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BIOACCUMULATION OF CU AND PB IN FRESHWATER FISH SAMPLES FROM SUNGAI KELANTAN

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ABSTRACT

This study determine the level of copper (Cu) and lead (Pb) in the meat and gills of freshwater fish from 3 different locations along Sungai Kelantan. Sampling was carried out in wet and season and the fish were caught using gill nets. The bioconcentration of heavy metals were determined using Inductive Coupled Plasma Mass Spectrometry. Highest mean concentrations of Pb in meat of fish during the wet and dry seasons are 0.027±0.036 mg/kg (S3) and 0.064±0.020 (S1), while for Cu are 0.028±0.028 mg/kg (S3) and 0.038±0.009 (S1). Highest mean concentrations of Pb in gills during the wet and dry seasons are 0.031±0.006 mg/kg (S3) and 0.064±0.020 (S1), while for Cu are 0.03±0.005 mg/kg (S3) and 0.038±0.009 (S1). The level of Pb and Cu showed significant different ($p < 0.05$) in gills of fish during monsoon seasons, which not exceed the permissible limit of heavy metals according to MFA and FAO.

Keywords: Sungai Kelantan; heavy metals; freshwater fish; water quality.

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1. INTRODUCTION

Sungai Kelantan is the longest river in the state of Kelantan, spanning a length of 248 km long and drains an area of 11,900 km² which occupy more than 85% of the State of Kelantan [1].

For years, Sungai Kelantan has been used as medium for transportation, irrigation, industrial and food source for the local people. In recent years, the water quality of Sungai Kelantan is deteriorating which as reported by Department of Environment (DOE) Malaysia as polluted based on the suspended solid index. Among most of the pollutants discharged into the aquatic environment, heavy metals are regarded as one of the most serious pollutants due to their environmental persistence and tendency to accumulate in aquatic environment [7]. Heavy metals in water may accumulate in aquatic species, enter the food chain and cause serious harm to human health when the contamination content and exposure are significant [12]. In recent years, focus has been directed to the level of bioaccumulation of heavy metals in aquatic organisms like freshwater fish and their risk to the human health. Detection of bioaccumulation of heavy metal in aquatic organisms is one of the ways of identifying the impact of heavy metals presence in river ecosystem. Fish can absorb an increased amount of heavy metals that present in the water directly through the epithelial absorption via their gills which will be deposited in the tissues of their body. The entry of heavy metals contaminants into the environment can be caused by human and natural activities that come from municipal, industrial and agricultural activity [29]. Although some of the heavy metals are essential as micronutrients to fish, high concentration in the food chain can cause toxicity and create environmental impacts that endanger aquatic ecosystems and human consumers [17].

Fish transfer the heavy metals from the environment into humans when they are consumed, and therefore form the final link for the transfer of heavy metals in the water to humans [1]. Consumption of heavy metals above the permissible limit can lead to toxic effect to the human body as they possess carcinogenic, mutagenic and neurotoxic properties. Heavy metals lead when absorbed in the human body will be deposited in teeth, bone structure, liver, lungs and other organs in high concentration and could be transported throughout the body system [15]. Copper (Cu) is important for the formation of bone and preserving the integrity of connective tissues. However, in high doses, copper can cause irritation to the nose, eyes, mouth, vomiting, diarrhoea and other unpleasant pain. This may also lead to liver and kidney damage. Lead (Pb) is a non-essential element and is toxic to humans when present in high doses. Consumption of fish that is contaminated with heavy metals is a serious issue because

people who lives near the river and uses the river in daily basis consume fish everyday as a source of protein. High content of beneficial nutrients in fish help elevate good health in humans. Chronic toxicity of heavy metals is dangerous because it possesses high risk to human health.

The present study was carried out to determine the bioaccumulation level of (Cu) and (Pb) in freshwater fish from Sungai Kelantan.

2. RESULTS AND DISCUSSION

A total of 45 fishes from nine different species were caught during the sampling period and were tabulated in the table below:

Table 1. Distribution of fish caught in three sampling station in both wet and dry seasons

Species (n)	Common Name	S1	S2	S3
Hemibagrus nemurus (4)	Baung	+	+	-
Hampala macrolepidota (3)	Sebarau	+	+	-
Hemibagrus wyckii (7)	Baung	+	-	-
Barbonymus schwanenfeldii (5)	Lampam sungai	+	-	-
Barbonymus gonionotus (6)	Lampam jawa	+	+	-
Puntius daruphani (7)	Ikan krai	-	+	-
Pangasius micromenus (6)	Ikan juara	-	+	+
Clarias gariepinus (5)	Keli	-	-	+
Channa striatus (2)	Haruan	-	+	+

Note: + present, - not present, (n) number of individual fish caught

All the fish were collected from three stations along Sungai Kelantan which are Station 1 (Gua Musang), Station 2 (Tanah Merah) and Station 3 (Kota Bharu) during wet season and dry season. The concentration of heavy metals in the freshwater fishes in this study was determined on dry weight basis because the values were more reliable and consistent than the wet weight value [24].

During the sampling in wet season, fishes that were collected at Station 1 (Gua Musang) include *Hemibagrus nemurus, Hampala macrolepidota, Hemibagrus wyckii* and *Barbonymus*

schwanenfeldii. At Station 2 (Tanah Merah), *Hampala macrolepidota, Barbonymus gonionotus* and *Puntius daruphani* were collected and at Station 3 (Kota Bharu), collected fishes were *Pangasius micromenus* and *Clarias gariepinus.* Metal concentration in meat and gill of each fishes collected were measured. During sampling in dry season, species of fishes that collected in Station 1 (Gua Musang) were *Barbonymus schwanenfeldii, Barbonymus gonionotu, Hampala macrolepidota* and *Hemibagrus wyckii* while at Station 2 (Tanah Merah), *Hemibagrus nemurus, Pangasius micromenus and Puntius daruphani* species were collected. At Station (Kota Bharu), *Clarias gariepinus, Channa striatus* and *Pengasius micromenus* species were collected. Metal concentration in meat and gill of each fishes collected were measured. The concentration of heavy metals Copper and Lead were detected in the muscle and gill tissues of fish samples collected from Sungai Kelantan and are summarized in the table below:

Table 2. Heavy metals content (mg/kg) in meat and gills of fish in three sampling stations

Station	**Monsoon**		**Dry**	
	Pb	**Cu**	**Pb**	**Cu**
		Gua Musang		
Meat	0.021±0.007	0.013±0.011	0.064±0.020	0.038±0.009
Gill	0.024±0.005	0.015±0.009	0.064±0.020	0.038±0.009
		Tanah Merah		
Meat	0.018±0.015	0.017±0.008	0.030±0.007	0.030±0.007
Gill	0.018±0.015	0.017±0.008	0.030±0.007	0.030±0.007
		Kota Bharu		
Meat	0.028±0.001	0.028±0.001	0.036±0.009	0.028±0.001
Gill	0.031±0.006	0.031±0.005	0.036±0.009	0.028±0.001

In the wet season, the highest Pb concentration in meat was found at Station 3 at 0.028 mg/kg and the lowest concentration of Pb was found in Station 2 at 0.018 mg/kg. Station 3 also was found the highest Pb levels in gills at 0.031 mg/kg, while Station 2 showed the lowest value at 0.018 mg/kg. During the dry season, the highest Pb level in meat was observed at Station 1 (0.064 mg/kg) and the least value of Pb was found in Station 2 (0.030 mg/kg) and for gills,

the highest concentrations of Pb were found at Station 1 at 0.064 mg/kg while Station 2 showed the lowest value at 0.030 mg/kg. It was found by [27] that there are increased presence of heavy metals in the river during the wet season, and this was the result of increased surface runoff due to increased amount of downpour during the wet season. Heavy downpour in the wet season will lead to the draining of the farms where large amounts of pesticides containing heavy metals are brought to the surface via runoff from the farms directly into the river and highly contributed by farming pollution, especially chemical fertilizers that contain Cu and Pb. Station 1 showed the highest Pb concentration in gills compared to other stations. This may be caused by increasing accumulation of heavy metal because of increased activity, ventilation, metabolic rate and feeding sessions [20]. During the wet seasons, the level of Pb showed no significant different ($p < 0.05$) for all the three stations. But it was found that during the dry seasons, there are significant different ($p < 0.05$) in meat and gill of fishes for the three stations.

During the wet season, the highest Cu level in meat was found at Station 3 (0.028 mg/kg) and the lowest concentration of Cu was found in Station 1 (0.013 mg/kg). In the gills, it was found that highest Cu concentration was found at Station 1 (0.038 mg/kg) and the lowset was found at Station 3 at 0.028 mg/kg. During the dry season, the highest Cu level in meat was found at Station 1 (0.038 mg/kg) and the lowest concentration of Cu was found in Station 3 (0.028 mg/kg) and for the gills, Highest concentration of Cu was found at Station 1 (0.038mg/kg) and the lowest concentration of Cu was found at Station 3 (0.028 mg/kg). The result indicates that Station 1 contained the highest Cu in meat and gills than other stations. This is due to accumulation Cu in fish depending on many factors including species, local elements, organisms, concentration and water quality [9]. The level of Cu showed no significant different ($p < 0.05$) in meat and gill of fishes in three different stations. From the result, it was indicated that the fishes that were caught in Kota Bharu during the wet season contain the highest Cu level meat and gills of fishes among the 3 stations. This may be due to the rapid urbanization along the river bank and sand mining that is observed at Kota Bharu which can disrupt the sedimentation in the river bed and increases the availability of heavy metals in the water. Depending on several properties of the water like hardness and pH, the toxicity effect

of copper may varies in which copper is more toxic in soft and low pH water. Fish will absorb more heavy metals, especially during the wet season where the flow of river is faster, resulting in movement of heavy metals in the river. During the dry season, the level of Cu showed no significant different ($p < 0.05$) in meat but showed significant different ($p < 0.05$) in gill of fishes in three different stations along Sungai Kelantan. In water, fishes are constantly exposed to aquatic and particulate heavy metals as water continuously flows through the gills and food sources. Bioaccumulation of heavy metals in different tissues uses different paths of bioaccumulation factors [11].

The level of Pb and Cu is different between meat and gill because of the differences in function of both organs in a fish. Gills are classified as target organs like intestine and are metabolically active parts that can accumulate heavy metals in higher concentration. The absorption of metals onto the gill surface as the first target for pollutants in water could also be a significant influence in the total metal levels of the gills. This explains the higher concentration of heavy metals found in the gills of the fish samples compared to the meat. Several factors such as sex, age, time of year, reproducing period, availability of food habitats, pollutant exposure and phylogenetic differences in regulatory mechanisms may influence the uptake, withholding and bioaccumulation of trace contaminants in fish muscles and other organs [19]. There are several other factors that can affect the accumulation of heavy metals. It also due to detoxifying ability of liver that tends to accumulate large amount of Pb and Cu in tissues [2]. The higher concentration of both heavy metals during the dry season may be related to higher temperatures, which increased fish movement and motion, metabolic rate and feeding sessions [20]. During the wet season, lower concentration of heavy metals were associated with higher volume of water in the river which dilute the heavy metals as heavy rains increases the river water volume [22].

Table 3. Heavy metals (mg/kg) in meat samples of fish species in Sungai Kelantan

Species	Pb	Cu
Hemibagrus nemurus	0.014±0.000	0.007±0.010
Hampala macrolepidota	0.032±0.009	0.014±0.014
Hemibagrus wyckii	0.058±0.035	0.033±0.008
Barbonymus schwanenfeldii	0.046±0.019	0.036±0.020
Barbonymus gonionotus	0.006±0.008	0.012±0.000
Puntius daruphani	0.030±0.005	0.029±0.008
Pangasius micromenus	0.036±0.009	0.028±0.001
Clarias gariepinus	0.028±0.001	0.028±0.001
Channa striatus	0.045±0.000	0.030±0.000

Mean concentration of Pb in fish species in ascending order were *B. gonionotus* < *H. nemurus* <*C. gariepinus* < *P. daruphani* < *H. macrolepidota* < *P. micromenus* < *C. striatus* < *B. schwanenfeldii* < *H. wyckii*. The highest mean concentration of Pb was detected in *H. wyckii, P. daruphani and C. striatus* with concentration of 0.058 mg/kg, 0.046 and 0.045 mg/kg respectively but still below permissible limit. Species that have the lowest concentration of Pb is *B. gonionotus* with concentration of 0.006 mg/kg. This result may be related to their feeding behavior and habitat. Being an herbivorous fish, *B. gonionotus* eats algae and submerged plants in the river [4] and is considered as primary consumer at a lower tropic level. This may explain the lower bio magnification and thus a lower bioaccumulation level of Pb compared to other species at a higher tropic level. From a previous study, it was found that fish from several species originated from Sungai Kelantan has been detected to have concentration of Pb slightly below permissible limit in the range of 0.10-0.16 mg/kg [27]. This finding contradicts the result of this experiment where the highest concentration of Pb was found to be 0.058 mg/kg, which is much lower than the previous findings. This may be due to several factors such as environmental factors, size and age of the fish and also timing of the sampling. Overall, Pb concentration in all species was below the permissible limit and is considered safe to be consumed. Pb is a non-essential element and is toxic to humans when present in high doses. Presence of Pb in trace amount in human body will interfere with

essential nutrients of similar characteristics such as copper and zinc. Pb can also be the reason for renal failure and liver damage in humans [28].

Mean concentration of Cu in fish species were found to be in ascending order of *H. nemurus* < *B. gonionotus* < *H. macrolepidota* < *P. micromenus* < *C. gariepinus* < *P. daruphani* < *C. striatus* < *H. wyckii* < *B. schwanenfeldii.* The highest mean concentration of Cu was detected in *B. schwanenfeldii* with 0.036 mg/kg and the lowest was *H. nemurus* with the level of 0.007 mg/kg. The different concentration of Cu among different species may be due to several factors, such as location of sampling sites where heavy metals accumulates differently in the environment and also the rate of which heavy metals in taken into the body system. Other than that, heavy metal intakes by fish in different aquatic environment depend on ecological necessities, metabolisms and other aspects such as salinity, water pollution level, nourishment and sediment [13]. When comparing this result with the guidelines of permissible limits, it was found that for Copper, the concentration is lower than the permissible limit recommended by Malaysia Food Act (1983) [18] and Regulation (1985), WHO/FAO (2004) [14], USFDA (1993) and EC (2001) [10]. From a previous study, it was found that in *Tilapia sp.* caught at Tasik Mutiara, Puchong, the concentration of Cu is lower than the permissible level recommended by Malaysian Food Act 1983 at the range of 3.49 to 0.53 [16]. In Bangi, it was also found that the concentration of Cu in *Oreochromis niloticus* is higher in culture pond when compared to rivers and lakes around Bangi, Selangor [8]. The distribution patterns of Pb, Cd, Zn, Ni, Cr and Cu in the three organs of the tilapia in followed the order: liver > gills > muscles [8]. The bioaccumulation of any heavy metals happens in an organism when uptake rate by the organism exceeds the rate of which the heavy metals are excreted [23]. In low concentration, copper is one of the essential metals that are needed by the body to regulate the human metabolism. Copper controls normal biological activity of many enzymes and hemoglobin creation [25]. Nonetheless, if the level of exceeds the maximum residual limits, it will cause problem to human health. In fish, fish species vary in their sensitivity to Cu with the LD50 for 96-h exposure to copper sulphate reported to be in the order of 58 mg/L for Tilapia (*Oreochromis niloticus*) and 70 mg per liter for catfish (*Clarias gariepinus*). Chronic toxic effects may induce poor growth, decreased immune response, shortened life span,

reproductive problems, low fertility and changes in appearance and behaviour [30].

The concentration of Pb in meat in this study is lower compared to [27, 31]. In [27] has found that the concentration of Pb in meat in fish caught from Sungai Kelantan to be slightly below the limits of the permissible level with the range of 0.100-0.169 mg/kg. This difference may be due to the differences in fish species, fish size, and the timings of the sampling. This is because fish have different metabolic rate and hence they will have different concentration of heavy metals. Pb causes renal failure and liver damage in humans. In fish, Pb causes decreases in survival, growth, development, behaviour and metabolism, in addition to an increase in the formation of mucus [28] while Cu is an essential element to healthy metabolism and growth of all living organisms [9]

Table 4. Comparison of mean concentrations of heavy metals (mg/kg) in meat of fish from the Sungai Kelantan during the wet and dry seasons with previous study

Study	**Pb**	**Cu**
	Meat	**Meat**
Tualang	0.021±0.064	0.013±0.038
Tanah Merah	0.018±0.030	0.017±0.030
Kota Bharu	0.027±0.036	0.028±0.028
Kelantan River		
[27]	0.119±0.119	-
(Sungai Galas)		
[31]	0.004-0.05	-

The concentration of Pb in meat in this study is also lower when compared to previous study by [21]. According to [21], the concentration of Pb and Cu in gill of fish at Anambra River, is 0.24 and 10.20 mg/kg. The concentration of Pb in fish gill was found to be below the permissible limit according to [10], while concentration of Cu is exceeding permissible limit of fish according to Malaysia Food Act (1983) [18] and Regulation (1985). These differences in findings may be due to the different ecosystem where the experiment was conducted and other factors including different fish species, fish size, age and level of heavy metals contamination in the river. High level of Cu in water may damage the aquatic organisms. Cu

is highly toxic to aquatic organisms and may cause irreversible harm at concentrations just over that required for growth and reproduction [6].

Table 5. Permissible limit of heavy metals concentration (mg/kg) in fish

	Pb	**Cu**
Malaysia Food Act (1983) and Regulation (1985)	2	10
WHO/FAO (2004)	1.5	30
USFDA (1993)	0.5	-
EC (2001)	0.2-0.4	-

The limits of heavy metal concentration in fish have been set for public health precaution. For example, Malaysia, has set maximum limits of contamination for Cd and Pb based on permissible limits recommended by the Malaysian Food Act (MFA 1983) [18]. In this study, however, the heavy metal permissible levels were also compared with others such as the WHO (1985), USFDA (1993), EC (2001) [10] and FAO (2003) [12]. This study showed the Pb and Cu concentration in meat and gill of freshwater fishes along Kelantan River was below the permissible limit of Malaysia Food Act (1983) and Regulation (1985) [18], WHO/FAO (2004) [12], USFDA (1993) and EC (2001) [10]. It was also found that they are lower than the permissible limit recommended by FAO, WHO/FAO (2004) [12], USFDA (1993) and EC (2001) [10].

3. EXPERIMENTAL

3.1. Materials and Methods

Sampling for freshwater fish was carried out during the wet season (December 2016) and dry season (February 2017). Three stations were chosen along the Sungai Kelantan which covers all the length of Sungai Kelantan from upstream to downstream. The stations are located at Gua Musang (05.07’12.8, 102.01’11.5), Tanah Merah (05.46’31.6, 102.09’05.6) and Kota Bharu (06.09’07.0, 102.13’55.5).

3.2. Sample Collection

Fish were caught at each sampling station using several gill nets set up in the respective area which were left for 48 hours. The placement of the gill nets were chosen based on the ideal

location in the river based on several factors. The gill nets were checked every 24 hours and all the fish caught were measured for length and weight before the fish were immediately stored in ice box with ice and kept cool. Upon arrival at the laboratory, the fish were stored in the cool room at -20°C until further analysis.

Fig.1. Sampling stations along Sungai Kelantan

3.3. Samples Analysis

The fish samples were dissected using scissors, scalpel and knife. The meat and gills part of the fish were dissected and weighed at 10 g per samples before the samples were oven-dried at 100°C for 24 hours until they achieved constant weight. Samples were then allowed to cool before the dry weights of the samples were taken. Acid digestion method was used based on the Association of Official Analytical Chemists (AOAC), 1984 [32]. Each samples of dried meat were put in the digestion tube where 10 mL of 69% nitric acid were added and they were left overnight. Then, 10 ml of 70% nitric acid was added and left in room temperature overnight. On the next day, the samples were digested in block thermostat at 100^0C for 2 hours before were left to cool. After that, 2 ml of 30% hydrogen peroxide (H_2O_2) was added. The digestion tubes were heated again for 1 hour until the color of solution change from orange to yellow and form clear solution. Then, the digestion tubes were allowed to cool before the solutions were filtered through 0.5 nm filter paper into a 25 mL volumetric flask. Deionized water was top-up into the volumetric flask until the volume reached 25 mL. The

concentration of copper and lead were determined using Inductively Couple Plasma-Mass Spectrometry (ICP-MS).

3.4. Statistical Analysis

All analysis was done at significant level of $p < 0.05$. All statistical analysis was done using Microsoft Excel for Windows 7.

4. CONCLUSION

The result of this study showed that fish caught from Sungai Kelantan has been determined to contain concentrations of heavy metals Cu and Pb. During the wet season, the highest Cu level in meat was found at Station 3 (0.028 mg/kg) and In the gills, it was found that highest Cu concentration was found at Station 1 (0.038 mg/kg) while for Pb, In the wet season, the highest Pb concentration in meat was found at Station 3 at 0.028 mg/kg and the lowest concentration of Pb was found in Station 2 at 0.018 mg/kg. During the dry season, the highest Pb level in meat was observed at Station 1 (0.064 mg/kg) and for gills, the highest concentrations of Pb were found at Station 1 at 0.064 mg/kg and for Cu, the highest Cu level in meat was found at Station 1 (0.038 mg/kg) for the gills, highest concentration of Cu was found at Station 1 (0.038mg/kg). During the dry season, the level of Cu showed no significant different ($p < 0.05$) in meat but showed significant different ($p < 0.05$) in gill of fishes in three stations while during the wet season, the level of Cu showed no significant different ($p < 0.05$) in meat and gill of fishes. Pb showed no significant different ($p < 0.05$) for all the three stations during the wet seasons but it was found that during the dry seasons, there are significant different ($p < 0.05$) in meat and gill of fishes for the three stations.

Besides, the concentration of Pb and Cu in the fish was found to not exceed the permissible limit of heavy metals recommended by Malaysia Food Act (MFA, 1983), US Food and Drug Administration (USFDA, 1993), European Commission (EC, 2001) and Food and Agriculture Organization (FAO, 2012) and are considered safe to be consumed. Although the level of heavy metals that were detected is low there is a risk of future increases in heavy metals concentration along with the increasing human activity along Sungai Kelantan. A regular monitoring of heavy metals concentration in water and fish are needed to minimize heavy

metals toxicity in freshwater ecosystem.

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