



BIOACCUMULATION OF HEAVY METALS IN FISH CONSUMED BY STUDENTS IN BOARDING SCHOOLS AND FOUNDATION COLLEGES IN PENINSULAR MALAYSIA: A HEALTH RISK ASSESSMENT

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ARTICLE INFO

Article History:

Received: 28 October 2024

Revised: 6 July 2025

Accepted: 22 September 2025

Published: 15 March 2026

Keywords:

Heavy metals, bioaccumulation, health risk assessment.

ABSTRACT

This study intends to investigate the level of bioaccumulation of six heavy metals: As, Cr, Cu, Fe, Pb, and Zn in the muscle tissues of several fish species served to the students in boarding schools and foundation colleges in Peninsular Malaysia. The bioaccumulation of heavy metals was measured using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). As, Cr, and Pb were revealed to be under the detection limit. Heavy metals inside muscle tissue were ranked in the following order: Fe > Zn > Cu. The Estimated Daily Intake (EDI) of each heavy metal was calculated based on a fish consumption rate of 168 g.day⁻¹, which reflects the average intake in Malaysia. According to the Target Hazard Quotient (THQ) measurement, the fish given to the students posed no significant non-carcinogenic risks to human health for the Fe, Zn, or Cu. As demonstrated by the Hazardous Index (HI), the cumulative effect of all heavy metals also suggests no potential for non-carcinogenic risk. The study did not apply the carcinogenic risk assessment for Pb and As, as the two heavy metals fell below the detection level. Additionally, the risk of developing cancer during one's lifetime is negligible.

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Introduction

Heavy metal pollution is a major environmental concern in aquatic ecosystems. The scientific community has expressed concerns about these heavy metals when their concentrations surpass the permitted limits, especially with regard to their general origins, pervasiveness, inability to biodegrade, bioavailability, and toxic effects on living organisms (Guo *et al.*, 2020; Ishak *et al.*, 2020; Yu *et al.*, 2021; Fernández-Trujillo *et al.*, 2021). Heavy metals naturally exist in the environment due to the leaching process, the weathering of natural rock containing heavy metals, and volcanic eruptions (Khan *et al.*, 2016; Raza *et al.*, 2016). Nevertheless, since the industrial revolution in the 18th century, anthropogenic activities have been the main contributor to heavy metals in the environment. Industrialisation and application of these heavy

metals in various industrial processes led to increased heavy metal discharge to the soil and aquatic ecosystems (Salam *et al.*, 2021; Jamil Emon *et al.*, 2023). Heavy metal pollution has been documented in a number of aquatic ecosystems across the globe. One significant instance of this kind of incidence was the illness discovered in Minamata Bay, which was first reported in Japan in 1956 and was caused by consuming fish contaminated with mercury (Bosch *et al.*, 2016).

As a consequence, heavy metal concentrations in aquatic environments are now widely monitored by a number of authorities and research teams across the world (Manoj & Kawsar, 2020; Salam *et al.*, 2021; Chen *et al.*, 2022; Tek & Ng, 2024). Anthropogenic activities such as the smelting process, mining activities,

heavy industries, and agriculture are commonly linked to the release of heavy metal pollutants, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), and zinc (Zn). Although some of the heavy metals listed above (Cu, Fe, and Zn) are considered essential heavy metals and vital for the proper functioning of physiological processes such as enzymatic activities, homeostasis, and impulse transmission, exceeding the permissible limit of these metals can still cause significant health risks to humans (Bosch *et al.*, 2016; Jamil Emon *et al.*, 2023). This is far more concerning when dealing with non-essential heavy metals with no known biological function such as lead (Pb) and mercury (Hg), which might cause toxicity even at low levels of concentration (Tengku Nur Alia *et al.*, 2020).

Fish and other aquatic organisms may absorb heavy metal accumulation in water columns via their gills, as well as by adsorption and ingestion. The combination of both bioaccumulation and biomagnification processes within the fish might lead to the heavy metal accumulating to a toxic level and causing significant health risks to humans (Ishak *et al.*, 2020; Khalil *et al.*, 2020; Rizk *et al.*, 2022). Fish consumption is globally popular at various social levels since this food is acknowledged to be an affordable protein source, as well as being nutrient-rich since it contains vitamins and Omega-3 fatty acids (Rahman *et al.*, 2020; Maulu *et al.*, 2021; Tek & Ng, 2024).

On the other hand, this also increases the possible risk of heavy metal toxicity. The consumption of fish among Malaysians is ranked only second to Japan and is considered the highest among Southeast Asian nations, where the Malaysian consumption rate is 168 g.day⁻¹ (Nurul Izzah *et al.*, 2016). This is noticeably important when dealing with children and youth, as they are in the process of growing, which requires far more nutrients and is susceptible to heavy metal toxicity effects. Thus, it is paramount for both non-essential and essential heavy metals to be evaluated to minimise the possible health risks due to heavy metal exposure through fish consumption.

Fish is a major component of the daily diet among students in boarding schools and foundation colleges in Malaysia, where meals are centrally prepared and served multiple times per day. Given that these institutions typically serve fish up to six times per week as an affordable source of protein, students may be at risk of higher cumulative exposure to heavy metals if contamination is present. This makes them a particularly relevant and vulnerable population for dietary risk assessment. Fish may be contaminated with heavy metals as a result of environmental pollution or bioaccumulation processes, which might pose health risks and raise societal problems.

Over the last four decades, Malaysia's economy has transitioned from one centred on agriculture to one centred on mixed industrialisation. This has resulted in increased contamination of the aquatic ecosystem. Due to their potentially poisonous qualities, heavy metals have been included in the types of contaminants, which have detrimental effects on aquatic biota like fish while possible dangers to human health could arise if heavy metal-contaminated fish is consumed (Tengku Nur Alia *et al.*, 2020; Jamil Emon *et al.*, 2023). Malaysia has been dealing with heavy metal pollution since 1990 and since then, the heavy metal pollution in this country has been concerning (Fathi Alhashmi *et al.*, 2012; Koh *et al.*, 2019; Yunus *et al.*, 2020). This country's high fish consumption rate could expose Malaysians, especially students, to a higher risk due to heavy metals' nonbiodegradable and biomagnification nature from one trophic level to the next (Tengku Nur Alia *et al.*, 2020).

Therefore, all foods given to the students must be assessed to measure their safety and quality so that levels of heavy metals can be kept within safe limits. While fish is a key and nutritious source of food, the health of students could be endangered by the intake of heavy metal-based contaminants. Students may unknowingly expose themselves to elevated levels of heavy metals without adequate monitoring and analysis, potentially leading

to adverse health effects such as heavy metal poisoning or long-term health implications. Therefore, this study aims to assess the concentrations of selected heavy metals in fish served to students in MARA boarding schools and MARA foundation colleges across Peninsular Malaysia and to evaluate the associated health risks based on Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), Hazardous Index (HI), and Carcinogenic Risk (CR).

Materials and Methods

Fish Samples Collection

Fish specimens were collected between November and December 2023 from nine boarding schools and foundation colleges under Majlis Amanah Rakyat (MARA) located across Peninsular Malaysia. These MARA institutions were selected based on their large student populations, which represent a significant demographic for dietary exposure studies, as well as their wide geographical distribution across multiple states in Peninsular Malaysia. The institutions involved in this study included Kolej MARA Kulim (KMK) and Kolej MARA Kuala Nerang (KMKN) in the state of Kedah, Kolej MARA Seremban (KMS) in Negeri Sembilan, Kolej MARA Banting (KMB) in Selangor, as well as several Maktab Rendah Sains MARA (MRSM) campuses, namely MRSM Kota Putra (MRSM-KP) in Terengganu, MRSM Transkrian (MRSM-TK) in Pulau Pinang, MRSM Batu Pahat (MRSM-BP) in Johor, and MRSM Pasir Tumbuh (MRSM-PT) in Kelantan. At these institutions, students are provided with five meals per day and fish is included in the menu on at least six occasions each week.

All fish specimens were collected directly from the cold storage facilities at the respective institutions. Based on interviews conducted, all fish supplies were sourced locally, as stated in the procurement records. The fish was preserved in the icebox and brought to the Biology laboratory in Kolej MARA Kulim for further processing. The collected fish were rinsed with deionised water and then stored individually,

based on their species, in clean polyethene bags. The fish samples were stored at -20°C until they were ready for analysis. Heavy metal analyses were performed on raw muscle tissue of the fish samples to prevent any alterations in metal content that could result from the cooking process. A board for measuring and an electronic balance (model lab 634) were used to measure the fish for weight in grammes and total length in centimetres, respectively. Four fish species such as *Atule mate* (weight: 116.39 ± 5.02 g, length: 23.58 ± 0.31 cm), *Rastrelliger kanagurta* (weight: 122.14 ± 10.98 g, length: 21.66 ± 0.72 cm), *Parastrumateus niger* (weight: 135.57 ± 5.77 g, length: 17.64 ± 0.62 cm), and *Oreochromis niloticus* (weight: 225.26 ± 6.30 g, length: 21.94 ± 0.37 cm) were used in this study to assess heavy metal concentrations. These species were selected based on their frequent inclusion in meals served to students under MARA institutions.

Digestion and Analyses of Heavy Metals in Fish

Overall, the study included four distinct specimens of identical size and belonged to the same species. Only muscle tissue without bones were used for the analyses of heavy metals in this study. The homogenised muscle tissue of the fish was split in half from each individual fish, with each piece put in a petri dish so that the procedure could be repeated. This procedure was repeated for the rest of the fish species. The samples from each petri dish were accurately measured for 10 g using an analytical balance and placed into dry and clean crucibles that had been prewashed with 10% HNO_3 to remove any impurities.

The muffle furnace was preheated to 400°C to 600°C and the crucible containing the samples was carefully placed into the furnace. After a few hours, every sample was allowed to cool down before being placed into a desiccator. The samples were left to cool in the desiccator. This digestion process was conducted according to the method proposed by Radojevic and Bashkin (2006) and EPA Method 3052 (EPA

2007). The aqua regia solution for the digestion process was prepared according to the standard method (Abdullah *et al.*, 2022). Agilent’s 5110 Synchronous Vertical Dual View (SVDV) ICP-OES was used to analyse the heavy metal contents. In order to validate the accuracy of the analytical procedure used during this study, a certified reference material, DORM 5 from the National Research Council of Canada (NRCC) was analysed using the same digestion and analytical method to confirm the analysis of heavy metals. The result of DORM 5 is listed in Table 1.

Based on Table 1, the percentage recovery of the heavy metals is considered acceptable and the method used for the digestion process of the sample is appropriate and accurate.

Assessment of Fish Indexes and Human Risk Assessment

The detrimental human health effects of heavy metals have led to the development of indices to assess the possible risk. These include the Hazardous Index (HI), Target Hazard Quotient (THQ), and Cancer Slope Factor (CSF), which were developed by scientific teams and regulatory agencies such as the USEPA and WHO (Varol & Sünbül, 2018; Han *et al.*, 2021). The indices were proven to be efficient and user-friendly for rapid interpretation of health risks, whether they are carcinogenic or non-carcinogenic. The health index was calculated from the bioaccumulation of heavy metal in the muscle tissue in the wet weight, as this is the form of fish normally consumed by the students.

Estimated Daily Intake (EDI)

The EDI of heavy metals is calculated based on the equation below (Tek & Ng, 2024):

$$EDI = C \times IR/BW \tag{1}$$

where:

- C - Average concentration of heavy metals in samples (mg.kg⁻¹)
- IR - Daily ingestion rate of a specific fish
- BW - Average body weight for young adults in Malaysia (64 kg)

Estimated Weekly Intake (EWI)

The EWI of heavy metals is calculated based on the equation below:

$$EWI = EDI \times 7 \text{ days}/BW \tag{2}$$

where:

- EDI - Estimated Daily Intake
- BW - Average body weight for young adults in Malaysia (64 kg)

Percentage of Provisional Tolerable Weekly Intake (% PTWI)

Percentage PTWI of heavy metals in the fish specimens is calculated based on the equation below:

$$\% PTWI = EWI/PTWI \text{ reference value} \times 100 \tag{3}$$

where:

- EWI - Estimated Weekly Intake
- PTWI - Reference value of heavy metals

Table 1: Measured and certified value of certified reference material of DORM-5 (fish protein) and the percentage of recovery (n = 3)

Heavy Metals	Certified Value (mg.kg ⁻¹)	Found Value (mg.kg ⁻¹)	Mean Recovery (%)
As	13.3 ± 0.7	12.71 ± 0.0	95.56
Cr	0.5 ± 0.1	0.44 ± 0.0	88.00
Cu	3.30 ± 0.1	3.45 ± 0.3	104.55
Fe	113 ± 8.0	104.01 ± 2.1	92.04
Pb	0.06 ± 0.0	0.07 ± 0.0	103.45
Zn	28.7 ± 1.0	29.72 ± 0.1	103.55

Target Hazard Quotient (THQ)

THQ is a method developed by the USEPA to evaluate the possible non-carcinogenic health effects of pollutants (Popovic *et al.*, 2018; Salam *et al.*, 2020). This is determined by comparing the exposure dose to the Reference Dose (RfD). The RfD is a measure of the daily amount of substance that can be ingested orally by the human population without posing a major health risk (Korkmaz *et al.*, 2017; Miri *et al.*, 2017; Ullah *et al.*, 2017). Table 2 displays the RfD values for heavy metals.

The THQ is a ratio that evaluates the possible risk of non-carcinogenic effects from a single heavy metal due to long-term exposure through fish consumption.

Table 2: The Reference Dose (RfD) for selected heavy metals

Heavy Metals	RfD (mg.kg ⁻¹ .day ⁻¹)
As	3.0×10 ⁻⁴
Cr	1.5
Cu	4.0×10 ⁻²
Fe	7.0×10 ⁻¹
Pb	3.5×10 ⁻²
Zn	3.0×10 ⁻¹

A THQ value below one suggests that the population that consumes the fish is unlikely to experience significant health complications. On the other hand, if the THQ value is equal to or greater than one, there may be health concerns that necessitate additional interventions and remedial measures. The THQ index can be determined using the following equation:

$$THQ = (Efr \times ED \times FiR \times C/RfD \times BW \times TA) \times 10^{-3} \tag{4}$$

where:

- Efr - Total exposure rate, which is equivalent to 365 days year⁻¹
- ED - Exposure time (70 years)
- FiR - Rate of fish consumption (mg.kg⁻¹.day⁻¹)
- C - Average concentration of heavy metals in samples (mg.kg⁻¹)

RfD - Oral reference dose according to the value stated by USEPA

BW - Average body weight of young adults in Malaysia (64 kg)

TA - Average exposure time (365 days year⁻¹ × ED)

Hazardous Index (HI)

The following equation is used to determine the HI, which depends on the sum of the hazard quotients of all the heavy metals examined during the study. If the value of HI ≥ 1, there is a possible health risk due to the accumulation effects of the heavy metals (Han *et al.*, 2021).

$$HI = \sum THQ = THQ (As) + THQ (Cu) + THQ (Cr) + THQ (Pb) + THQ (Fe) + THQ (Zn) \tag{5}$$

Carcinogenic Risk (CR)

This index assesses the lifetime probability of developing cancer due to consuming carcinogenic metals. The assessment of CR focused solely on Pb and As, utilising the cancer slope factor (CSF) of 8.5 E⁻³ mg.kg⁻¹.day⁻¹ for Pb and 1.5 mg.kg⁻¹.day⁻¹ for As (Ullah *et al.*, 2017). Based on the USEPA regulations, a tolerable CR value range is regarded as ≤ 10⁻⁶ while a CR value ≥ 10⁻³ would be deemed unacceptable. The following equation was used to calculate CR:

$$CR = CSF \times EDI \tag{6}$$

where:

CSF - Cancer Slope Factor by USEPA (mg.kg⁻¹.day⁻¹)

EDI - Estimation Daily Intake

Statistical Analysis

All the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 20 for Windows. The assessment of normality and equality of variances was assessed using Kolmogorov-Smirnov and Levene tests. Transformation of data such as log₁₀ and other transformation functions were conducted on the data to achieve the normal

distribution and meet the assumptions of the parametric statistical analyses. One-way analysis of variance (ANOVA) was conducted to assess the heavy metal in various fish species at various locations.

Results and Discussion

Concentration Level of Heavy Metals in Fish

The results of heavy metals analyses using ICP-OES were expressed in mg.kg⁻¹ dry weight. Out of six heavy metals analysed, only three were detected: Cu, Fe, and Zn. Conversely, the remaining metals - Pb, Cr, and As were deemed to be under the level of detection. The overall levels of Cu, Fe, and Zn are shown in Table 3. This study shows that the descending level of heavy metals in fish is as follows: Fe > Zn > Cu.

The bioaccumulation of heavy metals in the muscle tissue of fish obtained from these institutions in Peninsular Malaysia is shown in Table 4. ANOVA test for Cu showed no significant difference across fish species. However, Fe showed a significant difference, indicating that distinct mean Fe concentrations were observed across the different fish species. For example, *A. mate* exhibited the highest mean Fe concentration at 53.39 mg.kg⁻¹ while *O. niloticus* had the lowest mean Fe concentration at 13.72 mg.kg⁻¹. The overall mean Fe concentration across all fish species was calculated to be 31.37 mg.kg⁻¹, with a standard deviation of 17.09. The distribution of Fe concentrations within the dataset is summarised in these summary

statistics. The ANOVA test yielded a significant F-statistic of 34.24, accompanied by a p-value of 0.001. This suggests that at least one fish species significantly differed from others in terms of mean Fe concentration. In simpler terms, the variation in Fe concentration observed across different fish species is unlikely to be due to random chances. By examining individual fish species, it becomes evident that certain species exhibited significantly higher mean Fe concentrations compared to others. For instance, *A. mate* stood out with a notably higher mean Fe concentration of 53.39 mg.kg⁻¹ while *O. niloticus* and *P. niger* had comparatively lower mean Fe concentrations.

The analysis of variance (ANOVA) was employed to examine the influence of different fish species (*A. mate*, *O. niloticus*, *R. kanagurta*, and *P. niger*) on the mean concentration of Zn. The results demonstrated significant variations in Zn concentration among the fish species. Distinct mean Zn concentrations were observed among the various fish species. For instance, *A. mate* had the highest mean Zn concentration at 30.48 mg.kg⁻¹ while *P. niger* exhibited the lowest mean Zn concentration at 17.27 mg.kg⁻¹.

The overall mean Zn concentration across all fish species was calculated to be 23.01 mg.kg⁻¹, with a standard deviation of 8.07. These summary statistics provide an overview of the distribution of Zn concentrations within the dataset. The ANOVA test yielded a significant F-statistic of 12.55, accompanied by a p-value of 0.001. This indicates that at least one fish

Table 3: Descriptive analysis of heavy metal concentration in all fish species

Heavy Metal	Permissible Limit (mg.kg ⁻¹) (MFA)	Permissible Limit (mg.kg ⁻¹) (WHO)	Mean (mg.kg ⁻¹) d.w	Min.	Max.
As	1.0	2.0	BDL	-	-
Cr	0.2	0.6	BDL	-	-
Cu	30	30	1.71 ± 2.30	0.35	16.41
Fe	0.5	0.5	32.80 ± 21.34	8.68	111.35
Pb	2	1.5	BDL	-	-
Zn	3.0	100	22.94 ± 8.4	11.60	43.31

Note: *BDL = Below Detection Level; MFA = Malaysian Food Act (1983) and Regulation (1985); WHO = World Health Organisation (1985).

species significantly differed from others in terms of mean Zn concentration. For example, *A. mate* stood out with a notably higher mean Zn concentration of 30.48 mg.kg⁻¹ while *O. niloticus* mg.kg⁻¹ and *P. niger* had comparatively lower mean Zn concentrations.

The levels of heavy metals in fish species according to the respective boarding schools and foundation colleges are shown in Table 5. All heavy metals detected in this study were found to be significantly different between institutions and fish species. The provided data outlines the results of an ANOVA conducted to investigate the relationship between different combinations of locations, fish species, and Cu concentrations. In this study, the ANOVA results revealed a statistically significant difference in Cu concentrations among the various combinations of locations and fish species ($F(31, 32) = 3.98, p = 0.001$). Upon closer examination, substantial variability in Cu concentrations across different combinations was evident. For instance, the mean Cu concentration for KMB *A. mate* was 0.56 mg.kg⁻¹ while for KMK *A. mate*, it was 1.71 mg.kg⁻¹.

The Zn concentrations among the various combinations of locations and fish species were found to be statistically significantly different ($F(31, 32) = 32.29, p = 0.001$). A more thorough analysis revealed significant variation in zinc contents across various combinations. For example, the mean Zn concentration for KMB *A. mate* was 19.88 mg.kg⁻¹, whereas for KMK *A. mate*, it was 42.29 mg.kg⁻¹.

Significant differences in Fe concentrations were observed among the different combinations of locales and fish species in this study ($F(31, 32) = 10.6, p = 0.001$). Further examination revealed notable variability in Fe concentrations across the different combinations. For instance, the mean Fe concentration for KMB *A. mate* was 32.2 mg.kg⁻¹, whereas KMK *A. mate* had a mean concentration of 57.18 mg.kg⁻¹. These findings suggest distinct patterns or trends in Cu, Fe, and Zn concentrations across different combinations of locations and fish species, with some combinations exhibiting higher Cu levels compared to others.

Table 4: Descriptive of heavy metals bioaccumulation (mg.kg⁻¹ d.w) of the muscle tissue of fish collected from various MARA institutions in Peninsular Malaysia

Fish Species	Heavy Metals	Min.	Max.	Mean (n = 27)
<i>A. mate</i>	Cu	0.50	3.01	1.05 ± 0.74
	Zn*	18.55	43.31	30.48 ± 7.75
	Fe*	280	111.35	53.39 ± 20.44
<i>O. niloticus</i>	Cu	0.94	5.66	2.42 ± 1.23
	Zn*	12.55	26.81	18.75 ± 5.40
	Fe*	8.68	20.89	13.72 ± 3.24
<i>R. kanagurta</i>	Cu	0.35	5.85	1.10 ± 1.25
	Zn*	12.97	40.59	24.53 ± 8.32
	Fe*	27.97	95.75	43.62 ± 15.20
<i>P. niger</i>	Cu	0.58	16.41	2.45 ± 4.07
	Zn*	11.6	26.68	17.27 ± 4.30
	Fe*	9.38	23.56	16.75 ± 4.04

Note: * significant, $p < 0.05$.

Table 5: Heavy metals concentration (mg.kg⁻¹ dw) in different fish species across various institutions

Fish Species	Institution	Heavy Metals Concentration (mg.kg ⁻¹ dw)		
		Cu	Zn	Fe
<i>A. mate</i>	KMK	1.71 ± 1.19 *	35.39 ± 2.29 *	57.18 ± 5.00 *
	KMS	0.73 ± 0.33 *	27.71 ± 1.89 *	48.51 ± 3.22 *
	KMB	0.56 ± 0.06 *	19.88 ± 1.88 *	32.20 ± 5.94 *
	KMKN	1.84 ± 1.65 *	42.29 ± 1.45 *	90.48 ± 29.51 *
	MRSM.KP	0.87 ± 0.49 *	35.60 ± 2.71 *	45.64 ± 5.69 *
	MRSM.TK	0.89 ± 0.16 *	34.78 ± 4.04 *	71.66 ± 11.33 *
	MRSM.BP	1.22 ± 0.64 *	27.33 ± 1.32 *	39.44 ± 1.53 *
	MRSM.PT	0.61 ± 0.04 *	20.91 ± 0.80 *	42.04 ± 1.51 *
<i>O. niloticus</i>	KMK	2.10 ± 0.72 *	24.96 ± 0.58 *	12.99 ± 0.09 *
	KMS	1.88 ± 0.08 *	12.85 ± 0.42 *	9.38 ± 0.98 *
	KMB	1.01 ± 0.09 *	16.49 ± 0.02 *	12.21 ± 1.40 *
	KMKN	2.44 ± 0.05 *	14.73 ± 0.01 *	14.98 ± 1.64 *
	MRSM.KP	4.22 ± 2.04 *	13.65 ± 0.21 *	12.92 ± 0.75 *
	MRSM.TK	1.96 ± 0.56 *	22.54 ± 0.11 *	13.48 ± 0.11 *
	MRSM.BP	3.35 ± 1.16 *	26.08 ± 1.04 *	20.07 ± 1.17 *
	MRSM.PT	2.10 ± 0.72 *	24.96 ± 0.58 *	12.99 ± 0.09 *
<i>R. kanagurta</i>	KMK	0.90 ± 0.15 *	15.56 ± 3.66 *	35.49 ± 5.08 *
	KMS	0.83 ± 0.31 *	24.90 ± 0.37 *	37.45 ± 3.22 *
	KMB	0.47 ± 0.04 *	19.33 ± 2.55 *	41.83 ± 2.24 *
	KMKN	1.53 ± 0.65 *	28.86 ± 4.63 *	31.20 ± 4.57 *
	MRSM.KP	0.80 ± 0.30 *	38.93 ± 2.35 *	56.40 ± 2.13 *
	MRSM.TK	0.57 ± 0.13 *	19.36 ± 0.15 *	37.04 ± 3.80 *
	MRSM.BP	3.10 ± 3.89 *	35.55 ± 1.31 *	70.64 ± 35.52 *
	MRSM.PT	0.96 ± 0.37 *	16.56 ± 1.52 *	47.69 ± 1.05 *
<i>P. niger</i>	KMK	1.35 ± 0.08 *	26.56 ± 0.18 *	16.66 ± 0.86 *
	KMS	1.51 ± 1.08 *	15.81 ± 1.24 *	11.08 ± 2.40 *
	KMB	0.92 ± 0.27 *	18.18 ± 4.24 *	18.70 ± 0.45 *
	KMKN	1.24 ± 0.27 *	16.17 ± 0.01 *	18.68 ± 5.72 *
	MRSM.KP	1.13 ± 0.44 *	14.46 ± 2.37 *	12.46 ± 1.80 *
	MRSM.TK	0.59 ± 0.01 *	12.12 ± 0.73 *	18.04 ± 0.30 *
	MRSM.BP	11.96 ± 6.30 *	16.90 ± 0.18 *	17.29 ± 5.65 *
	MRSM.PT	0.94 ± 0.39 *	17.99 ± 0.92 *	21.15 ± 3.42 *

Note: * significant, $p < 0.05$.

Assessment of Heavy Metals in Edible Part of Fish on Human Health Risks

The calculation of health risk indices was based on the wet weight, as the fish is normally eaten fresh. The assessments of EDI, EWI, and PWTI for the heavy metals Cu, Fe, and Zn are shown in Table 6. The descending rank order of EDI, EWI, and % PWTI for each species are as follows: *A. mate* is Fe > Zn > Cu; *O. niloticus* Zn > Fe > Cu; *R. kanagurta* Fe > Zn > Cu; and *P. niger* Zn > Fe > Cu.

Table 7 presents the Target Hazard Quotient (THQ) and Hazardous Index (HI) of fish species in this study. The table lists the calculations of THQ and HI, which were obtained using the heavy metal concentrations given as their wet weights. The THQ is a ratio that evaluates the possible risk of non-carcinogenic effects from a single heavy metal due to long-term exposure through fish consumption. HI represents the sum

Table 6: The Estimated Daily Intake (EDI), Estimated Weekly Intake (EWI), and Provisional Weekly Tolerable Intake (PWTI) of heavy metals based on different fish species

Fish Species	Heavy Metals	EDI	EWI	% PWTI
<i>A. mate</i>	Cu	3.28×10^{-4}	2.30×10^{-3}	6.56×10^{-2}
	Fe	1.67×10^{-2}	1.17×10^{-1}	2.09
	Zn	9.53×10^{-3}	6.67×10^{-2}	9.53×10^{-1}
<i>O. niloticus</i>	Cu	7.56×10^{-4}	5.29×10^{-3}	1.51×10^{-1}
	Fe	4.29×10^{-3}	3.00×10^{-2}	5.36×10^{-1}
	Zn	5.86×10^{-3}	4.10×10^{-2}	5.86×10^{-1}
<i>R. kanagurta</i>	Cu	3.44×10^{-4}	2.41×10^{-3}	6.88×10^{-2}
	Fe	1.36×10^{-2}	9.54×10^{-2}	1.70
	Zn	7.67×10^{-3}	5.37×10^{-2}	7.67×10^{-1}
<i>P. niger</i>	Cu	6.13×10^{-4}	4.29×10^{-3}	1.23×10^{-1}
	Fe	4.19×10^{-3}	2.93×10^{-2}	5.23×10^{-1}

Table 7: Target Hazard Quotient (THQ) and Hazardous Index (HI) of fish species

Fish Species	Heavy Metals	THQ	HI	Status
<i>A. mate</i>	Cu	3.28×10^{-4}	6.4×10^{-2}	No adverse health risk
	Fe	1.67×10^{-2}		No adverse health risk
	Zn	9.53×10^{-3}		No adverse health risk
<i>O. niloticus</i>	Cu	7.56×10^{-4}	4.4×10^{-2}	No adverse health risk
	Fe	4.29×10^{-3}		No adverse health risk
	Zn	5.86×10^{-3}		No adverse health risk
<i>R. kanagurta</i>	Cu	3.44×10^{-4}	5.4×10^{-2}	No adverse health risk
	Fe	1.36×10^{-2}		No adverse health risk
	Zn	7.67×10^{-3}		No adverse health risk
<i>P. niger</i>	Cu	6.13×10^{-4}	6.0×10^{-3}	No adverse health risk
	Fe	4.19×10^{-3}		No adverse health risk
	Zn	4.32×10^{-3}		No adverse health risk

of all THQs of different heavy metals found in a single sample. HI provides an overall indication of combined risk from multiple heavy metals. The result shows that none of the fish species being served to the students in these institutions posed a significant non-carcinogenic risk based on the standard daily intake.

Carcinogenic Risks

All the analysed samples showed no detectable concentration of Pb and As. Therefore, the carcinogenic index could not be determined and this suggests that there is no possible carcinogenic risk to the students due to the consumption of fish.

Bioaccumulation of Cu

Cu is a heavy metal that is important for numerous physiological functions, including the creation of haemoglobin, enzymatic reactions, metabolism, and the immune system (Rodríguez *et al.*, 2017; Mangalagiri *et al.*, 2020). The assessment of the statistics demonstrated that the species of fish used in the research did not differ significantly in terms of their Cu bioaccumulation levels. Nevertheless, there was still noticeable variation in Cu levels across fish species. For example, the concentration of Cu in the muscle tissue of *P. niger* in this study was higher ($2.45 \pm 4.07 \text{ mg.kg}^{-1}$) than the reported value of Cu in *P. niger* from the coast of Morib, Malaysia, which recorded $0.36 \pm 0.21 \text{ mg.kg}^{-1}$. In contrast, the same study reported that the Cu in *P. niger* in Kuala Selangor was found to be below detection level (Tek & Ng, 2024).

Another study conducted on the same species within the coastal neighbouring country also recorded a lower value of Cu, which ranged from 0.76 to 1.28 mg.kg^{-1} (Charisma *et al.*, 2013). A similar result was also recorded by *P. niger* caught in the Persian Gulf, where the Cu level in the muscle tissue was recorded at $0.25 \pm 0.03 \text{ mg.kg}^{-1}$ (Hosseini *et al.*, 2018). Despite the Cu concentrations in *P. niger* in this research being greater than the value reported, the Cu level did

not exceed the allowable limits established by the WHO and MFA (30 mg.kg^{-1}), so humans would not be endangered if they consumed this fish. For *R. kanagurta*, the concentration of Cu recorded in this study ($1.10 \pm 1.25 \text{ mg.kg}^{-1}$) was within the range of the reported value of Cu in *R. kanagurta* ($0.22 - 15.61 \text{ mg.kg}^{-1}$) reported in various studies within this country (Mokhtar *et al.*, 2015; Octavianti & Jaswir, 2017; Shukri *et al.*, 2020; Mat Beyi *et al.*, 2021; Yap & Al-Mutairi, 2022). The level of Cu *R. kanagurta* in this study was also below the permissible limit according to WHO and MFA. The concentration of Cu in the muscle tissue of *O. niloticus* in this study was higher ($2.42 \pm 1.23 \text{ mg.kg}^{-1}$) than the reported value of *O. niloticus* from Bukit Merah Reservoir, which was recorded at $0.06 \pm 0.00 \text{ mg.kg}^{-1}$ (Abdullah *et al.*, 2022).

Other studies on the bioaccumulation of Cu in *O. niloticus* in this country showed that the range of Cu was $2.65 - 0.03 \text{ mg.kg}^{-1}$ (Shuhaimi-Othman & Ahmad, 2011; Amirah *et al.*, 2013; Zarith Sufiani & Mohd Yusoff, 2015; Alam *et al.*, 2015; Taweel Yusoff *et al.*, 2018; Abdullah *et al.*, 2022). However, the level of Cu in *O. niloticus* was still within the permissible limit. The level of Cu in *A. mate* in this study ($1.05 \pm 0.74 \text{ mg.kg}^{-1}$) was lower than the reported value of Cu in the same species from the coast of Terengganu as reported by Chuan *et al.* (2018), which ranged from $3.32 \pm 0.42 \text{ mg.kg}^{-1}$ to $6.67 \pm 0.22 \text{ mg.kg}^{-1}$. However, Azmi *et al.* (2019) and Yap and Al-Mutairi (2022) reported a lower value of Cu in *O. niloticus* (0.92 ± 0.0 and 0.39 ± 0.3 , respectively).

In summary, the research conducted in this study demonstrated that the muscle tissue of all fish species examined had a Cu bioaccumulation rate that was significantly lower than the limit of 30 mg.kg^{-1} set by the MFA and WHO. Consequently, the health risk assessment generated by the Cu-related health risk indices for each fish species suggests that it is doubtful that consuming these fish would result in any harm.

Bioaccumulation of Fe

Fe is considered an essential heavy metal and is a significant constituent of hemoglobin and other proper functions and physiological processes. Insufficiency of iron will lead to anemia, weakness, and a loss of focus. However, an elevated level of iron in freshwater can have detrimental effects on the well-being of organisms, including fish, and ultimately, humans. Hemochromatosis or iron overload can occur in humans when there is a significant accumulation of iron. This disease will impact the joints, heart, fertility, and cause persistent fatigue (Akinbile *et al.*, 2013). Elevated iron levels have been associated with the development of type 2 diabetes, as well as age-related conditions like Parkinson's and Alzheimer's disease and liver cancer (Matouke & Abdullahi 2020; Alipour *et al.*, 2021).

The bioaccumulation of Fe in all fish species in this present study was found to be above the permissible limit according to MFA (0.5 mg.kg⁻¹). *A. mate* recorded the highest concentration of Fe compared to the other fish species in this study. As a predatory pelagic fish positioned at a higher trophic level, *A. mate* typically accumulates more heavy metals. However, this is the least concern, as Fe toxicity is rare, and Fe is more associated with deficiency. Throughout the country, reports have revealed that sea-based and freshwater-based types of fish have high Fe levels.

The fish tissues accumulate such levels because Fe is a vital heavy metal. A direct comparison based on specific fish species is rather difficult, as many studies on Fe conducted in this country normally excluded the element Fe from their studies. However, a few studies on *O. niloticus* in this country showed that the level of Fe ranged between 3.50 mg.kg⁻¹ – 0.73 mg.kg⁻¹ (Yap *et al.*, 2015; Abdullah *et al.*, 2022). The significant differences in Fe concentrations across fish species underscore the importance of understanding the role of fish species in accumulating Fe from their environment.

Further investigation into the ecological and environmental factors influencing Fe

accumulation in different fish species could provide valuable insights into potential sources of contamination and ecosystem health. In summary, the results of the ANOVA demonstrate the importance of the fish species in determining the concentrations of Fe. This highlights the necessity of targeted strategies to monitor and manage any aquatic ecosystem in order to ensure its health and sustainability. The indices utilised to evaluate the health risk associated with Fe in all fish varieties confirmed that students would not be exposed to non-carcinogenic hazards if they consumed the five species despite the Fe levels exceeding the MFA's permissible limit.

Bioaccumulation of Zn

Zn, as one of the essential heavy metals plays a significant role in physiological processes such as hundreds of enzymatic activities that serve as an important cofactor (Briffa *et al.*, 2020). However, if the level of Zn exceeds the permissible limit, it may have adverse effects on the reproductive system (Mahboob *et al.*, 2014). For *A. mate*, the bioaccumulation of Fe in this study (30.48 mg.kg⁻¹) was higher than the reported value of Zn within the same species (8.49 ± 4.0 mg.kg⁻¹) (Azmi *et al.*, 2019). There is a scarcity of information regarding the bioaccumulation of Zn in this species in this country and its neighbouring countries.

The higher concentration of Zn in *A. mate* may be attributed to the fact that it belongs to the Carangidae family and is primarily a pelagic predator that feeds on small fish, invertebrates, and plankton. Predatory fish are known to accumulate higher levels of heavy metals and Zn, being an essential trace element tends to accumulate in greater concentrations. Meanwhile, the bioaccumulation of Zn in *O. niloticus* (18.75 ± 5.40 mg.kg⁻¹) in the study was found to be within the reported value of Zn in *O. niloticus*, which ranged from 0.53 to 31.00 mg.kg⁻¹ (Taweel *et al.*, 2011; Amirah *et al.*, 2013; Alam *et al.*, 2015; Zarith Sufiani & Mohd Yusoff, 2015; Yusoff *et al.*, 2018; Abdullah *et al.*, 2022).

The bioaccumulation of Zn in the muscle tissue of *P. niger* in this study (7.27 ± 4.30 mg.kg⁻¹) was found within the reported value of Zn in *P. niger* in this country, which ranged from 5.12 – 9.21 mg.kg⁻¹ (Tek & Ng, 2024; Azmi *et al.*, 2019). A similar result was obtained for the bioaccumulation of Zn in *R. kanagurta* (24.53 ± 8.32 mg.kg⁻¹), which was well within the reported value of Zn in *R. kanagurta* in this country, that ranged from 9.08 to 38.95 mg.kg⁻¹. For each species of fish used in the research, the Zn bioaccumulation was under the 100 mg.kg⁻¹ limits allowed by the WHO and MFA. Consequently, the health risk assessment, as determined by health risk indices for Zn in all fish species, indicates that there was no discernible harm associated with consuming these fish species.

Conclusions

The research has shown that in the fish that the students were served, the heavy metals were found to be below the limit allowed by the WHO and MFA. The below-detection levels prevented the detection of some heavy metals (As, Cr, Pb). The calculation of health risks based on detected heavy metals showed no possible non-carcinogenic or carcinogenic health risks to the students. Therefore, it is possible to conclude that for each type of fish utilised in this study (*A. mate*, *O. niloticus*, *P. niger*, and *R. kanagurta*) and served to students in these institutions, the bioaccumulation of each type of heavy metal was under the allowable limits stipulated by the national- and international-level food guidelines, so, consuming these species would not pose a danger to health.

In light of these findings, the current types and frequency of fish consumption in these institutions can be considered safe and may be continued without modification. These preliminary data provide essential information to shed light on the aspect of food security (fish) for the students. Based on the results obtained in this study, future researchers should take several recommendations into consideration. These

suggestions include programmes to monitor the bioaccumulation of and contamination by heavy metals in every species of fish served to the students. Regular checking is required, particularly if the fish source comes from areas by the coast, which are more likely to be polluted and experience anthropogenic activities. A more comprehensive study in the future needs to cover a wide range of other heavy metals such as mercury (Hg) and cadmium (Cd). These two heavy metals are known to cause severe health problems to humans and fish consumption is considered one of the leading causes of these heavy metals' contamination in humans. The type of speciation of each heavy metal also needs to be considered, as it highly influences the toxicity level. For example, methylmercury is more carcinogenic than inorganic mercury and the same is true for the element As, where inorganic arsenic is more potent than organic As.

Acknowledgements

I extend my sincere gratitude and appreciation to Majlis Amanah Rakyat (MARA), especially Innovation & Creative Technology Division (BITK) for providing the necessary funding and resources essential for carrying out this research endeavour. Last but not least, I would like to express my heartfelt thanks to Mr. Muhammad Yusri Mohd Zaini for his help during the sample collection. This research was supported by Research and Innovation Grant 2023 (MARA-600-6/4/3 JLD 2(74)) under Innovation & Creative Technology Division (BITK), Majlis Amanah Rakyat (MARA).

Conflict of Interest Statement

The author declares that they have no conflict of interest.

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