RESEARCH ARTICLE

Health risk assessment of heavy metals in Indian Mackerel (*Rastrelliger kanagurta*) sold in selected wet markets in Hulu Langat District, Malaysia

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Abstract:

Indian Mackerel (*R. kanagurta*) is valued for its nutritional benefits and contribution to a healthy diet. However, increasing anthropogenic activities have raised concerns about elevated concentrations of heavy metals (HMs) in marine ecosystems, which may pose health risks through their accumulation in marine species. This study aimed to assess the concentrations of HMs in *R. kanagurta* and evaluate the associated health risks based on estimated fish intake among Malaysian adults and children. Samples were collected from selected wet markets in Hulu Langat, Malaysia, and analyzed for Cd, Cu, Cr, and Zn using atomic absorption spectroscopy (AAS). The concentrations of HMs in the analyzed fish samples were found to be within acceptable limits set by FAO, WHO and Malaysian Food Regulations 1985, and ordered as Zn > Cu > Cr > Cd. Despite the concentrations being within permissible limits, the Target Hazard Quotient (THQ) values indicated low hazard risks for both groups (THQ<1). The study recommends further investigation, including the analysis of bioavailable metal forms, for a more precise risk assessment. Continuous monitoring of HM levels in *R. kanagurta* is crucial to ensure regulatory compliance, provide data for risk assessment, and serve as an early warning system to ensure consumer safety.

Keywords: *Rastrelliger kanagurta*, Risk Assessment, Target Hazard Quotient, Wet Market

1. INTRODUCTION

Fish is one of the important sources of protein for consumers in Malaysia (Ahmad et al., 2016; Kamaruddin et al., 2023). Fish does not only provide protein, but also contains various minerals, vitamins, and polyunsaturated omega-3 fatty acids which are able to lower the risk of cardiovascular diseases and some types of cancer (Zhu et al.,2015). Given its high nutritional value, it is recommended to consume one dish of fish per day (MOH, 2020). With an average consumption of at least 46.9 kilograms per person per year, Malaysia ranks second in Southeast Asia for the highest fish consumption (Kamaruddin et al., 2023). However, environmental pollution is becoming more serious in some coastal areas and estuaries with rapid industrial development. These activities have significantly increased the amount of heavy metals (HMs) that naturally occur in the environment, particularly the marine ecology. Worryingly, marine organisms such as fish may store these pollutants in their muscle tissue and pose a threat to human health, especially for individuals who consume large amount of fish (Bosh et al., 2016 ; Han et al., 2021).

HMs have been considered as a serious global environmental threat (Han et al., 2021). The most common HMs found in aquatic environments include cadmium (Cd), nickel (Ni), zinc (Zn) and copper (Cu) (Han et al., 2021; Luczynska, Paszczyk and Luczynski, 2018; Mangalagiri et al., 2020; Sedeghi et al., 2021; Naji, Khan and Hashemi., 2016; Joseph et al., 2022). These contaminants are concerning because of their difficulty in degrading and high potential for bioaccumulation in the aquatic environment (Chen & Chau, 2016). Consequently, marine fish will absorb toxic metals from the water and soil around them as well as from their food (Han et al., 2021). Previous studies have confirmed that their country's marine fish is contaminated with HMs. For instance, it was discovered that the levels of chromium and arsenic in samples of marine fish collected from sites near Hainan, China, did not meet the food safety standards established by the Joint FAO/WHO Expert Committee on Food Additives (Liu et al., 2015). Arsenic, cadmium, chromium, mercury, and lead were also found in samples of marine fish collected from the coastal areas of Zhejiang, China (Han et al., 2021). Therefore, there is a possibility that marine fish in other countries including Malaysia has been contaminated with heavy metals.

R. kanagurta is one of the marine fish that Malaysians often consume and can be found in most wet markets around the country (Rahman, Hajar & Yunus., 2020). This fish species is popular among Malaysians because it has a low market price, high abundance and is easy to catch (Rahman & Hafzath, 2012). However, several studies revealed that *R. kanagurta* in their region has been contaminated with HMs (Han et al., 2021; Mangalagiri et al., 2020; Khander et al., 2015; Akila et al., 2022; Mziray & Kimirei 2016). In Malaysia, Yap & Al-Mutairi (2022) identified the presence of Cu and Zn in *R. kanagurta* caught from Setiu, located on the East Coast of Peninsular Malaysia. Foods that contain hazardous metals above the permitted levels are regarded as being potentially harmful to human health. Hence, a health risk assessment was carried out in this study to calculate potential risk posed by the HMs found in the *R.kanagurta* samples. This is concerning because exposure to HMs can have negative consequences on one's health. Once entering the body, HMs are transported and compartmentalized into body cells and tissues, where they bind to proteins and nucleic acids, causing them to be destroyed and disrupting their cellular activities (Azeh et al., 2019). HMs may lead to several consequences in the human body such as mental disorders, damaging the blood constituents, and damage to organs such as the lungs, liver, kidney, and other organs which can lead to several disease conditions (Azeh et al., 2019 ; Jaishankar et al., 2014). Other than that, accumulation of HMs for a long time may lead to slowing progression of physical, muscular, and neurological degenerative processes which mimic diseases such as Parkinson's disease and Alzheimer's disease (Azeh et al., 2019; Jaishankar et al., 2014; Balali-Mood et al., 2021; Islam et al., 2022; Branca, Morucci & Pacini et al., 2018).

2. MATERIALS AND METHODS

2.1. Study area and sample collection

The study was conducted in three wet markets located in the Hulu Langat district, Malaysia. This district encompasses an area of approximately 829.44 km² and has a population of 1,156,600. The ethnic composition of the population includes Malays (50.73%), Chinese (30.76%), Indians (9.84%), and others (0.99%) (Pejabat Daerah Tanah Hulu Langat, 2018). Hulu Langat is an urban area characterized by commercial buildings, shopping complexes, roads, and private hospitals. The selected wet markets were chosen due to their high population density compared to other areas within the Hulu Langat district. In these markets, there were five stalls that sold Indian Mackerel (*R. kanagurta*). From each stall, three samples of *R. kanagurta* were collected and placed into high-density polyethylene (HDPE) plastic bags. The samples were then transported to the laboratory in ice boxes, where the temperature was maintained at 4ºC, in order to preserve their quality. FERC/FSK/EM/2022/0021

2.2. Sample preparation and analysis

Prior to sample preparation, the fish were thoroughly cleaned with distilled water to remove any blood. The fish were then dissected, and approximately 5 grams of muscle tissue were extracted for analysis. The samples were placed in crucibles and heated on a hot plate at 150-200ºC until all smoke ceased. Subsequently, the crucibles were transferred to a muffle furnace and ashed at 450ºC for 8 hours. After ashing, 5 ml of 1% HNO3 was added to each ash sample, which was then gently boiled to dissolve the ash. The resulting solution was filtered using a cellulose nitrate filter (Whatman) and diluted to 100 ml with 1% HNO3 in volumetric flasks (Kalagbor & Opusunju, 2015). The prepared solutions were then analyzed using Atomic Absorption Spectrophotometry (AAS) to determine the concentration of heavy metals (Naji, Khan & Hashemi, 2016; Ishak, Zuhdi & Aziz, 2020).

2.3. Health risk assessment

The data from the findings were used to calculate the target hazard quotient (THQ) for non-carcinogenic risk using the following equation:

$$THQ = \frac{E_{FR} \times Ed \times F_{IR} \times C}{\text{RfD} \times B_{Wa} \times ATn} \times 10^{-3}$$

For adult, the average fish ingestion rate (F_{IR}), exposure frequency (E_{FR}), exposure duration (Ed), body weight (B_{Wa}) and averaging time (AT_n) values was obtained from previous studies (Khandaker et al., 2015; Praveena & Omar, 2017). The values of F_{IR}, E_{FR}, Ed and B_{Wa} for Malaysian adult were 130 g/day, 365 days/year, 74 years and 62.65 kg, respectively. On the other hand, the values of F_{IR}, E_{FR}, Ed and B_{Wa} for Malaysian children were 70 g/day (equivalent to one serving size), 365 days/year, 74 years and 19.5 kg, respectively obtained from studies done by Praveena & Omar (2017) and Ministry of Health (2023). When the THQ < 1, the non-carcinogenic health effects are not expected to happen. However, if the THQ> 1, adverse health effects are possible to be experienced (Abbas, Ismail & Easa, 2008).

2.4. Ethical consideration

This study was classified as exempt by the Faculty Ethics Review Committee of the Faculty of Health Sciences, Universiti Teknologi MARA (Ref. No.: FERC/FSK/EM/2022/0021).

2.5. Statistical analysis

The data obtained in this study were statistically analyzed using SPSS version 28.0. Comparison of heavy metals concentration on *R. kanagurta* samples from three different wet markets were verified by the non-parametric test Kruskal-Wallis. The significance level for the analysis was set at 0.05.

3. RESULTS AND DISCUSSION

Table 1 summarized the concentration level of selected heavy metals detected in *R. kanagurta* samples. Based on Table 1, Zn (5.55 ± 2.16 mg/kg) is the highest mean concentration of HMs in the sample, followed by Cu (0.50 ± 0.21 mg/kg), Cr (0.14 ± 0.32 mg/kg) and Cd (0.09 ± 0.12 mg/kg). Thus, the mean of HMs concentration in the sample from all wet markets can be ranked as Zn > Cu > Cr > Cd. When the concentration is compared with the standard limit, only Cd (0.09 ± 0.12 mg/kg) has the concentration which exceeds the permissible limit of FAO and WHO (0.05 mg/kg). However, when the concentration is compared with Malaysian Food Regulations 1985 (1.00 mg/kg), the value is still within the permissible limit. Meanwhile, the concentration of Cu, Cr and Zn in all sample are still within the permissible limit of FAO, WHO and Malaysian Food Regulations 1985. Based on the result, Zn has the highest concentration of HMs as compared to other HMs since it is the second most trace element presents in almost all living organisms including fish (Lall & Kaushik, 2021). The abundance of Zn in the cells assists in biological functions for vertebrates which are catalytic, metabolism, growth function and restraining free radical oxygen in fish (Prabhu, Schrama & Kaushik, 2016). Other than that, Zn also plays an important role in structural function, which involves modifying enzyme activities and stabilizing tertiary structures of enzyme. For example, in Cu-Zn superoxide dismutase, Zn acts as a structure for this enzyme (Muralisankar et al., 2014). Marine fish like *R. kanagurta* are mainly ingesting sea water for their internal body fluid needs, which may also absorb inorganic elements like Zn in their gastrointestinal tracts (Lall & Kaushik, 2021). This explains why the content of Zn is higher than other HMs.

Statistical analysis revealed that only Cd and Zn concentrations were significantly different between wet markets ($p<0.05$). This could be due to different places where the fish were caught. In the study, sample from M2 is known to be originated from Kuala Sepetang coast which is located in west coast. This area was well-known for its seafood catches such as cockles and fish, but it has declined lately due to some possible factors (Zhi et al., 2017). The factors are due to algal blooms (caused by overabundance nutrient), mariculture and man-made pollution, specifically plastic waste. Illegal dumping has contaminated the village river with rubbish (Chen & Chau, 2016). Cr might be present in plastic waste, since chromium compounds are used in plastic production, as it is resistant to discoloration and corrosion (Ahimbisibwe et al., 2022). Hence, this explains the presence of chromium in fish collected in Kuala Sepetang coast. Next, in the sample from M3, it is known to have originated from Kuala Selangor coast which is also located on the west coast. A possible source of Cd on the coast might be due to leakage of leachate from Jeram Sanitary landfill. The landfill is connected with Sungai Sembilang which ends in Kuala Selangor coast. A study revealed that Cd is the pollutant present in leachate along with Cr, Cu, Fe, Ni and Pb (Ma et al., 2022). As the river flows until meets the Kuala Selangor coast, it also carries the pollutant onto the coast. The concentration of Cu in the sample is low, it might happen because this trace element has already been diluted by large quantity of rainfall (Karnan et al., 2021). Alongside the river, there are some factories such as latex products and furniture factories which are possible to contaminate the river with its effluent and by product. Hence, this could be the main reason for the presence of Cu on the coast. Last, the sample from M1 was known to have originated from Pangkor Island coast and Kuala Perlis. According to a study, although there is no pollution was detected in Kuala Perlis coast, a high concentration of Zn along with As and Pb were found in the mouth of Kuala Perlis River (Wong & Yong, 2020). The river ends in the coast, which could be the main contributor to the presence of Zn.

Table 1. Concentrations of heavy metals in *R. Kanagurta* samples

Heavy Metals	Wet Market			Mean±SD (mg/kg)	*p*-value
	Bandar Baru Bangi	Kajang	Semenyih		
	Mean±SD (mg/kg)	Mean±SD (mg/kg)	Mean±SD (mg/kg)		
Cr	BDL*	0.35±0.18	BDL*	0.14±0.32	0.075
Cd	BDL*	0.13±0.06	0.19±0.03	0.09±0.12	0.041**
Cu	0.43±0.08	0.57±0.11	0.48±0.02	0.50±0.21	0.756
Zn	4.28±0.21	7.07±1.15	5.05±0.24	5.55±2.16	0.024**

*BDL – below detection limit (Cr=0.0015 mg/kg; Cu=0.0015mg/kg; Cd=0.0008 mg/kg; Zn=0.0015 mg/kg)
***p*-value is significant at 0.05

Based on Table 2, the THQ for adult and child of all HMs from the wet markets are in the ranged of 2.25×10^{-2} to 6.93×10^{-1}. The values were below 1, which can be considered as safe to consume and no risk of human health effects. Even though the concentration of HMs detected in this study were the permitted limit, there is still a risk of health, especially if higher the amount of contaminated fish with the HMs is consumed. Hence, health effects such as nausea, stomachache, diarrhoea, and kidney issues that are raised from the consumption are expected to happen. Other than these effects, Cd may lead to health problems such liver dysfunction, pulmonary edema, testicular damage, osteomalacia, and adrenal and hematopoietic damage (Tinkov et al., 2018). Ingestion of cadmium can lead to cancers such as bladder, prostate and pancreas cancer, since it has been categorized as a human carcinogen (group I of International Agency for Research on Cancer classification) (IARC, 1997). Besides, excessive exposure of HMs through ingestions may also cause health problems. For example, copper toxicity which can disrupt normal cell function and acute poisoning may happen from excessive intake amount of Cu (Yap & Al-Mutairi, 2022). Overexposure to zinc may cause dizziness and abdominal pain shortly after the exposure. In addition, chronic effects after long-term exposure to high levels of Zn also cause weakened immune function, low levels of good cholesterol, and copper deficiency (National Institutes of Health, 2022).

Table 2. THQ values for Cd, Cr, Cu and Zn in this study

Wet Market	Group	Cr	Cd	Cu	Zn
WM1	Adult	-	-	2.25×10^{-2}	2.96×10^{-2}
	Child	-	-	3.89×10^{-2}	5.12×10^{-2}
WM2	Adult	2.40×10^{-1}	2.70×10^{-1}	2.96×10^{-2}	4.89×10^{-2}
	Child	4.15×10^{-1}	4.67×10^{-1}	5.12×10^{-2}	8.46×10^{-2}
WM3	Adult	-	4.00×10^{-1}	2.49×10^{-2}	3.49×10^{-2}
	Child	-	6.93×10^{-1}	4.31×10^{-2}	6.04×10^{-2}

4. CONCLUSION

The present study shows that *R. kanagurta* sold in selected wet markets in Hulu Langat do accumulate HMs (Cr, Cd, Cu, Zn). The HMs were found to be in the order of Zn > Cu > Cr > Cd. The content of HMs in the samples were within the permissible limit of the FAO, WHO and Malaysian Food Regulations 1985. The calculated THQ for adult and child were found to be less than 1 (THQ<1), which suggested low hazard risks for both groups. This should be concerned as fish is taken daily as source of protein, which may higher the risk of carcinogenic effects by consumers in Malaysia. For more precise risk assessment, the study suggests further investigation, including bioavailable metal form analysis. It is recommended that HMs levels in *R. kanagurta* be continuously monitored in order to ensure the regulatory compliance and guarantee consumer safety.

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