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Levels of trace metals in edible fish species of Permanent Forest Reserve (PFR), Merapoh, Pahang: a threat to Batek tribe

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Abstract. Monitoring metals in fish can give concise information on the water quality status than only monitoring of sediments and water column. The evaluation of metals in the fish can be used as an indicator to know the metals transfer to the human body through fish consumption. This study was carried out to evaluate the distributions of metals elements (Ni, Cu, Zn, and Cd) in fish species *Cyclocheilichthys apogon*, *Rasbora elegans*, and *Barbodes lateristriga* sampled from two rivers in Permanent Forest Reserve, Merapoh, Pahang. Metal elements were analysed using atomic absorption spectrometry and consumption rates advisory were estimated to assess the safety of fish consumption within Batek tribe were estimated. In risk and safety assessment, the metal concentrations in the edible muscles of fish samples were found to be below the established limits (Malaysian Food Act, 1983), except for Zn and As. However, target hazard quotient (THQ) values suggested avoiding consumption of *C. apogon* from Sungai Jalang and Sungai Kalong to reduce the potential risk to Batek tribe as the values of arsenic and cadmium approached unity. It is suggested that Cd and As are the best descriptors in characterising the potential of *C. apogon* as a bioindicators of metal pollution in aquatic ecosystem.

1. Introduction

Indicators of environmental quality enclose a number of environmental aspects such as biotic life, air, water, and soil. One example, of the fundamental human needs is access to clean food and water sources [1]. Additionally, rivers and streams play an important role in the lives of Batek, due to their dependence on them as sources of food and water. The Bateks are a group of gatherers and egalitarian hunters, who live in the forest as nomadic lifestyle [2]. In aquatic environments, metals are introduced by various sources, such as inputs from physical breakdown, natural atmospheric deposition, and human activities [3]. Metals whose concentration exceed the established limits are considered pollutants and they significantly impact the quality of aquatic ecosystem, due to their persistency and bio-accumulation potential via food chain [4]. Among aquatic biota, fishes are the most suitable species to use in assessing the contamination in streams and rivers. Previous study has established that fish can accumulate in concentration several times higher than the water column and sediment [5]. Apart from that, fish can be regarded as a good bioindicator because it is easily sampled, found in abundant population, has the ability to accumulate metals and has a long life span with an optimum size for analysis [6, 7]. In the present study, distribution of metals in *Cyclocheilichthys apogon*,



Rasbora elegans, and *Barbodes lateristriga*, from rivers located in Permanent Forest Reserve, Merapoh, Pahang was investigated. The site-specific metal distribution patterns in study samples were further examined. This aims to demonstrate the capability of fish species in providing information about the degree of metal pollution in the aquatic system and to reduce the risk of detrimental health effects to Batek community due to their dependence of on the mentioned species as a source of food.

2. Materials and Methods

2.1. Study area

Sungai Kalung and Sungai Jalang are located in Permanent Forest Reserve, Merapoh, Pahang, which is located in Central Forest Spine-Primary Linkage, interconnecting National Park and Titiwangsa range. These areas support four forest types which are lowland, upper hill and mountain forest. Generally, the Bateks collect food sources from these jungles to feed the community and rely on animals for protein by hunting in the forest using traps, spears and blowpipes. Furthermore, their favourite foods are made from the animal products of fish, birds, monkeys, and deer [2].

2.2. Sample collection and prepossessing

Field study and sampling were conducted from August until November 2020. The *Cyclocheilichthys apogon*, *Rasbora elegans*, and *Barbodes lateristriga* were provided by Batek people, and were caught from Sungai Kalung and Sungai Jalang, Merapoh, Pahang. The specimens (5 specimens for each fish species) were wrapped individually in polyethylene sampling bags, kept in an ice box, and transported to the laboratory on the same day. The total fish lengths and weights were recorded before they were stored at a temperature -20 °C until dissection could be performed. Muscles that had been removed were cleaned several times with deionized water. Then, these tissues were dried in the oven at 110 °C to achieve constant weight. In order to improve the sample homogeneity, each dissected fish sample was ground using pestle and mortar, and stored in a desiccator.

2.3. Laboratory procedure

All chemicals used were of analytical grade where sixty-five percent HNO₃ solution were of suprapur quality (Merck). Certified Reference Material ERM®- BB422 for trace metals were from European Commission, Joint Research Centre Institute for Reference Materials and Measurements (IRMM) Geel, Belgium. The acid digestion method was used based on the analytical method for flame atomic absorption spectrophotometer (AAS). About 1.0 g of dried samples were weighed directly into 50 ml beaker and digested with 10 ml of HNO₃ for 60 minutes on a hot plate to prevent vigorous reactions. Then, the temperature was raised to 150 °C and maintained for another 3 hours. Once the digestion was completed all tissue samples were completely dissolved in the acid. The sample was cooled down before adding double-distilled water into 250 ml volumetric flask. The clear sample was filtered through 0.45 µm PTFE membrane. All the digestate solutions were stored into centrifugal tubes below 8 °C and were analysed by AAS.

2.4. Calculation and analyses

All calculation and statistical analyses were performed with Microsoft® Excel 2010 and SAS® JMP® version 9 software package. An analysis of variance (ANOVA) was carried out to determine the significant differences between the studied elements [8]. The condition factors (CFs) was used to quantify the condition of fish samples which include the nourishment and degree of maturity, [9] CFs can be estimated by the following equation:

$$CF = \left[\frac{W}{L^3} \right] \times 100$$

where W is the weight of fish body in grams, and L is the length of fish in centimetres. In this study, the methodology of estimation target hazard (TH) provided indication of human health risk due to contaminant exposure. The equation used to estimate the target hazard quotient is as follow [10]:

$$THQ : \frac{EDI}{RfD}$$

3. Results and Discussion

Certified reference material (ERM®- BB422) was used to check the accuracy of acid digestion/extraction method. Table 1 demonstrates that the determined elemental concentrations were in good agreement with the certified reference material.

Table 1. Analysis of certified reference material.

		ERM®- BB422	
	Certified / mg/kg	Found / mg/kg	Recovery / %
Ni	-	-	-
Cu	1.67	1.0	60
As	12.7	12.82	100
Zn	16.0	18.7	116
Cd	0.0075	-	-

- not certified

Morphometric data of *Cyclocheilichthys apogon*, *Rasbora elegans*, and *Barbodes lateristriga* samples are summarised in Table 2. In order to overcome the potential of metal variability, an effort was made to analyse fish species with a comparable size from both sites. Although there are differences in size variation, no significant difference was observed in their CF values which means that no substantial differences were noted in the effect of metal concentration in studied fishes [6,8 11].

Table 2. Biometric parameters of studied fishes.

	Sungai Kalung (4°37'09.6"N 101°58'46.4"E)			Sungai Jalang (4°42'14.9"N 101°59'44.9"E)		
	<i>C. apogon</i>	<i>R. elegans</i> ,	<i>B. lateristriga</i>	<i>C. apogon</i>	<i>R. elegans</i> ,	<i>B. lateristriga</i>
Length (cm)	37 ± 0.3	11 ± 0.3	26 ± 0.4	17 ± 0.8	5.0 ± 0.8	29 ± 0.2
Weight (g)	14 ± 0.3	10 ± 0.8	11 ± 0.1	10 ± 0.5	7.0 ± 0.2	12 ± 0.7
Conduction factor (CF)	1.0 ± 0.3	0.9 ± 0.1	1.0 ± 0.8	1.0 ± 0.3	1.0 ± 0.5	1.0 ± 0.5

The mean metal concentrations in muscle tissues of studied fish samples are summarised in Table 3. The results demonstrated that studied fishes from both sites generally contained higher concentrations of Zn. Essential metals such as Zn are needed in fish metabolism, while other elements such as As and Cd have no function in the biological system. Due to storage, excretion, regulation, and absorption mechanisms, studied fishes show significant differences ($p < 0.005$) in metals accumulation rates [3]. One of the noteworthy observations was the mean concentration of As found were higher in *C. apogon* from both sites. It is believed that the significant amount of As found in *C. apogon* could be linked to the mining activities in Merapoh, Pahang [12]. Such elements are persistent and toxic in humans [13], thus their accumulation in fish muscles can threaten Batek tribe if the fish is consumed.

Overall, metal concentrations found were generally to be below the maximum permissible limit [14]. However, concentrations of Zn and As in *C. apogon* reportedly exceeded the maximum permissible limit set by Malaysia Food Act 1983 (Zn-100 mg/kg ; As-1 mg/kg). This could be an alarming finding in the general public view if the total As is considered as hazardous. The toxicity of As rely on its chemical species where the organic As, which is found mostly in fish species, is innocuous compared to inorganic As, which accounts for less than 10 % of total As, is toxic. Thus, it is important to consider both metal intakes and doses that can lead to harmful human effects due to ingestion of metals [15].

Table 3. Metal concentration in fish samples for both sites.

mg/kg	Sungai Kalung			Sungai Jalang		
	<i>C. apogon</i>	<i>R. elegans,</i>	<i>B. lateristriga</i>	<i>C. apogon</i>	<i>R. elegans,</i>	<i>B. lateristriga</i>
Ni	2.0 ± 0.1	1.0 ± 0.3	7.0 ± 0.2	11 ± 0.9	7 ± 0.2	-
Cu	2.0 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	9 ± 0.3	1.0 ± 0.1	-
As	8.0 ± 0.4	-	-	50 ± 1.0	-	-
Zn	140 ± 0.0	66 ± 0.8	63 ± 0.7	145 ± 0.5	92 ± 0.8	56 ± 0.7
Cd	1.0 ± 0.3	0.6 ± 0.1	0.8 ± 0.0	1.0 ± 0.3	0.4 ± 0.0	0.6 ± 0.1

All reported values are referred to dry base, mean concentration ± s.d, $n = 35$

- not detected

The non-carcinogenic health risks associated with fish consumption are evaluated based on target hazard quotients (THQs). If THQs levels assume below of one exposure (THQ <1), it is unlikely human will experience any adverse effects. Higher THQ values (THQs >1) mean experiencing a higher probability of non-carcinogenic effects in a long term [8, 16, 17]. The THQ values in this work are shown in Table 4.

Table 4. The target hazard quotient for metal ingestion.

mg/kg	Sungai Kalung			Sungai Jalang		
	<i>C. apogon</i>	<i>R. elegans,</i>	<i>B. lateristriga</i>	<i>C. apogon</i>	<i>R. elegans,</i>	<i>.lateristriga</i>
Ni	0.12	0.07	0.4	0.7	0.04	-
Cu	0.06	0.01	0.02	0.01	0.03	-
As	>1	-	-	>1	-	-
Zn	0.52	0.25	0.24	0.54	0.34	0.21
Cd	1.4	0.74	0.92	1.4	0.46	0.74

- not reported

As can be seen, THQ values are significantly higher than 1 for As and Cd for *C. apogon* from both sites, thus it can be concluded that the evaluated metal concentration in the edible parts of *C. apogon* from studied sites pose adverse health effects to Batek community in Merapoh, Pahang. This implies that the consumption of *C. apogon* from Sungai Kalung and Sungai Jalang over a lifetime is likely to cause harmful effects to Batek tribe based on the THQ values. It is shown that the sources of As pollution are derived from mining activities [12]. In addition, exposures to Ni, Cu and Zn via fish ingestion could be deemed as non-significant since each of them has small THQ values [18]. However, it is advisable to regulate the intake of *C. apogon* to reduce the risk of deleterious health effects.

4. Conclusion

This study showed that metal distribution in studied fishes was strongly associated with the type of species and fish habitat. The variability in study area and diet was regarded as the main factor for the observed differences. More attention should be given to the consumption of *C. apogon* from both study areas as it was observed that values of THQ for Cd and As were more than 1. It is suggested that As and Cd are the best descriptors in characterizing the potential of *C. apogon* as potential bioindicator of metal pollution in freshwater ecosystem.

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