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An Assessment of Trace Metal Accumulation in the Fish Genus *Barbonymus* sp. from a Former Mining Lake in Kg. Gajah, Perak, Malaysia, and Its Potential Human Health Risk

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ABSTRACT

Ex-mining lakes are known to have elevated metal levels from past mining activities, thus, consuming fish originating from these lakes may pose potential health risks. The ability of fish to accumulate metals from the surrounding environment raised public concern about the health risks posed when consuming fish from former mining lakes. An investigation was carried out to quantify the concentrations of iron (Fe), zinc (Zn), and lead (Pb) in the water and organs (gills and muscle) of twenty *Barbonymus* sp. found in a former mining lake. Metal levels were measured using ICP-MS, and the results obtained were compared with their respective standards. A comparable Fe>Zn>Pb pattern was observed in the metal concentrations of both samples. Although the concentration of Pb in the water samples surpassed the limit of 0.166 mg/L, the levels of Fe and Zn were within the range set by the National Lake Water Quality Standards for Malaysia (NLWQS). The concentrations of iron in the fish muscles and gills are beyond the established thresholds set by the World Health Organisation (WHO) and Food and Agriculture Organisation (FAO). The concentration of Zn in the fish gills exceeded the FAO standard limit, and the levels of Pb in both organs exceeded the acceptable limits set

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by all regulations, including the Malaysian Food Act 1983 (MFA). Notably, the incremental life cancer risk (ILCR) for lead (Pb) was determined to be within the threshold limit, and the hazard index (HI) of consuming *Barbonymus* sp. is less than 1, thus indicating a low potential health risk.

Keywords: Bioaccumulation, cancer risk, freshwater fish, heavy metals, non-cancer risk, target hazard quotient

INTRODUCTION

The lakes in Sg. Galah, Kg. Gajah, Perak, Malaysia, are the result of tin ore mining operations that lasted until 1984. Mining activities resulted in the formation of nine interconnected man-made lakes that serve as habitats for various fish, aquatic plants, and organisms. The lakes are now used as a source of income for local inland fishermen. Fishermen rely on lake fish catches for food and income from selling the fish at markets or to fishmongers.

Correspondingly, former mining lakes pose a significant trace metal risk. The common trace metals linked to former mining water bodies are arsenic (As), iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) (Baharim et al., 2022; Pistelli et al., 2017). These trace metals, if present in high concentrations, may endanger human health either directly through ingestion or dermal contact with the water (Koki et al., 2018; Low et al., 2016; Medunić et al., 2019) or indirectly through the consumption of fish caught from the lakes that may have bioaccumulated the metals over time (Ashraf et al., 2012; Dalzochio et al., 2018; Saat et al., 2014). Aside from the common metals found in lakes due to mining operations, the lakes in the study area receive continuous metal input from agricultural activities in the surrounding areas (Kamari et al., 2017; Okereafor et al., 2020). Runoffs containing metal fertilisers and pesticide residues may increase metal contamination in lakes (Hembrom et al., 2019; Müller et al., 2020; Xie et al., 2016).

The assortment of macro and micronutrients that is present in fish renders it an indisputable protein source in a well-balanced human diet (Liu et al., 2020; Mishra et al., 2007; Tacon & Metian, 2013). Their ability to accumulate trace metals in their tissues, however, has sparked worldwide concern, and the health risk posed by eating fish has been called into question. The amount of trace metal accumulation in fish varies according to metal, fish species, and tissues of concern (Petrović et al., 2013). Sex, age, size, reproductive cycle, swimming habits, eating behaviour, and habitat quality are all other factors that influence metal uptake in fish.

Therefore, determining the levels of metal pollution in the ex-mining lake water and fish is critical in order to analyse bioaccumulation events and predict the potential human health risk caused by metal contamination, particularly to ensure food security when consuming fish.

The Cyprinidae family contains the most abundant genera and species of freshwater fish in Malaysia (Kamarudin & Esa, 2009). The genus *Barbonymus*, formerly referred to as *Barbus, Barbodes, Puntius*, or *Systomus*, can be classified into ten species: *B. altus, B. balleroides, B. collingwoodii, B. gonionotus, B. schawenfeldii, B. belinka, B. mahakkamensis, B. platysoma*, and *B. sunieri* (Batubara et al., 2021, Kottelat, 2013; Yang et al., 2012; Zheng et al., 2016). Two species, the *Barbonymus schawenfeldii* (tinfoil barb) and the *Barbonymus gonionotus* (Java barb), are frequently encountered in the freshwater ecology of Malaysia (Kusmini et al., 2021; Rashid, 2014). These species have been primary targets for inland fishers as food and ornamental fish due to the stunning colours of their caudal and ventral fins (Eslamloo et al., 2012; Isa et al., 2012; Muchlisin et al., 2015). The Malaysian Department of Fisheries (DOF, 2021) has confirmed that the total number of *Barbonymus* sp. landings, particularly in the public water bodies in Perak, exhibited an upward trend from 2013 to 2020, with a slight decrease in 2015. *Barbonymus* sp., or pointedly to river carp and Javanese carp, was discovered in more ex-mining lakes, lakes, and rivers in Perak alone in 2020 than in any other year before, with landings totalling up to 352.77 tonnes that year. The *Barbonymus* sp. was chosen for this study because of its large population and the local community's keen attention.

The objectives of this present study are to:

(i) quantify the concentrations of trace metals in the water of the lake and in the muscles and gills of *Barbonymus* sp., a regularly encountered fish species in this lake.

(ii) investigate the relationship between metal concentrations in water and fish.

(iii) compare metal concentrations in water to the National Lake Water Quality Standard (NLWQS) and in fish organs to the World Health Organisation (WHO), 2004, Food and Agriculture Organisation (FAO), 1983, and Malaysian Food Act (MFA), 1983.

(iv) utilising the incremental life cancer risk (ILCR) for prospective cancer effects and the hazard index (HI) for undesirable non-cancer effects to calculate the potential harm to human health. The results provide an initial assessment of the magnitude of trace metal contamination in the study region, as well as the possible risk to human health from fish ingestion.

MATERIALS AND METHODS

Sampling Location

Figure 1 depicts Sg. Galah, Kg. Gajah's public waters for inland fishermen, which consist of nine interconnected lakes (Lake A – Lake I). The Kinta River near Lakes B, H, and I supply the lakes' water through three culverts. However, due to a lack of management in the culvert areas, siltation, solid debris, and invasive macrophyte infestation significantly restrict the flow of water entering and exiting the lakes. As a result, except during river flooding, which occurs once a year during an extraordinarily high rain event, the water within the lakes is circulated between them. Furthermore, in recent years, the invasion of invasive macrophytes, particularly water hyacinth (*Pontederia crassipes*), has become more serious. This invasive weed covers 70% of the lake areas, preventing local inland fishermen from fishing. Lake B was the only one still open for fishing. Because the lakes are interconnected and have limited fishing access, this study assumes that one lake can serve as a representative of all lakes due to the natural migration of fish between them. Therefore, in this study, the sampling at Lake B is representative of all the lakes.

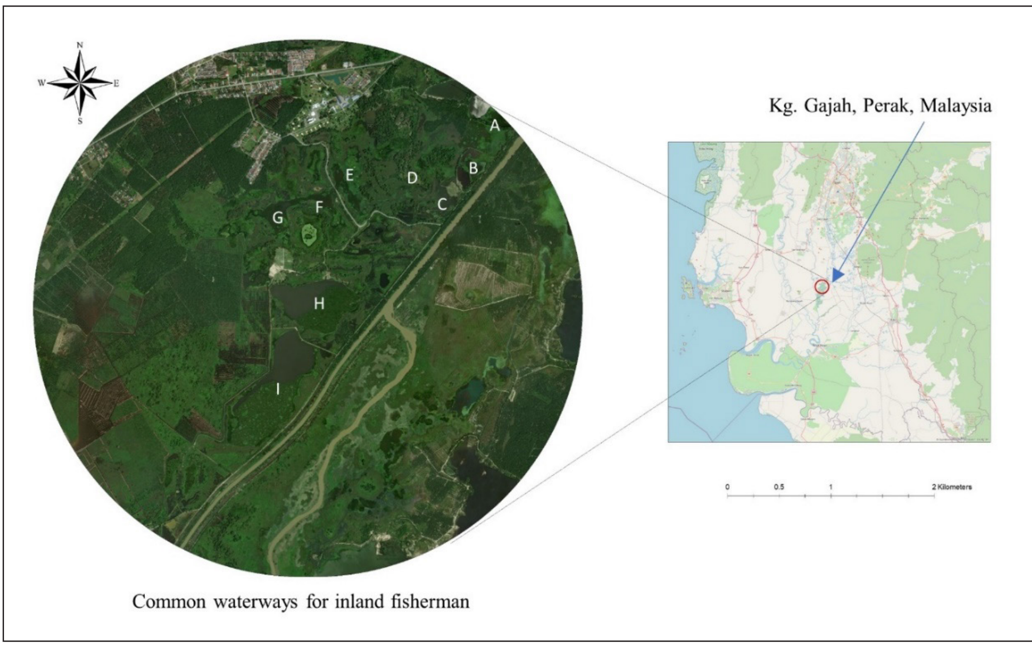

Figure 1. Ex-mining lakes of Sg. Galah, Kg. Gajah, Perak

Water Quality Determination and Water Sample Collection

Acid-washed bottles were used to collect water samples at three different locations, each representing Lake B's inlet, middle, and outlet. The samples were kept in a cool box at 4^{o}C until further analysis. These samples were stored at a pH level below 2 in order to minimise the incidence of precipitation, adsorption, or microbiological activity by the addition of nitric acid (HNO_3). The quality of the lake water was assessed using a handheld multiparameter instrument model 556 (YSI, USA) to measure in-situ water quality parameters such as pH, temperature, salinity, dissolved oxygen (DO), conductivity (EC), and total dissolved solids (TDS); turbidity was assessed using a turbidity meter model 2100P (HACH, USA).

Fish Sampling

Fish samples were collected using gill nets with the assistance of local inland fishermen, washed with distilled water, and placed in separate polyethylene bags. The fish were measured for length (mm) and weight (g), and fish with a similar average length and weight were collected. Uneven numbers of fish in each species prevented species-level identification. As a result, the fish samples will be reported as *Barbonymus* sp. samples. Around 20 fish samples were collected. All the samples were stored at -20 °C until they were further analysed.

Acid Digestion of the Water and Fish Samples

Prior to the analysis for trace metals using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), both the fish and water samples underwent acid digestion (USEPA, 1991).

The water samples were allowed to evaporate up to one-fourth of their original volume after being boiled for 5-10 minutes on a hot plate through a 0.45 µm cellulose nitrate membrane filter. The samples were subsequently filled to a volume of 10 mL by washing the vials with a 2% HNO_3 solution to prevent any possibility of sample loss. The samples were then placed in a refrigerator for two weeks to allow the metals to stabilise. Trace metal concentrations were then analysed for Fe, Zn, and Pb in triplicate using ICP-MS (USEPA, 1991).

The fish samples were pre-treated by being washed in distilled water, and the muscle and liver were dried in an oven at 105°C for 24 hours. The fish bones and scales were removed after drying, and the muscles and gills were collected. These fish parts were then ground separately with a mortar and pestle before being dried in a crucible and stored until further digestion.

Approximately 0.5 g of fish muscles and 0.2 g of fish gills, respectively, were added with 5 mL of HNO_3 and, correspondingly, subjected to heating on a hot plate within a digestive tube. The sample was then heated to 40°C for one minute and then gradually heated to 100°C for 10 minutes until it reached the maximum temperature of 140°C. The digestion continued for 3 hours at a temperature of 140°C. Before dilution with distilled water, the digested residues were cooled and then filtered into a 50 mL centrifuge tube using a 0.45 µm nylon syringe filter. The filtrates were then refrigerated before ICP-MS metal analysis of Fe, Zn, and Pb.

Trace Metal Analysis Using ICP-MS

The presence of trace metals Fe, Zn, and Pb in digested samples was determined using ICP-MS. The trace metal levels were given in mg/kg dry weight for the fish samples and mg/L for the water. The following standard concentrations were used: 10 ppb, 30 ppb, 50 ppb, 100 ppb, and 300 ppb to prepare the calibration curve.

The per cent recovery for the fish extraction was calculated using the Equation 1

$$Recovery\ (\%) = \left[\frac{x-y}{z}\right] \times 100 \qquad [1]$$

Where x represents the average concentration of trace metal after a spike, y denotes the average concentration of trace metal before a spike, and z represents the concentration of trace metal that has been spiked.

The recovery percentages for Fe, Zn, and Pb were 81.8%, 82.8%, and 83.3%, respectively, which constituted acceptable results.

Analytical blanks, consisting of reagents without the sample matrix, were processed alongside the samples to monitor for potential contamination. Internal standards and calibration curves with $R^2 > 0.999$ were used for instrument validation.

Bioconcentration Factor and Health Risk Assessment

The ratio of the fish's steady-state metal ion concentrations to the concentration in the water is known as the bioconcentration factor (BCF). BCF values were measured according to Equation 2 proposed by Gobas et al., (2009):

$$BCF = \frac{Concentration\ \ of\ trace\ metal\ in\ fish\ (\frac{mg}{kg})}{Concentration\ \ of\ trace\ metal\ in\ water\ (\frac{mg}{L})} \qquad [2]$$

The BCF values were calculated to demonstrate the possibility of metal uptake by the fish from the metal present in the lake water.

Finally, a health risk assessment was carried out. The potential health risks associated with fish eating were calculated by utilising the data on trace metal concentration in the fish and the anticipated consumption rate according to USEPA (2012) guidelines (Ashraf et al., 2012; Azmi et al., 2019). Therefore, the consumption rate is predicted based on the following assumptions:

- the ingestion rate (IR) of fish per day was 0.16 kg/day/person (FAO, 2009; Idriss & Ahmad, 2015).
- both men and women in Malaysia weigh an average of 62 kg for their adult body weight (BW) ((Ahmad et al., 2016)).
- bioavailability and maximum absorption rate are at 100%.

The Target Hazard Quotient (THQ) was determined for the non-carcinogenic risk using the Equation 3 (Khoshnood et al., 2014; Javed & Usmani, 2019):

$$Target\ Hazard\ Quotient\ (THQ) = \frac{EDI}{RfD} \qquad [3]$$

To calculate the Estimated Daily Intake (EDI), the following equation (Equation 4) was used:

$$Estimated\ Daily\ Intake\ (EDI) = \frac{[EFr \times ED \times IR \times C]}{[BW \times AT]} \quad [4]$$

In the present study, the exposure frequency was set at 365 days per year and is denoted as EFr. According to USEPA (2012), the ED stands for the exposure duration, which is set at 70 years, to evaluate the impacts of the non-carcinogens. The IR represents the daily fish ingestion rate assumed to be 0.16 kg/day/person for Malaysians. The C represents the metal concentrations (mg/kg wet weight) in the muscles and gills of the fish samples. The average body weight (BW) for Malaysian adults was set at 65 kg. The averaging time (AT) for non-carcinogens was 365 days/year × ED. The oral reference dose (RfD) for each metal is expressed as mg/kg/day (Table 1).

From the THQ of each metal, the Hazard Index was determined using the Equation 5.

$$Hazard\ Index\ (HI) = \ sum\ of\ THQs\ of\ every\ metals \quad [5]$$

Values greater than 1 indicate that the exposure concentration exceeds the reference concentration and may have significant negative consequences. Values less than 1 suggest that the population being exposed is unlikely to suffer any detrimental health effects.

The Incremental Life Cancer Risk (ILCR) was calculated using the Equation 6 (Bacigalupo & Hale, 2012, Cao et al., 2015; Sultana et al., 2017), to determine the potential target cancer risk for metals that exceeded the standards.

$$Incremental\ Life\ Cancer\ Risk\ (ILCR) = CDI \times CSF \quad [6]$$

The CDI, similar to EDI in the THQ calculation, estimates the average daily dose of exposure to the metal carcinogen over a person's lifetime and is measured as the chronic daily intake of the chemical carcinogen in milligrams per kilogram of body weight per day. CSF is an abbreviation for the cancer slope factor. Only Pb was measured for the ILCR with the CSF of 8.5×10^{-3} mg/kg/day (Ahmad et al., 2016; USEPA, 1989; Orisakwe et al., 2017). The ILCR value that ranges between 1.0×10^{-6} to 1.0×10^{-4} (around 1 probability risk out of every 1,000,000 lifetime exposures) is recommended by USEPA (2012).

Table 1
Non-cancer oral reference dose (RfD) for the investigated metals

Element	RfD (mg/kg/day)	References
Fe	0.7000	Harmanescu et al., 2011
Zn	0.3000	Harmanescu et al., 2011; Korkmaz et al., 2017; USEPA, 1989
Pb	0.0035	Harmanescu et al., 2011; Orisakwe et al., 2017

Data Analysis

All samples collected and metal analysis were conducted with a minimum of three replicates. Descriptive statistics, including mean and standard deviation, were then determined. An assessment was conducted to compare the water quality parameters and trace metal concentrations with the National Lake Water Quality Standard (NLWQS) established by the National Hydraulic Research Institute of Malaysia (NAHRIM) for Category C lakes, which are designated for the conservation of aquatic life and biodiversity. The fish muscles and gills were analysed for trace metal concentration in accordance with the guidelines established by the World Health Organisation (WHO) in 2004, the Food and Agriculture Organisation (FAO) in 1983, and the Malaysia Food Act (MFA) in 1983.

The correlation between trace metal levels in lake water, fish muscles and gills was conducted using Pearson Product Moment Correlation in SPSS. Statistical analysis using one-way ANOVA and Kruskal-Wallis tests was employed to ascertain the presence of any statistical disparity between the sampling locations and water quality parameters.

RESULTS AND DISCUSSION

Water Quality Status of the Lake

The physicochemical properties of water are necessary for the monitoring of water quality (Alonso Castillo et al., 2013; Javed et al., 2016). With the exception of pH and DO, the physicochemical characteristics of the water samples shown in Table 2 were found to be generally within Malaysia's NLWQS permissible level for Category C lakes.

The pH of the limnetic zone of the lake was below pH 6.0, which was acidic and slightly contaminated. These acidic conditions might have originated from the commonly produced sulphide from the previous mining operations (Khalid et al., 2017). Higher or lower pH influences the water taste as well as the damage to the fish skin and eyes (Dirisu et al., 2016), while pH levels above 9.0 are similarly toxic to fish and other aquatic animals (Stone et al., 2013; Wurts, 2003). Although the effect of pH does not directly correspond

Table 2
In-situ water quality parameters of the lake

Point	Inlet	Middle	Outlet	NLQWS (NAHRIM) Category C
pH	6.77±0.03	3.89±0.50	6.57±0.01	6.0 – 9.0
EC (μS/cm)	161.33±0.49	159.30±0.70	167.50±0.00	2000
Salinity (%)	0.10±0.00	0.10±0.00	0.10±0.00	<1
Temperature (°C)	30.17±0.06	29.70±0.10	28.30±0.00	28
Turbidity (NTU)	8.98±0.14	9.30±0.41	11.43±0.61	70
DO (mg/L)	1.44±0.76	0.52±0.02	0.49±0.04	55-130

to human health, the pH level does impact metal solubility and concentration (Miao et al., 2021; Muhammad et al., 2011). Under slightly acidic circumstances (pH=5.0), higher metal solubilities were detected, which rose when pH was held at 3.3 (Başak & Alagha, 2010; Chuan et al., 1996). Therefore, the acidic conditions of the water were deemed unfavourable for the fish and may impact the lake ecosystem in the long run.

It was found that the dissolved oxygen (DO) levels in some areas of the lake were below the ideal threshold established by the NLWQS. Low DO is common in ex-mining ponds and lakes (Orji et al., 2013; Srivastava et al., 2009). Low DO values could be due to a decrease in aquatic plant activity and a high level of organic material content (Breitburg et al., 1997; Seitaj et al., 2017). Conversely, aquatic plants proliferate in the lake ecosystem in the study area. Therefore, as the DO level measures the water's assimilative capacity, its depletion may also indicate pollution of biological or chemical origins (Chiejine et al., 2015). Depleted DO levels threaten aquatic life as DO is crucial for the metabolism of all aquatic organisms. If low DO conditions are prolonged in the study area, metal contamination might not be the only concern.

EC, pH, and temperature were significantly different among the sampling points ($p<0.05$). A drastic decrease in pH levels in the middle of the lake compared to the inlet and outlet could be due to water flowing into the lake and passing over or through soil or bedrock of different mineral compositions. In terms of EC, although the differences between the level measured at the middle point and the two other points were significant, the difference was less than 5%, hence it was still considered negligible. Equally, the temperature measurements varied by around 1-2°C across each location and may have been influenced by the shadowing caused by cloud cover on the lake surface during the sampling process. In contrast, turbidity and dissolved oxygen levels measured for all points were similar ($p>0.05$). Therefore, the condition for the whole lake was representative of the values measured for both parameters.

Trace Metals in Water

Ex-mining waters are usually enriched with many elements. Determining the metal concentrations in the lakes is essential for the risk assessment, especially if the water is being used for human consumption. Table 3 shows the results of metal concentration that are detected in the ex-mining lake for the inlet, middle, and outlet. It is noticeable that there are differences between the Fe, Zn, and Pb concentrations in the inlet, middle, and outlet ($p<0.05$).

The major element in the mining area sediment and soil is usually Fe, which is present in high concentrations compared to other trace metals because in most of the earth's upper and lower crust, Fe is one of the most abundant elements. This is true because Fe has the most significant quantities of trace metals among the tested metals. Agriculture wastewater,

Table 3
Trace metal concentration in water

Point	Inlet	Middle	Outlet
Fe (mg/L)	0.80±12.02	0.57±15.11	0.64±22.74
Zn (mg/L)	0.10±0.28	0.53±0.34	0.18±2.47
Pb (mg/L)	0.13±0.38	0.18±0.01	0.19±0.09

prior mining and metallurgical operations that used zinc, as well as the use of commercial products containing zinc, contributed as anthropogenic sources of zinc in the water (ATSDR, 2005). Despite being lower than the guideline's value, Zn was detected in high concentration in the middle part of the lake as compared to the inlet and outlet ($p<0.05$). This could be due to the poor water flow, whereby a higher concentration of pollutants accumulated at the lake's centre. Different flow rates can significantly affect water quality parameters at any point in the lake (Pourfallah Koushali et al., 2021).

The comparison between metal concentrations in water and NLWQS is illustrated in Figure 2. The metal levels in the lake were all within the NLWQS's acceptable limits, except for Pb at 0.121 mg/L. It was discovered that the water's Pb levels were 33.3% higher than the recommended limit. Possible sources of Pb could include leftover residual metals from the mining activities (Paul, 2017) and agricultural runoff (Hamzah et al., 2018).

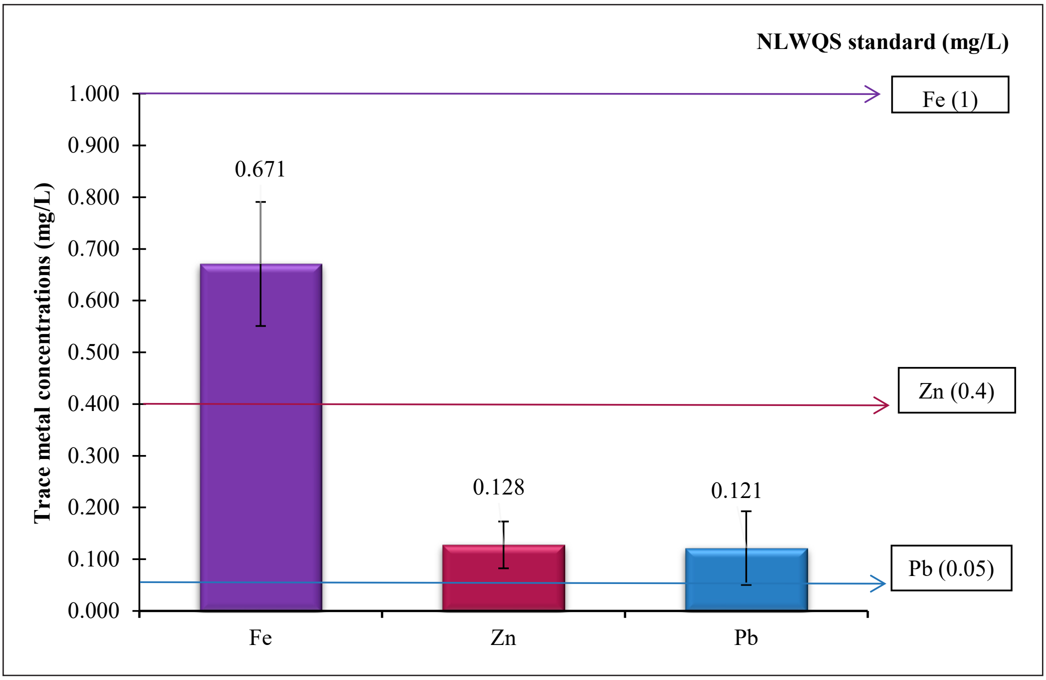

Figure 2. A comparison of the amount of trace metals in water with NLWQS

The present study has focused on the analysis of Fe and Zn because they are essential trace elements for biological functions but can pose a danger at elevated levels. Pb was included as well due to its known toxicity and prevalence in anthropogenic environments, especially in mining and industrial areas. On top of that, these metals are commonly monitored in food safety and environmental health guidelines, making them relevant indicators for risk assessment. However, the study acknowledged that the exclusion of other highly toxic metals, such as As and Cd, limits its ability to fully assess the accumulative health risk associated with metal contamination in the studied fish species. Therefore, future studies should cover a larger range of metals to provide a more complete risk assessment.

Trace Metal Concentration in Fish and the Bioconcentration Factor

The mean concentration of trace metals in the muscles and gills of the *Barbonymus* sp. followed a decreasing order of Fe > Zn > Pb. Metal concentrations observed in the *Barbonymus* sp. gills and muscles were compared to WHO, FAO, and MFA guidelines in Table 4. The Fe concentration in the muscles and gills of the *Barbonymus* sp. exceeded the maximum limits set by the FAO and World Health Organisation. The concentration of Pb recorded in this study also surpassed the safe range set by MFA, WHO, and FAO. Zn concentration in the *Barbonymus* sp. gills was less than the MFA and FAO limits of 100 mg/kg and 30 mg/kg, respectively. However, the amount of Zn in the *Barbonymus* sp. muscle was slightly greater than the FAO limit of 30 mg/kg, but this was still considered to be low compared to the values in MFA.

Table 5 presents the bioconcentration factors (BCF) of trace metals computed for the gills and muscles of *Barbonymus sp.* The BCF values among the studied trace metals showed a descending trend of Fe>Zn>Pb for its gills and Zn>Fe>Pb for the muscles of the *Barbonymus* sp. For both the gills and muscles of *Barbonymus* sp., the BCF values of all studied metals were greater than 1.0, indicating the propensity to accumulate Fe, Pb, and Zn from the water (Ju et al., 2017).

Metal accumulation in fish is a complex process influenced by both internal and external variables (Griboff et al., 2017; Jabeen & Chaudhry, 2010). Metal bioavailability, alkalinity, and ambient temperature are listed as external factors, whereas the feeding

Table 4
Trace metal concentrations in Barbonymus *sp. (mg/kg)*

Metal	Mean concentration		WHO	FAO	MFA
	Gills	Muscles			
Fe	267.66	132.76	43	2.5	-
Zn	18.65	31.85	-	30	100
Pb	9.82	10.19	1.5	0.5	2

habit of the fish is an example of internal factors affecting the metal accumulation in fish. Major pathways for metals to enter the fish are through surface exposure to water, the food chain, and respiratory activity (Adegbola et al., 2021).

Table 5
Bioconcentration Factor (BCF) of trace metal in muscles and gills of Barbonymus *sp.*

Trace metals	Organs	BCF
Iron (Fe)	Gills	398.90
	Muscles	197.85
Lead (Pb)	Gills	81.16
	Muscles	84.21
Zinc (Zn)	Gills	145.70
	Muscles	248.83

Gills are chosen in this study for assessing metal accumulation, as they reflect the metal pollution in water. Higher metals can usually be found in the gills as they are more exposed to the outer environment (water) than the muscle (Bebianno et al., 2004; Rajeshkumar & Li, 2018). Moreover, metal accumulation in gills is also due to the larger surface areas, which allow rapid metal diffusion and metal ion exchange process from the surrounding aquatic environment (Bebianno et al., 2004; El-Moselhy et al., 2014). Since muscles are frequently consumed when people eat fish, it was chosen as the organ of concern, even though it is not thought to be an active tissue in terms of accumulating trace metals (Agah et al., 2009; Aytekin et al., 2019; Mohammad Ali et al., 2021). In this study, a statistically significant difference was observed in Fe levels ($p < 0.05$), while no significant difference was found in Zn levels, although the concentration in the muscles was somewhat higher ($p < 0.05$). However, there were no statistically significant differences in Pb levels between the organs ($p > 0.05$). Kalay et al. (1999) argued that after a contaminant has passed through the body's defence barrier, it will begin to accumulate in the fish muscle.

It should be emphasised that, due to sampling constraints, this study has grouped all the fish samples under *Barbonymus* sp., which restricts the ability to generalise the results because various species within the genus can exhibit differing physiological traits and ecological niches. These changes may alter heavy metal uptake, accumulation, and detoxification mechanisms, potentially resulting in variations in the observed patterns (Cordeli et al., 2023; Oros, 2025). Future research on particular species within the genus may provide more detailed insights into metal accumulation.

Human Health Risk Assessment

The rate of fish consumption reported in Malaysia (both for inland and marine sources) in 2016 was about 59 kg per capita, making it among the world's highest demands for fish (FAO, 2020). As the demand for fish rises, it becomes increasingly important to assess the health risks connected with eating seafood that has been contaminated with trace metals (Mansour et al., 2009). Table 6 shows the estimated possible health hazards that are associated with consuming *Barbonymus* sp. that is polluted with Fe, Zn, and Pb from the former mining lake. All THQ values for metals analysed were less than 1, indicating that the people consuming *Barbonymus* sp. from the former mining lake were not exposed to health risk (Ahmad & Sarah, 2015; Lemly, 1996; Wang et al., 2005). However, humans exposed to the effects of the combination of more than one metal or interactive effects can be higher (Li et al., 2013).

Table 6
Target Hazard Quotient (THQ) and Increment Lifetime Carcinogenic Risk (ILCR) by consuming Barbonymus *sp.*

Element	Risk Assessment	
	THQ	ILCR
Fe	0.0014	-
Zn	0.0004	-
Pb	0.0141	$1.05E^{-06}$

Due to their toxicity and frequent association with ex-mining metal contamination, the only carcinogenic risk for Pb was investigated pertaining to the consumption of *Barbonymus* sp. (Ghnaya et al., 2015; Oke & Vermeulen, 2017). According to Silbergeld et al. (2000), Pb poisoning can cause oxidative DNA damage, direct DNA damage, and suppression of DNA synthesis. It can also produce reactive oxygen species. The US EPA's tolerable risk range, which is 1×10-6 to 1×10-4, is still within the increased lifetime cancer risk (ILCR) for Pb, 1.05 ×10-6, indicating negligible carcinogenic risk to the consumer (USEPA, 1989). Nevertheless, Pb contamination remains particularly hazardous to vulnerable populations such as children and pregnant women. Their physiological differences, including rapid development in children and the unique vulnerability of the fetus, can lead to greater sensitivity to Pb exposure, potentially amplifying the long-term health consequences that are associated with even low-level contamination. Children are known to be more affected by Pb than adults, even at a low concentration. The risk to the infant from a pregnant woman should not be overlooked, as Pb can cross the placental barrier, potentially causing harm to the developing neurological system of the newborn (ATSDR, 2017). As a result, while the immediate carcinogenic risk to the general population may appear to be small, the possibility of long-term harm, particularly to the developing systems of children and the unborn, necessitates ongoing monitoring and risk communication.

Other similar studies on the health risk assessment of fish from the ex-mining lake also reported coinciding results. According to the evaluation by Ishak et al. (2020), while the computed HQ levels for both Pb and Cd showed no known health risk to humans, it is important to exercise caution, as there is still the possibility of other trace metals being present in the lake which potentially endanger human health if not monitored.

It should be noted that vulnerable individuals who are susceptible to long-term trace element exposure, such as children, pregnant or nursing mothers, and their infants, are not taken into account in the human health risk assessment in this study (Javed & Usmani, 2019).

CONCLUSION

This study sets out to evaluate the accumulation of trace metals in the populations of *Barbonymus* sp. that are obtained from a former mining lake in Sg. Galah, Kg. Gajah, Perak, Malaysia. Analysis revealed that the gills of *Barbonymus sp*. contained higher levels of Fe, Zn, and Pb than the muscles. However, both organs exhibited the same pattern of Fe > Zn > Pb accumulation. Iron (Fe) and lead (Pb) concentrations in the gills and muscles of *Barbonymus sp*. surpassed many established thresholds. Nevertheless, according to the findings of the health risk assessment, it can be concluded that the potential of harmful effects (including cancer and non-cancerous conditions) on human health that are linked to prolonged consumption of fish is still low. However, it is important to note that the present study is based on samples that have been collected at a single time point, which may not account for potential seasonal variations. Given the tropical environment of the study area in Perak, future research should consider the potential impact of seasonal fluctuations in precipitation and temperature on the solubility and bioavailability of metals in the lake, as these factors could subsequently affect their accumulation patterns in fish tissues. Other investigations on the movement of metals between different trophic levels or mediums are necessary to enhance the obtained findings.

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