Determination of heavy metal accumulation in fish species in Galas River, Kelantan and Beranang mining pool, Selangor

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Abstract

Concentrations of heavy metals namely Cu, Zn, Pb, Ni, Mn and Cd were detected in tissue of six freshwater fish species (\textit{Hampala microlepidota}, \textit{Barbonymus schwanenfeldii}, \textit{Mystacoleucus marginatus}, \textit{Hemibagrus nemurus}, \textit{Cyclocheilichthys apogon} and \textit{Oreochromis niloticus} from Galas River and Beranang mining pool. The samplings were carried out from June to November 2014. Heavy metals in fish were analyzed by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) and Atomic Absorption Spectrophotometer (AAS). Irrespective of fish species, range for muscle samples are Zn> Ni>Cd>Cu>Mn>Pb. Levels of Zn and Pb in the tilapia muscle from mining pool were higher than fish from Galas river. \textit{M. marginatus}, \textit{H. macrolepidota} and \textit{B. schwanenfeldii} showed highest concentration for Mn, Cu and Ni. The range for heavy metals in Galas river are Zn>Mn>Pb>Ni>Cu. Concentrations of heavy metals in water samples and fish were lower than permissible limit set by Food Act 2003 and Malaysian Food Regulations 1983.

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Keywords: Heavy metals; Galas River; mining pool; fish

1. Introduction

Heavy metal pollution in rivers gives threat to public water supplies and also to consumer of fishery sources [24]. Heavy metals constitute a core group of aquatic pollutants via its bio-accumulative and non-biodegradable properties in food [17]. Human may be contaminated by organic and inorganic pollutants associated to aquatic systems by consumption of contaminated fish and other aquatic foods from this environment [2].

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Studies on bioaccumulation of pollutants in fish are important in determining different content of trace metal in fish species from bio-magnifications of food chains, metabolic capability and feeding habits [6]. The accumulation of metal is a tool for identifying the impact of metal in aquatic ecosystem, and therefore shows an adverse effect in organism [7]. Kelantan River where Galas is a tributary was reported as polluted by Department of Environment (DOE) Malaysia based on the suspended solid index. Rahman [22] stated that number of polluted river in Malaysia is rapidly increasing. Study by Ahmad [4] found levels of heavy metal in sediments of Kelantan river such as Pb, Zn, Cu, and Cd. Rohasliney [23] showed an order of Pb>Ni>Cd in total mean concentration of fish from Kelantan river. Mazlin et al., [20] found that Orechromis sp has greater capacity for metal bioaccumulation of Pb and Cd. Kah Hin Low et al., [15] found level of Cd in tilapia fish were higher than other metals, where the mean composition was similar to maximum level specified by Commission Regulation [9]. According to the Priority List of Hazardous Substances established by the Agency for Toxic Substances and Disease Registry, the descending order of heavy metals threatening to human health were Pb>Cd>Ni>Zn>Cu>Mn.

The objective of this study is to analyzed heavy metals concentrations in muscle tissue of freshwater fish namely Hampala microlepidota (Sebarau), Barbonymus schwanenfeldii (Lampam sungai), Mystacoleucus marginatus (Sia), Hemibagrus nemurus (Baung), Cyclocheilichthys apogon (Temperas) and Oreochromis niloticus (Tilapia)).

2. Materials and methods

2.1. Study site

The site at Galas river (4°52'22.56" N 101°57'20.4" E) is formed by the junction of Nenggiri and Pergau river with the catchment area of 7,770 km² (Fig.1.). Another study site is a disused mining pool in Beranang, Selangor (2°53'26"N 101°48'1"E) (Fig.2.). Part of this disused mining pool is now an aquaculture project for fish farming using net pen culture system and sport fishing.

(a) Fig. 1. Galas River; (b) Fig.2. Map showing the locality of Beranang, Selangor.
2.2. Sample collection

Fish samples from both sites were caught using gill nets from October to November 2014 and analysis were carried out according to APHA [5]. The nearest length (mm) and weight (gram) of each fish were measured. The fishes were washed using deionized water and place in separated polyethylene bags with ice. The samples were kept frozen at -20°C until ready for analyses.

For water sampling, polyethylene bottles were acid-washed with 10% concentrated nitric acid HNO₃ (v/v) and rinsed thoroughly with distilled deionized water. All glassware and equipment used were acid–washed. The bottles were rinsed three times and immersed about 10 cm below the water surface before sampling. Three 500 ml of water samples were taken at each sampling point, and keep in ice while been transported to laboratory. The samples were filtered through 0.45 µm micropore membrane filter and acidified with concentrated HNO₃ (65%) to a pH less than 2. The samples were kept at 4°C before treatment.

2.3. Sample preparation for water

About 9ml of concentrated HNO3 were added to filtered water sample and heated gently at 70°C until the solution become transparent [5]. The solutions were allowed to cool and filtered using 0.45µm micropore membrane filter. The solutions were then add up with ultra-pure of water to 100ml and analyzed for trace metal concentration.

2.4. Sample preparation of fish

All the glassware and plastics were soaked overnight in 10% (v/v) nitric acid rinsed with distilled water and deionized water and dried before being used. Five gram of boneless muscle tissue was removed using stainless steel knife and was digested to a strong acid digestion (H₂O₂+HNO₃ conc.) mixture at 1: 3 ratios [11] at 150°C for 20 minutes and allowed to cool at room temperature. Samples were processed in duplicate and then diluted to a total 50ml with ultra-pure water and filtered through 0.45 µm micropore membrane filter paper for analyses.

2.5. Reagents

All reagents were of analytical reagent grade. Ultra-pure water was used for all dilutions. The element standard solutions from Perkin Elmer that were used for the calibrations were prepared by diluting stock solutions of mg/mL.

2.6. Heavy metal analysis

All samples were digested in concentrated HNO₃ and H₂O₂ in a beaker. The samples were then diluted until 50 ml with ultra-pure water. After filtration, the prepared samples were determined for Zn, Cu, Pb, Ni, Mn, and Cd by using atomic absorption spectrophotometer (AAS) and Inductively Coupled Plasma (ICP-MS). Heavy metals included in this study are: Zinc (Zn), Copper (Cu), Lead (Pb), Nickel (Ni), Manganese (Mn) and Cadmium (Cd). Element standard solution from Perkin Elmer was prepared by diluting stock solutions of 100mg/mL of each element based on Abdulali et al., [1]. The concentrations of heavy metals were presented in mg/kg, wet weight (ww) for fish sample and µg/L for water.

3.0 Results and discussion

The comparison among fish species in Galas river and Beranang mining pool according to their metal accumulation levels in tissues was given in Table 1 and Table 2 respectively. Zn showed highest concentrations in all tissues of the species, followed by Ni and Cd, Mn, Cu, and Pb. Metal accumulation orders in muscles of B. schwanenfeldii and H. macrolepidota were Zn>Ni>Cu>Mn>Pb. The result shows that the mean concentrations of heavy metals in tissues were different among species (Table1). This indicated that different species from the same
area contained different levels of heavy metals in their tissues

Table 1 Heavy metal contents (mg/kg wet weight) of fish species from Galas River, Kelantan

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>B. schwanenfeldii</th>
<th>H. macrolepidota</th>
<th>M. marginatus</th>
<th>C. apogon</th>
<th>H. nemurus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.313±0.105</td>
<td>0.274±0.079</td>
<td>0.333±0.12</td>
<td>0.260</td>
<td>0.136</td>
</tr>
<tr>
<td>Cu</td>
<td>0.018±0.009</td>
<td>0.046±0.049</td>
<td>0.018±0.007</td>
<td>0.012</td>
<td>0.027</td>
</tr>
<tr>
<td>Pb</td>
<td>0.011±0.011</td>
<td>0.005±0.003</td>
<td>0.004±0.004</td>
<td>0.010</td>
<td>0.011</td>
</tr>
<tr>
<td>Ni</td>
<td>0.072±0.011</td>
<td>0.067±0.01</td>
<td>0.064±0.005</td>
<td>0.058</td>
<td>0.058</td>
</tr>
<tr>
<td>Mn</td>
<td>0.014±0.003</td>
<td>0.018±0.005</td>
<td>0.028±0.007</td>
<td>0.021</td>
<td>0.012</td>
</tr>
<tr>
<td>Cd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

nd=not detected

These results were comparable to other studies from different river in Malaysia. Rohasliney [23] reported B. schwanenfeldii from Kelantan river with the maximum Cd, Ni and Pb concentrations of 0.03, 0.10 and 0.10 µg/g respectively and H. nemurus µg/g dry weight with 0.023, 0.056 and 0.103 respectively.

Table 2 Heavy metal contents (mg/kg wet weight) of the species in Beranang mining pool, Selangor

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>O. niloticus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.434±0.105</td>
</tr>
<tr>
<td>Cu</td>
<td>0.030±0.012</td>
</tr>
<tr>
<td>Pb</td>
<td>0.053±0.048</td>
</tr>
<tr>
<td>Ni</td>
<td>nd</td>
</tr>
<tr>
<td>Mn</td>
<td>nd</td>
</tr>
<tr>
<td>Cd</td>
<td>0.016±0.003</td>
</tr>
</tbody>
</table>

Table 3 Permissible limit of metal concentration in fish

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>MFA1</th>
<th>FAO2</th>
<th>EC3</th>
<th>USFDA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>100</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>30</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pb</td>
<td>2</td>
<td>0.5</td>
<td>0.2-0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Ni</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cd</td>
<td>1</td>
<td>0.5</td>
<td>0.05</td>
<td>0.01-0.21</td>
</tr>
</tbody>
</table>

Table 4 Metal concentrations (mean with SD) in the water (µg/L) at Galas River and Beranang mining pool, Selangor

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Galas river</th>
<th>Beranang Mining Pool</th>
<th>EPA</th>
<th>NWQS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.003±0.001</td>
<td>nd</td>
<td>120</td>
<td>5000</td>
</tr>
<tr>
<td>Cu</td>
<td>0.145±0.101</td>
<td>nd</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>Pb</td>
<td>0.092±0.187</td>
<td>0.036</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>Ni</td>
<td>0.016±0.006</td>
<td>nd</td>
<td>470</td>
<td>50</td>
</tr>
<tr>
<td>Mn</td>
<td>0.108±0.079</td>
<td>nd</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Cd</td>
<td>nd</td>
<td>0.011</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

3.1 Concentrations of trace elements in fishes and comparison with international dietary standards and guideline

3.1.1 Non-essential elements

Concentration of Cadmium in O. niloticus sample was recorded at 0.02 mg/kg. Abdulali et al. [1] reported Cd in O. niloticus from Langat River at a level of 0.03mg/kg while Mazlin et al., [20] detected Cd in O. niloticus from aquaculture pond at a level of 0.006-0.015µg/g. The mean value of Cd from this study was also lower than reported values from others studies namely [15], [22]. The FAO sets a Cd concentration limit of 0.5 mg/kg in fish while a maximum Cd content of 1mg/kg was set by the Malaysian Food Act (MFA) [19].
The concentration of Pb measured in this study varied from 0.004 to 0.05mg/kg. Maximum Pb values were present in *O. niloticus* (0.05mg/kg). The lowest concentration was detected in *M. marginatus* (0.004mg/kg). For all the samples analyzed and irrespective of species, the concentrations of lead in fish samples were below the 0.5 mg Pb/kg wet weight limit stipulated by the FAO. The Pb level in fish of this study was also lower than reported by others such as by [1], [16] and [18]. The MFA acceptable limit for Pb concentration is 2 mg/kg. All the Pb concentrations of fish species were also found to be lower than the acceptable limit suggested by MFA and FAO.

Ni concentrations in all samples ranged from 0.06 to 0.07 mg/kg wet weight. All fish species sampled for Ni concentrations showed lower than the permissible levels recommended by the MFA and the FAO.

### 3.1.2 Essential elements

Excessive Zn intake is detrimental to human health and can cause poisoning, diarrhoea and fever [8]. In this study, the highest concentration of Zn was detected in *O. niloticus* (0.43mg/kg) followed by *M. marginatus* (0.33mg/kg) and *B. schwanenfeldii* (0.31mg/kg). The lowest values of Zn were observed in *H. nemurus* (0.14mg/kg) and *C. apogon* (0.26mg/kg). The mean value of Zn for *O. niloticus* tissue samples examined in this study was 0.434 mg/kg. The high Zn concentration detected in Tilapia (0.43 mg/kg) could be due to their feeding on benthic worms and crustaceans. The mean Zn concentration from fish in this study was lower than other studies including Abdulali et al., [1] in Langat river (20.58ug/g) and Muiruri et al., [21] in Kenya (28.49.5mg/kg). MFA permissible level of Zn for human consumption is 100mg/kg wet weight [19].

Cu plays a vital role in enzymatic processes and are essential for the synthesis of haemoglobin. However, very high intake will cause health problems [10], [3]. The concentration of Cu in fish samples varied between 0.01 and 0.05mg/kg. The highest concentration of Cu was detected in *H. macrolepidota* (0.05 mg/kg) and the lowest value was detected in *C. apogon* (0.01mg/kg). The concentrations of Cu in all the samples were below the MFA and FAO limits. The observed values of Cu in fish tissues were lower than those by Kah et al., [15] (0.16–0.27mg/kg) and Abdulali et al., [1] for *O. niloticus* (1.46-1.69 µg/g).

The levels of manganese in the fish samples ranged from 0.01 to 0.03mg/kg wet weight. Daily intake of small amounts of manganese is needed for growth and good health in children. However Mn deficiencies can cause severe skeletal and reproductive abnormalities. The maximum concentration of Mn in this study was in *M. marginatus* (0.03mg/kg) followed by *C. apogon* and *H. microlepidota* (0.02mg/kg). The lowest was observed in *B. schwanenfeldii* and *H. nemurus* (0.01mg/kg). No Mn concentration was detected from *O. niloticus* in this study.

### 3.2 Metal concentration in water

Metal concentrations in water (Table 4) were compared with National Recommended Water Quality Criteria by U.S. EPA and Malaysian Interim Water Quality Standard (NWQS). Results show that Zn, Cu, Ni, Pb, Mn and Cd concentrations in this study were lower and within the range of EPA criteria and Class III of NWQS standards.

### 4. Conclusion

Differences were observed among muscle of different fish species in accumulating heavy metals. The concentration of metals in fish from Galas and Beranang were within the acceptable limits according to FAO and MFA. The concentrations of Zn and Pb in the muscles tissue of Tilapia from Beranang mining pool were remarkably higher than those from fishes of Galas River. To sustain a healthy freshwater ecosystem and prevent health problem to the locals, concentration of heavy metals should be monitored regularly.

### References

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