Determination of heavy metal and biochemical status of fish facilitates biomonitoring of coastal swamp pollution of South Kalimantan, Indonesia

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Abstract. The increasing ecological pressures on aquatic environments require complex biomonitoring techniques. The objective of this study was to evaluate heavy metal accumulation and oxidative stress biomarkers in fish as indicators of ecological stress in the coastal swamps of Kuala Lupak and Kuala Tambangan. Atomic absorption spectrometry was employed to quantify heavy metal concentrations (Cr, Hg, Pb, Cd, Fe, Zn, Cu). Biomarkers of oxidative stress, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation (LPO) were measured in muscle, liver, and skin tissues of various fish species. The results revealed significant differences in heavy metal concentrations in water and sediment between the two locations and varying levels of heavy metals in different fish species and tissues. These differences were reflected in the activities of antioxidant enzymes (CAT, SOD, GPx) and the levels of lipid peroxidation across species and tissues, indicating potential oxidative stress in fish due to heavy metal contamination. The study highlights the potential impact of monitoring heavy metal levels in environmental matrices and biota, integrated with biomarker analysis, for assessing ecological stress and understanding the biological responses to pollution in coastal swamp ecosystems.

Key Words: oxidative stress biomarkers, coastal swamp conservation, fish bioaccumulation.

Introduction. The coastal swamp ecosystem of South Kalimantan plays a crucial role as a buffer for biodiversity, including fisheries resources, in the more expansive coastal landscape. However, this ecological transition area is threatened by increasing anthropogenic activities from surrounding settlements, agriculture, and industry, leading to pollution of the Java Sea (Sofarini et al 2012; Santoso et al 2021). The importance of a comprehensive assessment of the pollution status of this vital but vulnerable ecosystem is urgent, especially considering the significant and persistent threat of heavy metal contamination that can accumulate in sediments and marine biota, adversely affecting aquatic organisms and human health (Marques et 2019; Roveta et al 2021; Albuquerque et al 2023).

Recent environmental monitoring emphasizes the importance of using fish as bioindicators and applying an integrated multi-marker approach to assess the impact of aquatic pollution (Kumar et al 2021; Hamada et al 2024; Prabakaran et al 2024). Global studies have shown that integrating the analysis of heavy metal accumulation in fish tissues with measurements of biochemical responses, especially those related to oxidative stress, provides a more comprehensive understanding of environmental contamination (Tabrez et al 2021; Aljaryan et al 2024; Ukasha et al 2024). Heavy metal exposure, for example, has been shown to induce oxidative stress in fish, characterized by changes in antioxidant enzyme activities and increased lipid peroxidation (Ju et al 2024; de Macedo et al 2024; Sousa & Sun 2024). However, heavy metal accumulation and biochemical responses vary across fish species, influenced by physiology, diet, and habitat differences (Mnkandla et al 2019; Nayak et al 2021; Agri et al 2022). Therefore, a multi-marker strategy provides a more sensitive and ecologically relevant assessment of pollution impacts than water or sediment chemistry alone (Abdallah et al 2024).

Preliminary research carried out by Santoso et al (2024) detected significant levels of heavy metal pollution, especially Fe, and worrying levels of Pb and Cr in waters and commercial fish in the coastal swamp ecosystem of South Kalimantan, with minimal regular monitoring. These findings provide an important reference for the conservation of previously undocumented habitats. Therefore, this second phase of research, part of a two-year project, analyzes oxidative stress biomarkers in fish tissues in complement to the initial findings. An integrated approach combining annual heavy metal levels and biological responses provides a holistic perspective on the impacts of pollution. This study is a continuation of the previously published article entitled "Assessing the health of South Kalimantan coastal swamp wetlands using measurements of heavy metals in commercial fish species" (Santoso et al 2024). The rationale for this continuation lies in the need to understand the biological effects of the observed contamination by analyzing oxidative stress biomarkers in fish, which serve as early indicators of environmental stress and sublethal toxicity. The results of this study will provide a strong scientific basis for the management and conservation of these ecosystems.

This study thoroughly examines the crucial problem of pollution characterization in the coastal swamps of Kuala Lupak and Kuala Tambangan, South Kalimantan. Despite its high ecological significance, a comprehensive understanding of heavy metal pollution and its impacts on fish biota in these locations is still limited. This gap hinders effective environmental management and conservation. This study assesses heavy metal contamination and associated biochemical responses in fish as indicators of environmental stress in the coastal swamp ecosystems of Kuala Lupak and Kuala Tambangan, South Kalimantan. Using a problem-oriented approach, concentrations of heavy metals (Hg, Pb, Cd, Fe, Zn, Cu, Cr) were measured in water, sediment, and tissues (liver, muscle, skin) of three economically and ecologically significant fish species—*Arius sagor*, *Mugil cephalus*, and *Plotosus lineatus*. In addition, activities of antioxidant enzymes —catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)— and lipid peroxidation (LPO) levels were evaluated to determine oxidative stress as a biomarker of exposure to metal pollution. Based on the premise that biochemical changes can detect pollutant exposure earlier, this approach can potentially influence and shape future environmental policies (Cacciatore et al 2022; Nagarani et al 2023; Mustafa et al 2024).

Thus, this study aimed to provide the first baseline assessment of heavy metals and oxidative stress biomarkers (CAT, SOD, GPx, LPO) in key fish species from the coastal swamps of Kuala Lupak and Kuala Tambangan, South Kalimantan, to support biomonitoring and ecosystem management.

Material and Method

Description of the study sites. This study, carried out in July 2024, compared two locations in the coastal swamps of South Kalimantan to analyze the ecosystem pollution gradient. The reference location was Kuala Lupak, a mangrove conservation area with minimal human activity. In contrast, Kuala Tambangan, a fishing settlement with intensive maritime activities near the Java Sea estuary, served as the comparison location. The differences in geographical conditions of the two locations – Kuala Lupak (3°27′28.223″ S 114°22′04.780″ E and 3°28′02.383″ S 114°21′31.282″ E) and Kuala Tambangan (3.968195″ S 114.632223″ E and 3.968005″ S 114.629447″ E) – allow for the evaluation of the impact of human activities on the coastal zone and the formulation of effective conservation strategies. The distribution of research locations and sampling is illustrated in Figure 1.

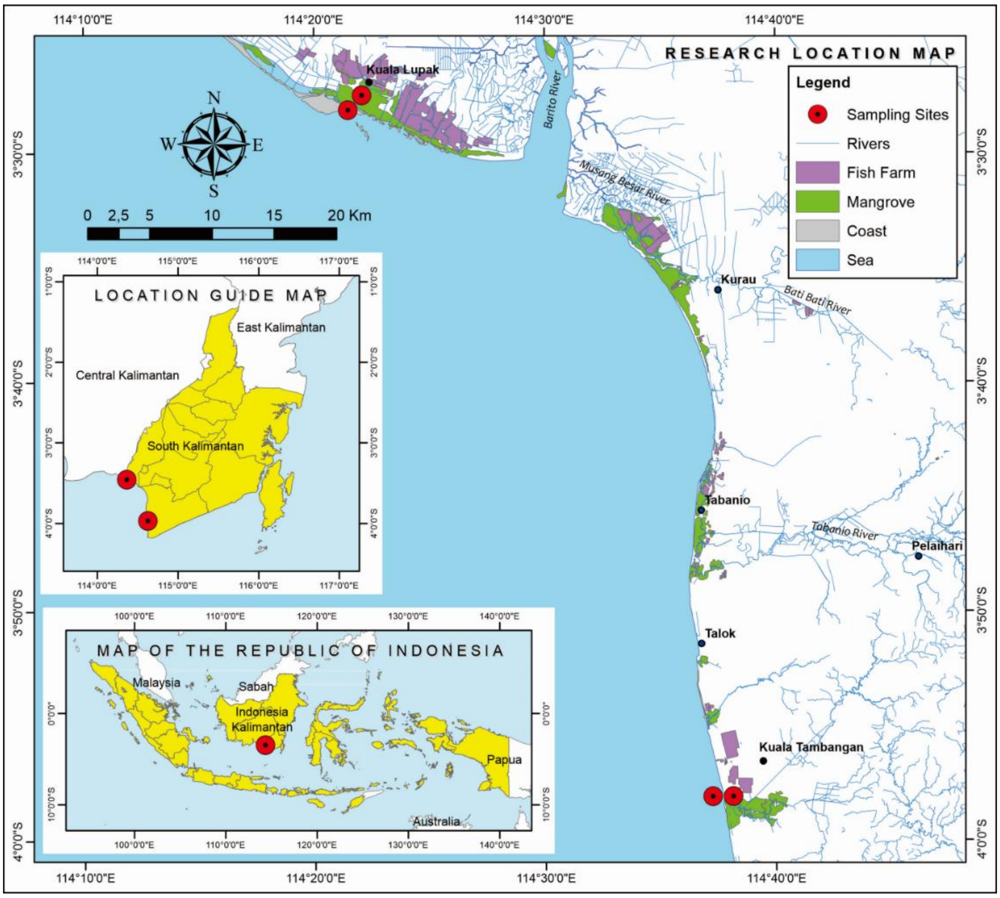

Figure 1. Study map and sampling sites in Kuala Lupak estuary and Kuala Tambangan, South Kalimantan, Indonesia.

Sample collection and analysis. Water, sediment, and fish sampling was carried out once in July 2024 using a purposive sampling method. Analysis of heavy metal concentrations (Pb, Hg, Cd, Zn, Fe, Cu, and Cr) in water samples was carried out using the liquid-liquid extraction method (Jayaprakash et al 2015). Surface water sampling was carried out at two locations, each with two sampling stations, and repeated twice per station using an Aqua trap. A total of 2 mL of concentrated HNO_3 was added to 1 L of filtered water sample, then dissolved heavy metals were determined. In the unfiltered water sample (100 mL), 2 mL of 2% APDC solution was added and extracted with 10 mL of IBMK. The water phase was then treated with concentrated HNO_3 and pure water for further extraction. Heavy metal concentrations (mg L^{-1}) were measured using Thermo Scientific ICE series 3500 atomic absorption spectrometry (AAS) after evaporation of traces of organic solvents on a low-temperature hot plate. Sediment samples were collected at four estuary stations using a Van Veen sampler, with three samples taken from each station. The samples were dried at 40°C to constant weight, then powdered for homogeneity and increased surface area. One gram of dry powdered sample was digested by adding a mixture of acids. The acid composition was HNO_3 and HCl, with a ratio and concentration of 20 mL of HNO_3 (ultrapure) and 5 mL of HCl (ultrapure). Digestion was carried out using a hot plate (80–90°C) for 10 minutes until a clear solution or white smoke appeared. After cooling, the digestion results were filtered (Whatman filter paper 0.45 μm), diluted to 25 mL with deionized water, and analyzed for heavy metal concentrations using Flame Atomic Absorption Spectrometry (FAAS). Quality control included reagent blanks and duplicate samples. The analysis results were expressed in mg kg^{-1} dry weight of sediment.

Local fishermen collected fish samples (*A. sagor*, *P. lineatus*, and *M. cephalus*) from coastal swamp wetlands. The samples were immediately transported to the laboratory in insulated containers with ice to maintain their condition. In the lab, a meticulous dissection was undertaken, with various tissues, including the liver, muscles, and skin, carefully dissected using clean stainless-steel surgical tools and ice applied to preserve tissue integrity. Approximately 0.5 g of each tissue was accurately weighed and placed in a digestion tube for heavy metal analysis. A mixture of concentrated nitric acid and sulfuric acid was added to initiate digestion, which was carried out in a hot block digester at 60°C for 30 minutes. After cooling, more nitric acid was added, and the temperature was increased stepwise to 120°C and 150°C until the mixture turned dark. Hydrogen peroxide was then added to obtain a clear solution, ensuring complete digestion. The digested samples were then filtered using 0.45 µm filter paper and diluted to a known volume with deionized water. Finally, the concentrations of heavy metals were determined using Flame Atomic Absorption Spectrophotometry (FAAS), with the instrument calibrated using standard solutions. Quality control measures were implemented to ensure accurate results, which were expressed in mg kg^{-1} wet weight.

Sample preparation for enzymatic analysis. Biochemical assays were conducted at 4°C for enzymatic analysis. Tissues (liver, muscles, and skin) were homogenized using a tissue homogenizer in pH 7.4 phosphate buffer containing one mM EDTA, 250 mM sucrose, 150 mM KCl, and one mM DTT, at a ratio of 1:5 w/v (liver, muscles) and 1:3 w/v (skin). A SIGMA-FAST™ protease inhibitor (1 µL mL^{-1}) was added to the buffer to prevent protein degradation. The homogenate was then centrifuged at 12,000 g for 30 minutes at 4°C to obtain the S12 fraction. 600 µL aliquots of the supernatant were collected and stored at -80°C for subsequent analysis. All reagents were sourced from Sigma-Aldrich (St. Louis, USA) (Aouini et al 2018).

Antioxidant enzymes and lipid peroxidation. This study analyzed several key biomarkers in fish tissues (liver, muscle, and skin) to assess oxidative stress. Following homogenization and centrifugation to obtain the S12 fraction, enzymatic assays were performed at 4°C. Superoxide dismutase (SOD) activity was determined using the method of Misra & Fridovich (1972), which involves monitoring the inhibition of epinephrine auto-oxidation to adrenochrome at 480 nm. Similarly, lipid peroxidation (LPO) levels, catalase (CAT), and glutathione peroxidase (GPx) activities were also measured to provide a comprehensive oxidative stress profile. Protein concentrations in the S12 fractions were quantified using the Lowry method to normalize enzyme activities, ensuring precise and accurate sample comparisons. To measure catalase (CAT) activity in muscle, liver, and skin tissues, a reliable tissue-specific homogenization was performed (1:5 w/v for liver/muscle, 1:3 w/v for skin) in an appropriate buffer. The CAT activity was determined spectrophotometrically by mixing 2.45 mL of 50 mM phosphate buffer (pH 7), 50 µL of tissue homogenate (S12 fraction), and 1 mL of hydrogen peroxide substrate solution. The decrease in absorbance was monitored at 240 nm for 3 minutes at a controlled temperature (e.g., 25°C). One unit of CAT activity is defined as µmol of H_2O_2 decomposed per minute per mg of protein, with protein concentration determined using the Lowry method. The method applied was based on Kumar et al (2021).

To quantify LPO in liver, muscle, and skin tissues, begin with the previously prepared tissue homogenates (S12 fraction). To inhibit further oxidation during the assay, 0.25 mL of the homogenate was immediately mixed with 25 µL of 10 mM butylated hydroxytoluene (BHT). Subsequently, 3 mL of 1% phosphoric acid and 1 mL of 0.67% thiobarbituric acid (TBA) reagent were added to the mixture. The solution was then incubated in a shaking water bath at 90°C for 45 minutes. Following incubation, the samples were cooled to room temperature, and the absorbance of the supernatant was measured spectrophotometrically at 535 nm. LPO levels are expressed as malondialdehyde (MDA) equivalents per mg of protein, with protein concentrations determined using the reliable and widely accepted Lowry method. LPO was found in the liver, muscle, and skill tissues by applying the Kumar et al method (2021). To accurately measure GPx activity, the S12 fraction of tissue homogenates was utilized, following the methodology of Aouini et al (2018) adapted for a microplate reader by McFarland et al (1999). This process was conducted with thoroughness, ensuring the validity of the results. Specifically, a dilution of 1:3 v/v for the muscles, liver, and skin tissues was prepared. The assay was conducted in triplicate by adding samples, blanks, and standards to wells containing an assay mixture composed of 50 mM phosphate buffer (pH 7.6), 0.1 mM EDTA, 0.15 mM sodium azide, three mM of reduced glutathione (GSH), 0.25 mM NADPH, and 0.67 U mL^{-1} glutathione reductase (GR). The enzymatic reaction was initiated by adding cumene hydroperoxide after a 2-minute pre-incubation at 28°C. The absorbance was monitored at 340 nm over 5 minutes, with readings taken every 30 seconds. GPx activity was expressed as μmol of NADPH oxidized per minute per mg of total protein, with protein concentrations determined using the Lowry method, employing bovine serum albumin as the standard (Nakano et al 1992).

Statistical analysis. The data were studied statistically applying SPSS/Statistical Package for Social Sciences ver. 22. The findings, expressed as the mean ± mean standard deviation, were tested for normality and homogeneity of variance using Shapiro–Wilk and Levene tests, respectively. The one-way ANOVA/analysis of variance was employed to analyze the treatment effect, and the results were found to be significant. Posthoc honestly significant difference tests were used to identify significant variations between the means, with comparisons made at the 5% probability level. All statistical analyses were carried out at an alpha level of 0.05, underscoring the importance of the findings.

Results and Discussion

Heavy metals concentrations in water, sediment, and fish. The pollution of aquatic ecosystems by anthropogenic waste, particularly organic and inorganic contaminants, is an urgent global issue that significantly impacts fish populations. This study, which analyzed the levels of heavy metals in the tissues of several fish species in the swamp waters of Kuala Lupak and Kuala Tambangan, revealed a grave situation. The results showed the negative impact of heavy metal concentrations on stress bioindicators in fish. Figures 2 and 3 and Table 1 and 2 show the average concentrations of heavy metals in water, sediment, and tissues of *P. lineatus*, *A. sagor*, and *M. cephalus*, underscoring the severity of this problem and the need for immediate action.

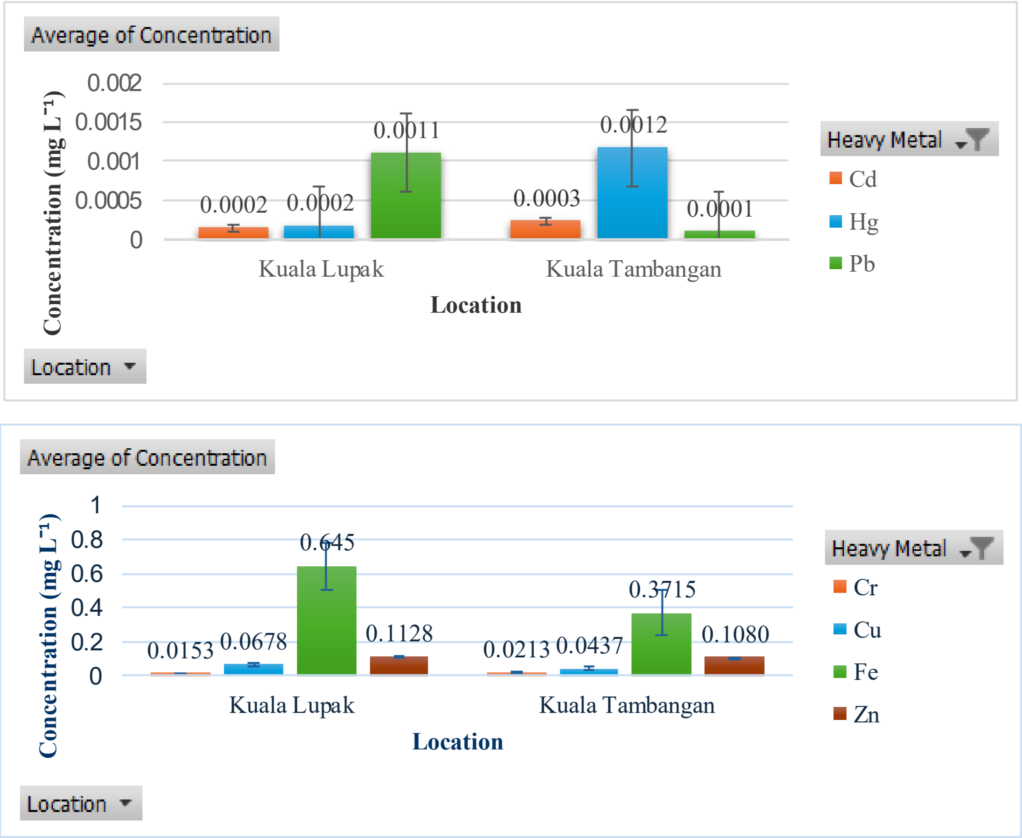

Figure 2. Average of heavy metal concentration in water.

Figure 2 quantitatively assesses seven heavy metals (Hg, Pb, Cd, Fe, Zn, Cu, and Cr) in two distinct coastal areas of South Kalimantan, Indonesia: Kuala Lupak and Kuala Tambangan. Within each location, measurements were taken at two stations: a coastal swamp area and an adjacent coastal area. This allows for a comparative analysis of heavy metal levels between these closely situated but potentially different aquatic environments. Cadmium levels showed some variability, with slightly higher concentrations observed in the coastal swamp of Kuala Tambangan (0.0003 mg L^{-1}) compared to other stations. Some values in Kuala Tambangan are slightly below the Indonesian Government Regulation (0.001 mg L^{-1}). Concentrations of mercury were generally low across all stations but were notably higher in Kuala Tambangan (0.0012 mg L^{-1}) compared to Kuala Lupak (0.0002 mg L^{-1}). In Kuala Tambangan, the concentrations exceeded the Indonesia Government Regulation No. 22 of 2021 (0.001 mg L^{-1}) and the WHO 2008 guideline (0.001 mg L^{-1}). Lead concentrations were relatively low and consistent across all sampling stations, ranging from 0.0001 to 0.0011 mg L^{-1}. All measured values were below the regulatory limits provided. Chromium concentrations were generally low, ranging from 0.0153 to 0.0213 mg L^{-1}. Kuala Tambangan (0.0213 mg L^{-1}) levels were higher than Kuala Lupak (0.0153 mg L^{-1}). All concentrations exceeded the Indonesian Government Regulation (0.005 mg L^{-1}). Copper concentrations varied between the locations and stations. Kuala Lupak showed higher levels (0.0678 mg L^{-1}) than Kuala Tambangan (0.0437 mg L^{-1}). Several concentrations exceeded the Indonesian Government Regulation (0.008 mg L^{-1}). Iron exhibited the highest concentrations among the measured heavy metals at all stations, ranging from 0.3715 to 0.645 mg L^{-1}. Concentrations were generally higher in Kuala Lupak (0.645 mg L^{-1}) than in Kuala Tambangan (0.3715 mg L^{-1}). Some concentrations in Kuala Lupak exceeded the Indonesian Government Regulation (0.30 mg L^{-1}), USEPA (2020) (0.30 mg L^{-1}), and WHO 2008 (0.30 mg L^{-1}) guidelines. Zinc concentrations were relatively consistent across all stations, ranging from 0.1080 to 0.1128 mg L^{-1}. All measured values were above the Indonesian Government Regulation No. 22 of 2021 (0.05 mg L^{-1}).

Furthermore, the study noted a general trend where the average order of heavy metal concentrations in the coastal swamp water bodies from the largest to smallest was Fe>Zn>Cu>Cr>Pb>Cd>Hg. Interestingly, the results indicated a statistically significant difference in heavy metal concentrations between the coastal swamp water bodies of Kuala Lupak and Kuala Tambangan for most metals, except for lead (Pb). However, the study also found no substantial difference in the average concentration of heavy metals between coastal and coastal swamp stations within the exact location. The study reveals spatial variation in waterborne heavy metals. Kuala Tambangan generally exhibited higher Hg, Cd, Fe, and Cr levels, exceeding regulatory limits for some. Conversely, Kuala Lupak showed higher Cu concentrations. Pb and Zn were relatively consistent. These differences likely stem from distinct anthropogenic activities and/or natural geochemical characteristics between the coastal swamp ecosystems.

These findings suggest that both locations are influenced by heavy metal contamination to varying degrees, with some metals exceeding established regulatory guidelines for water quality. The differences observed between Kuala Lupak and Kuala Tambangan could be attributed to varying anthropogenic pressures or natural geochemical differences between the coastal swamp ecosystems (Gebeyew et al 2022). The higher levels of Fe, Zn, Cu, and Cr compared to other metals indicate potential sources of these contaminants in the surrounding environment. This data on waterborne heavy metal concentrations provides a crucial baseline for understanding potential bioaccumulation in aquatic organisms, including the fish species analyzed in this study.

Figure 3 provides an assessment of heavy metal contamination in sediments, major reservoirs for pollutants in aquatic ecosystems, and potential sources for bioaccumulation in benthic organisms and the broader food web. This study measured the average concentrations of 7 heavy metals (Hg, Pb, Cd, Fe, Zn, Cu, and Cr) in coastal marshes and adjacent coastal stations in the Kuala Lupak and Kuala Tambangan areas of South Kalimantan, Indonesia.

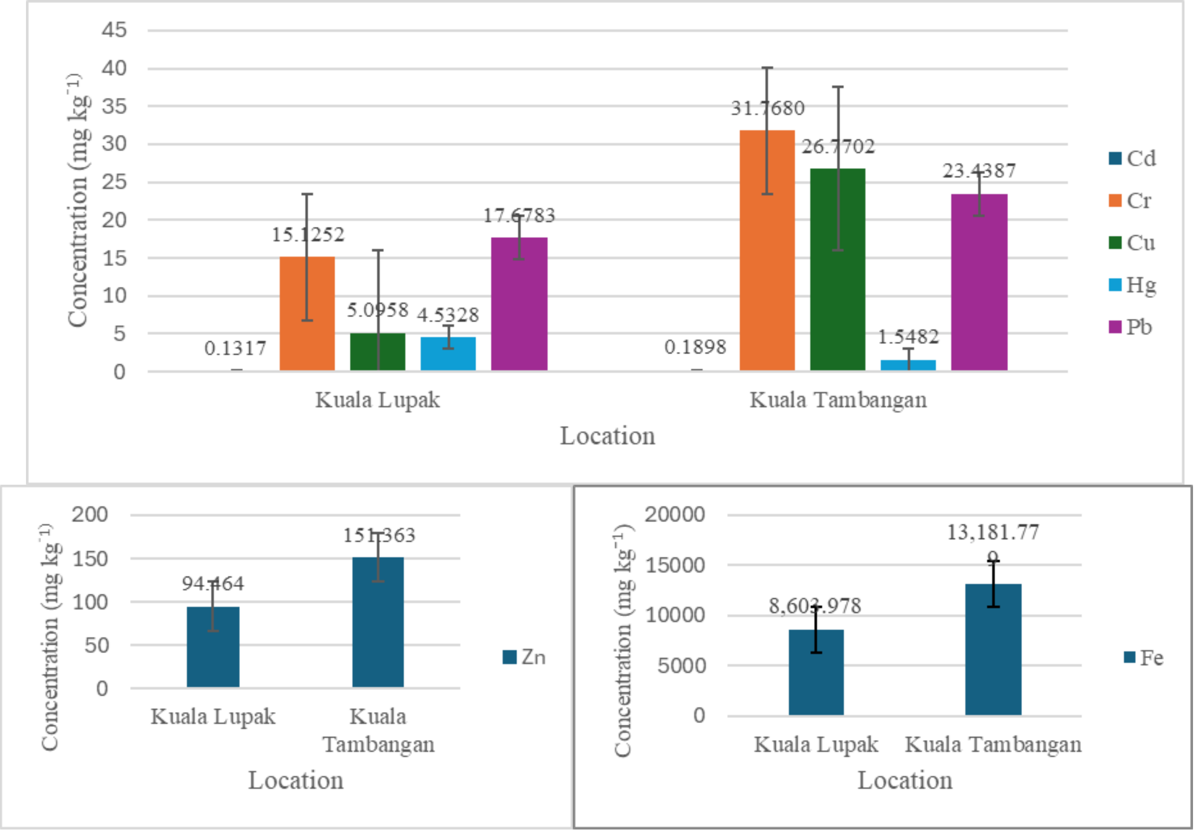

Figure 3. Average concentration of heavy metals in sediment.

Key findings from Figure 3 include overall high concentrations. The data reveals generally high concentrations of several heavy metals, mainly Fe (13,181.77 mg kg^{-1}) and Zn (151.363 mg kg^{-1}), in the sediments of both locations. This suggests a significant input of these metals into the coastal swamp ecosystems. The average order of heavy metal concentrations in the coastal swamp sediments, from highest to lowest, was consistently Fe>Zn>Cr>Pb>Cu>Hg>Cd in both Kuala Lupak and Kuala Tambangan. This pattern may indicate similar sources or biogeochemical processes influencing metal deposition in both areas. A significant difference was found in the heavy metal concentrations in sediments between Kuala Lupak and Kuala Tambangan for all analyzed metals. Kuala Tambangan exhibited substantially higher mean Pb, Cd, Fe, Zn, Cu, and Cr concentrations in coastal swamps and stations than in Kuala Lupak. For example, Fe concentrations in Kuala Tambangan were approximately 1.5 times higher than in Kuala Lupak. Similarly, Zn, Cu, and Cr showed elevated levels in Kuala Tambangan. Conversely, Hg concentrations were higher in the Kuala Lupak sediments than in Kuala Tambangan. Compared to the Interim Sediment Quality Guidelines (ISQG) for Canada, concentrations of several heavy metals, mainly Fe and Zn, significantly exceeded these guidelines at both sites, indicating potential risks of adverse effects on sediment-dwelling organisms. Based on ISQG standards, the maximum permissible concentration limits for Hg, Pb, Cd, Zn, and Cu are 0.17 mg kg^{-1}, 35 mg kg^{-1}, 0.6 mg kg^{-1}, 123 mg kg^{-1}, and 35.7 mg kg^{-1}, respectively. Despite the significant differences between the two locations, the study found no substantial variation in heavy metal concentrations between the coastal and coastal swamp stations within the exact location. This suggests that the factors influencing heavy metal deposition are more related to the overall geographical area (Kuala Lupak vs. Kuala Tambangan) rather than the immediate proximity to the swamp environment within each area. These findings strongly suggest that both Kuala Lupak and Kuala Tambangan coastal swamp ecosystems are impacted by heavy metal contamination in their sediments, with Kuala Tambangan generally showing higher levels of most metals (Pb, Cd, Fe, Zn, Cu, Cr), while Kuala Lupak has higher mercury levels. The exceedance of sediment quality guidelines for several metals raises concerns about the health of the benthic community and the potential for bioaccumulation of these contaminants into higher trophic levels, including the fish species studied (Zhang et al 2023). The significant differences in sediment metal concentrations between the two locations warrant further investigation into these contaminants' specific sources and pathways to implement effective mitigation strategies. Despite the similar levels between coastal and coastal swamp stations within each location, the overall high sediment contamination underscores the need for environmental monitoring and management in these South Kalimantan coastal ecosystems.

Further analysis shows different trends in water and sediment, indicating higher heavy metal concentrations in sediments. This increase in concentration is due to high industrial runoff, domestic waste disposal, and ship transportation, causing a significant rise in metal accumulation in sediments through a binding process facilitated by organic matter and fine sediment particles. The retention of heavy metals in sediments is affected by natural geochemical cycles (Gebeyew et al 2022). The high sediment binding capacity in the coastal swamps of Kuala Tambangan could explain the spatial variation in metal distribution, a phenomenon that warrants further investigation. This aligns with the study showing that sediment type and organic content significantly affect metal adsorption and retention patterns (Tanhan et al 2023). The spatial differences underscore how environmental and anthropogenic factors interact to drive patterns of heavy metal accumulation in coastal ecosystems (Lozano-Bilbao et al 2023).

Table 1 shows the Hg, Pb, and Cd concentrations in the tissues of *A. sagor*, *M. cephalus*, and *P. lineatus* fish from Kuala Lupak and Kuala Tambangan. These data indicate exposure and absorption of heavy metals from the environment across species, tissues (muscle, liver, skin), and locations (coastal and coastal swamps). The data provides a detailed insight into the uptake, distribution, and potential ecological and health implications of metal contamination in these estuarine environments.

The analysis of mercury (Hg) revealed that the Hg concentration in the *M. cephalus* liver (1.347 mg kg^{-1}) and *A. sagor* (0.195 mg kg^{-1}) was significantly higher than that in their respective muscles and skin. Furthermore, the concentration of Hg in the liver of *M. cephalus* and *A. sagor* was also much higher than that in *P. lineatus* (0.032 mg kg^{-1}). Despite minor variations, the concentration of Hg in the muscle and skin of the three species in Kuala Lupak and Kuala Tambangan were generally not significantly different. The $p\text{-value}<0.05$ indicates an overall significant difference in Hg concentration between the groups studied, underscoring the potential impact of these findings on marine conservation and the need for further research and action. This is due to the role of the liver as the primary site of detoxification and accumulation of heavy metals. This showed the significant role of the liver in metal detoxification, storage, and metabolic processing. Livers across species often accumulate higher levels of toxic metals due to their substantial binding capacity through metallothioneins and other proteins. By metabolizing and binding metals, previous studies reported that the liver became a primary storage site for bioavailable contaminants, including Hg and Cd, particularly in estuarine and coastal species (Lozano-Bilbao et al 2023).

The main findings from Table 1 on Pb concentration in coastal swamps, *M. cephalus* muscle, showed a higher Pb concentration (0.002 ± 0.0^a mg kg^{-1}) compared to its muscle in the coastal area and both locations in Kuala Tambangan (0.001 ± 0.0^b mg kg^{-1}). This significant difference underlines the importance of our study. The concentration of Pb in the muscles of *M. cephalus* fish in the coastal swamps of Kuala Lupak (0.002 ± 0.0^a mg kg^{-1}) was significantly higher than in other locations (0.001 ± 0.0^b mg kg^{-1}). This is strongly suspected to be caused by anthropogenic activities around Kuala Lupak, such as waste disposal and agricultural runoff, which increase lead input into the environment. Differences in sediment composition, water characteristics, and feeding behavior of *M. cephalus* in the Kuala Lupak swamps can also potentially increase the bioavailability and accumulation of lead in fish at that location.

Table 1

Concentrations of Hg, Pb, and Cd in various tissues of different fish species (mg kg^{-1})

Fish species	*Organ*	*Location*	*Station*	*Hg*	*Pb*	*Cd*
Arius sagor	Muscles	Kuala Lupak	Coastal swamp	0.107±0.000^{fgh}	0.001±0.0^{b}	0.005±0.000^{m}
			Coastal	0.106±0.001^{fgh}	0.001±0.0^{b}	0.004±0.001^{m}
		Kuala Tambangan	Coastal swamp	0.061±0.000^{ghi}	0.001±0.0^{b}	0.095±0.001^{fg}
			Coastal	0.059±0.002^{ghi}	0.001±0.0^{b}	0.094±0.001^{fg}
	Liver	Kuala Lupak	Coastal swamp	0.195±0.000^{cd}	0.001±0.0^{b}	0.073±0.000^{fghijk}
			Coastal	0.192±0.003^{cd}	0.001±0.0^{b}	0.072±0.001^{fghijk}
		Kuala Tambangan	Coastal swamp	0.076±0.001^{ghi}	0.001±0.0^{b}	0.071±0.002^{fghijk}
			Coastal	0.070±0.005^{ghi}	0.001±0.0^{b}	0.062±0.001^{ghijkl}
	Skin	Kuala Lupak	Coastal swamp	0.221±0.000^{c}	0.001±0.0^{b}	0.033±0.007^{klm}
			Coastal	0.221±0.010^{c}	0.001±0.0^{b}	0.026±0.004^{lm}
		Kuala Tambangan	Coastal swamp	0.069±0.002^{ghi}	0.001±0.0^{b}	0.152±0.000^{de}
			Coastal	0.068±0.000^{ghi}	0.001±0.0^{b}	0.151±0.002^{de}
Plotosus lineatus	Muscles	Kuala Lupak	Coastal swamp	0.069±0.002^{ghi}	0.001±0.0^{b}	0.039±0.000^{hijklm}
			Coastal	0.062±0.001^{ghi}	0.001±0.0^{b}	0.037±0.001^{jklm}
		Kuala Tambangan	Coastal swamp	0.095±0.001^{ghi}	0.001±0.0^{b}	0.096±0.000^{fg}
			Coastal	0.094±0.001^{ghi}	0.001±0.0^{b}	0.095±0.001^{fg}
	Liver	Kuala Lupak	Coastal swamp	0.054±0.000^{ghi}	0.001±0.0^{b}	0.072±0.000^{fghijk}
			Coastal	0.039±0.016^{i}	0.001±0.0^{b}	0.071±0.002^{fghijk}
		Kuala Tambangan	Coastal swamp	0.105±0.000^{fgh}	0.001±0.0^{b}	0.279±0.009^{c}
			Coastal	0.103±0.002^{gh}	0.001±0.0^{b}	0.260±0.022^{c}
	Skin	Kuala Lupak	Coastal swamp	0.051±0.001^{hi}	0.001±0.0^{b}	0.072±0.000^{fghijk}
			Coastal	0.039±0.006^{i}	0.001±0.0^{b}	0.071±0.001^{fghijk}
		Kuala Tambangan	Coastal swamp	0.090±0.000^{ghi}	0.001±0.0^{b}	0.174±0.067^{d}
			Coastal	0.087±0.006^{ghi}	0.001±0.0^{b}	0.104±0.005^{fg}
Mugil cephalus	Muscles	Kuala Lupak	Coastal swamp	0.109±0.000^{efg}	0.002±0.0^{a}	0.078±0.001^{fghij}
			Coastal	0.106±0.001^{fgh}	0.001±0.0^{b}	0.062±0.006^{ghijkl}
		Kuala Tambangan	Coastal swamp	0.055±0.000^{ghi}	0.001±0.0^{b}	0.083±0.000^{fgh}
			Coastal	0.040±0.007^{i}	0.001±0.0^{b}	0.082±0.001^{fghi}
	Liver	Kuala Lupak	Coastal swamp	1.347±0.21^{a}	0.001±0.0^{b}	12.177±0.006^{a}
			Coastal	1.264±0.101^{b}	0.001±0.0^{b}	12.120±0.036^{b}
		Kuala Tambangan	Coastal swamp	0.070±0.000^{ghi}	0.001±0.0^{b}	0.109±0.002^{ef}
			Coastal	0.060±0.010^{ghi}	0.001±0.0^{b}	0.106±0.004^{fg}
	Skin	Kuala Lupak	Coastal swamp	0.165±0.000^{cde}	0.001±0.0^{b}	0.038±0.000^{ijklm}
			Coastal	0.161±0.002^{def}	0.001±0.0^{b}	0.037±0.001^{jklm}
		Kuala Tambangan	Coastal swamp	0.082±0.000^{ghi}	0.001±0.0^{b}	0.099±0.000^{fg}
			Coastal	0.081±0.002^{ghi}	0.001±0.0^{b}	0.072±0.009^{fghijk}

Fish species	*Organ*	*Location*	*Station*	*Hg*	*Pb*	*Cd*
			P value	0.000	0.10	0.000
	Permissible limits in fishes			0.5 (WHO) 0.0005 (US FDA)	0.3 (WHO) 0.3 (SNI 7387:2009)	0.05 (WHO) 0.50 (FAO)

The numbers shown are mean ± standard deviation. Numbers followed by similar letters in similar metal types indicate no significant difference based on the posthoc honestly significant difference test at the 95% confidence level. FAO (Food and Agriculture Organization of the United Nations) (1983); WHO (Expert Committee on Food Additives) (1989); US FDA (United States Food and Drug Administration) (1993); SNI 7387:2009 (Indonesian National Standard: Maximum limits for heavy metal contamination in food) (2009).

Table 2

Concentrations of Fe, Zn, Cu, and Cr in various tissues of different fish species (mg kg^{-1})

Fish species	*Organ*	*Location*	*Station*	*Fe*	*Zn*	*Cu*	*Cr*
Arius sagor	Muscles	Kuala Lupak	Coastal swamp	14.38±0.00^{d}	5.6±0.00^{n}	0.59±0.00^{jkl}	0.002±0.000^{f}
			Coastal	14.19±0.03^{d}	3.70±0.19^{op}	0.52±0.07^{kl}	0.002±0.001^{f}
		Kuala Tambangan	Coastal swamp	25.81±0.00^{d}	15.64±0.00^{i}	0.74±0.00^{ijk}	0.813±0.006^{e}
			Coastal	25.56±0.22^{d}	14.87±0.68^{i}	0.63±0.09^{jkl}	0.800±0.010^{e}
	Liver	Kuala Lupak	Coastal swamp	177.36±0.00^{c}	172.00±0.00^{b}	7.16±0.00^{f}	0.002±0.000^{f}
			Coastal	176.94±0.51^{c}	171.05±1.06^{b}	7.10±0.06^{f}	0.001±0.001^{f}
		Kuala Tambangan	Coastal swamp	142.83±0.07^{c}	212.51±0.06^{a}	5.28±0.01^{g}	0.519±0.000^{ef}
			Coastal	142.51±0.40^{c}	212.54±0.01^{a}	5.19±0.08^{g}	0.512±0.006^{ef}
	Skin	Kuala Lupak	Coastal swamp	45.83±0.02^{d}	40.30±0.00^{d}	1.82±0.01^{h}	0.002±0.000^{f}
			Coastal	44.67±0.54^{d}	39.88±0.56^{d}	1.78±0.04^{h}	0.002±0.000^{f}
		Kuala Tambangan	Coastal swamp	12.17±0.12^{d}	7.88±0.03^{m}	0.01±0.00^{l}	0.389±0.181^{ef}
			Coastal	12.41±0.41^{d}	7.79±0.12^{m}	0.01±0.00^{l}	0.387±0.129^{ef}
Plotosus lineatus	Muscles	Kuala Lupak	Coastal swamp	24.70±0.58^{d}	3.66±0.00^{op}	0.01±0.00^{l}	0.002±0.000^{f}
			Coastal	23.54±0.50^{d}	3.47±0.18^{op}	0.01±0.00^{l}	0.001±0.001^{f}
		Kuala Tambangan	Coastal swamp	30.46±0.12^{d}	4.35±0.00^{o}	1.28±0.00^{hi}	0.941±0.000^{e}
			Coastal	30.28±0.09^{d}	4.30±0.08^{o}	1.19±0.02^{hi}	0.792±0.134^{e}
	Liver	Kuala Lupak	Coastal swamp	243.08±0.00^{b}	26.10±0.54^{f}	6.68±0.00^{f}	0.029±0.000^{f}
			Coastal	242.09±1.35^{b}	25.16±0.85^{f}	6.64±0.04^{f}	0.027±0.001^{f}

Fish species	Organ	Location	Station	Fe	Zn	Cu	Cr
		Kuala Tambangan	Coastal swamp	15.68 ± 0.00^{d}	149.78 ± 0.00^{c}	35.21 ± 0.01^{b}	0.031 ± 0.001^{f}
			Coastal	15.23 ± 0.25^{d}	149.54 ± 0.48^{c}	35.03 ± 0.78^{b}	0.029 ± 0.007^{f}
	Skin	Kuala Lupak	Coastal swamp	21.83 ± 0.00^{d}	9.46 ± 0.00^{l}	0.05 ± 0.00^{l}	0.002 ± 0.000^{f}
			Coastal	21.79 ± 0.06^{d}	9.43 ± 0.03^{l}	0.05 ± 0.00^{l}	0.001 ± 0.001^{f}
		Kuala Tambangan	Coastal swamp	17.69 ± 0.06^{d}	8.55 ± 0.04^{lm}	9.07 ± 0.03^{e}	0.488 ± 0.388^{ef}
			Coastal	17.29 ± 0.53^{d}	8.55 ± 0.02^{lm}	9.39 ± 0.47^{e}	0.271 ± 0.056^{ef}
Mugil cephalus	Muscles	Kuala Lupak	Coastal swamp	261.71 ± 0.06^{b}	12.64 ± 0.29^{j}	12.91 ± 0.06^{d}	15.892 ± 0.006^{a}
			Coastal	260.63 ± 0.05^{b}	11.53 ± 0.61^{k}	12.32 ± 0.53^{d}	13.529 ± 1.185^{b}
		Kuala Tambangan	Coastal swamp	21.49 ± 0.58^{d}	3.08 ± 0.06^{p}	0.01 ± 0.00^{l}	0.560 ± 0.057^{ef}
			Coastal	20.89 ± 0.74^{d}	3.03 ± 0.02^{p}	0.01 ± 0.00^{l}	0.532 ± 0.012^{ef}
	Liver	Kuala Lupak	Coastal swamp	2345.78 ± 0.56^{a}	38.11 ± 0.00^{e}	21.23 ± 0.00^{c}	12.864 ± 0.002^{b}
			Coastal	2332.00 ± 77.7^{a}	38.10 ± 0.00^{e}	21.05 ± 0.07^{c}	10.865 ± 0.500^{c}
		Kuala Tambangan	Coastal swamp	273.74 ± 0.04^{b}	22.11 ± 0.00^{g}	149.50 ± 0.26^{a}	0.592 ± 0.006^{ef}
			Coastal	273.52 ± 0.21^{b}	22.06 ± 0.07^{g}	149.45 ± 0.42^{a}	0.525 ± 0.058^{ef}
	Skin	Kuala Lupak	Coastal swamp	172.63 ± 0.00^{c}	17.93 ± 0.04^{h}	0.96 ± 0.00^{ijk}	7.876 ± 0.000^{d}
			Coastal	171.94 ± 0.72^{c}	17.54 ± 0.47^{h}	0.91 ± 0.01^{ijk}	7.681 ± 0.152^{d}
		Kuala Tambangan	Coastal swamp	25.44 ± 0.00^{d}	5.86 ± 0.01^{n}	1.04 ± 0.00^{ijk}	0.412 ± 0.000^{ef}
			Coastal	24.59 ± 1.06^{d}	5.70 ± 0.28^{n}	1.03 ± 0.01^{ijk}	0.219 ± 0.104^{ef}
			P value	0.000	0.000	0.000	0.000
	Permissible limits in fishes			100 (WHO) 1 (SNI 7387:2009)	30 (FAO) 50 (WHO) 100 (BPOM RI)	10 (WHO) 30 (FAO)	0.15 (WHO) 1 (US FDA) 2.5 (BPOM RI)

The numbers shown are mean ± standard deviation. Numbers followed by similar letters in similar metal types indicate no significant difference based on the posthoc honestly significant difference test at the 95% confidence level. FAO (Food and Agriculture Organization of the United Nations) (1983); WHO (Expert Committee on Food Additives) (1989); US FDA (1993); BPOM RI (2018); SNI 7387:2009 (Indonesian National Standard: Maximum limits for heavy metal contamination in food)

The analysis results in Table 1 show that the concentration of Cd in the liver of *M. cephalus*, *A. sagor*, and *P. lineatus* fish at both study sites was significantly higher than in their muscles and skin. The highest Cd concentrations were found in the livers of the three species (12.177±0.006^{a} mg kg^{-1}, 0.073±0.000^{fghijk} mg kg^{-1}, and 0.279±0.009^{c} mg kg^{-1}, respectively). Although the Cd concentrations in the livers of *M. cephalus* and *A. sagor* were generally higher in Kuala Lupak, statistical analysis revealed a complex and intricate distribution pattern, with significant but inconsistent differences between sample group comparisons ($p<0.05$). The Cd content in the liver of different species and locations showed relatively similar results, as indicated by various letter codes (e.g., 0.073±0.000^{fghijk} in *A. sagor* at Kuala Lupak and 0.072±0.000^{fghijk} in *P. lineatus* at Kuala Lupak). This indicates that there were no statistically significant differences between the measurement results with the same letter codes. The higher accumulation of Cd in the liver is consistent with the function of the liver in detoxifying and metabolizing heavy metals, primarily through active filtration of contaminants from the blood (Ju et al 2024). Although Kuala Lupak data tended to show higher Cd concentrations in the livers of *M. cephalus* and *A. sagor*, the distribution of Cd was influenced by environmental factors specific to each location.

Table 2 shows the Fe, Zn, Cu, and Cr concentrations in the tissues of *A. sagor*, *M. cephalus*, and *P. lineatus* fish from Kuala Lupak and Kuala Tambangan. These data indicate exposure and absorption of heavy metals from the environment across species, tissues (muscle, liver, skin), and locations (coastal and coastal swamps). Table 2 presents statistically significant differences in Fe concentrations among species, tissues, and locations. In *M. cephalus*, liver Fe concentrations in Kuala Lupak (coastal swamp: 2,345.78±0.56^{a} mg kg^{-1}; coastal: 2,332.00±77.72^{a} mg kg^{-1}) were significantly higher than in Kuala Tambangan (273.74±0.04^{b} mg kg^{-1}). Similar trends were seen in *A. sagor* and *P. lineatus*, which also showed significantly higher liver Fe concentrations in Kuala Lupak (177.36±0.00^{c} mg kg^{-1} and up to 243.08±0.00^{b} mg kg^{-1}, respectively) than in Kuala Tambangan (142.83±0.07^{c} and 15.68±0.00^{d} mg kg^{-1}). The study highlights the significant role of the liver in iron (Fe) metabolism and storage, as it showed significantly higher Fe concentrations in the liver of *M. cephalus*, *A. sagor*, and *P. lineatus* in Kuala Lupak. The consistently high levels of Fe in the liver at these locations in all three species underscore the potential influence of local environmental factors on increasing the bioavailability or absorption of iron. Although Fe levels in muscle and skin were generally lower, significant variations indicated different patterns of iron accumulation and distribution between tissues ($p<0.05$).

Analysis from Table 2 shows that Zn concentration in *M. cephalus*, a species commonly found in coastal areas, was significantly higher in the muscle of fish from the Kuala Lupak coastal swamp (12.64±0.29^{j} mg kg^{-1}) than in those from Kuala Tambangan (3.08±0.06^{p} mg kg^{-1}), a similar trend was also seen in coastal areas (Kuala Lupak: 38.10±0.00^{e} mg kg^{-1}; Kuala Tambangan: 22.06±0.0^{g} mg kg^{-1}). In *A. sagor*, a species known for its adaptability to various habitats, Zn concentration in the liver was significantly higher in Kuala Tambangan (149.78±0.00^{c} mg kg^{-1} in coastal swamp area) than in Kuala Lupak (26.10±0.54^{f} mg kg^{-1} in coastal swamp), with a similar pattern in muscle and skin. Similar results were seen in *Plotosus lineatus*, a species commonly found in coastal areas, with higher Zn concentrations in the liver in Kuala Tambangan (149.54±0.48^{c} mg kg^{-1} in the coastal area) compared to Kuala Lupak (26.10±0.54^{f} mg kg^{-1} in the coastal swamp); this trend also applied to muscle and skin. The p-value<0.05 indicated that the overall difference in Zn concentrations was highly significant. The significantly higher Zn concentrations in the muscle of *M. cephalus* in Kuala Lupak, in contrast to the higher Zn concentrations in the liver, muscle, and skin of *A. sagor* and *P. lineatus* in Kuala Tambangan, indicated a Zn accumulation pattern influenced by species and observation location. Although Zn is an essential micronutrient, this difference may be due to differences in dietary intake and environmental conditions in the two locations. For example, differences in dietary sources or bioavailability of Zn in the Kuala Tambangan sediments could explain the higher Zn uptake in *A. sagor* and *P. lineatus*.

The analysis of Table 2 uncovers significant differences in Cu concentrations between species and locations ($p<0.05$). For instance, in *M. cephalus*, the liver Cu concentrations were notably higher in the coastal swamps of Kuala Tambangan

(149.50 ± 0.26^{a} mg kg^{-1}) and coastal areas (149.45 ± 0.42^{a} mg kg^{-1}) compared to Kuala Lupak (≤ 1 mg kg^{-1}). Similarly, *A. sagor* and *P. lineatus* exhibited significantly higher liver Cu concentrations in Kuala Tambangan (up to 212.51 ± 0.01^{a} mg kg^{-1} and 171.05 ± 1.06^{b} mg kg^{-1} in the coastal area). Even though muscle and skin Cu concentrations were relatively low across species, there were still statistically significant variations. However, there are measurement that show the same results. For example, the Cu content in the skin of M cephalus in both Kuala Lupak and Kuala Tambang showed no statistically significant difference, as indicated by several identical letter codes (e.g. 0.96 ± 0.00^{ijk} and 1.04 ± 0.00^{ijk}). These findings underscore a species-specific response to Cu availability in their respective environments. The liver Cu concentrations of *M. cephalus*, *A. sagor*, and *P. lineatus* in Kuala Tambangan were significantly higher than in Kuala Lupak ($p<0.05$), indicating differences in Cu bioavailability and uptake based on location. The significant increase in Cu concentrations in the liver of *M. cephalus* in Kuala Tambangan is a result of previous studies that suggested the possibility of increased Cu due to regional pollutant inputs such as industrial waste. Conversely, the lower Cu levels in Kuala Lupak and muscle and skin of all species suggest differences in Cu accumulation strategies and environmental exposures across species and locations. These findings significantly impact understanding species-specific responses to Cu availability in different environments.

The analysis of Table 2 reveals statistically significant differences ($p<0.05$) in Cr concentrations in fish livers at the study sites. Notably, Cr concentrations in the livers of *M. cephalus*, *A. sagor*, and *P. lineatus* in Kuala Lupak (coastal swamp and coastal area) were significantly higher compared to Kuala Tambangan. While Cr concentrations in muscle and skin were relatively low in all sites and species, this finding underscores the liver's pivotal role in detoxification, with higher Cr accumulation in fish livers, particularly in Kuala Lupak. Our statistical analysis ($p<0.05$) further confirms significant differences in Cr concentrations in fish in Kuala Lupak and Kuala Tambangan. *M. cephalus*, *A. sagor*, and *P. lineatus* in Kuala Lupak exhibited higher Cr accumulation in the liver, indicating a fascinating location-dependent Cr bioavailability and absorption. This discovery piques interest in the environmental factors influencing the fish. Conversely, the low Cr concentrations in muscle and skin underscore the liver's central role in Cr accumulation in these species in the study environment.

The data indicates tissue-specific bioaccumulation patterns for all seven metals. The liver generally serves as a significant accumulation site for Hg, Pb, Cd, and Fe, highlighting its role in detoxification and storage. Muscle tissue, being the edible part, generally had lower concentrations, but these levels are crucial for assessing human health risks. Skin tissue often exhibited the lowest concentrations, suggesting limited accumulation or rapid turnover. The current research has uncovered significant species-specific variations in metal accumulation, findings that have profound implications for environmental science and marine biology. For instance, *A. sagor* showed higher liver mercury, *P. lineatus* accumulated more liver lead and iron in some cases, and *M. cephalus* tended to have higher cadmium and zinc levels, especially in its liver. These differences likely reflect variations in feeding habits, metabolic rates, and physiological mechanisms for metal uptake and elimination. While some subtle differences in metal concentrations were noted between Kuala Lupak and Kuala Tambangan (e.g., slightly higher Zn and Cu in *A. sagor* muscle from Kuala Lupak), the overall trends in tissue-specific and species-specific accumulation were broadly consistent across the two locations. This suggests that broader regional contamination patterns might influence metal bioavailability and uptake. The lack of consistently significant differences in metal concentrations between the coastal swamp and adjacent coastal stations for most metals and species suggests that the immediate habitat type within these coastal areas might have a less pronounced effect on metal bioaccumulation in these fish species compared to other factors such as location and species-specific physiology. The detected levels of certain heavy metals, particularly mercury, lead, and cadmium, especially in the liver and muscle of these commercially important fish species, warrant further investigation regarding potential risks to both the fish themselves and human consumers. Comparing these concentrations with established safety guidelines is crucial for a thorough risk assessment. The findings emphasize the importance of considering tissue type, species-specific characteristics, and regional environmental factors when assessing the impact of metal contamination in aquatic environments.

This study showed that the concentration of heavy metals in several fish samples from Kuala Tambangan far exceeded the safe limits set by WHO and SNI 7387:2009, indicating significant health risks. For instance, there were concentrations of Cd 0.279 mg kg^{-1} (exceeding the WHO threshold of 0.05 mg kg^{-1}) in the liver of *P. lineatus*, Pb 14.71 mg kg^{-1} (exceeding the WHO threshold, and SNI 0.3 mg kg^{-1}) in the muscle of *M. cephalus*, Zn 212.51 mg kg^{-1} (exceeding the WHO threshold of 100 mg kg^{-1} and SNI 1 mg kg^{-1}) in the liver of *A. sagor*, and Cr 0.412 mg kg^{-1} (exceeding the WHO threshold of 0.15 mg kg^{-1}) in the skin of *A. sagor*. These findings underscore the importance of this study and the need for a comprehensive risk evaluation. Similar studies in Pakistani waters showed that Fe and Pb levels were above WHO permissible levels, posing a risk to fish and human health through bioaccumulation (Afzaal et al 2022). A similar situation also occurred in the Niger Delta in Nigeria, reporting significant concentrations of Cd, Cr, and Zn in water, sediments, and tissues of farmed fish, exceeding the permissible limits set by global health organizations, thus endangering public health due to consumption of fish contaminated with heavy metals (Ehiemere et al 2022).

Studies in Haizhou Bay, China (Zhang et al 2023), and rivers in Brazil (Francisco et al 2023), as well as a global review (Yunusa et al 2023), have shown significant accumulation of heavy metals (Zn, Cu, Pb, Hg, and As) in aquatic ecosystems. Bioaccumulation of these metals, especially in benthic organisms and fish, poses health risks characterized by genotoxicity and entry of heavy metals into the food chain. These findings emphasize the urgency of implementing strict regulations and increasing monitoring efforts to reduce heavy metal pollution and protect human health.

Stress biomarkers in fish species. The research on oxidative stress enzymes, such as CAT, SOD, and GPx, has practical implications as these enzymes act as biomarkers by indicating increased free radical levels (Abdallah et al 2024). These enzymes enhance the defense response in aquatic animals, mainly fish. SOD catalyzes the scavenging capacity, while catalase effectively removes hydrogen peroxide. The role of CAT and SOD as the first-line defense system against pollution (Sousa & Sun 2024) is not only fascinating but also crucial for understanding how these enzymes protect cells from the toxic impacts of hydrogen peroxide by decomposing it into water. Table 3 presents the results of measuring the activity of antioxidant enzymes CAT and SOD in the liver, muscle, and skin tissues of *A. sagor, P. lineatus*, and *M. cephalus*. Meanwhile, LPO levels are presented in Table 4.

Table 3

CAT and SOD in muscles, liver, and skin tissue of different fish species collected from coastal swamp waters of Kuala Lupak and Kuala Tambangan

Fish species	*Organ*	*Location*	*Station*	*CAT Units mg protein^{-1}*	*SOD Units mg protein^{-1}*
Arius sagor	Muscles	Kuala Lupak	Coastal swamp	3.516±0.004^{gh}	0.003±0.000^{hi}
			Coastal	3.431±0.116^{ij}	0.001±0.000^{i}
		Kuala Tambangan	Coastal swamp	7.312±0.002^{b}	0.001±0.000^{i}
			Coastal	7.089±0.012^{c}	0.001±0.000^{i}
	Liver	Kuala Lupak	Coastal swamp	0.972±0.007^{tu}	0.005±0.004^{ghi}
			Coastal	0.931±0.022^{tu}	0.002±0.001^{i}
		Kuala Tambangan	Coastal swamp	8.532±0.015^{a}	0.004±0.001^{ghi}
			Coastal	7.403±0.223^{b}	0.001±0.000^{i}
	Skin	Kuala Lupak	Coastal swamp	4.732±0.002^{f}	0.001±0.000^{i}
			Coastal	4.577±0.038^{f}	0.001±0.000^{i}

Fish species	Organ	Location	Station	CAT Units mg protein^{-1}	SOD Units mg protein^{-1}
		Kuala Tambangan	Coastal swamp	5.990 ± 0.007^{d}	0.010 ± 0.000^{defgh}
			Coastal	5.257 ± 0.047^{e}	0.010 ± 0.000^{defgh}
Plotosus lineatus	Muscles	Kuala Lupak	Coastal swamp	1.013 ± 0.002^{stu}	0.007 ± 0.001^{fghi}
			Coastal	0.983 ± 0.02^{stu}	0.004 ± 0.001^{ghi}
		Kuala Tambangan	Coastal swamp	1.228 ± 0.001^{qr}	0.004 ± 0.001^{ghi}
			Coastal	0.997 ± 0.004^{stu}	0.002 ± 0.000^{i}
	Liver	Kuala Lupak	Coastal swamp	0.845 ± 0.021^{uv}	0.007 ± 0.000^{fghi}
			Coastal	0.756 ± 0.004^{v}	0.003 ± 0.001^{hi}
		Kuala Tambangan	Coastal swamp	1.347 ± 0.006^{pq}	0.003 ± 0.001^{hi}
			Coastal	1.240 ± 0.010^{qr}	0.001 ± 0.000^{i}
	Skin	Kuala Lupak	Coastal swamp	3.966 ± 0.004^{g}	0.006 ± 0.001^{fghi}
			Coastal	3.684 ± 0.019^{h}	0.004 ± 0.001^{ghi}
		Kuala Tambangan	Coastal swamp	2.106 ± 0.003^{n}	0.002 ± 0.000^{i}
			Coastal	2.471 ± 0.138^{l}	0.001 ± 0.000^{i}
Mugil cephalus	Muscles	Kuala Lupak	Coastal swamp	1.152 ± 0.001^{rs}	0.008 ± 0.001^{efghi}
			Coastal	1.038 ± 0.053^{stu}	0.003 ± 0.001^{hi}
		Kuala Tambangan	Coastal swamp	3.334 ± 0.047^{j}	0.015 ± 0.000^{de}
			Coastal	2.912 ± 0.057^{k}	0.024 ± 0.003^{c}
	Liver	Kuala Lupak	Coastal swamp	2.310 ± 0.026^{lm}	0.064 ± 0.012^{a}
			Coastal	2.253 ± 0.054^{mn}	0.052 ± 0.006^{b}
		Kuala Tambangan	Coastal swamp	0.757 ± 0.006^{v}	0.007 ± 0.000^{fghi}
			Coastal	0.350 ± 0.010^{w}	0.004 ± 0.001^{ghi}
	Skin	Kuala Lupak	Coastal swamp	1.591 ± 0.005^{o}	0.016 ± 0.003^{d}
			Coastal	1.480 ± 0.022^{op}	0.012 ± 0.001^{defg}
		Kuala Tambangan	Coastal swamp	3.336 ± 0.003^{j}	0.014 ± 0.001^{def}
			Coastal	3.478 ± 0.018^{ij}	0.016 ± 0.001^{d}
			P value	0.000	0.000

The numbers shown are mean ± standard deviation. Numbers followed by similar letters in similar columns indicate that they are not significantly different based on the posthoc honestly significant difference test at the 95% confidence level

The analysis of Table 3 reveals statistically significant differences in CAT and SOD enzyme activities ($p<0.05$) among fish species, tissues, and study locations. In *M. cephalus*, the highest liver CAT activity was discovered in the coastal swamps of Kuala Lupak, a significant departure from other locations and tissues; muscle CAT activity was lower and varied between locations. SOD activity in this species also exhibited significant differences, with the highest value in the liver at the Kuala Lupak location. *A. sagor* displayed significant differences in CAT and SOD activities between tissues and locations, with the highest muscle CAT activity in Kuala Tambangan, while liver SOD activity was highest in Kuala Lupak. In *P. lineatus*, the highest liver CAT activity was found in Kuala Tambangan, while muscle SOD activity was generally low and varied between locations. The letters in Table 4 (posthoc test, 95% confidence) indicate differences in species- and tissue-specific antioxidant enzyme activities in response to environmental conditions and potential heavy metal stress in Kuala Lupak and Kuala Tambangan.

The research findings on fish exposed to heavy metals at various locations, including Hadejia-Nguru Wetland and Warri River, support the previous studies. They show increased SOD and CAT enzyme activities as an adaptive response to Cd and Pb exposure exceeding WHO thresholds (Musa & Sabiu 2022; El-Shenawy et al 2021). These findings are in line with previous studies on *Clarias gariepinus* and *Oreochromis niloticus*, which also showed increased SOD and CAT enzyme activities as biological markers of antioxidant defense mechanisms against metal stress (Abdullahi et al 2022; El-Shenawy et al 2021). Increased activity of CAT and SOD enzymes helps fish neutralize reactive oxygen species (ROS), such as hydrogen peroxide and superoxide radicals, thereby protecting cells (Magnuson et al 2023). The variations in antioxidant responses, which are influenced by metal concentration and tissue type, indicate a crucial role for the liver in detoxifying and adapting species to pollution. Specifically, the liver plays a key role in metabolizing and excreting heavy metals, thereby reducing their toxic effects on other tissues and organs. This discovery opens up a promising avenue for the use of CAT and SOD as effective bioindicators to assess the health of aquatic ecosystems and the impact of heavy metals on aquatic organisms, instilling hope for the future of aquatic health assessment.

Table 4

GPx and LPO level in muscles, liver, and skin tissues of different fish species collected from coastal swamp waters of Kuala Lupak and Kuala Tambangan

Fish species	*Organ*	*Location*	*Station*	*GPx (units mg protein^{-1})*	*LPO (nmol mg protein^{-1})*
Arius sagor	Muscles	Kuala Lupak	Coastal swamp	0.183±0.001^{p}	299.07±0.06^{e}
			Coastal	0.124±0.003^{pq}	279.50±0.45^{g}
		Kuala Tambangan	Coastal swamp	0.642±0.001^{j}	257.00±0.01^{i}
			Coastal	0.360±0.018^{n}	253.19±0.33^{i}
	Liver	Kuala Lupak	Coastal swamp	1.851±0.046^{c}	295.52±0.41^{e}
			Coastal	1.647±0.056^{e}	288.56±2.82^{f}
		Kuala Tambangan	Coastal swamp	1.770±0.026^{d}	222.67±0.58^{kl}
			Coastal	1.105±0.051^{i}	220.40±0.61^{l}
	Skin	Kuala Lupak	Coastal swamp	0.462±0.007^{m}	200.03±0.02^{s}
			Coastal	0.338±0.033^{n}	200.01±0.00^{s}
		Kuala Tambangan	Coastal swamp	0.293±0.002^{no}	207.67±0.58^{pq}
			Coastal	0.141±0.035^{pq}	199.00±1.00^{s}
Plotosus lineatus	Muscles	Kuala Lupak	Coastal swamp	0.257±0.007^{o}	233.00±0.01^{j}
			Coastal	0.128±0.023^{pq}	225.47±0.49^{k}
		Kuala Tambangan	Coastal swamp	0.518±0.001^{lm}	416.00±0.01^{a}
			Coastal	0.347±0.030^{n}	399.99±0.01^{b}
	Liver	Kuala Lupak	Coastal swamp	1.301±0.021^{g}	291.29±0.35^{f}
			Coastal	1.186±0.028^{h}	271.32±0.31^{h}
		Kuala Tambangan	Coastal swamp	0.091±0.001^{q}	273.67±0.58^{h}
			Coastal	0.086±0.003^{q}	272.33±0.58^{h}

Fish species	*Organ*	*Location*	*Station*	*GPx (units mg protein^{-1})*	*LPO (nmol mg protein^{-1})*
Mugil cephalus	Skin	Kuala Lupak	Coastal swamp	0.596±0.002^{jk}	208.34±0.58^{pq}
			Coastal	0.482±0.013^{m}	202.07±0.05^{rs}
		Kuala Tambangan	Coastal swamp	1.907±0.000^{c}	205.67±0.58^{qr}
			Coastal	2.008±0.012^{b}	211.00±1.00^{nop}
	Muscles	Kuala Lupak	Coastal swamp	1.871±0.002^{c}	200.56±0.97^{s}
			Coastal	1.775±0.022^{d}	177.33±1.15^{t}
		Kuala Tambangan	Coastal swamp	1.226±0.001^{h}	214.33±0.58^{mn}
			Coastal	1.062±0.036^{i}	211.33±0.58^{mnop}
	Liver	Kuala Lupak	Coastal swamp	2.082±0.023^{a}	346.10±0.00^{c}
			Coastal	2.048±0.033^{ab}	338.07±6.26^{d}
		Kuala Tambangan	Coastal swamp	2.083±0.002^{a}	212.99±0.01^{mno}
			Coastal	2.010±0.001^{ab}	210.77±0.68^{nop}
	Skin	Kuala Lupak	Coastal swamp	1.753±0.003^{d}	209.00±0.00^{opq}
			Coastal	1.468±0.019^{f}	202.11±0.12^{rs}
		Kuala Tambangan	Coastal swamp	0.533±0.006^{klm}	211.33±0.58^{mnop}
			Coastal	0.577±0.006^{jkl}	215.33±0.58^{m}
			P value	0.000	0.000

The numbers shown are in the form of average±standard deviation values. Numbers followed by similar letter in similar column showed no obvious difference based on a posthoc-Honestly significant difference test at a 95% confidence level.

Table 4 demonstrates statistically significant differences in GPx and LPO activities ($p<0.05$) in *M. cephalus*, *A. sagor*, and *P. lineatus*. In *M. cephalus*, the highest liver GPx activity was found in Kuala Lupak and Kuala Tambangan, significantly higher than muscle and skin. The level of muscle LPO in *M. cephalus* was lower in Kuala Lupak. *A. sagor* showed the highest muscle LPO activity in Kuala Lupak, significantly different from other locations, and liver GPx activity was higher in Kuala Lupak. *P. lineatus* had very high muscle LPO activity in the coastal swamps of Kuala Tambangan, indicating significant oxidative damage, while liver GPx activity tended to be higher in Kuala Lupak. Different letters in Table 4 indicate statistically significant differences (post-hoc test). These results indicate species- and tissue-specific variations in antioxidant capacity (GPx) and oxidative damage (LPO) levels in response to environmental conditions at both sample locations. This response aligns with global studies where fish in metal-contaminated locations showed increased antioxidant enzyme activities to mitigate oxidative damage (Tanhan et al 2023). Our findings have practical implications for understanding and managing the health of fish populations in diverse environmental conditions.

A study carried out in 2024 in Kuala Lupak and Kuala Tambangan revealed significant differences in antioxidant enzyme activities (CAT, SOD, GPx) and lipid peroxidation (LPO) levels between fish species and tissues, indicating potential oxidative stress due to heavy metal pollution (Zahran et al 2025). The findings of this study are crucial as they shed light on the potential negative impact of pollution on fish health in coastal wetland ecosystems. *Mugil cephalus* fish in Kuala Lupak showed increased liver CAT and SOD, indicating a higher antioxidant response, possibly due to more significant liver stress. In contrast, *A. sagor* in Kuala Lupak showed the highest muscle LPO, indicating substantial oxidative damage despite increased liver GPx. *P. lineatus* in Kuala Tambangan showed very high muscle LPO, potentially related to higher Pb and Cd levels, indicating an overwhelmed antioxidant defense system. These results suggest that fish species and tissues respond differently to environmental stressors. Similarly, previous studies reported high LPO in response to metal-induced stress, particularly in heavily polluted waters (Zahran et al 2025). Increased LPO indicates significant oxidative damage in certain species and locations, indicating the potential negative impact of pollution on fish health in coastal wetland ecosystems. This biomarker is an important tool for assessing ecological stress.

Differences in tissue responses to pollutant exposure are significant, with the liver as the main detoxification organ showing the highest glutathione peroxidase (GPx) activity due to its role in xenobiotic metabolism. Skin and muscle variations in enzymatic activity complicate understanding tissue responses influenced by exposure type and metal binding capacity (Atli et al 2020). The geographic location of the fish also plays a role; fish living in coastal wetlands, with higher pollutant accumulation due to low water flow and high sedimentation, show higher values of oxidative stress markers than those living in open coastal locations (Li et al 2023). GPx and lipid peroxidase (LPO) are effective as bioindication biomarkers of metal contamination, providing information on sublethal impacts on fish and supporting their use in biomonitoring (Nayak et al 2021; Rizzo et al 2024). Due to their effectiveness in local and global monitoring, these indicators of oxidative stress hold the potential to become universal biomonitoring tools, offering hope for a more comprehensive assessment of the impact of pollution on aquatic biota (Kumar et al 2019).

Conclusions. The results of the current study in the coastal swamp ecosystem of South Kalimantan showed significant differences in heavy metal levels (Hg, Cd, Cr, and Fe) between species and fish tissues (liver, muscle, skin), as well as between environmental media (water and sediment) in Kuala Lupak and Kuala Tambangan. These differences in heavy metal concentrations were positively correlated with increased oxidative stress in fish, as indicated by changes in antioxidant enzyme activity (CAT, SOD, GPx) and lipid peroxidation levels (LPO). Therefore, integrated monitoring of heavy metal levels in water, sediment, and biota, combined with oxidative stress biomarker analysis, is critical to assess the impact of pollution and support sustainable environmental management in these ecosystems.

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