


Figure 3. Fe content trend in some tissue of giant mudskipper fish period 2020-2022.

The correlation coefficient between Fe levels in the water and sediment was 0.77. A highly significant positive correlation among the concentrations present in the water, sediment, and tissues of the giant mudskipper fish, as indicated by the outcomes of this investigation. Based on these observations, the accumulation in water and sediment has augmented Fe levels within the tissues of the fish. This correlation was in line with the consistently high concentrations in water and sediment. Figure 3 further shows a robust positive correlation between sediment-bound Fe concentrations and those in the kidney, skin, gills, liver, and muscle tissues, with correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81, respectively. These results underscored the effect of sediment-bound Fe accumulation on the corresponding values within the

tissues of the fish. This correlation was in line with the consistent elevation of sediment-bound Fe concentrations over 3 years, exhibiting an annual increment, as presented in Figure 1. The study also underscored a significant positive correlation across various organs of the giant mudskipper fish. This indicated a uniform distribution across diverse organs after Fe uptake into the body of the fish. In Figure 3, a close positive correlation is evident between the Fe concentration in the skin, serving as an entry point for Fe uptake. In the kidney, gills, muscle, and liver, the level of this metal exhibited correlation coefficients of 1.00, 0.99, 0.99, and 0.99, respectively. Similarly, a positive, strong correlation existed among the concentration in the gills, another site of Fe entry, and the Fe levels in the liver, muscle, skin, and kidney.

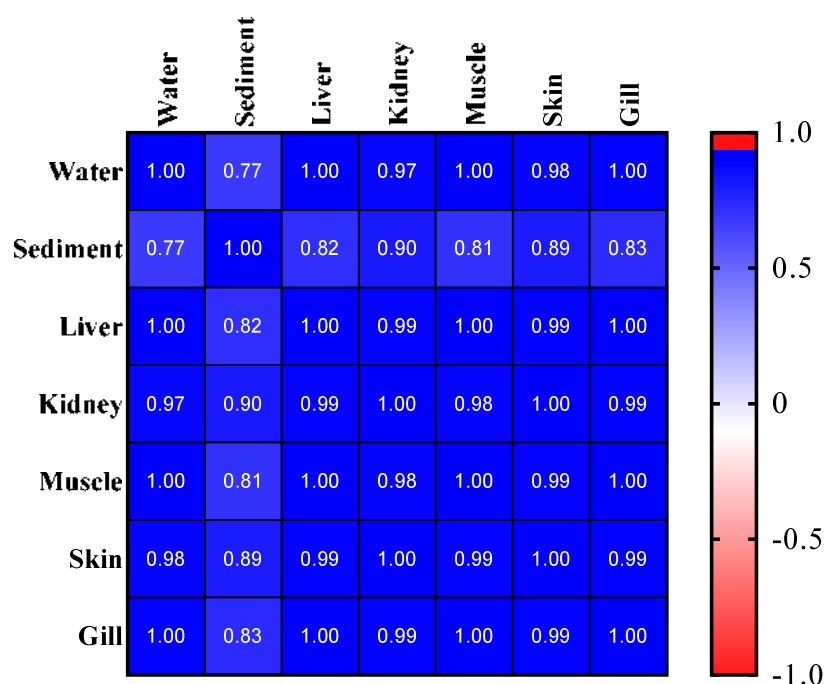


Figure 4. Pearson correlation of Fe content in some tissue of mudskipper fish period 2020-2022.

These featured coefficients of 1.00, 1.00, 0.99, and 0.99, respectively. The results showed that elevated Fe concentrations in the skin and gills precipitate were directly proportional to the liver, kidney, and muscle tissues.

## Discussion

### *Fe contamination in estuarine waters: Sources and implications*

The heightened prevalence of Fe within the Kuala Lupak estuary in 2022 was attributed to the effects of extreme rainfall and substantial flooding in the region of Kalimantan Selatan from December 2020 to February 2021. This event led to the dissolution of heavy metal deposits from upstream areas, resulting in their transport into the estuarine environment and eventual deposition within the estuary. This result was in line with the study conducted by (Čmelík et al., 2019), which identified a substantial increase in heavy metal concentrations in both water and sediment along the course of the Bilina River in the Czech Republic, directly attributed to extreme rainfall events. However, it is important to note that floods had a comparatively shorter-lived influence on the quality of water and sediment compared to the cumulative impacts of anthropogenic activities.

A study by Supriyantini and Endrawati (2015) also reported elevated concentrations within water bodies, sediment, and tissues of the green mussel (*Perna viridis*) in the coastal rivers of Tanjung Emas Semarang. In this investigation, the elevated Fe content in the estuary stemmed from various sources,

including soil and anthropogenic activities occurring on land. These activities comprise household waste containing Fe, water reservoirs sourced from Fe, industrial waste deposits, and corrosion from Fe-containing pipes transported into the estuary by river flow. According to Haeruddin et al. (2020), the highest heavy metal concentrations within sediment were proximal to contamination sources.

Fe contamination within estuarine waters emanates from natural processes, such as geological weathering, rock decomposition, and anthropogenic activities. These anthropogenic sources include Fe release from ore materials and volcanic eruptions that discharge Fe-rich materials into water bodies through runoff, erosion, and flooding. Human activities such as coal transportation and mining operations contribute significantly to this pollution (Dalu et al., 2020). Anthropogenic Fe sources also include land-based activities, such as the discharge of household waste containing Fe, Fe-laden water reservoirs, industrial waste deposits, and the corrosion of Fe-containing pipes, releasing  $\text{Fe}^{2+}$  and sulfuric acid due to the oxidation of Fe pyrite ( $\text{FeS}_2$ ) present in coal layers (Jaishankar et al., 2014).

The escalating temporal increase in Fe concentrations in both water bodies and sediments, as observed in this study, was supported by an investigation conducted by Weber et al. (2020) concerning the contamination in Brazil's Piranga and Doce rivers. Both rivers were contaminated with Fe due to the collapse of the Samarco mine tailings dam in Brazil in 2015. Environmental catastrophes such as dam failures that release tailings profoundly impacted the landscape and aquatic environments, progressively

deteriorating the water quality of the Doce and Piranga rivers over time. Another source of this contamination stems from mineral weathering/erosion and elevated Fe content in soil. The contamination of these rivers has led to the bioaccumulation of Fe in *Hoplias intermedius* and *Hypostomus affini* fish. This caused histopathological lesions in the liver, including vacuolization, hepatocyte hypertrophy, and elevated necrotic areas compared to fish from non-tailings-contaminated rivers. This study strengthens the notion that fish living in heavy metal-contaminated waters experience declining aquatic health quality, leading to heavy metal bioaccumulation and histopathological lesions.

#### ***Accumulation of iron in sediments: Multifaceted sources and ecological implications***

In this study, sediments in the Kuala Lupak estuary accumulate higher levels of heavy metals than the water column. This has rendered the sediments a significant potential source and a primary repository for all pollutants (Weber et al., 2013). These particles can store diverse heavy metals in substantial and consistent quantities, hence, they become valuable indicators for assessing water pollution status (Haeruddin et al., 2020). Liline et al. (2022) reported that sediments in the Rutong, Waai, and Poka mangrove areas in Maluku function as a medium for transferring heavy metals such as chromium (Cr) and Fe to mudskipper fish. The heavy metals in estuarine sediments result from sedimentation processes that retain significant amounts of adsorbed metal particles, leading to their deposition at the bottom. Wastes containing these metallic elements were deposited in sediments upon entering estuaries. This was facilitated by reduced environmental conditions, low dissolved oxygen, and elevated pH, causing metal concentration or enrichment (Carvalho Neta et al., 2019). The result was in line with the study by Dalu et al. (2020), stating that heavy metal content in sediment was significantly higher than in the water column, as suspended particles were absorbing it. High Fe content in sediment can lead to contamination of water bodies, which can have negative impacts on the health of aquatic biota and the overall ecological balance of aquatic ecosystems.

Sediments serve as indicators of land-based pollution by acting as "sinks" for pollutants. The rise in heavy metal levels in sediments suggests that heavy metals in the water column are deposited along with suspended solids, posing a potential threat to estuarine and coastal ecosystems. These sediments, serving as sinks for various human activities, may also act as sources of heavy metals, underscoring the ongoing necessity for monitoring to evaluate their role as pollution sources. Heavy metals are prone to accumulating in sediments, resulting in consistently higher concentrations than those in the water column. Suspended sediment particles quickly transport these metals into water through the movement of water masses (Weber et al., 2013). The high volume of

merchant and coal transport vessels contributes to water mass movement in the Barito River estuary.

The accumulation of heavy metal in sediments was attributed to a variety of sources, including leaded gasoline emissions, chemical manufacturing industries, motorized transportation, underground pipe corrosion, coal-based thermal power plants, and urban waste. The results of this study were in line with the report of Jayaprakash et al. (2015), indicating that heavy metal concentrations in water were consistently lower than in sediment within contaminated water bodies. This is because sediment particles tend to absorb metals from the water. In sediment-rich environments, organisms such as the giant mudskipper fish accumulated heavy metals from their food and water through gills, mouth, and skin mucus, further underscoring the potential for metal bioaccumulation. The intricate interplay between suspended sediment particles and their propensity to absorb heavy metals from the aqueous phase was a crucial mechanism underlying the contrasting concentrations of these metals in water and sediment. This phenomenon accentuated the role of sediments as repositories for heavy metals, specifically in aquatic environments subject to anthropogenic influences and diverse pollutant sources.

The congruence between the report of Jayaprakash et al. (2015) and this study underscores the broader ecological implications of metal accumulation in sediments. The distinct levels of heavy metals within water and sediment reflect the differential interactions between these metals and particulate matter suspended in water. Consequently, sedimentary environments act as crucial hubs for storing heavy metals, providing a pertinent context for comprehending the potential ecological ramifications of anthropogenic pollution in aquatic ecosystems. High Fe content in sediment can alter the physical and chemical properties, such as color, texture, and pH. This could also affect the habitat of benthic organisms and the overall ecological balance of aquatic ecosystems (Viana et al., 2021). These results elaborate on the necessity for robust monitoring practices to assess the extent and impacts of contamination within sediment systems.

Direct observations during this study, as well as interviews with residents, led to the identification of sources of coastal swamp water pollution in Kuala Lupak. These sources include residential waste and the waste products of factories situated along the banks of the Barito River estuary. Factories, such as plywood processing plants perform wood washing activities as well as generate solid waste such as wood fragments, bark, sawdust, and sanding residues. Other sources of contamination were believed to arise from gold mining activities in the Barito River basin, the use of paint in shipyards, agricultural activities including the application of pesticides and fungicides, converting mangrove areas to fish ponds, and the application of explosives for fishing. Fuel companies discharging



waste were also suspected sources of pollution. These companies distributed diesel fuel through water transport routes that traverse the Barito River estuary. Additionally, the high density of commercial and coal transport ships, alongside coal spillage and debris entering the river, contributed to the degraded water quality of the river. According to the National Transportation Safety Committee, approximately 7.101 units of coal barges navigate the Barito River (Mitra et al., 2023).

In addition to anthropogenic activities, Fe is sourced from natural processes comprising regular biogeochemical cycles, volcanic activities, soil erosion, continental runoff, shelf inputs, as well as atmospheric deposition from volcanic events and wildfires (Soltani et al., 2021). Furthermore, as the most dominant transition metal in the Earth's crust, it undergoes multiple natural cycles (Jaishankar et al., 2014). Anthropogenic pollution of trace metals, including Fe, in estuarine waters has deteriorated water quality and negatively impacted fish health (Inayat et al., 2023).

#### ***Bioaccumulation potential and ecological impact of iron in giant mudskipper fish***

Many studies have documented that indicator species accumulate contaminants in their tissues at higher levels than the surrounding habitat, such as water and sediment. This characteristic makes them valuable as potential bioindicators within ecosystem health biomonitoring programs. According to Table 2, the concentrations of Fe in the muscle, kidney, liver, and skin of giant mudskipper fish exceeded those in the surrounding aquatic environment. The elevated concentrations within fish tissues were attributed to the increased levels present in both sediment and water. These results corroborate with those of Gawad (2018), who posited that high concentrations of heavy metals in sediment and water allow aquatic organisms to accumulate metals biologically and transport them through the food chain. The ability of giant mudskipper fish to accumulate heavy metals in tissues stems from their position at the pinnacle of the food chain. These organisms reside benthically in mud within the intertidal coastal zone and mangrove forest floor; hence, they are susceptible to direct exposure to pollutants within the estuarine environment. According to Ferreira et al. (2019), estuaries represent habitats with continuous and direct exposure to heavy metal pollutants.

Estuaries have gained wide recognition as net sinks for trace metals, including Fe, which precipitate rapidly within the environment (Crerar et al., 1982). While rivers transport substantial amounts of dissolved and particulate Fe into the sea, a significant portion is retained and accumulated within sediments (Daneshvar et al., 2015; Zhu et al., 2018). This phenomenon is attributed to the exceptional capacity of the sediments as a final trap and adsorptive reservoir for heavy metals in aquatic environments. Fe colloids

are immobilized within surface estuarine sediments and can become mobilized by organic matter and reduction agents. When these values exceed a certain threshold, the mobility and deposition of Fe lead to pollution, thereby posing toxicity risks to fish. This risk arose primarily because of the persistent, non-biodegradable, accumulative, and diverse sources of trace metals (Shafie et al., 2015). Continued and significant discharges of trace metal pollutants into the sea can damage estuarine and coastal ecosystems, given their chronic toxicity, non-biodegradability, and bioaccumulation in biota, including fish. Trace metals can transfer and biomagnify through food chains, posing severe threats to ecosystem health and human well-being. Furthermore, the contamination of metal within estuarine ecosystems can engender toxic effects in fish and humans, primarily by consuming contaminated food sources (Prasad et al., 2023).

The investigation into the toxic effects of Fe on fish inhabiting estuarine ecosystems proved a compelling inquiry for biologists in South Kalimantan, attributable to the robust positive correlation between Fe levels in the water, sediment, and fish organs. The heightened concentrations of this metal were suspected to be a consequence of its extensive usage (Bat and Arici, 2018). Furthermore, Fe is a prevalent component of industrial and mining waste, frequently discharged into aquatic environments, hence, exhibiting a pronounced correlation with aquatic biota, including fish. Its elevated contents in water and sediment facilitated a more pronounced accumulation within fish organs. Fe in its ferrous form ( $\text{Fe}^{2+}$ ) had heightened toxicity towards fish compared to when it was ferric ( $\text{Fe}^{3+}$ ).  $\text{Fe}^{2+}$  exhibited toxic effects by binding to gill surfaces and undergoing oxidation to form insoluble  $\text{Fe}^{3+}$ , thereby inciting cellular damage, ultimately culminating in respiratory dysfunction (Singh et al., 2019).

The bioconcentration of Fe within fish tissues attained its zenith within the liver and gonads, subsequently diminishing in the brain, muscles, and heart (Authman, 2015). The study conducted by Omar et al. (2014) substantiated the liver as a prime target organ for Fe-induced toxicity in fish. Furthermore, the toxicity of this metal within gills disrupts respiration due to the physical obstruction of the surfaces (Dalzell et al., 1999). The alkaline nature of the gill surfaces promoted the facile oxidation of soluble Fe, forming insoluble Fe compounds that cloak gill lamellae, impeding respiratory function.

Excessive Fe absorption led to poisoning in *Labeo rohita*, catalyzing the generation of ROS via Fenton reactions, which damaged biomolecules, cells, and tissues through oxidative stress mechanisms (Singh et al., 2019). This metal also potentiates the toxicity of other chemical agents, such as paraquat or 2,3,7,8-tetrachlorodibenzo-p-dioxin (Sevcikova et al., 2011). In a study by Kaloyianni et al. (2020), the toxicity of magnetite nanoparticles ( $\text{Fe}_3\text{O}_4$ ) was reported in *Danio rerio* and *Carassius gibelio* fish,

evoking oxidative, proteolytic, genotoxic, and apoptotic effects, accompanied by elevated malondialdehyde and carbonyl protein levels.

## Conclusion

In conclusion, the study elucidated substantial positive associations in Fe levels across water, sediment, and tissues of the giant mudskipper fish (*Periophthalmodon schlosseri*) during the 2020-2022 period. These associations underscored the dynamic interplay between environmental Fe buildup and its assimilation within fish tissues. The graphical representation accentuated a strong positive correlation (correlation coefficient 1) between waterborne concentrations and those in the liver, muscle, and gills. This indicated that higher Fe levels in water correspond to elevated levels in these fish tissues. Similarly, noteworthy positive correlations were observed between Fe levels in water as well as kidney and skin tissues, with correlation coefficients of 0.97 and 0.98, respectively.

Sediment-bound Fe also correlated positively with kidney, skin, gills, liver, and muscle tissues, with sequential correlation coefficients of 0.90, 0.89, 0.83, 0.82, and 0.81. The strong positive correlation within the various fish organs implied uniform Fe dispersion post-uptake. The skin and gills, primary entry points for this metal, exhibited robust positive correlations with kidney, gills, muscle, liver, and skin tissues. This inquiry into the detrimental impacts of Fe on estuarine fish in South Kalimantan assumed significance due to the strong positive correlations between the concentrations of this metal in water, sediment, and fish organs. The widespread dispersion of Fe from industrial and mining activities underscored its prevalence in aquatic ecosystems, often resulting from industrial and mining by-products infiltrating water bodies.

The intricate associations between Fe concentrations in water, sediment, and fish tissues illuminated the intricate relationship between environmental Fe accumulation and biological assimilation in estuarine ecosystems. The study underscored the importance of comprehending the impact of heavy metal contaminants, such as Fe, on aquatic organisms and habitats, with potential implications for ecosystem health and human well-being. Therefore, future studies should comprise a multifaceted approach, including scientific investigations, policy formulation, public awareness, and collaborative endeavors among stakeholders to safeguard the well-being of aquatic ecosystems and human populations.

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