E3S Web of Conferences **158**, 05003 (2020) https://doi.org/10.1051/e3sconf/202015805003
ICEPP 2019

Concentration of copper (Cu) in tinfoil barb fish (*Barbonymus schwanenfeldii*) of Kuantan River and Pinang River, Pahang, Malaysia

Nadzifah Yaakub[1,*], *Shu Qi* Choong[1] *and Wan Marlin* Rohalin[1]

[1]School of Animal Science, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, 22200 Besut, Terengganu, MALAYSIA

Abstract. The study was conducted to determine of copper (Cu) in muscle tissue of Tinfoil Barn Fish (*Barbonymus schwanenfeldii*) and surface water at Kuantan River and Pinang River, Pahang. The study also determine the water quality parameters and water quality index (WQI). The fish was caught by using gill net and the were digest using acid digestion method and analysed by Inductive Coupled Plasma Micro Spectrometer (ICP-MS). The mean concentration of Cu in fish muscle was 0.5070 ± 0.01748 mg/kg for Kuantan River and 0.4732 ± 0.01807mg/kg for Pinang River which below the permissible limit set by Malaysia Food Act (MFA) and Food and Agriculture Organization (FAO). Cu concentration were 0.0052 ± 0.0004390 mg/kg in Kuantan River and 0.0017 ± 0.00006669 mg/kg in Pinang River. The level of Cu in both rivers were not harmful to the fish as the concentrations are below the permissible limit set by US Environmental Protection Agency (USEPA) and National Water Quality Standard (NWQS). There was no significant difference ($p < 0.05$) in the concentration of muscle tissue for Cu between rivers. In contrast, there showed a significant difference ($p < 0.05$) in the concentration of Cu in water between rivers. Kuantan River and Pinang River have been classified in Class II according to the Department of Environment (DOE) that the water must require conventional treatment for water supply purpose.

1 Introduction

Fish is one of the important animal protein sources for human consumption. Due to the lower content of fat or carbohydrate in white-fleshed fish compared to other animal meat makes they become an important part of a healthy diet [1]. In several decades, the issue of heavy pollution in water bodies, particularly in the freshwater system concerns in society. Heavy metals are mostly toxic although some heavy metals are essential at a low level for enzymatic activity and other biological processes. If this essential metal exceeds the required level of living beings, they will become toxic and lethal to the living organism [2]. In the heavy metals polluted water bodies, the heavy metal is distributed in the water bodies, tended solids and sediments and finally enter the human food chain. During the lifetime of fish in heavy metals polluted water, they can accumulate large amounts of heavy metal in the water through food, water and sediments since they are at the top of the aquatic food chain. The metals also can bioaccumulate in fish by ingestion directly through food, diffusion across the skin, and breathing through gills [3]. Then these heavy metals are transferred through the bloodstream to the organs. Through bonding with metal-binding proteins in tissues, it can reach high concentrations and stay for a long time. Considering the high demand for fish in human consumption, intake of contaminated fish could have adverse effects on human health. This may include serious threats such as renal failure, damage to the liver, cardiovascular diseases, and death.

Malaysia had raised the issue of contamination of heavy metal in aquatic ecosystems such as the river. The emergence of this problem is due to heavy metal water pollution that releases from anthropogenic activities such as bauxite mining. Bauxite mining started on a small scale at Pahang in 2013. Irresponsible parties used unregulated and unscrupulous mining practices in Pahang's bauxite mining to produce more income due to the high demand for bauxite[4].

Detection of toxicity of heavy metal in freshwater fish at Pahang is vital to provide information about the current level of heavy metal in fish to the local of Pahang as that area may have a potential risk of toxicity of heavy metal in fish. This information can be consulted by society about the potential risk in consuming fish that accumulate heavy metal above a certain level in their diet. This study determines the concentration of copper (Cu) in *Barbonymus schwanenfeldii* (Lampam Sungai) and water of Kuantan River and Pinang River. Besides, the water quality parameters and water quality index (WQI) were also determined for both Kuantan River and Pinang River.

2 Materials and methods

* Corresponding author: nadzifah@unisza.edu.my

Kuantan River and Pinang River were selected as the study area because it is believed that this river had been polluted by mining-related activities which took place at upstream of Kuantan River. Moreover, the Kuantan River basin is the main source of domestic water supply and fishing activities are commonly carried out by the nearest fishing village. Kuantan River is located at 03°50.9°N latitude and 103°29.2°E longitude in Pahang [5] while Pinang River located at 03°84'41.51"N and longitude 103°26'26.09"E.

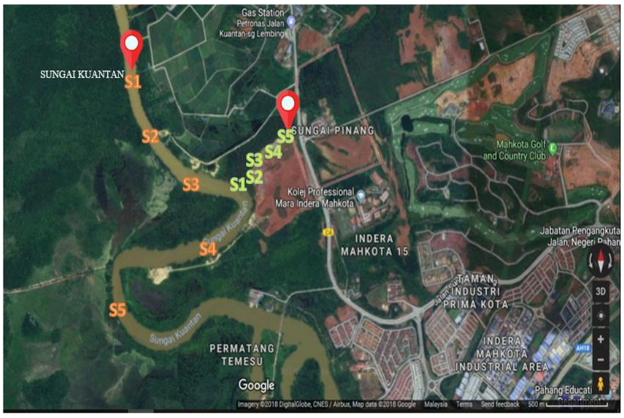

Figure. 1. Map of Kuantan River and Pahang River. Source from Google Maps. (2015)

2.1. Fish and water sampling

In five randomly selected stations along the river, water samples were collected. The water samples were collected in a polyethylene container and placed in an ice box for further analysis to the laboratory. The parameters in situ were taken directly. The water sample was placed in a refrigerator below 4 ° C to reduce the organisms ' actions and metabolism in the water [6]. The fish sample were caught by using gill nets which set up at sampling stations for 48 hours. The gill nets were checked every 24 hours. The sample will be stored in the freezer before carry out further laboratory analysis.

2.2. Laboratory analysis for copper

2.2.1 Fish sample

During dissection, the fish muscle was removed by using a sterile knife, 10g of the boneless fish muscle is placed into labeled petri dish then covered by aluminium foil. Each fish sample is prepared for 3 trials. After that, the samples are dried at 80°C in microwave oven for overnight until samples reach constant weight. The dried samples are placed in desiccators for cooling, then the dried samples are weighed and recorded. After that, the samples are crushed into smaller particles and added into the test tube.

2.2.2 Digestion

Acid digestion method was used to digest the muscle samples based on the Association of Official Analytical Chemists. 10ml of 70% nitric acid is added into the samples at room temperature for overnight. The next day, the samples are soaked in nitric acid then digested by using lab dry bath for 2 hours at 100°C and left it cool. After the samples cool, 2ml of 30% hydrogen peroxide (H^2O^2) is added and digested in lab dry bath for another 1 hour. The samples then left to cool after digestion. Afterward, the solution of digested samples was filtered into 25 ml volumetric flask by using glass funnel and filter paper. The test tube and glass funnel are rinsed with deionised water and makeup to 25 ml by adding deionised water. The digest solution is analysed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Cu concentration.

2.2.3 Water sample

Water samples collected were filtered through a membrane filter. Then, the samples were acidified with 70% nitric acid (HNO_3) to make the pH of the solution less than pH 2 to avoid metal deposition. The samples were analysed by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Cu concentration.

2.3. Metal calculation

The concentration of Cu in fish meat were calculated by using the formula [7] below:

$$C = A \times B/M \quad (1)$$

Where,
C= Concentration of test samples (mg/kg)
A= Reading from ICP (mg/kg)
B= Volume of the final solution (L)
M= Weight of sample (kg)

2.4. Water quality analysis

Water quality analysis consists of 10 parameters. These parameters are differentiated into two groups, in-situ parameters and ex-situ parameters. In- situ parameters are measured and recorded by using multi sensor probe YSI model 449D during water sampling, which is pH, temperature, salinity, DO, TDS, BOD, and NH3-N. On the other hand, the rest parameters are ex-situ parameters, include turbidity, TSS, and COD, are analysed in the laboratory. Turbidimeter (HACH 21000 Q) is used to measure turbidity of water. Multipara meter Handheld Colorimeter (HACH DR 900) is used to measure the TSS in water. To measure COD in water, 2ml of water sample in the vial that contain COD digestion reagent is digested by DRB 200 Digital Reactor Block for two hours and the reading is taken by using DR2800 Spectrophotometer. After the water quality parameters are obtained, water quality index is calculated by using the following formula DOE 2017: WQI= 022 SI DO + 0.19 SI BOD + 0.16 SI COD + 0.16 SI SS + 0.15 SI AN + 0.12 SI pH (DOE, 2017) where SI refers to sub index function for each parameter.

2.5. Statistical analysis

All the data collected were analysed by using One-way analysis of variance (ANOVA) to indicate significant differences of heavy metal levels in fish and water between Kuantan River and Pinang River.

3 Results and discussions

3.1. Bioconcentration of Cu in fish muscle tissue

A total of 10 individuals of *Barbonymus schwanefelldi* were used in this study. In terms of size, the samples ranged between 20 to 28 cm in length. For the wet weight, the tissue samples ranged between 10.09-10.26g respectively. Dry weight recorded was in a range of 2.70-1.91g.

As referred in table 1, the mean concentration of Cu in Kuantan River was 0.5070 ± 0.01748 mg/kg which was slightly higher than the Pinang River with value of 0.4732 ± 0.01807 mg/kg. Geographically the water flow of river was carried from upstream to downstream start from Kuantan to Pinang River. By receiving industrial effluents generated by upstream, freshwater fish in Kuantan tend to accumulate higher Cu in muscle tissue. The previous study shows the heavy metal content in aquatic organisms may be affected by their habitats and different ecosystems [8]. Essential metals are essential for normal fish metabolism[9], but they have been reported toxic to humans at one time after ingestion of as little as 10 to 15 mg. [10]. Comparison to the permissible limit set by [11] and [12] tabulated in Table 1, the concentration of Cu in muscle tissue from both rivers are below the permissible limit. Thus, the fish caught from both rivers are safe to be consumed without the fear of poisoning from this metal. Statistically, since $p > 0.05$, there was no significant difference in the concentration of Cu in muscle tissue of *B. schwanenfeldii* between Kuantan and Pinang rivers.

Table 1. Mean concentration (mg/kg) ± SE of Cu in fish muscle tissue from Kuantan River and Pinang River and the permissible limit of heavy metal for fish (mg/kg).

River	Mean concentration of heavy metal in fish muscle tissue (mg/kg) ± SE dry weight
	Cu
Kuantan River	0.5070 ± 0.01748
Pinang River	0.4732 ± 0.01807
p-value (One-Way ANOVA)	0.07221
Permissible limit of heavy metal for fish (mg/kg)	
Malaysia Food Act (1983)	10.0
FAO (1983)	30.0

3.2. Bioconcentration of Cu in water

Based on the result obtained, the concentration of Cu in surface water of Kuantan River is slightly higher than Pinang River, which recorded 0.0052 ± 0.0004390 mg/L and 0.0017 ± 0.00006669 mg/L respectively. This result is in agreement with heavy metals in *B.scwanenfeldii* as Cu concentration in fish from Kuantan river was higher than Pinang river. Contamination of drinking water with high level of copper may lead to chronic [13]. The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions. When compared to the permissible limit set by [14] for the concentration of Cu in river water, it is shown that both rivers were below the value of CMC and CCC, which were 0.013mg/L and 0.009 mg/L respectively. As compared to the concentration of Cu set by NWQS as tabulated in Table 3, both rivers had the concentration of Cu lower than the permissible limit, which were 0.02 mg/L at Class I. Statistically, since $p < 0.05$, there was significant difference in concentration of Cu in water between Kuantan and Pinang rivers.

Table 2. Mean concentration (mg/L) ± SE of Cu in the water of Kuantan River and Pinang River and the permissible limit of heavy metal in river water (mg/L)

River	Mean concentration of heavy metal in water (mg/L) ± SE
	Cu
Kuantan River	0.0052 ± 0.0004390
Pinang River	0.0017 ± 0.00006669
p-value (One-Way ANOVA)	0.000883581*
Permissible limit of heavy metal in river water (mg/L)	
USEPA (2014)	CMC - 0.013 CCC - 0.009

Table 3. NWQS of Malaysia for heavy metals.

Parameter	Unit	Class				
		I	IIA/IIB	III	IV	V
Al	mg/L	Natural level	-	0.06#	0.5	>0.5
Cu	mg/L	Natural level	0.02	-	0.2	>0.2

2.4. Water Quality Parameters and Water Quality Index (WQI)

The result of water quality parameters and water quality index (WQI) of each river were tabulated in Table 4. Based on the result, the water temperature of Pinang is 26.40 ± 0.00°C, which was slightly higher than Kuantan River, 25.42 ± 0.3707°C. According to [15], the temperature of the water may not be as important because of the wide range of temperature tolerance in aquatic life. The TDS level recorded was significantly higher in Pinang River (40.30 ± 0.00mg/L) compared to Kuantan River (25.48 ± 0.5957 mg/L). A high value of TDS indicates pollution of water. However, the TDS reading of both Pinang River and Kuantan River was below the limit at Class I set by NWQS (Table 5). TSS of Kuantan River (70.20 ± 1.960mg/L) was below the NWQS limit at Class III while TSS of Pinang River (5.60 ± 1.166mg/L) was marked below the NWQS limit at Class I. This is because Kuantan River flows through 4 administrative regions that include forests, agricultural areas, small villages, and main urban area of Kuantan city [16]. The pH of water in Kuantan River is 7.05 ± 0.3250 which was below the NWQS limit at Class I. Meanwhile the pH of water in Pinang River was 6.40 ± 0.00 which was slightly lower compared to Kuantan River and was below the limit at Class II. Low pH prevails because of low water movement; with faster rates of movement, the pH of water is increased [17]. Next, the BOD value of Kuantan River was higher than Pinang River as the BOD of Kuantan obtained is 0.67 ± 0.01960mg/L while the BOD value of Pinang River was 0.46 ± 0.00mg/L. The reading of COD obtained for Kuantan River is 25.80 ± 1.5621mg/L which was higher than the COD reading obtained for Pinang River, 15.40 ± 0.6mg/L. Based on the NWQS limit, the COD value of Kuantan River is below Class III and Pinang River is below Class II. According to the result obtained, DO of water in Kuantan River was higher than Pinang river. The reading of DO in water of Kuantan River (6.07 ± 0.5145mg/L) and Pinang River (3.30 ± 0.1059mg/L) were below the NWQS limit at class II and III respectively. The value of TSS has a direct relationship with the value of turbidity. Comparatively the turbidity value in the Kuantan River (274.20 ± 2.059 NTU) was higher than the turbidity value in the Pinang River (12.04 ± 0.14 NTU), just as it was with the TSS. NH^3 -H concentration in water is the measure of the nitrogen present as NH3- N. The concentration of NH3-H is 0.098 ± 0.01497mg/L for Kuantan River and it is higher than the concentration of NH^3-H for Pinang River which recorded 0.058 ± 0.003742mg/L. The salinity of Kuantan River and Pinang River are 0.02 ± 0.00 and 0.03± 0.00 respectively. Statistically, there were significant difference ($p > 0.05$) between Kuantan River and Pinang River for all water parameters except for pH and salinity.

Table 4. Mean ± SE physical-chemical parameters of the waters and water quality index (WQI) of rivers.

Water parameters	Kuantan River	Pinang River
Temperature (°C)	25.42 ± 0.37	26.40 ± 0.00
pH	7.05 ± 0.32	6.40 ± 0.00
DO (mg/L)	6.07 ± 0.51	3.30 ± 0.10
TDS (mg/L)	25.48 ± 0.59	40.30 ± 0.00
AN (mg/L)	0.098 ± 0.014	0.058 ± 0.003
Salinity (ppt)	0.02 ± 0.00	0.03± 0.00
BOD (mg/L)	0.67 ± 0.01960	0.46 ± 0.00
COD (mg/L)	25.80 ± 1.5621	15.40 ± 0.6
TSS (mg/L)	70.20 ± 1.960	5.60 ± 1.166
Turbidity (NTU)	274.20 ± 2.059	12.04 ± 0.14
WQI	82.59 ± 1.25	79.85 ± 0.45s
Class	II	II

Water Quality Index (WQI) also used to indicate the level of pollution and water classes. Based on the result, WQI of Kuantan River and Pinang River were 82.59 ± 1.255 and 79.85 ± 0.4586 respectively. Both rivers were considered at Class II (Table 4) in which the water required conventional treatment for water supply or can be used for recreational with body contact. It also can be used for fishery sector for sensitive aquatic species.

Table 5. National Water Quality Standards for Malaysia. (DOE 2017)

Parameters	Unit	Classes					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/L	0.1	0.3	0.3	0.9	2.7	>2.7
BOD	mg/L	1	3	3	6	12	>12
COD	mg/L	10	25	25	50	100	>100
DO	mg/L	7	5-7	5-7	3-5	< 3	<1
pH		6.5-8.5	6.0-9.0	6.0-9.0	5-9	5-9	-
Salinity	%	0.5	1	-	-	2	-
TSS	mg/L	25	50	50	150	300	300
TDS	mg/L	500	1000	-	-	4000	-
Temperature	°C	-	Normal + 2°C	-	Normal + 2°C	-	-
Turbidity	NTU	5	50	50	-	-	-
Water Quality Index (WQI)	mg/L	>92.7	76.5-92.7		51.9-76.5	31.0-51.9	<31.0

4 Conclusions

The mean concentration of Cu in fish muscle tissue was 0.5070 ± 0.01748 mg/kg for Kuantan River and 0.4732 ± 0.01807mg/kg for Pinang River. Generally, the fish is considered safe to be consumed this is because the concentration of Cu does not exceed the permissible limit set by Malaysia Food Act (1983) and FAO (1983). Furthermore, the level Cu in the water of Kuantan River and Pinang River were determined. The level of Cu in the water of both rivers was not critical to the fish as the concentrations are below the permissible limit set by USEPA (2014) and NWQS. Next, the WQI of Kuantan River was 82.59 which is considered clean and it has been classified at Class II according to the DOE (2017) water quality classification. While the WQI of Pinang River was 79.85 which is considered slightly polluted although it has been classified in Class II. The uses of water from Class II river is suitable for the fishery sector for rearing sensitive aquatic species or recreational use with body contact. If the water used as water supply, it required conventional treatment before supply to the household.

References

1. Ibrahim, A., Khan, M. A., Norrakiah, A., & Fazleen, I. Z. (2014). Fresh water aquaculture fish consumption in Malaysia and heavy metals risk exposure to consumers. International Food Research Journal, 21(6), 2109.
2. Pandey, G., & Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. Research Journal of Animal, Veterinary and Fishery Sciences, 2(2), 17-23.
3. Rajeshkumar, S., & Li, X. (2018). Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicology reports, 5, 288-295.
4. Academy of Sciences Malaysia. (2017). Sustainable Mining: Case Study for Bauxite Mining in Pahang. Dowloaded from: https://issuu.com/asmpub/docs/sustainable_mining_bauxite
5. Rosli, M., Samat, S., Yasir, M., & Yusof, M. (2018). Analysis of Heavy Metal Accumulation in Fish at Terengganu Coastal Area, Malaysia. Sains Malaysiana, 47(6), 1277-1283.
6. APHA., Eaton, A. D., AWWA., & WEF. (2017). Standard methods for the examination of water and wastewater. Washington, D.C: APHA-AWWA-WEF.
7. APHA., Eaton, A. D., AWWA., & WEF. (2005). Standard methods for the examination of water and wastewater. Washington, D.C: APHA-AWWA-WEF.
8. Fidan, A. F., Ciğerci, İ. H., Konuk, M., Küçükkurt, İ., Aslan, R., & Dündar, Y. (2008). Determination of some heavy metal levels and oxidative status in Carassius carassius L., 1758 from Eber Lake. Environmental monitoring and assessment, 147(1-3), 35-41.
9. Canli, M., & Atli, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental pollution, 121(1), 129-136.
10. Scheinberg, I.H. and I. Sternlibe. 1960. Copper metabolism. Pharmacol. Rev. 12:355-381.
11. Malaysian Food Act (MFA). (1983). Malaysian food and drug. Kuala Lumpur: MDC Publishers Printer Sdn. Bhd
12. FAO. (1983). Compilation of legal limits for hazardous substances in fish and fishery products. FAO fisherycircular No. 464, pp. 5–100
13. Iqbal, M. A., Chaudhary, M. N., Zaib, S., Imran, M., Ali, K., & Iqbal, A. (2011). Accumulation of Heavy Metals (Ni, Cu, Cd, Cr, Pb) in Agricultural Soils and Spring Seasonal Plants, Irrigated by Industrial Waste Water. Journal of Environmental Technology and Management, 2(1).
14. USEPA. (2014). National recommended water quality criteria. United States Environmental Protection Agency', Office of Water, Office of Science and Technology.
15. Appavu, A., Thangavelu, S., Muthukannan, S., Jesudoss, J. S., & Pandi, B. (2016). Study Of Water Quality Parameters Of Cauvery River Water In Erode Region. Journal of Global Biosciences, 5(9), 4556-4567.
16. Kozaki, D., Rahim, M. H. b. A., Ishak, W. M. F. b. W., Yusoff, M. M., Mori, M., Nakatani, N., & Tanaka, K. (2016). Assessment of the river water pollution levels in Kuantan, Malaysia, using ion-exclusion chromatographic data, water quality indices, and land usage patterns. Air, Soil and Water Research, 9, ASWR. S33017.
17. Sorenson, J. R., Campbell, I. R., Tepper, L. B., & Lingg, R. D. (1974). Aluminum in the environment and human health. Environmental Health Perspectives, 8, 3-95.