Field-Based Assessment of Aluminum Industry Effluent: Hematological and Biochemical Responses of Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT

The presence of unacceptable levels of heavy metals from untreated effluents and other contaminants can destabilize ecosystems, leading to physiological changes in aquatic organisms. To investigate this concern, water samples and Nile tilapia (*Oreochromis niloticus*) were collected from three locations along the Mbaa River to assess heavy metal concentrations in the water and the physiological profiles of the fish. The sampling sites, designated as Site A, B and C, were spaced 50 m apart. Site A was located upper stream; Site B was situated at the point source of an aluminium industry and represented the midstream area, while Site C was down-stream. A fish farm at the Federal University of Technology, Owerri, served as the control site. A significant difference ($p<0.05$) was observed in the concentrations of lead, zinc, cadmium, copper, and iron in water samples from Site B compared to the other sites and the control. Morphological indices of the fish showed no significant difference in body weight, liver weight, or fork length across all stations. However, compared to the control, fish from Site B exhibited a decrease in red blood cell (RBC) count and packed cell volume (PCV), along with a significant increase ($p<0.05$) in white blood cell (WBC) count. Serum levels of alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase did not show significant variation among fish from different sites. The findings of this study contribute to environmental monitoring and conservation efforts, highlighting the need for proper treatment of industrial effluents prior to discharge into aquatic environments in order to mitigate their impact on fish populations.

Keywords: Blood, Conservation, Enzymes, Fish, Heavy metals

INTRODUCTION

Contamination by untreated effluents containing heavy metals and other anthropogenic sources –such as industrial and domestic wastewater, the use of agrochemicals in crop production, geological disintegration of the earth's crust, and atmospheric deposition –can negatively impact the integrity of aquatic ecosystems (Ajima *et al*., 2015; Popovic *et al*., 2023). Due to their environmental persistence and potential to bioaccumulate in aquatic organisms, heavy metals are important contaminants in aquatic environments, capable of posing serious threats to resident aquatic biota as well as public health (Fu *et al*., 2019; Adebayo *et al*., 2022; Ahmadijokani *et al*., 2022). The presence of hazardous substances, including heavy metals in high concentrations in water bodies can alter the chemical and biological characteristics of aquatic ecosystems, leading to ecological imbalance. Continuous exposure to heavy metals may also result in genetic changes capable of inducing biochemical alterations in various fish organs (Mehmood *et al*., 2019; Okoro and Tawari-Tufeyin, 2024).

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Environmental stressors are known to alter physiological and morphological indices of fish (Canosa and Bertucci, 2023). Among the notable morphological indices, the condition factor (K) and hepatosomatic index (HSI) are frequently used in studies related to environmental stress (Melefa *et al*., 2022), particularly toxicant exposure (Morado *et al*., 2017). Serum biochemical parameters are important biomarkers used to assess the toxicity of xenobiotics on target organs in fish, as well as the general health status of stressed organisms (Ismail and Mahboub, 2016).

Heavy metals, such as cadmium have been reported to cause an increase in GSI and reduction in K and HSI in Nile tilapia (Ciftci *et al*., 2015), and to reduce HSI in *Labeo rohita* (Fiaz *et al*., 2024). Lead (Pb) and copper (Cu) have elevated levels of AST, ALT and ALP in *O. niloticus* (Alsulami *et al*., 2025). Ullah *et al*. (2021) found increased glucose concentrations and decreased total protein levels in *Tor putitora* exposed to heavy metals.

Nile tilapia (*O. niloticus*) is a globally significant food fish, known for its resilience and adaptability to diverse ecological conditions. Consequently, it is widely used in aquaculture. Several studies (Abiona *et al*., 2019; Mahboob *et al*., 2020; Hossain *et al*., 2021; Getnet *et al*., 2024; Reda *et al*., 2025) have reported the impacts of heavy metals on Nile tilapia, including alterations in the gills, liver, spleen, and muscle, and immune system. Additionally, changes in the expression of genes related to muscle growth (MyoD, IGF-1), and immune response (TNFa, IL6) have been observed (Shaalan, 2024).

Reports have also shown that heavy metals primarily affect serum biochemistry and enzymes related to oxidative stress in Nile tilapia by inducing the production of reactive oxygen species (ROS) through redox cycling. This leads to oxidative stress by disrupting the balance between ROS generation and the body's antioxidant defense mechanisms (Alm-Eldeen *et al*., 2018; Ghannam *et al*., 2021; Sherif *et al*., 2024; Gupta *et al*., 2025).

Haematological parameters are considered good indicators of physiological changes and health status in fish (Burgos-Aceves *et al*., 2019). Kim *et al*. (2019) found a substantial reduction in haemoglobin and haematocrit levels in juvenile olive flounder (*Paralichthys olivaceus*) exposed to waterborne zinc, while Abdel-Warith *et al*. (2020) noted a significant increase in haemoglobin and haematocrit values in *O. niloticus* exposed to sub-lethal concentrations of Pb and Cu.

However, the effects of heavy metal contaminations on both haematological and morphological profiles of Nile tilapia in Mbaa River have not been investigated. This study aims to assess the site-specific impacts of aluminium industry effluents on haematological and biochemical responses in Nile tilapia, with particular emphasis on multivariate biomarker analysis.

MATERIALS AND METHODS

Study area

The Mbaa River, located near Inyishi (the study region), lies between latitudes 5° 31' N and 5° 39' N and longitudes 7° 05' E and 7° 13' E (Figure 1). The primary occupation of the local population is farming, which is supplemented by fishing activities. Untreated effluents from an aluminum industry, situated just a few meters from the river bank, are frequently discharged into the Mbaa River.

Collection of water and fish samples

Water and fish samples were collected from three sampling stations along the Mbaa River, designated as Site A, B and C, each separated by approximately 50 m. Site A (upstream; 5°39’09”N, 7°10’00”E), Site B (midstream; near the aluminum industry effluent discharge point; 5°38’00”N, 7°09’13”E), and Site C (downstream; 5°36’30”N, 7°09’00”E) were selected for analysis. In addition, a control site – a fish farm near the Federal University of Technology, Owerri (5°30’20”N, 7°03’45”E), was included for comparison (Figure 1).

At each sampling station, water was collected using a 1-liter acid-leached polythene bottle. A total of 0.5 liters of water was taken from three different positions just below the surface. The samples were acidified with 10% HNO_3, stored in an ice bath, and transported to the laboratory for heavy metal analysis. Concentrations of lead (Pb), zinc (Zn), iron (Fe), cadmium (Cd), and copper (Cu) were determined using an atomic absorption spectrophotometer (Unicam 919, Analytical Technology Inc., Cambridge, UK).

For aluminium (Al) analysis, 1-liter water samples were collected in polypropylene Nalgene bottles pre-washed with 5% nitric acid for 48 h and rinsed thoroughly with ultrapure water before use. To preserve the samples, 2 mL of 69 –70% nitric acid was added. The samples were distilled and stored at 4 °C until further analysis. Aluminum concentrations were measured using a Perkin Elmer ELAN 9000 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), following the method described by Domínguez Renedo *et al*. (2019).

A total of 12 fish (mean weight = 40.23± 3.00 g; mean length = 12.0±0.6 cm) were collected from each of the three river sites and the control site, yielding a total of 48 fish. The specimens were immediately transported to the laboratory in a plastic tank for further analysis.

Morphological indices

The body weight (wet weight), liver weight, and fork length of fish from each sampling station were measured and recorded. The condition factor (K) and hepatosomatic index (HSI) for each fish were calculated following Hammock *et al*. (2022), using the following formula:

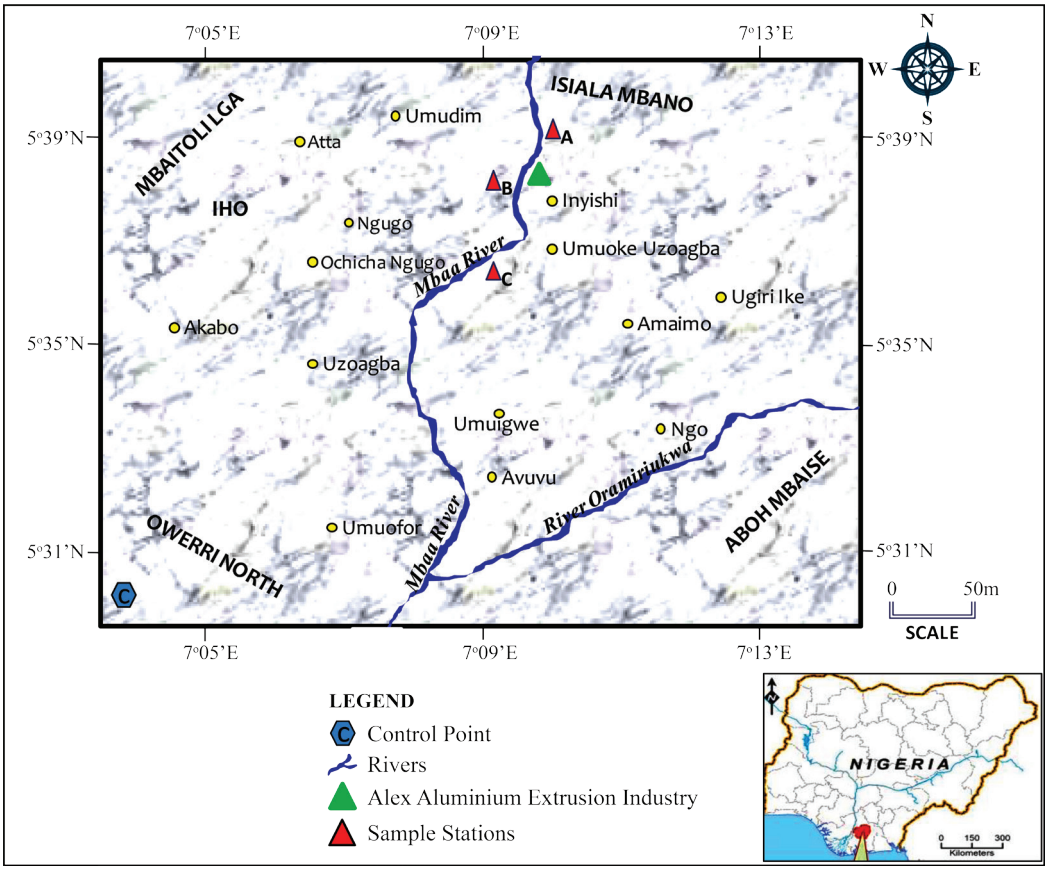

Figure 1. Map of Mbaa River showing the study area (Modified from NGSA, 2016).

$$\text{Condition factor (K)} = \frac{b}{f^3} \times 100$$

$$\text{Hepatosomatic Index (HSI)} = \frac{l}{b} \times 100$$

where,
b = body weight (g)
f = fork length (cm)
l = liver weight (g)

Collection of blood samples

Fish were sedated using clove oil at a concentration of 50 µL per liter of water prior to blood collection. Blood was drawn from the caudal vein using syringes pre-rinsed with 2.7 % ethylenediaminetetraacetate (EDTA) solution. The collected blood was immediately transferred into EDTA test tubes and gently mixed to prevent haemolysis. For serum collection, blood samples from a separate batch of fish were transferred directly into dried Eppendroff tubes. The tubes were positioned at an angle at room temperature to allow clotting. Once clot formation was completed, the samples were centrifuged at 10,000×g for 5 min, and the serum was carefully collected for biochemical analysis (Campbell, 2015).

Haematological and biochemical analyses

Red blood cells (RBC) and total white blood cell (WBC) counts were determined using a Neubauer-type hemocytometer. Toisson's solution was used as the diluting fluid for RBCs, while Turk's solution was used for WBCs, following the method of Rusia and Sood (1992). Differential leukocyte counts–including neutrophils, monocytes, lymphocytes, eosinophils, and basophils –were determined from stained blood smears according to Anderson (2003). Haemoglobin (Hb) concentration was measured using the cyanmethemoglobin method (Blaxhall and Daisley, 1973). Packed cell volume (PCV) was determined by centrifuging heparinized blood samples in glass capillaries at 14,000 × g for 5 min at room temperature (Nelson and Morris, 1989) using a microhematocrit centrifuge (Hawkesley and Sons, Ltd., Lancing, UK). PCV values were then read with a microhematocrit reader and expressed as a percentage of total blood volume. Standard formulas were used to calculate the following blood indices:, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC):

$$\text{Mean corpuscular volume (MCV)} = \frac{(PVC \times 10)}{RBC}$$

$$\text{Mean Corpuscular Haemoglobin (MCH)} = \frac{(Hb \times 10)}{RBC}$$

$$\text{Mean Corpuscular Haemoglobin Concentration (MCHC)} = \frac{(Hb \times 100)}{PVC}$$

Serum glucose, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were measured using commercial diagnostic kits (ERBA Diagnostic Mannhelm GmbH, Germany and Transasia Biomedical Ltd, India), following the manufacturers' instructions.

Statistical analysis

Data were analysed using SPSS version 20.0 (SPSS Inc., Chicago, Illinois, USA). Results are presented as mean±standard deviation. Normality was assessed using the Shapiro-Wilks test, and homogeneity of variance was tested using Levene's test. One-way analysis of variance (ANOVA) was performed to evaluate differences between sampling stations and the control group, followed by Duncan's multiple range tests to identify significant differences between means at $p<0.05$. Pearson correlation analysis was conducted using XLSTAT® 2014 to examine relationships between haematological and biochemical parameters. Principal component analysis (PCA) was also performed to assess variation in biomarkers among fish from different sampling sites.

RESULTS

Heavy metals concentration in water sample

Table 1 presents the concentrations of heavy metals in water samples collected from different sampling stations. As expected, Station B exhibited significantly higher ($p<0.05$) levels of all detected metals compared to the other stations and the control site. Site A had higher concentrations of Al, Pb and Zn than Site C, while Cd and Cu levels did not differ significantly between these two sites. Conversely, Fe concentration was higher at site C than at Site A. Notably, heavy metal concentrations at the control site were below detectable limits.

Morphological profile

The morphological indices of Nile tilapia in the Mbaa River (Table 2) showed no significant differences ($p\geq0.05$) in body weight, liver weight, or fork length across all sampling stations when compared with the control. However, fish from Station B exhibited significantly lower ($p<0.05$) condition factor and hepatosomatic index relative to the control and other stations, suggesting potential stress or health impairment due to environmental conditions at this site.

Haematological and biochemical parameters

The haematological indices of Nile tilapia from the Mbaa River (Table 3) showed no significant differences ($p>0.05$) in haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) across all sampling sites. However, a significant decrease ($p<0.05$) in RBC and PCV was observed in fish from Station B compared to the other sites and the control.

Table 1. Heavy metals concentrations detected in water samples collected from three different sites along the Mbaa River (Site A, B, and C) and a control site.

Heavy metal	Stations			Control	Maximum permissible limit WHO* (mg·L^{-1})
	A (mg·L^{-1})	B (mg·L^{-1})	C (mg·L^{-1})		
Al	0.25±0.12^{b}	2.87±0.05^{a}	0.62±0.08^{c}	ND	0.100
Pb	1.08±0.43^{b}	2.84±1.08^{a}	1.02±0.22^{c}	ND	0.010
Zn	0.69±0.01^{b}	1.74±0.05^{a}	0.78±0.06^{c}	ND	3.000
Cd	0.79±0.01^{b}	2.78±0.78^{a}	1.06±0.01^{b}	ND	0.003
Cu	0.82±0.04^{b}	1.78±1.03^{a}	0.83±0.54^{b}	ND	1.000
Fe	0.99±0.02^{b}	2.08±1.09^{a}	1.01±0.22^{c}	ND	0.300

Note: Mean±SD within a row superscripted with different lowercase letters significantly differ ($p<0.05$); ND = not detected; *Nik-Abdul-Ghani *et al.* (2021)

Table 2. Morphological indices of Nile tilapia collected from three sites along the Mbaa River (Site A, B, and C) and the control site.

Parameter	Stations			Control
	A	B	C	
Body weight (g)	35.06±0.37^{a}	34.83±0.81^{a}	34.74±0.63^{a}	34.86±0.13^{a}
Liver weight (g)	0.29±0.05^{a}	0.30±0.04^{a}	0.30±0.03^{a}	0.30±0.02^{a}
Fork length (cm)	12.84±1.27^{a}	12.87±1.14^{a}	12.65±1.17^{a}	12.82±0.05^{a}
K	1.69±0.08^{a}	1.45±0.03^{b}	1.67±0.79^{a}	1.73±0.15^{a}
HSI	0.84±0.11^{a}	0.59±0.04^{b}	0.87±0.99^{a}	0.97±0.05^{a}

Note: Mean±SD within a row superscripted with different lowercase letters are significantly different ($p<0.05$); condition factor, HSI = hepatosomatic index

Table 3. Haematological parameters of Nile tilapia collected from three sites along the Mbaa River (Site A, B, and C), and a control site.

Parameter	Stations			Control
	A	B	C	
Haemoglobin ($g \cdot dL^{-1}$)	6.20±0.20[a]	6.27±0.75[a]	6.23±1.15[a]	6.37±0.20[a]
RBC ($\times 10^6$ $cell \cdot mm^{-3}$)	1.38±0.28[a]	1.25±0.25[b]	1.38±0.23[a]	1.37±0.08[a]
PCV (%)	22.70±0.13[a]	20.67±0.15[b]	22.27±0.06[a]	21.66±0.05[a]
MCV ($fl \cdot cells^{-1}$)	151.66±1.03[a]	150.90±1.06[a]	149.82±1.07[a]	152.03±1.02[a]
MCH (pg)	41.74±0.08[a]	41.81±0.07[a]	41.78±0.05[a]	42.02±0.04[a]
MCHC ($g \cdot dL^{-1}$)	28.57±0.03[a]	28.89±0.59[a]	27.94±0.06[a]	29.21±0.01[a]
WBC ($\times 10^4$ $cell \cdot mm^{-3}$)	4.05±1.45[a]	4.89±1.32[b]	4.02±1.44[a]	4.00±1.51[a]

Note: Mean±SD within a row superscripted with different lowercase letters significantly differ ($p<0.05$).

Conversely, white blood cell (WBC) counts were significantly elevated ($p<0.05$) in fish from Station B relative to the control and other stations, possibly indicating an immune response to environmental stressors.

The mean values of neutrophils, lymphocytes, eosinophils, and basophils did not vary significantly among sampling sites ($p\geq0.05$). However, monocyte levels tended to be higher at Sites A and B and lower at Site C compared to the control, although these differences were not statistically significant ($p\geq0.05$) (Table 4).

Levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in fish from Site B were not significantly different ($p\geq0.05$) from those in other stations and the control. However protein levels were significantly lower ($p<0.05$), while glucose levels were significantly higher ($p<0.05$) in fish from Station B compared to other stations and the control (Table 5).

Pearson correlation analysis revealed significant relationships between several biochemical and haematological parameters (Table 6). PCV had a strong positive correlation with RBC ($r = 0.905$, $p<0.05$), while MCH was positively correlated with Hb ($r = 0.985$, $p<0.05$). In contrast, WBC exhibited a significant negative correlation with RBC ($r = -0.993$, $p<0.05$) and PCV ($r = -0.851$, $p<0.05$). LDH activity was negatively correlated with RBC ($r = -0.906$, $p<0.05$) but positively correlated with WBC ($r = 0.913$, $p<0.05$), suggesting a potential link between immune response and metabolic stress. Additionally, protein levels showed a significant negative correlation with MCV ($r = -0.982$, $p<0.05$) (Table 6).

Table 4. Differentials white blood cell counts of Nile tilapia collected from three sites along the Mbaa River (Site A, B, and C), and a control site.

Parameter	Stations			Control
	A	B	C	
Neutrophil	22.99±1.42[a]	22.76±1.57[a]	21.34±1.05[a]	21.08±1.58[a]
Lymphocytes	18.33±0.92[a]	18.56±1.02[a]	18.38±0.96[a]	18.14±0.05[a]
Monocytes	5.16±0.13[ab]	5.19±0.17[ab]	5.06±0.07[a]	5.02±0.08[b]
Eosinophils	3.31±0.02[a]	3.29±0.17[a]	3.32±0.05[a]	3.24±0.11[a]
Basophils	1.96±0.07[a]	1.86±0.06[a]	1.89±0.03[a]	1.65±0.04[a]

Note: Mean±SD within a row superscripted with different lowercase letters significantly differ ($p<0.05$).

Table 5. Biochemical parameters of Nile tilapia collected from three sites along the Mbaa River (Site A, B, and C) and a control site.

Parameter	Stations			Control
	A	B	C	
ALT (iu·L^{-1})	85.12±1.12^{a}	85.09±1.13^{a}	85.61±1.11^{a}	85.44±1.15^{a}
AST (iu·L^{-1})	120.54±0.08^{a}	121.01±0.07^{a}	120.98±0.05^{a}	121.01±0.06^{a}
LDH (iu·L^{-1})	50.44±0.03^{a}	51.02±0.01^{a}	49.99±0.09^{a}	50.27±0.07^{a}
Glucose (mg·dL^{-1})	40.44±1.04^{c}	45.45±0.09^{a}	43.01±1.07^{b}	41.08±1.07^{c}
Protein (g·dL^{-1})	35.24±0.07^{b}	33.28±0.07^{a}	37.21±0.08^{b}	41.08±0.07^{c}

Note: Mean±SD within a row superscripted with different lowercase letters significantly differ ($p<0.05$); LDH = lactate dehydrogenase; ALT = alanine aminotransferase; AST = aspartate aminotransferase

Table 6. Pearson correlations between haematological and biochemical parameters of Nile tilapia collected from the Mbaa River.

Variables	Hb	RBC	PCV	MCV	MCH	MCHC	WBC	ALT	AST	LDH	Glucose	Protein
Hb	–											
RBC	-0.096	–										
PCV	-0.488	**0.905**	–									
MCV	**-0.822**	-0.476	-0.063	–								
MCH	**0.985**	0.073	-0.331	**-0.899**	–							
MCHC	0.747	-0.353	-0.528	-0.383	0.709	–						
WBC	-0.020	**-0.993**	**-0.851**	0.574	-0.187	0.274	–					
ALT	0.256	0.582	0.300	-0.640	0.328	-0.433	-0.621	–				
AST	0.641	-0.399	-0.698	-0.421	0.550	0.160	0.320	0.474	–			
LDH	0.009	**-0.906**	-0.733	0.549	-0.129	0.514	**0.913**	**-0.864**	0.028	–		
Glucose	-0.036	**-0.862**	-0.798	0.455	-0.200	-0.078	**0.866**	-0.169	0.610	0.586	–	
Protein	0.702	0.632	0.248	**-0.982**	0.806	0.256	-0.717	0.694	0.292	-0.681	-0.579	–

Note: Values in bold are significantly different from zero ($p<0.05$).

Principal component analysis in blood and biochemical parameters of the fish

Figure 2a presents the PCA correlation circle, which illustrates the relationships among haematological and biochemical parameters in *O. niloticus* across the sampling sites. The plot shows that white blood cell (WBC) count, lactate dehydrogenase (LDH), and glucose are positively associated, indicating a co-occurrence of stress-related responses. These variables load strongly along the positive axis of PC1, suggesting they are major contributors to the separation of physiological profiles.

In contrast, red blood cell (RBC) count and packed cell volume (PCV) load in the opposite direction along PC1, showing a negative correlation with stress indicators. Haemoglobin (Hb), mean corpuscular haemoglobin (MCH), and serum protein exhibit moderate loadings and are located away from the cluster of stress-related markers, reflecting their potential association with normal physiological status. The pattern observed in the loading plot supports the hypothesis that fish exposed to industrial effluent (particularly at Site B) experience haematological alterations consistent with physiological stress.

The PCA biplot (Figure 2b) further illustrates the separation of fish sampled from different sites based on their haematological and biochemical profiles. Fish from Site B (middle stream) are associated with elevated glucose, WBC, and LDH levels, while those from the control site exhibit higher protein, Hb, and MCH. RBC and PCV were negatively correlated with stress-related biomarkers, suggesting physiological alterations due to heavy metal exposure near the effluent discharge point.

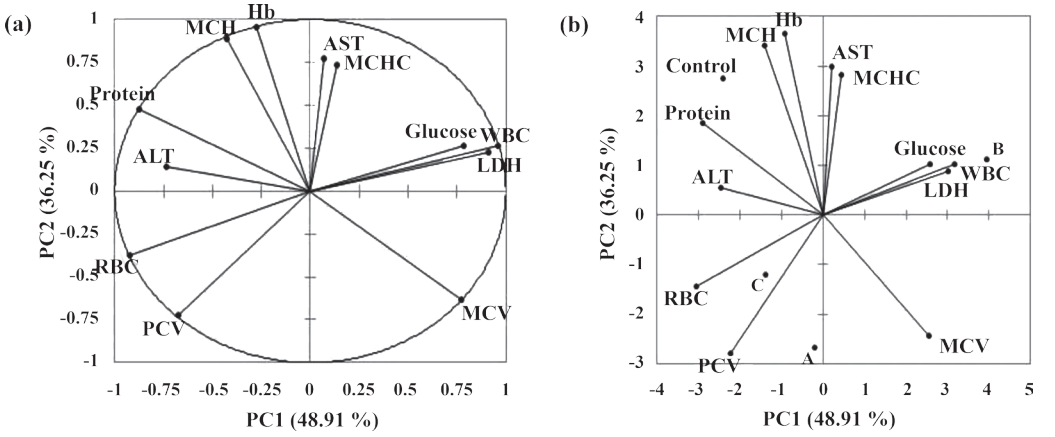

Figure 2. PCA of haematological and biochemical parameters in Nile tilapia from three sites along the Mbaa River: (a) the PCA correlation circle representing the relationships among haematological and biochemical parameters; (b) Biplot illustrating variable loadings and sample clustering by site.

DISCUSSION

This study was conducted out of concern for the potential impacts of wastewater discharge from an aluminum factory into the river, which could ultimately affect aquatic animals and humans. Results revealed that the concentrations of Al, Pb, Cd, and Fe in water from all three sites exceeded the WHO (2017) standards, with the highest levels detected at Site B, located nearest to the wastewater discharge point. Similarly, Cu and Zn concentrations were elevated at Site B, although only Cu exceeded the recommended safety threshold.

The detection of these metals is consistent with the nature of aluminum production. Aluminum is extracted from bauxite ore, which naturally contains impurities such as iron (Fe) and trace amounts of heavy metals including lead (Pb) and cadmium (Cd) (Sharma *et al*., 2019). During processing, these metals can be released into wastewater streams. Copper (Cu) and zinc (Zn), though not directly used in the aluminum smelting process, may originate from industrial equipment corrosion, piping systems, or auxiliary materials used in the facility. Inadequate treatment of industrial effluent can lead to the accumulation of these metals in surrounding aquatic environments, as observed in this study (Xue *et al*., 2016).

Morphological indices as indicators of stress

While body sizes were not different, K and HSI were significantly lower in the fish collected from Site B. Morphological parameters such as the condition factor (K) and hepatosomatic index (HSI) are useful as exposure indices for environmental pollutants (Elias *et al*., 2020). The decrease in K at Site B indicated that fish were in poorer condition (Froese, 2006; Abowei, 2010), and similar findings have been reported in fish exposed to environmental contaminants (Meador *et al*., 2018).

The hepatic enzyme responsible for detoxifying hazardous chemicals in exposed fish is correlated with liver size, represented by HSI (Ardeshir *et al*., 2017; Weinrauch *et al*., 2021). Increased HSI is typically associated to detoxification activity in response to xenobiotics (Long *et al*., 2020). In this study, the significant reduction in K and HSI of fish from Station B compared with the control and other stations indicates a decline in fish growth and condition attributable to the metabolic effects (Popovic *et al*., 2023) of multiple heavy metals including cadmium and lead, in close proximity to the aluminum industry. Heavy metals such as cadmium, lead, and chromium, commonly found in industrial discharges and municipal landfills, negatively affect fish growth, survival, and biochemical indices due to their high toxic potential (Carolin *et al*., 2017; Thitiyan *et al*., 2021; Swangneat *et al*., 2024). Factors that prevent the allocation of energy toward somatic growth, along with additional energy demands under stress, may contribute to reduced health condition in animals (Maulvault *et al*., 2017; Anacleto *et al*., 2018). Under stress, energy reserves are utilized, which may result in decreased HSI (Ciftci *et al*., 2015). Heavy metals may contribute to reduced K and HSI in fish by damaging the liver which is a key organ for detoxification, and causing oxidative stress (Kumar *et al*., 2024). Similarly, Ciftci *et al*. (2015) reported a reduction in K and HSI in Nile tilapia treated with cadmium.

The present study also revealed alterations in blood parameters of fish from the river, including a reduction in red blood cell (RBC) counts. A decrease in RBC may indicate anemia, possibly due to inhibition of erythropoiesis by metallic ions from industrial sources. Changes in haematological parameters could be compensatory responses to maintain gas exchange in the gill lamellae and may also indicate variations in blood congestion (Dias *et al*., 2023; Kanu *et al*., 2023). This depletion may result from impaired osmoregulation across the gill epithelium due to xenobiotic accumulation (Zhang *et al*., 2018). Comparable results were observed in *O. niloticus* exposed to verapamil (Ajima *et al*., 2017), *Paralichthys olivaceus* exposed to waterborne zinc (Kim *et al*., 2019), and in *C. gariepinus* treated with agrochemicals (Amaeze *et al*., 2020). Haematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are important in evaluating anemia and the oxygen-carrying capacity of fish (Iheanacho and Odo, 2020).

The main role of WBCs is to protect the body from infections and foreign substances and to produce and transport antibodies during immune responses. Increases in WBC counts in fish may indicate an immune response to the harmful effects of metals in the river. Elevated WBCs can also be associated with increased antibody production to counteract heavy metal toxicity. Elevated WBC levels have been reported in fish exposed to hexavalent chromium (Sivakumar *et al*., 2020), methyltestosterone (Sayed and Moneeb, 2015), drugs (Ajima *et al*., 2021), and industrial effluents (Sharma *et al*., 2023).

A general decrease in PCV and RBC, alongside increased WBC in fish at Station B, suggests physiological stress, potentially due to the toxic effects of cadmium and lead, while the elevated WBC indicates immune system activation in response to heavy metal toxicity.

Alterations in differential leukocyte counts are often noted as indicators of acute stress (Fazio *et al*., 2019; Ligina *et al*., 2022). In this study, increased neutrophils, lymphocytes, eosinophils, monocytes, and basophils were observed in exposed fish compared to the control, although the increases were not statistically significant. These changes may reflect an immune response to toxic metal exposure. In fish, differential leukocytes are found in connective tissue and are often elevated during chronic inflammation or periods of immune activation (da Silva Correa *et al*., 2017; Guerrera *et al*., 2021). Monocytes, which can differentiate into macrophages, play a key role in innate immunity by phagocytosing pathogens, and also acting as antigen-presenting cells to activate the adaptive immune system (Hodgkinson *et al*., 2015). An increase in monocytes levels among other WBC subtypes at the station suggests that the fish's immune system is actively responding to the impact of heavy metals from the aluminum industry.

Heavy metals may also cause blood system changes by directly affecting bone marrow precursors, inhibiting enzymes responsible for cell division, or causing immune-mediated cell destruction (Han *et al*., 2019). Similarly, da Silva Correa *et al.* (2017) found no significant differences in total leukocytes, lymphocytes, neutrophils and basophils, but observed increased monocytes and eosinophils in *O. niloticus* sampled in Brazil. Sivakumar *et al*. (2020) also documented a significant elevated neutrophils and lymphocytes with no significant variation in eosinophil, monocytes and basophils counts in *Labeo rohita* treated with hexavalent chromium.

Transaminases such as AST and ALT are typically present at low concentrations but may increase in circulation following organ damage (Ajima *et al*., 2021; Wang *et al*., 2022). In this study, AST, ALT and LDH in fish serum did not differ significantly from the control, indicating that the contaminants may not have caused severe physiological dysfunction in the fish liver. In contrast, Mohamed *et al*. (2019) observed suppressed AST, and ALT activity in *Tilapia zillii* and *Mugil capito* in Qarun Lake, possibly due to toxicant interference with enzyme synthesis.

Although ALT and AST are commonly used to assess liver damage their unchanged levels, along with reduced HSI, in this study may indicate impaired liver growth rather than overt necrosis. This could be due to adaptive responses such as reduced cell proliferation or apoptosis, without significant membrane damage (Soler *et al*., 2020).

Pollutants, including heavy metals, may lead to increased glucose and decreased protein levels in fish due to oxidative stress and disruption of normal metabolism (Ajima *et al*., 2018). The increased glucose concentration observed at Site B may be attributed to stress-induced mobilization of glycogen stores as part of the fish's adaptive response (Cui *et al*., 2017). This increase in glucose could be linked to enhanced carbohydrate metabolism, particularly the activation of hepatic glucose 6-phosphatase enzymes, which catalyze glucose catabolism to meet elevated energy demands under stress caused by heavy metal exposure from the aluminum industry.

Consistent with our findings, *T. putitora* from the Panjkora River also exhibited increased glucose and decreased total protein levels due to heavy metal contamination from hydropower construction effluents (Ullah *et al*., 2021). Shah and Wondisford (2023) further explained that the gluconeogenic pathway contributes to glucose synthesis from amino acids through hepatic enzymes, especially under stressful conditions. In general, organisms experiencing stress often exhibit altered carbohydrate metabolism as a physiological response to shifting energy requirements (Shivaraj and Asiya, 2018).

Proteins are vital for tissue building in organisms and play a crucial role in energy metabolism, especially under stressful conditions (de Magalhães *et al*., 2020). The physiological status of an organism can be assessed through its protein metabolism (Rajeswari and Sunita, 2021). The observed decrease in total serum protein levels at Site B may indicate pathological changes in hepatic and renal tissues due to heavy metals exposure from the nearby aluminum industry, as well as overall physiological stress (Nwani *et al*., 2014; Samtiya *et al*., 2020; Emon *et al*., 2023). Similar reductions have been reported in *C. gariepinus* exposed to drugs (Ogueji *et al*., 2017) and in *O. niloticus* from Buriganga River, where heavy metal pollution significantly reduced glucose and serum protein levels (Hossain *et al*., 2021).

Correlation analysis showed strong relationship between haematological, biochemical, and environmental parameters. PCV was strongly correlated with RBC, and MCH with Hb. In contrast, LDH activity and glucose levels were negatively correlated with RBC but positively correlated with WBC, indicating a potential link between immune activation and metabolic stress.

PCA results supported these findings, indicating distinct physiological patterns among sampling sites. Fish from Site B, with the highest concentrations of heavy metals, were associated with elevated glucose, WBC, and LDH, and reduced PCV and RBC. The control group, by contrast, showed high protein, MCH, Hb, and ALT levels.

Overall, physiological dysfunction in fish at Site B appears primarily attributable to Al, which was detected at the highest concentration among all the measured metals.

CONCLUSIONS

This study showed that *O. niloticus* from the Mbaa River exhibited significant haematological and morphological alterations compared to those from a control site, indicating contamination with heavy metals, particularly Al, Pb and Cd, from a nearby aluminum industry. Detected metal concentrations exceeded WHO (2017) safety limits, posing a clear risk to aquatic life and potentially to human health through fish consumption.

To deepen understand of heavy metal pollution and propose practical mitigation strategies for sustainable aquatic ecosystems, future research should explore tissue histology, DNA damage, molecular responses, and toxicological mechanisms.

These findings emphasize the need for regular monitoring and demonstrate the critical importance of proper treatment of industrial effluents before discharge to minimize environmental and public health risks.

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