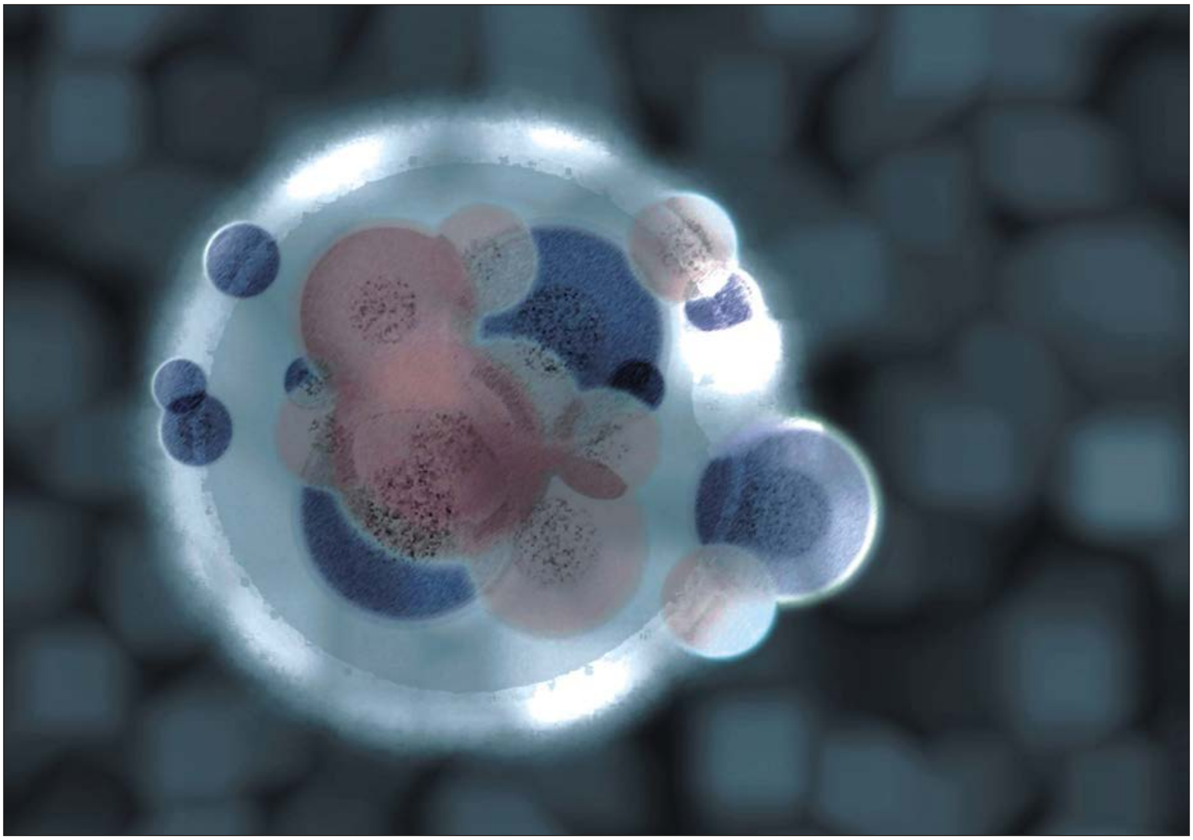

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Levels of Some Heavy Metals in Fishes From Pahang River Estuary, Pahang, Malaysia

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Abstract: In this study, the concentration of Pb, Cu and Zn were determined in muscle, gills and stomach of six fish species caught from Pahang river estuary. These metals concentration were measured by ICP-MS in order to assess the fish contamination with these metals. This study showed that all catfishes (*Arius* sp.) presented the highest metals content. Tissue analysis revealed that the stomach accumulated the highest level of these metals. Based on the results, metal concentration in the edible part of the examined fish (muscle) were in the safety permissible levels for human consumption.

Key words: Pahang river estuary, Pb, Cu, Zn, fishes, ICP-MS

INTRODUCTION

Fish constitute an important source of protein for many people throughout the world and fish consumption has increased in importance among health-conscious because it provides a healthy, low cholesterol sources of protein and other nutrients (Burger and Gechfeld, 2005; Agusa *et al*., 2005). Heavy metals such as copper and zinc are essential for fish metabolism while some others such as lead has no known role in biological systems. For the normal metabolism of fish, the essential metals must be taken up from water, food or sediment. However, similar to the route of essential metals, non-essential ones are also taken up by fish and accumulate in their tissues (Canli and Atli, 2003; Turkmen *et al*., 2005; Dural *et al*., 2007).

It has been recognized for many years that the concentrations of metals found in coastal areas, whether they are in the dissolved or particulate phase may be derived from a variety of anthropogenic and natural sources (Dalman *et al*., 2006). In most circumstances, the major part of the anthropogenic metal load in the bottom sediment and organisms has a terrestrial source from mining and intensive aquaculture and municipal wastewaters, untreated effluents, harbour activities, urban and agricultural runoff along major rivers and estuaries. On the other hand, the residual content of contaminants (metals and organics) and Cu, which is commonly used in antifouling chemical has drawn little attention regarding impacts to the marine environment (Mazet *et al*., 2005).

Many studies have been conducted on the contamination of different fish species with heavy metals in different parts of the world (Hulya and Erhan, 2000; Al-Jedah and Robinson, 2001; De Souza Lima *et al*., 2002; Tuzen, 2003; De Mora *et al*., 2004; Wang *et al*., 2005; Jung *et al*., 2006). The aim of this pilot study was to assess the concentrations of lead, copper and zinc in six different fish species. This can serve as an indicator for the extent of pollution in the Pahang River estuary.

MATERIALS AND METHODS

Fish sample: Samples of *Ilisha melastoma*, *Ilisha elongate*, *Otolithes biauritus*, *Arius sumatranus*, *Arius tenuispinis* and *Arius thalassinus* were collected from the Pahang river estuary (Fig. 1) in May 2008. One hundred and ninty one samples of different sizes were collected, placed in an icebox, transported to the laboratory and kept in freezer -20°C prior to the analysis. The fish samples were then defrosted and their standard length and weight were recorded. Whole fish were dissected on a clean bench shortly after thawing with the aid of a stainless steel knife which had been cleaned with acetone and hot distilled water prior to use. The fish samples were finally preserved in clean dry polyethylene bottle prior to analysis.

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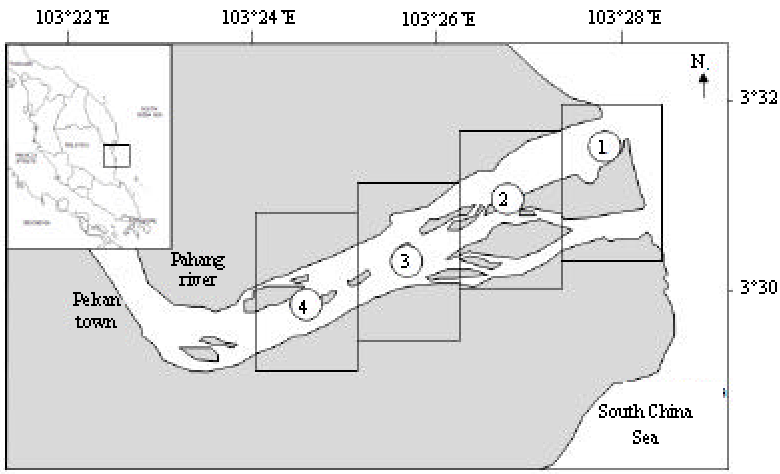

Fig. 1: Map of study area at Pahang river estuary, Pahang

Chemical analysis: Dissected parts (muscle, stomach and gill) were processed for heavy metals analysis. An accurate sample dry weight, 0.5 g of fish tissue was gently digested for 3 h in 10 mL concentrated HNO_3 followed by 6 mL mix acid ($3HCl:1H_2O_2:2H_2SO_4$) and heated at 60°C until the digestion was completed. Then, 3 mL mix acid ($2HNO_3:1H_2O_2$) was added to the solution and then heated to dryness. After cooling, the solution was then transferred to a 50 mL polypropylene vial and completed with 5% HNO_3. For each series of 20 samples, two analytical blanks were prepared in a similar manner without sample to check the possible contamination. Finally, the samples were analyzed for Pb, Cu and Zn concentration by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agusa *et al*., 2004). Analytical quality was assessed using standard reference materials, DORM (dogfish muscle: National Research Council Canada). Recoveries of all the elements ranged from 96 to 105% of the certified value.

Statistical analysis: One half of the value of the respective limit of detection was substituted for those values below the limit of detection and use in statistical analysis. All data were tested for fitness to a normal distribution by Kolmogorov-Smirnov's one sample test. Most of the variables were not normally distributed, therefore the data were log transformed and subjected to parametric statistics. Pearson correlation coefficient was used to measure the strength of the association between trace element concentration in muscle, gill and stomach and body length. Scheffe method, along with one-factor ANOVA, was conducted for the detection of specific accumulation of trace element (Feldlite *et al*., 2008). A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Figure 2a-c show the concentration of Pb, Cu and Zn in six fish species from Pahang river estuary. A total of 191 fishes were analyzed with a mean size and weight varying from 9.8-33.5 mm and 10.2-520.5 g, respectively. Earlier studies also indicated that different contents of heavy metals in different fish species might be a result of different ecological needs, metabolism and feeding patterns (Al-Saleh and Shinwari, 2002; Yilmaz, 2003). Roméo *et al*. (1999) and Dusek *et al*. (2005) pointed out that Cd, Cu and Zn contents in edible muscles of pelagic fish species were lower than for benthic fish species. Similarly, this study showed that the Cd, Cu and Zn contents in muscles of *Ilisha melastoma* and *Ilisha elongata* (pelagic fishes) were lower than those *Arius thalassinus* and *Arius sumatranus* (benthic). *Ilisha melastoma* and *Ilisha elongata* both inhabit coastal waters, mud banks or lagoons and feeds on plankton, small crustaceans and fishes. Both *Arius thalassinus* and *Arius sumatranus* feeds on invertebrates and small fishes selectively (Law and Singh, 1991).

Heavy metals concentration in muscle, gill and stomach: Bioaccumulation in stomach was observed in most metals since, it is the major organ involved in xenobiotic metabolism in fish (Rashed, 2001; Fernandes *et al*., 2007). Zn accumulated the most in all the fishes followed by Cu and Pb, Cu and Zn are essential elements and are carefully regulated by physiological mechanisms in most organisms. Many studies have demonstrated that diet is the most important route of Cu accumulation in aquatic animals (Sindayigaya *et al*., 1994). Pb is a natural component in the marine ecosystem. It is present at low concentrations in clean seawater, sediments and tissue of

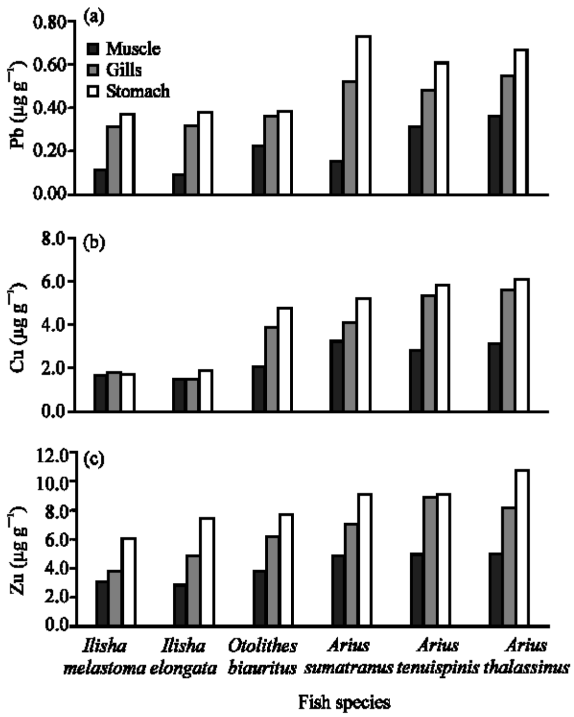

Fig. 2: (a-c) Pb, Cu and Zn concentrations (ug g^{-1} dry weights) in different tissues of six fish species collected in Pahang Estuary

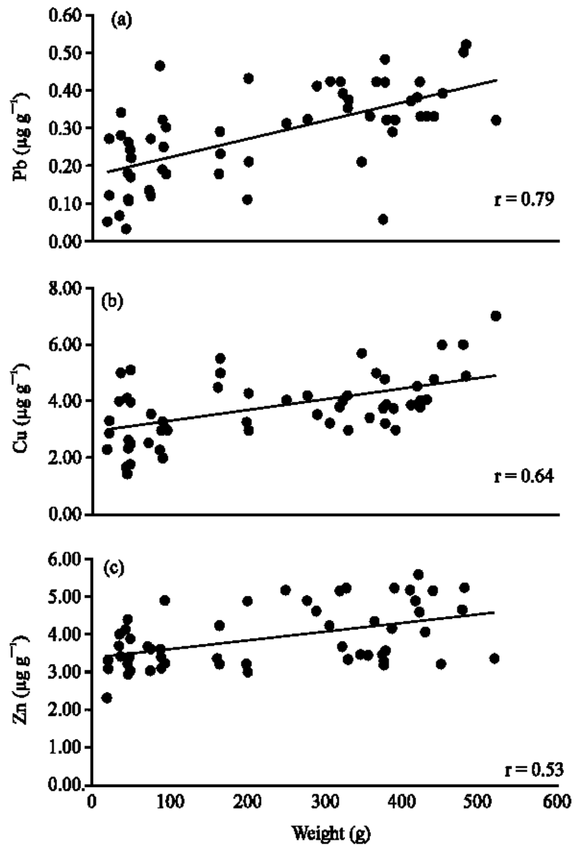

Fig. 3: (a-c) Relationship of Pb, Cu and Zn concentrations in fish species versus weight

marine plants and animals. Although, fish bioaccumulate Pb from seawater in proportion to its concentration in solution, Pb is not very bioavailable or toxic to marine animals (Dalman *et al*., 2006). Liver, stomach and gill in fish are more often recommended as environmental indicator organs of water pollution than any other fish organs. Muscle parts are tested as it is the part which is consumed by public (Zauke *et al*., 1999; Agusa *et al*., 2005).

Relationship between body size and trace element concentrations: In all the fish species, significant positive correlations between total length and total weight were found ($p<0.05$) (Fig. 3a-c). Significant positive correlation were found between body weight and concentration of Pb ($r = 0.79$; $p<0.05$), Cu ($r = 0.64$; $p<0.05$) and Zn ($r = 0.53$; $p<0.05$) indicated that the metal concentration increase with increasing body length of the fish.

Estimation of potential health risk: To assess whether metal levels found in fish samples from the Pahang river estuary are safe for human consumption, a comparison is made to reference values for fish muscle as this tissue is the most important part to be used for human consumption. For this purpose, the health risk to Pekan people through consumption of fish, intake rates of trace elements were estimated on the basis of the concentrations of trace elements in muscle of fish and daily fish consumption. The potential hazards of metals transferred to humans are probably dependent on the amount (g wet weight) of fish consumed by an individual. The daily and weekly intakes were estimated for economically important fish species consumed by adult people. The average daily fish consumption in Malaysia is 15.8 g person^{-1} (Laurenti, 2002). This is also equivalent to 110.6 g/person/week. The EWI (estimated weekly intake) and EDI (estimated daily intake) values presented in Table 1 were estimated by assuming that an adult will consume 15.8 g fish day^{-1} which is equal to 110.6 g fish week^{-1}. For example, an adult who consumed 12 g day^{-1} of *Otolithes biauritus* daily from Pahang river estuary would take in approximately 2.64 µg (0.22 µg $g^{-1}\times12$ g) of Pb each day. If the consumer were to take the fish for 7 days, then he would consume 18.48 µg Pb (2.64 µg×7 days). This is lower than the recommended limit for the provisional tolerable weekly intake of Pb (50 µg adult^{-1}) (FAO/WHO, 1984).

Table 1: The estimated daily and weekly intakes for the fish species consumed by adult in Pahang

	Metal		
Intakes	Pb	Cu	Zn
PTWI[1]	250	3500	7000
PTDI[2]	35.7	500	1000
Ilisha melastoma EWI[3] (EDI[4])	12.17 (1.74)	182.49 (26.07)	346.18 (49.45)
Ilisha elongata EWI (EDI)	9.95 (1.42)	168.11 (24.02)	322.95 (46.14)
Otolithes biauritus EWI (EDI)	24.33 (3.48)	226.73 (32.39)	428.02 (61.15)
Arius sumatranus EWI (EDI)	16.59 (2.37)	356.13 (50.88)	544.15 (77.74)
Arius tenuispiuis EWI (EDI)	34.29 (4.90)	310.79 (44.40)	556.32 (79.47)
Arius thalassinus EWI (EDI)	35.56 (5.69)	343.97 (49.14)	561.85 (80.26)

[1]PTWI: Provisional permissible tolerable weekly intake in μg week^{-1}. [2]PTDI: Permissible tolerable daily intake in μg day^{-1}. [3]EWI: Estimated weekly intake in μg week^{-1}. [4]EDI: Estimated daily intake in μg day^{-1}

CONCLUSION

In conclusion, the Zn is the highest accumulated heavy metal among all the selected heavy metals tested in all the fish parts. Stomach is the organ which had the highest concentration of heavy metals compared to gills and muscle. According to our results, the examined fish were not associated with the enhanced metal content in their muscle and were safe within the limits for human consumption.

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