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RESEARCH ARTICLE

The bioconcentration of metals in *Oreochromis niloticus* and *Pangasius* sp. from a commercial fishpond

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ABSTRACT

The accumulation of metals in aquatic organisms is a growing environmental and public health concern, often linked to contamination from industrial and agricultural sources. This study investigated the concentrations of iron (Fe), zinc (Zn), and copper (Cu) in *Oreochromis niloticus* and *Pangasius* sp., collected from a commercial fishpond in Marang, Terengganu. Metal concentrations in fish tissues were determined using acid digestion followed by flame atomic absorption spectrophotometry (FAAS). Results showed that *Pangasius* sp., a bottom-dwelling species, accumulated significantly higher levels of Fe (271 mg/kg), Zn (50.6 mg/kg), and Cu (16.2 mg/kg) as compared to *O. niloticus*, a column feeder. These differences are likely due to species-specific habitat preferences and feeding behaviours, with *Pangasius* sp. being more exposed to sediment-associated metals. To evaluate potential human health risks, Target Hazard Quotients (THQs) were calculated, indicating possible non-carcinogenic effects. Importantly, the concentrations of all metals exceeded the permissible limits set by the World Health Organisation (WHO) and the Malaysian Food Act. These findings highlight the need for ongoing monitoring of aquaculture environments and the implementation of effective management strategies to minimise metal contamination and ensure food safety.
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1. INTRODUCTION

Fish, especially those occupying higher trophic levels, are prone to accumulating elevated concentrations of heavy metals through dietary intake and gill absorption, which may adversely affect their health and physiological functions (Huang et al., 2023). This issue raises particular concern in Malaysia, where fish consumption is significantly high, averaging approximately 168 g/day, the second highest globally after Japan (Che Abdullah et al., 2022). Fish are widely consumed as an affordable and accessible source of high-quality protein, essential amino acids, and beneficial polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Annette et al., 2018). However, the nutritional benefits of fish can be compromised when heavy metals accumulate in their tissues, potentially introducing harmful contaminants into the human diet. Moreover, fish are commonly regarded as reliable bioindicators of aquatic ecosystem health, providing important insights into environmental contamination and associated risks to public health (Roy et al., 2022). This is particularly relevant in the context of heavy metals, which are widely recognised as major pollutants in aquatic environments due to their toxicity, non-degradability, persistence, and capacity for bioaccumulation and biomagnification across trophic levels (Saravanan, 2024). Heavy metals can enter the environment through both natural sources and human-induced activities, including industrial effluents, agricultural drainage, and urban expansion. Fish and shellfish act as key accumulators within the aquatic food chain, making their consumption a major route of human exposure to these contaminants (Ray & Vashishth, 2024).

Essential trace elements, including iron (Fe), zinc (Zn), and copper (Cu), play critical roles in maintaining normal biological processes; however, when these and other toxic metals accumulate beyond permissible limits, they can exert detrimental effects on human health, even at minimal concentrations (Alipour et al., 2021). Prolonged exposure to contaminants such heavy metals has been linked to various physiological dysfunctions, including carcinogenic outcomes, with children particularly vulnerable to developmental impairments and other serious health risks (Varol et al., 2017; Ullah et al., 2017). Therefore, the bioaccumulation of heavy metals in fish represents a significant concern for food safety and public health, especially when concentrations exceed the permissible limits (Hossain et al., 2022).

Both *Pangasius sp.* and *O. niloticus* exhibit omnivorous feeding behaviours that play an important role in shaping their metal accumulation potential. *Pangasius* sp. is typically a bottom-feeding species that consumes a variety of organisms, such as algae, zooplankton, higher plants, insects, shrimps, and small fish, supporting its benthic omnivorous nature (Ali et al., 2005). Similarly, *O. niloticus* demonstrates omnivore with dietary shifts across its lifespan, where juveniles mainly feed on animal-based foods including zooplankton, phytoplankton, insects, and detritus, while adults shift towards plant-based diets dominated by macrophytes, followed by phytoplankton and detritus (Tesfaye et al., 2020).

Several factors determine the level of metal accumulation in fish, including their position within the food chain, the availability of metals for uptake, and the intensity of environmental contamination. In aquaculture systems, contaminated feed and poor water quality have been identified as key contributors to elevated metal levels in farmed fish (Fatema et al., 2019; Rajeshkumar & Li, 2018). This is particularly relevant in Malaysia, where freshwater aquaculture has been practiced for over five decades, with key species including river catfish (*Pangasius* sp.), freshwater catfish (*Clarias* sp.), and tilapia (*Oreochromis* sp.) commonly reared under monoculture systems (Hanafi et al., 2000). Amongst these, *Oreochromis niloticus* (tilapia) contributes significantly to national production, with yields reaching over 32,000 metric tonnes and generating a wholesale value of MYR 299.7 million in 2017. Similarly, *Pangasius* sp. is widely cultured due to its fast growth rate and consumer preference (Mohammad Ridzuan et al., 2020). Despite the growing demand, the heavy reliance on formulated commercial feeds, often incorporating fishmeal have raises additional concerns, as substandard feed ingredients and polluted water sources may introduce toxic metals into aquaculture systems (Kundu et al., 2017). This highlights the need for continuous monitoring of heavy metal contamination in farmed fish to safeguard consumer health.

Therefore, this study was conducted to determine the extent of heavy metal accumulation in *O. niloticus* and *Pangasius sp.* from commercial pond, and to analyse the related human health implications of consuming these species. These two species are widely cultivated and frequently consumed due to their rapid growth, adaptability to local aquaculture systems, and affordability as compared to other protein sources. Given their role as a staple and accessible source of dietary protein for the local population, understanding their safety and potential health implications is crucial for ensuring food security and protecting public health.

2. MATERIALS AND METHODS

2.1. Sampling area

The study was conducted at a fish farm located in Marang, Terengganu, situated at coordinates 5°39'00"N, 101°45'41"E. The pond is a soil-based aquaculture system, measuring approximately 25 m by 25 m with an average depth of 2.5 m (Figure 1). Aeration devices were installed to improve dissolved oxygen levels and maintain water quality. The pond was supplied with groundwater as the main water source, and water replenishment was conducted on a top-up basis during the culture period without complete water exchange. The fish stocked in this pond were juvenile *O. niloticus* and *Pangasius* sp., which were reared until marketable size under standard commercial aquaculture practices.

This pond was selected as the sampling site due to the absence of prior studies evaluating its metal contamination levels. Operational since 2019, it is primarily used for commercial freshwater fish farming. Fish harvested from this pond are distributed locally, including at the Marang fish market, serving as an important and affordable source of protein for the surrounding community. It should be noted that soil sample or water testing was not conducted in this study, and the assessment focused solely on metal concentrations in fish muscle tissues. Therefore, while metal accumulation in fish may originate from groundwater, commercial feed, or sediment interaction, the present study does not differentiate between these sources. This limitation is acknowledged and highlights the need for future studies to include parallel assessments of pond soil and water quality to better trace the sources of heavy metal exposure.

Figure 1: Sampling location of commercial pond

2.2. Sample collection

Specimens of *O. niloticus* (tilapia) and *Pangasius* sp. (river catfish) were purchased directly from a local fish farmer to ensure proper species identification and traceability. A total of twenty individuals from each species were collected for the analysis of trace metals in muscle tissues. This sample size was considered sufficient based on a study by Duran et al., (2014). To preserve sample integrity, all fish were immediately placed in low-density polyethylene (LDPE) sampling bags and transported to the laboratory in an icebox under chilled conditions. Upon arrival at the laboratory, the fish were thoroughly rinsed with deionised water to remove any external debris. Species identification was carried out according to the taxonomic guidelines of Kottelat (2013). The identification process involved examination of external morphological features such as body shape, colouration, fin configuration, scale patterns, and meristic counts (e.g., dorsal, anal, and pectoral fin rays). These diagnostic characteristics were compared against the reference descriptions by Kottelat (2013). Prior to sample preparation, the maturity stage of each fish was determined by measuring total length and weight, following the criteria described by Mohsin and Ambak (1991), which remains a widely cited and valid reference in Malaysian ichthyological and aquaculture research due to the absence of more recent standardised criteria for these species.

Only mature fish (weight & length; 100–150 g and 14–16 cm for *O. niloticus* and 200–250 g and 20–25 cm for *Pangasius* sp.), as determined according to the criteria described by Mohsin and Ambak (1991), were selected for the analysis of trace elements in this study (Muscle samples were obtained from the dorsal region, specifically from above the lateral line and between the dorsal fin and caudal peduncle, using sterilised stainless-steel scalpels. The collected muscle tissues were oven-dried at 110 °C for 24 h. After drying, the samples were ground into a fine, homogeneous powder by using a pestle and mortar and subsequently sieved through a 355-micron mesh to ensure uniform particle size. The processed samples were stored in amber glass containers and kept in a desiccator until further analysis, including acid digestion and metal concentration determination.

2.3 Sample digestion and metal analysis

Prior to analysis, all non-metallic laboratory equipment underwent pre-treatment by soaking overnight in 10% nitric acid, followed by multiple rinses with deionised water to prevent potential contamination. The concentrations of Fe, Zn, and Cu in the muscle tissues of *O. niloticus* and *Pangasius* sp. were quantified through acid digestion, followed by analysis by using Flame Atomic Absorption Spectrophotometer (FAAS). For the digestion procedure, 1.0 g of homogenized muscle tissue was accurately weighed and transferred into a 50 mL glass beaker. Subsequently, 10 mL of concentrated nitric acid (HNO_3, 69% w/w, analytical grade) was added. The beaker was then covered with a watch glass and gently heated on a magnetic stirrer hot plate. The temperature was first stabilised at 40 °C for 1 h to prevent excessive reactivity, followed by an increase to 140 °C, where it was maintained for an additional 3 h to facilitate complete digestion of organic content. After digestion, the solutions were visually inspected to confirm complete dissolution of the samples. Once cooled to room temperature, each digested sample was transferred into a 250 mL volumetric flask and topped up to the mark using double-distilled water. The resulting solutions were filtered through Whatman No. 1 filter paper, followed by further filtration through 40-micron syringe filters into 15 mL centrifuge tubes. All filtered extracts were kept at 4 °C prior to heavy metal determination by FAAS.

2.4. Estimation of health risk

Target Hazard Quotient

Several established methods are available to estimate the potential health risks associated with the consumption of heavy metals in fish. In this study, the Hazard Quotient (HQ) approach was employed to assess the level of human health risk resulting from exposure to Fe, Zn, and Cu through fish consumption. The calculation was based on the guidelines provided by the USEPA Region III Risk-Based Concentration Table (Amirah et al., 2013). The equation used to determine the Target Hazard Quotient (THQ) is as follows:

$$THQ = \frac{EF\ x\ ED\ x\ FIR\ x\ C}{RfD\ x\ WAB\ x\ TA} \text{ x } 10^{-3}$$

Where EF is exposure frequency (365 days/year), ED is exposure duration; 70 years (Agusa et al., 2007), FIR is fish ingestion rate; fish: 71 g day^{-1} $person^{-1}$ (FAO, 2008), C is metal concentration in muscle of studied fish; edible fish part (mg/kg; wet weight), RfD is oral reference dose (Amirah et al., 2013), WAB is average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Lim, 2000), TA is average time of exposure; 365 day/year multiplied by ED (Wei et al., 2014).

Hazard index (HI)

The cumulative non-carcinogenic health risk was expressed as the Hazard Index (HI), calculated as the sum of individual Target Hazard Quotients (THQs) for various metals, as described by the USEPA (2011), and is expressed as follows:

$$HI = THQ(\text{Fe}) + THQ(\text{Zn}) + THQ(\text{Cu})$$

2.5. Data analysis

All data were expressed as mean ± standard deviation. Statistical analyses were performed using Microsoft® Excel 2020 and IBM SPSS Statistics software. Prior to conducting the one-way analysis of variance (ANOVA), the data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Both assumptions were satisfied ($p > 0.05$), validating the use of ANOVA to assess significant differences in the concentrations of the analysed heavy metals (Razali & Wah, 2011).

2.6. Ethical consideration

All experimental procedures involving fish were conducted in accordance with the guidelines for the care and use of laboratory animals. The research protocol was reviewed and approved by Research and Innovation Committee, Faculty of Earth Science, Universiti Malaysia Kelantan (Approval No.: UMK/FSB/ACUE/0T/1/2025).

3. RESULT AND DISCUSSION

3.1. Fish morphometric data

Numerous studies have highlighted that, to a certain extent, the concentrations of accumulated metals in fish can be influenced by factors such as weight, length, and age (Li et al., 2020; Tengku et al., 2019; Hajeb et al., 2009). The significant relationship between body size (specifically length and weight), serves as a key indicator of fish growth and metal concentration is closely linked to metabolic processes and fat dilution effects (Li et al., 2020).

Thus, to reduce potential variability associated with size differences, efforts were made to sample fish of relatively similar sizes in this study, with their morphometric measurements summarised in Table 1. This approach aimed to minimise the possible influence of size and age differences on metal accumulation patterns. However, the results showed noticeable differences in the weight and/or length between the two fish species studied.

Many studies have shown that fish size (length and weight) may influence the accumulation of heavy metals in fish tissues. Notably, juvenile fish with smaller body sizes often exhibit higher metal concentrations, possibly due to the underdeveloped metabolic systems and organ functions that reduce their ability to regulate metal uptake efficiently (Mortazavi et al., 2012; Li et al., 2020).

Conversely, a negative correlation between heavy metal concentration and fish size has been widely reported, where metal concentrations tend to decrease with increasing fish size. For example, Farkas et al. (2003) observed reduced metal concentrations in larger fish. Similarly, Nussey et al. (2000) found that levels of chromium, nickel, and lead decreased with increasing fish length in *Labeo umbratus.*

This trend is commonly attributed to the growth dilution effect, whereby as fish grow, metals become more diluted within their larger body mass, coupled with possible declines in metabolic uptake rates (Mok et al., 2012). However, it should be acknowledged that in heavily polluted environments, this dilution effect may be overridden, leading to higher metal accumulation regardless of fish size (Balzani et al., 2022).

As shown in Table 1, the mean size of *Pangasius sp.* (1103.33 g; 75.0 cm) was substantially larger than that of *O. niloticus* (605.04 g; 57.43 cm). However, size variation in freshwater fish rarely shows a significant correlation with metal concentrations, since internal metal levels are typically regulated through biological mechanisms (Balzani et al., 2022). The condition factor (CF) values calculated for both species were 0.2 in *Pangasius sp.* and 0.3 in *O. niloticus*, indicating that despite being reared under the same culture environment, differences in condition exist between the two species. Table 2 presents the classification of fish condition based on CF values according to Barnham and Baxter (2003), where higher CF values represent better condition.

Table 1: Biometric information related to of *Pangasius* sp. and *Oreochromis niloticus*.

Fish species	*Pangasius* sp.	*Oreochromis niloticus*
Weight (g)	1103.33 ± 3.00	605.04 ± 1.20
Length (cm)	75.00 ± 2.5	57.43 ± 1.8
Condition factor (CF)	0.2 ± 0.02	0.3 ± 0.01

Mean ± standard deviation

Table 2: Condition factor (Barnham & Baxter, 2003)

Condition factor	Comments
1.60	Excellent condition, trophy class fish
1.40	A good, well-proportioned fish
1.20	A fair fish, acceptable to many anglers
1.00	A poor fish, long and thin
0.80	Extremely poor fish, resembling a barracouta, big head and narrow, thin body

Accordingly, tilapia (*O. niloticus*) displayed relatively better condition as compared to *Pangasius sp.*, which may reflect inherent biological and growth differences, such as tilapia showing a more proportional increase in weight and length, while *Pangasius* sp. allocates more energy to length growth.

3.2. Concentrations of heavy metals

Table 3 provides the concentrations of Fe, Zn, and Cu quantified in the muscle tissues of *Pangasius* sp. and *O. niloticus*, presented in mg/kg on a dry weight basis. For both species, the metals followed a consistent concentration trend of Fe > Zn > Cu (Figure 1), based solely on concentrations measured in muscle tissue. Results from the one-way analysis of variance (ANOVA) showed that the concentrations of Fe, Zn, and Cu differed significantly ($p < 0.05$) between *Pangasius* sp. and *O. niloticus.* The mean concentrations of Fe, Zn, and Cu in *Pangasius* sp. were 271.00 ± 0.60 mg/kg, 50.60 ± 0.05 mg/kg, and 16.20 ± 0.02 mg/kg, respectively, which were significantly higher compared to *O. niloticus*, which recorded 154.00 ± 0.25 mg/kg for Fe, 41.10 ± 0.03 mg/kg for Zn, and 14.50 ± 0.01 mg/kg for Cu. These results indicated that *Pangasius* sp. accumulated higher concentrations of heavy metals in muscle tissues compared to *O. niloticus*, despite both species being reared under similar aquaculture conditions.

Table 3: The mean heavy metal concentration of *Pangasius* sp. and *Oreochromis niloticus*.

Species	Mean heavy metal concentration (mg/kg d.w)		
	Fe	Zn	Cu
Pangasius sp.	271.00 ± 0.60	50.60 ± 0.05	16.20 ± 0.02
Oreochromis niloticus	154.00 ± 0.25	41.10 ± 0.03	14.50 ± 0.01
Permissible limit in fish			
FAO (2008)	-	40	30
MFA (1995)	-	100	30
USEPA (2000)	950	410	54

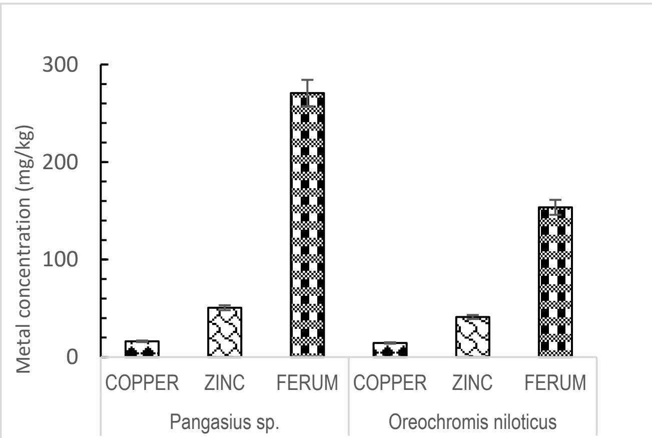

Figure 1: The mean concentration of metal in studied fishes

In this study, Fe was consistently detected at the highest concentrations in both *Pangasius* sp. and *Oreochromis niloticus*, while other metals were comparatively low. This pattern is consistent with the findings of Bashir et al. (2012), who reported Fe as the predominant metal in both water and fish tissues in the coastal waters of Kapar and Mersing. The elevated Fe accumulation may be linked to its natural abundance in soils and sediments, as well as its role as an essential micronutrient required for physiological processes such as haemoglobin synthesis. In soil-based pond systems, sediment resuspension and leaching further enhance Fe bioavailability, thereby amplifying its accumulation (Bashir et al., 2012). By contrast, the comparatively lower concentrations of other trace metals likely reflect their limited environmental inputs and the absence of significant anthropogenic contamination in the studied pond systems.

The distribution pattern reflects typical environmental bioavailability and aligns with previous studies, which reported that metal accumulation in fish tissues is not solely a function of environmental exposure, but also strongly influenced by species-specific physiological functions and biological traits (Uysal et al., 2008). Despite being cultured under identical pond conditions, *Pangasius* sp. consistently showed higher mean concentrations of all analysed metals than to *O. niloticus*. This suggests that interspecies differences in metal accumulation are not merely due to shared environmental factors but are also shaped by inherent biological and ecological differences. Supporting this, Kwok et al. (2014) highlighted that feeding behaviour, metabolic rate, body temperature, and bioconcentration capacity significantly affect metal bioaccumulation in fish.

A likely explanation for this disparity lies in the feeding strategies and habitat preferences of the two species. *Pangasius* sp. is a bottom-feeding omnivore, often foraging near pond sediments where metals from feed and surrounding water can settle and accumulate. In contrast, *Oreochromis niloticus* is a benthopelagic omnivore, feeding more within the water column and therefore potentially less exposed to sediment-associated contaminants (Mang & Jiraungkoorskul, 2020). The omnivorous diet of both species; consisting of aquatic insects, shrimp, small fish, algae, plankton, and detritus; also contributes to metal uptake, but the sediment-foraging behaviour of *Pangasius* sp. may enhance its exposure (Hashim et al., 2014). This pattern is consistent with the findings from Fatema et al. (2019) and Rajeshkumar & Li (2018), who reported higher metal loads in bottom-dwelling fish species due to direct interaction with contaminated sediments. Moreover, metal accumulation may also be influenced by aquaculture practices, particularly the use of commercial formulated feed. In many cases, substandard or contaminated raw ingredients used in feed manufacturing have been identified as potential sources of metal contamination in farmed fish (Das et al., 2018; Sabbir et al., 2018).

In addition to ecological and dietary factors, body size significantly influences metal accumulation, with larger fish generally exhibiting greater accumulation resulting from extended exposure durations. In this study, *Pangasius* sp. exhibited significantly greater mean body length and weight (75.00 ± 2.50 cm; 1103.33 ± 3.00 g) compared to *O. niloticus* (Table 1), which may have contributed to its higher metal burden. This is in line with observations by Hashim (2014) and Backstrom et al. (2020), who reported a positive correlation between fish size and metal accumulation. Taken together, these results underscore the multifactorial nature of metal bioaccumulation in fish. Feeding ecology, trophic behaviour, habitat preference, physiological characteristics, size, and feed quality all play interconnected roles. Collectively, these factors highlight the importance of understanding species-specific traits when assessing heavy metal risks in aquaculture systems (Wu et al., 2023).

3.3. Comparison with permissible limits

Table 3 presents the concentrations of iron (Fe), zinc (Zn), and copper (Cu) detected in *Pangasius* sp. and *O. niloticus,* which were evaluated against the permissible limits established by the Food and Agriculture Organisation (FAO, 2008), Malaysian Food Act (MFA, 1995), and the United States Environmental Protection Agency (USEPA, 2000). The mean Fe concentrations in both *Pangasius* sp. (271.00 ± 0.60 mg/kg) and *O. niloticus* (154.00 ± 0.25 mg/kg) were well below the USEPA (2000) permissible limit of 950 mg/kg, indicating that Fe levels in both species are within acceptable safety margins. However, no maximum permissible limits for Fe were reported by MFA (1995) and FAO (2008), and therefore these values were indicated as not available (NA) in Table 3.

For Zn, the FAO (2008) recommends a permissible limit of 40 mg/kg, while MFA (1995) and USEPA (2000) suggest 100 mg/kg and 410 mg/kg, respectively. The mean Zn concentration in *Pangasius* sp. (50.60 ± 0.05 mg/kg) slightly exceeded the FAO (2008) threshold but remained within the MFA (1995) and USEPA (2000) acceptable ranges (Figure 2). Meanwhile, *O. niloticus* recorded 41.10 ± 0.03 mg/kg, marginally above the FAO (2008) limit but significantly lower than MFA and USEPA standards. This suggests minimal potential health risks associated with Zn exposure from these species under local consumption patterns.

For Cu, the measured concentrations in *Pangasius* sp. (16.20 ± 0.02 mg/kg) and *O. niloticus* (14.50 ± 0.01 mg/kg) were well below all permissible limits, including FAO (30 mg/kg), MFA (30 mg/kg), and USEPA (54 mg/kg). This finding indicated that the Cu levels in both species are unlikely to pose a significant risk to consumers. Overall, while Fe, Zn, and Cu levels in the studied fish species are generally within safe limits according to most international guidelines, the slight exceedance of Zn concentration above the FAO (2008) limit in both species highlights the need for continuous monitoring, especially considering high fish consumption rates in Malaysia.

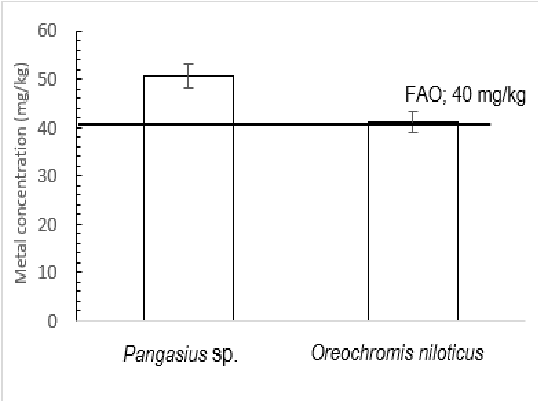

Figure 2: Concentration of Zinc in *Pangasius* sp. and *Oreochromis niloticus*

3.4. Health risk assessment of metals in fish

Based on the determined concentrations of Fe, Zn, and Cu in *Pangasius* sp. and *O. niloticus* from a commercial pond, a health risk assessment was conducted to evaluate their safety for human consumption. This assessment focused exclusively on the muscle tissue, which represents the edible portion of the studied fish species. The Target Hazard Quotient (THQ) method was employed to assess potential non-carcinogenic health risks associated with the consumption of these metals from both species. As shown in Table 4, the THQ values for Fe and Cu exceeded the threshold value of 1 in both species, indicating potential health risks from long-term consumption.

Notably, *Pangasius* sp. exhibited markedly higher THQ values for Fe (128.72) and Cu (17.94) compared to *Oreochromis niloticus* (Fe = 72.99; Cu = 45.57). In contrast, THQ values for Zn remained well below 1 for both species (0.56 in *Pangasius* sp. and 0.16 in *O. niloticus*). According to USEPA guidelines (USEPA, 2011), THQ values greater than 1 suggest that the estimated exposure surpasses the reference dose (RfD), potentially leading to adverse health effects with prolonged consumption.

In addition to individual metal risks, the cumulative non-carcinogenic risk was evaluated using the Hazard Index. The HI values were calculated at 147.22 for *Pangasius* sp. and 118.72 for *O. niloticus,* both substantially exceeding the safe limit of 1. These elevated HI values indicated a significant cumulative health risk associated with the combined effects of Fe, Zn, and Cu, particularly in *Pangasius* sp.

Table 4: Health risk estimates for Fe, Zn, and Cu from *Pangasius* sp. and *Oreochromis niloticus* based on the fish muscle tissue consumption.

Species	Hazard Quotient			Hazard index (HI)
	Fe	Zn	Cu	
Pangasius sp.	128.72	0.56	17.94	147.22
Oreochromis niloticus	72.99	0.16	45.57	118.72
RfD (mg/kg)	0.007	0.3	0.004	

The elevated THQ and HI values observed in this study were primarily driven by Fe and Cu, with Fe contributing the largest share of the overall risk. Meanwhile, the low THQ values for Zn are consistent with its essential biological function, as Zn is required in trace amounts for normal physiological processes (Dawood et al., 2022). The researcher's findings also align with previous studies that have reported elevated HI values associated with fish consumption. For instance, Biswas et al. (2023) found that the combined effect of multiple heavy metals (HI) exceeded the safe threshold (HI > 1) in several commercial fish species, with *Hilsa ilisha* recording the highest HI (26.7) and *Sillaginopsis panijus* the lowest (0.104). Similarly, Varol et al. (2017) documented even higher HI values, with *Liza mystaceus* from the Tigris River reaching 70.6. Compared to these studies, the HI values in the present study (147.22 for *Pangasius* sp. and 118.72 for *O. niloticus*) were substantially higher, highlighting a severe cumulative non-carcinogenic risk for consumers. Species-specific differences in metal accumulation, reflected in THQ and HI values, may be attributed to differences in feeding behaviour and habitat utilisation. *Pangasius* sp., a bottom-feeding omnivore, is more susceptible to ingesting sediment-bound metals, whereas *O. niloticus*, which primarily feeds in the water column, tends to accumulate comparatively lower metal concentrations (Fatema et al., 2019; Kwok et al., 2014).

In conclusion, this study results indicate that the regular consumption of these fish species, particularly *Pangasius* sp., may pose significant non-carcinogenic health risks, primarily due to elevated Fe and Cu concentrations, as evidenced by high THQ and HI values. Therefore, continuous monitoring of heavy metals in aquaculture environments and effective risk mitigation strategies are strongly recommended to ensure food safety and protect public health, particularly in high fish-consuming populations.

4. CONCLUSION

This study evaluated the concentrations of iron (Fe), zinc (Zn), and copper (Cu) in the muscle tissues of *Pangasius* sp. and *O. niloticus* cultured in a commercial pond, as well as the potential non-carcinogenic health risks based on the Target Hazard Quotient (THQ) and Hazard Index (HI). The findings revealed that THQ values for Fe and Cu exceeded the threshold value of 1 in both species, indicating possible health concerns with long-term consumption. Furthermore, the HI values for both fish were substantially higher than 1, suggesting cumulative non-carcinogenic risks from their intake.

Notably, *Pangasius* sp. exhibited higher mean concentrations of metals and greater THQ and HI values compared to *O. niloticus*, which may be attributed to differences in feeding behaviour and habitat preferences. Nonetheless, both species exhibited risk levels above permissible limits, suggesting that consumers may be equally exposed to health risks regardless of species preference. In summary, the consumption of *Pangasius* sp. and *O. niloticus* from the studied pond could contribute to non-carcinogenic health risks, predominantly linked to elevated Fe and Cu levels. These results emphasise the urgent need for regular monitoring of metal contamination in aquaculture systems and stricter quality control of feed inputs.

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