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Bioaccumulation of Heavy Metals in Fish Species Collected From Former Tin Mining Catchment

Ashraf, M. A.[1*], Maah, M. J.[1] and Yusoff, I.[2]

[1] Department of Chemistry, University of Malaya, Kuala Lumpur 50603 Malaysia

[2] Department of Geology, University of Malaya, Kuala Lumpur 50603 Malaysia

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ABSTRACT: This study has been carried out to determine the accumulation of heavy metals (arsenic, copper, lead, tin and zinc) in fish samples collected from former tin mining catchment. Total eight types of fish family having fifteen different species are identified. *Cyperinidae* is the most abundant family with eight different species found in the catchment. Fish samples were analysed by using inductively coupled plasma optical emission spectrophotometer ICP-OES. The accuracy of method is validated by certified reference material. The highest level of arsenic was observed in *Hampala macrolepidota* while *Osteochilus hasselti* shows the least. For Copper concentrations, the maximum was obtained in *Mastacembelus armatus* and the minimum in *Oxyeleotris marmorata*. High levels of lead, tin and zinc were found in the *Rasbora elegans, Trichogaster trichopterus, Oxyeleotris marmorata* respectively while *Macrobrachium resenbergii, Mastacembelus armatus, Rasbora elegans* had the least concentrations. The sequence of order of the heavy metals measured was Sn > Pb >Zn > Cu> As, respectively. The results showed elevated levels of tin, led and zinc in all the fish samples although copper and arsenic were available in relatively low concentration in the most samples. Sn, Pb, and Zn concentration in the samples were greater than Malaysia food act permissible levels. Hazard index < 1 suggests unlikely adverse health effects whereas HI > 1 suggests the probability of adverse health effects. Although the heavy metals analysed in the catchment did not pose any immediate health risk to humans but due to the bioaccumulation and magnification of these heavy metals in humans, it is essential to safeguard levels of the metals in the environment.

Key words: Species, Classification, Heavy metals, Permissible level, Health effects, Hazard index

INTRODUCTION

During recent years the concentrations of toxic metals in many ecosystems are reaching unprecedented levels. The increasing use of metals in industry and mining activities have lead to serious environmental pollution through effluents and emanations (Goldberg *et al*., 1978; Phillips, 1980; Sericano *et al*., 1995). Under certain environmental conditions, heavy metals may accumulate to a toxic concentration (Güven *et al*., 1999), and cause ecological damage (Harms, 1975; Jefferies & Freestone, 1984; Freedman, 1989).

Specifically aquatic systems are more sensitive to heavy metal pollutants and the gradual increase in the levels of such metals in aquatic environment, mainly due to anthropogenic sources, became a problem of primary concern (Meybeck *et al*., 1989; Allen *et al*., 1993). This is due to their persistence as they are not usually eliminated either by biodegradation or by chemical means, in contrast to most organic pollutants. Moreover, the decay of organic materials in aquatic systems together with detritus formed by natural weathering processes and uncontrolled mining activities provides a rich source of nutrients in both the bottom sediments and overlying water body (wade *et al.*,2008; Jafari & Gunale, 2006). Microorganisms, microflora and algae are capable of incorporating and accumulating metal species into their living cells from various supply sources (Jaffar *et al.*, 1988).

Consequently, small fish become enriched with the accumulated substances. Predatory fish again, general display higher levels than their prey. Eventually man, consuming the fish, inevitably suffers from the results of an enrichment taken place at each trophic level, where less is extracted than ingested (Forstner & Wittmann, 1983). Fish occupies the highest tropic level in aquatic system (APHA, 1981). Besides that, it has high economical values, thus fish is suitable for water quality symbol and easy to be interpreted by public. Fish can response to

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*Corresponding author E-mail: chemaqeel@gmail.com environmental changes that can be used for pollution indicator study. Fish is a good bio-indicator because it is easy to be obtained in large quantity, potential to accumulate metals, long lifespan, optimum size for analysis and easy to be sampled (Batvari *et al*., 2007). Heavy metal intakes by fish in polluted aquatic environment are different depends on ecological requirements, metabolisms and other factors such as salinity, water pollution level, food and sediment. Fish accumulates metals in its tissues through absorption and human can be exposed to metals via food web. This will cause acute and chronic effect to human (Yilmaz & Dogan, 2007; Fidan *et al*., 2007). The use of fish as bioindicator can determine the actual situation of pollution level before and during monitoring.

In general, studies on heavy metals by fish analysis can be important in two main aspects. First, from the public health point of view, where the attention has been drawn to the necessity of measuring the accumulation of heavy metals; particularly these metals which pose serious health hazards to humans (e.g. As, Pb, Hg). Second, from the aquatic environment view point, the main problem has been to prevent biological deterioration and to identify the sources which threaten ecological equilibrium. In this regard, the more abundant metals such as copper, zinc and manganese may sometimes represent greater hazard than lead, mercury and cadmium (Kinne, 1984).

Several techniques have been used for determination of metal concentrations in fish species such as flame atomic absorption spectrometry (Abedin, 1986; El-Mehdi, 1987; Ahmet, 1992; Petisleam, *et al.*, 2003; Petisleam, *et al.*, 2007; Bermejo-Barrera, *et al.*, 2001) graphite furnace atomic absorption spectrometry (Sperling, 1988; Mustafa, 2003; Botson *et al.*, 2004), electro-thermal atomic absorption spectrometry (Perez, *et al.*, 2001; Mendez, *et al.*, 2002), inductive coupled plasma (Chirila, *et al.*, 1999; Sanchez, *et al.*, 2003; Milne, *et al.*, 2010) and mass spectrometry (Sanchez, *et al.*, 2003; Petisleam, *et al.*, 2005). Different digestion methods were used as sample preparation methods for determination of heavy metals in fish samples (Olaifa *et al*., 2004; Huijuan *et al.*, 2005).

The present study was undertaken to study the concentrations of Sn, Cu, Pb, Zn and As in different tissues (bone, skin, flesh and tail) of fish species at former tin mining catchment Bestari Jaya using ICP-MS. concentration levels of selected trace metals in commercially important fish species and correlate the concentration of metals with respect to their weight and length.

Bestari Jaya catchment is located at 3^0, 24’ 40.41" N and 101^0 24’ 56.23" E. It is a part of Kuala Selangor district, located in Selangor, biggest state of the country. District Kuala Selangor has three main towns namely, Mukim Batang Berjuntai, Mukim Ulu Tinggi, Mukim Tg.karang. Bestari Jaya is located in Mukim Batang Berjuntai. Tin mining activities has ceased from last ten years, now sand mining. The catchment has total of 442 small and big mining lakes and ponds. Bestari Jaya has a tropical, humid climate, with very little variations in temperature throughout the year. The

Fig. 1. Map of Bestari Jaya Catchment

average temperature of the area is 32^0C during day and 23 ^{0}C at night. (Ashraf *et al.*, 2010).

MATERIALS & METHODS

Fish samples were collected from Bestari Jaya catchment with the help of local fishermen between March and April of 2011. After collection, the samples were cleaned with deionized-distilled water, weighed, measured, stored in pre-cleaned plastic bags, and kept frozen in an ice box. Fifteen (15) fish samples were double bagged in separate new plastic bags, sealed and labelled accordingly.

The physical characteristics of the sampled fish species were assessed. A measuring board made of perspex and a fixed meter scale was used to measure the total length (cm) of each individual samples. At the zero end of the scale, a suitable stop is placed at 90^o angle. The anterior end of the fish is placed against this stop. The total length is recorded for comparison to the known maximum size of the species. An electronic weight scale is used to weigh the individual mass of the fish. In addition, the total number and weight (g) for each batch and species was also determined to represent the overall abundance in the specific river. The weight of fish, together with its size distribution would indicate the fish life stages. The weight is measured in gram (g).

The ratio between the fish whole body weight to body length (head to tail) was examined to identify fishes with anomalously high or low ratios. The overall health of fish species in the catchment were assessed using the method developed by (Williams, 2000). A condition factor index (K) was calculated using the equation:

$$K = 100W/L^3$$

where, W = body weight in grams; L = body length (fork length) in cm

The frozen fish samples were partially thawed on cleaned plastic sheets using scalpels with steel blades and plastic forceps. Whole taxa of a designated length range were cut into small pieces with Teflon-tipped scissors. The samples were pooled and freeze-dried for 10 days to a constant weigh for the determination of metal content, 0.5 g dry muscle samples of the fish. (Moeller *et al*., 2001).

Two types of digestion procedures were applied. Optimum digestion conditions are given below. The samples were dried to constant weight at 110 ^{0}C.

For dry ashing analysis, sample (1 g) was placed in a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 ^{o}C in 1 h. The samples were ashed for about 4 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25% v/v) and the mixture, where necessary, was heated slowly to dissolve the residue. The solution was transferred to a 25 ml volumetric flask and made up to volume (Vaidya & Rantala, 1996). A blank digest was carried out in the same way. All metals were determined against aqueous standards.

For wet ashing analysis, the samples were solubilized using high-pressure decomposition vessels, commonly known as a digestion bomb. A sample (1 g) was placed into Teflon container and 5 ml of concentrated HNO3 was added. The system was heated to 130 ^{o}C for 90 min and finally diluted to 25 ml with deionized water. The sample solution was clear. A blank digest was carried out in the same way. All metals were determined against aqueous standards.

Inductively coupled plasma optical emission spectrometry ICP-OES (Perkin Elmer AA Analyst) was used to analyse the concentration of heavy metals in fish samples. All reagents used were of analytical reagent grade (Merck, Germany). Acid washed glassware analytical grade reagents and double distilled deionised water was used throughout the experiments. Standard stock solutions of arsenic, copper, tin, lead, and zinc were prepared from Titrasol (1000 mg/L). The working solution was freshly prepared by diluting an appropriate aliquot of the stock solutions. In order to check on the purity of the chemical used, a number of chemical blanks were run. There was no evidence of any contamination in these blanks. In order to validate the method for accuracy and precision, certified reference materials (fish: DORM-2, National Research Council, Canada) were analysed for each element. Accurate results are obtained as shown in (Table 2). The detection limit is defined as the concentration corresponding to 3 times the standard deviation of 10 blanks.

RESULTS & DISCUSSION

During sampling March-April 2011, the total number of fishes caught was 66 with 4064 grams. The main family observed in this survey, with 81 % (Fig. 2) of overall caught species was still the family of Cyprinidae, followed by the families of Palamonidae and Nandidae. The cyprinids were mainly represented by Terbul(68), Kawan(42), Rong(16) and Seluang dua titik (16). The condition factor is an estimation of the general well being of fish and crustaceans (Jones *et al*., 1999). It is based on the hypothesis that heavier individuals of a given length are in better condition than less weightier ones (Bagenal & Tesch, 1978). Condition factor has been used as an index of growth and feeding intensity. Most notably, fish measured with an exceptionally low weight-to-length ratio may indicate the organism is stressed, either by physical injury, disease, dietary limitations, or poor water quality. The sampled fish species recorded an average K factor of 1.7 (Table 1). Age factor or maturity of fish may influence the accumulation of heavy metals (Mohsin & Ambak, 1991). Growth rate is important to stabilize the accumulation of metals. Fish can accumulates high level of pollutants if it has a constant growth and live in polluted ecosystem. This study indicates that mature fish accumulated higher metals compared to juvenile and premature fish. *Labiobarbus cuvieri*, *Chela anommalura* and *Osteochilus hasseltii* (mature omnivour fish) accumulated higher concentrations of Zn rather than *Osteochilus vittatus* and *Channa striatus* (premature). Although, comparison between same species is more appropriate, however, the comparison made was based on similar feeding behaviour. This study was unable to obtain sufficient number of fish to make comparison between age for the same species.

Fishes collected from catchment are comprises of three different types of feeding behaviors; herbivor, omnivor and carnivor. *Hampala macrolepidota*, *Rasbora sumatrana*, *Rasbora elegans*, *Cyclocheilichthys apogon* are carnivores (consume small fishes); *Osteochilus vittatus*, *Trichogaster trichopterus* and *Pristoplepis fasciatus*, *Acanthopsis choirorhyhchos*, *Oxyeleotris marmorata* are herbivores (consume plants) while *Labiobarbus cuvieri*, *Chela anommalura*, *Osteochilus hasseltii*, *Mastacembelus armatus*, *Macrobrachium resenbergii* and, *Channa striatus* are omnivores (Mohsin & Ambak, 1991). Feeding behaviour is one of the decisive factors of heavy metals accumulation in fish. Although, many studies showed carnivores accumulate higher metal concentrations (Mohsin & Ambak, 1991; Amundsen *et al*., 1997) result of this study demonstrates that metal concentrations in three different feeding behavior are not significantly differ. Low metal concentrations in the ecosystem could limits the absorption of these metals in the organisms. As regards to (Mohsin & Ambak, 1991) classification, this study found that most of the fish samples were considered as an adult. The mean metal concentrations of Cu, Zn, Pb Sn and As (mg/kg) in 15 fish species are shown in (Fig. 3). Tin mean concentrations were the highest as compared to other metals and ranged from 56.34 to 153.45 mg/kg. Metals accumulation in fish was found in the order of Sn>Pb>As>Zn>Cu. Metals concentration in fish tissues were detected at small variations.

Most of the locations chosen for the fish sampling for the determination of heavy metals have abandoned mining activities for more than 10 years and have been converted to secondary land uses such as fish breeding and other aqua-culture activities. The mean elemental concentrations measured in the fishes during the study period are also presented in (Fig. 3). Reported values of the various elements are the results of the total concentrations (dry weight) measured in each individual whole fish. Almost all the fish samples collected from the catchment contained detectable amounts of the elements studied (Cu, Pb, Zn, As, Sn). These elements were present in all the fish samples and at varying concentrations. It must be noted that, varying concentrations of the heavy metals were measured in the sampled fishes with some fishes

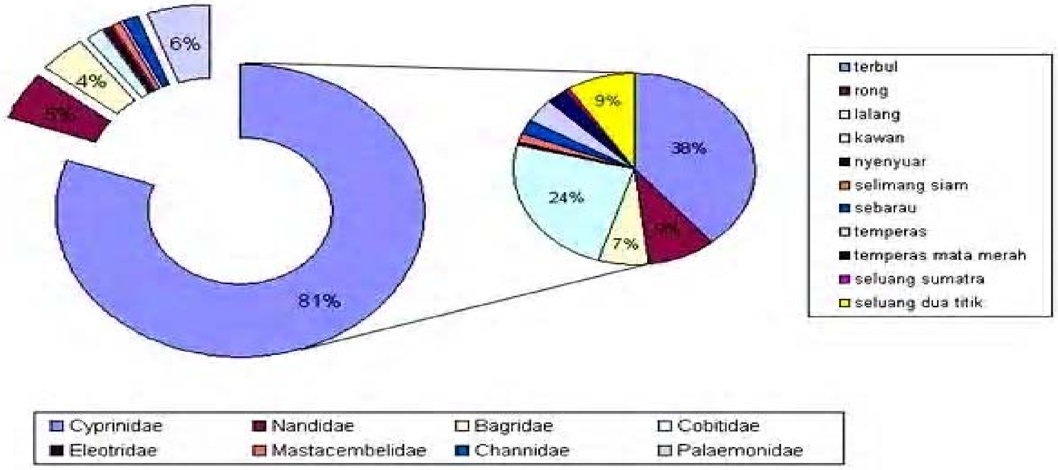

Fig. 2. Abundance of fish species in the catchment

Table 1. Physical characteristics & taxonomic classification of fish species in the catchment

S No.	Family	Species	Local Name	Length	*Maturity Size/Adult (cm)	Condition of Fish (K)	Status
1	Cyperinidae	*Osteochilus hasselti*	Terbul	14.5-17.0	6-20	1.8	Mature
2		*Osteochilus vittatus*	Rong	8-22.3	50.0	1.7	Mature
3		*Chela anommalura*	Lalang	7-15.8	7.0-20.0	1.5	Premature
4		*Labiobarbus cuvieri*	Kawan	9.4-18.2	10.0-20.0	1.7	Mature
5		*Hampala macrolepidota*	Sebarau	7.5-18.5	25.0	1.9	Mature
6		*Cyclocheilichthys apogon*	Temperas mata merah	18.5-19.7	28.0-100.0	1.5	Premature
7		*Rasbora sumatrana*	Seluang sumatra	8.8-10.7	48.0	1.7	Mature
8		*Rasbora elegans*	Seluang dua titik	14.4-17.4	12.0-18.0	1.8	Mature
9	Nandidae	*Pristoplepis fasciatus*	Patung	7.7-14.3	33.0	1.8	Mature
10	Cobitidae	*Acanthopsis choirorhyhchos*	Lali	7.5-12.5	20.0-35.0	1.6	Premature
11	Eleotridae	*Oxyeleotris marmorata*	Ketutu	13.6-17.6	10.0-12.0	2.0	Mature
12	mastacembelidae	*Mastacembelus armatus*	Tilan	30-41.2	12.0-19.0	2.0	Mature
13	Channidae	*Channa striatus*	Haruan	10.8-25	12.0-30.0	1.6	Premature
14	Palaemonidae	*Macrobrachium resenbergii*	Udang galah	4.5-13.5	10.0-30.0	1.8	Mature
15	Siluridae	*Trichogaster trichopterus*	Sepat padi	5.6-14.7	15.0-35.0	1.7	Mature

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3		*Chela anommalura*	Lalang	7-15.8	7.0-20.0	1.5	Premature
4		*Labiobarbus cuvieri*	Kawan	9.4-18.2	10.0-20.0	1.7	Mature
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6		*Cyclocheilichthys apogon*	Temperas mata merah	18.5-19.7	28.0-100.0	1.5	Premature
7		*Rasbora sumatrana*	Seluang sumatra	8.8-10.7	48.0	1.7	Mature
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11	Eleotridae	*Oxyeleotris marmorata*	Ketutu	13.6-17.6	10.0-12.0	2.0	Mature
12	mastacembelidae	*Mastacembelus armatus*	Tilan	30-41.2	12.0-19.0	2.0	Mature
13	Channidae	*Channa striatus*	Haruan	10.8-25	12.0-30.0	1.6	Premature
14	Palaemonidae	*Macrobrachium resenbergii*	Udang galah	4.5-13.5	10.0-30.0	1.8	Mature
15	Siluridae	*Trichogaster trichopterus*	Sepat padi	5.6-14.7	15.0-35.0	1.7	Mature

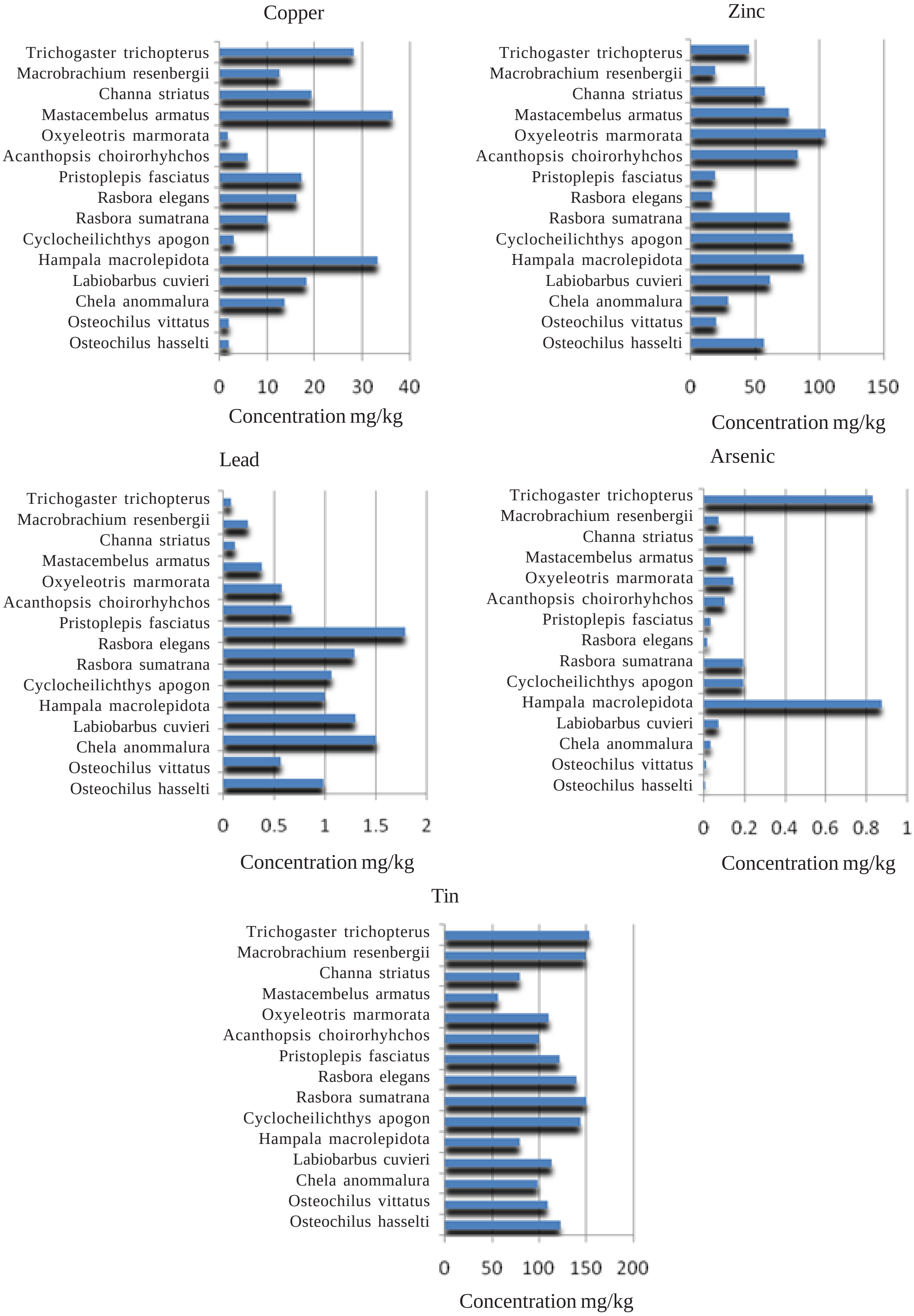

Fig. 3. Concentration of heavy metals in different fish species

reporting very high concentrations whilst other samples measured relatively lower concentrations and in some cases no concentrations of the elements. Zinc and copper tended to be the least concentrated in the fish as compared to other elements measured. Concentrations of copper varied from <1.51 to 36.31 mg/Kg (dry wt.). The low copper values in the catchment fish may be due to the high chlorides content of the catchment since salt-water organisms are known to be more resistant to copper poisoning than freshwater organisms (Asagba *et al*., 2008). The greatest sources of zinc in humans are sea foods and meats (Reigart & Roberts, 1999). Concentrations of Zinc in the fish samples were relatively high (16.25 to 104.23 mg/Kg). The maximum permissible chromium levels for fish are 102 mg/kg as reported by (FAO, 1983). Arsenic is one of the most toxic elements to fish. Acute exposures can result in immediate death. Chronic exposures can result in the accumulation of the metalloid to toxic levels. In fish, bizarre morphological alterations, as well as early neoplastic alterations are produced in the live. Total arsenic results are similarly low, with only 5 of the 15 fish tissue samples having a total arsenic concentration above the detection limit of 0.11 mg/kg. The highest total arsenic concentration observed was 0.87 mg/kg for *Hampala macrolepidota* from the catchment. Total concentration of arsenic in fish samples taken from mining ponds are ranged between a minimum of 0.0025 to a maximum of 0.83 mg/kg. The review concluded that arsenic concentrations in planktivorous fish are elevated over predatory and omnivorous fish. Arsenic does not biomagnify up the aquatic food chain as do some other chemicals. Concentration of lead ranged from 0.07 to 1.78 mg/kg. The highest concentration of zinc was recorded in the muscles of *Rasbora elegans* and *Trichogaster trichopterus*. Similarly concentration of tin were ranged from 56.34 to 153.45 mg/kg with highest concentration of tin were found in the *Trichogaster trichopterus*. The results showed that the concentrations of Pb and Sn, were higher in *Trichogaster trichopterus* fish, than in fish from other sampling locations showing that *Trichogaster trichopterus* is more susceptible to heavy metals accumulation but previously there are not reported any marked effect with high concentration of tin metal.

Health risk estimates in this study were calculated based on the integration of the data from heavy metals analysis and the assumed consumption rate based on US.EPA guidelines. The following assumptions were made based:

- Hypothetical body weights of 10 Kg for children between the ages of zero and one year, 30 Kg for children between the ages of one and eleven years and 70 Kg for adults
- Maximum absorption rate of 100% and a bioavailabilty rate of 100%
- Food consumption rate of fish in Malaysia given as 0.08 kg/day Hence, for each type of contaminat (heavy metal), the estimated daily dose (ED) (mg/Kg/day) was obtained using the equation detailed in the US. EPA handbook , 1989:

ED = Conc. of element of interest x Food consumtion rate / Body weight, kg

To estimate the health effects, the estimated lifetime average daily dose of each chemical was compared to its Reference Dose (RfD). The reference dose represents an estimate of a daily consumption level that is likely to be without deleterious effects in a lifetime. And the hazard index (HI) is estimated as the ratio of the estimated metal dose and the reference dose as depicted in US.EPA, 1996.

Hence, HI = ED/RfD

Where, ED = Estimated Dose and RfD = Reference Dose Estimated health risk associated with consumption of the fish is presented in (Table 3). Hazard index < 1 suggests unlikely adverse health effects whereas HI > 1 suggests the probability of adverse health effects. For adults, health risks estimates for copper, zinc, lead, arsenic and tin were lowest for the general public at the highest ingestion rate (Table 3) whilst the same elements were relatively

Table 2. Permissible levels of metals & validation of extraction and analysis with certified reference material

Sr. No.	Metals	Permissible Limits* mg/kg	Measured Value mean	Certified Value	Accuracy %
1	Lead	2	0.89±0.01	0.85±0.13	78
2	Arsenic	0.026	0.44±0.18	0.45±0.15	98
3	Copper	30	89.30±0.42	106±10.00	89
4	Zinc	100	183.80±1.70	180.00±6.00	98
5	Tin	156	198±2.0	204±10.00	95

*MFA, 1983

Table 3. Health risk estimates associated with heavy metals in fish species

Metal	RfD	Estimated Dose Time (mg/kg)			Hazard Index		
		0-1 Year	1-11 Year	Adult	0-1 Year	1-11 Year	Adult
Copper	3.70E-02	0.082	0.027	0.012	1.7	0.58	0.25
Zinc	4.70E-02	0.07	0.024	1.98	1.5	0.5	0.22
Lead	5.70E-05	0.088	0.056	2.56	1.92	0.71	3.44
Arsenic	7.00E-03	0.0065	0.0022	0.00093	0.9	0.007	0.0031
Tin 8.60E-03	0.4728	0.1576	0.0675	54.98	18.32	7.85	5.98

higher for children at the highest ingestion rate. Even though the values do not pose any health hazards for the ordinary consumer, the levels are likely to be higher for families of fishmen since their meals are more fish-dependent. The use of metal-based fertilizers by farmers in the ex-mining area should be monitored and regulated.

The accumulation of metals by the fish depends on the location, feeding behaviour, trophic level, age, size, duration of exposure to metals and homeostatic regulation activities of fish (Sankar *et al.*, 2006; Kargin, 1996) has listed multiple factors that influence metals accumulation in fish such as season, physical and chemical properties of water. Knowledge of metals concentration in fish is important to management for various purposes such as risk of taking fish as part of diet and metals pollution control strategies. Most of fish are at top in aquatic food chain and have potential to accumulate high metals content even in mild polluted conditions. Therefore, metals concentration in fish could be used as an index to estimate level of pollution especially in aquatic bodies (Karadede-Akin & Unlu, 2007) even in the lake system. Although, fish muscle was reported have lowest metal concentrations compare to bone, gill and liver, this study focus metals in fish muscle since, people eat fish muscle and not others. Various studies on metal concentrations in fish samples has done previously (Findan *et al.*, 2007; Chi *et al.*, 2007; Ahmad and Shuhaimi, 2010 ; Laar *et al.*, 2011). This study found Cu and Zn concentrations were almost as low as from unpolluted lake likes Eber (Findan *et al.*, 2007). However, Sn and Pb concentrations were higher than fishes from Eber Lake and Taihu Lake (Chi *et al.*, 2007). Both metals were probably contributed to the lake through various sources, such as agriculture activities likes oil palm and rubber plantations nearby catchment besides mining activities. A large scale of oil palm plantation was developed by Sime Darby Company nearby the catchment and the use of chemical fertilization could introduce Pb, Zn and Cu to the catchment. Excessive fertilizer may be transported to the catchment through surface run off. The catchment has several dredged out small and big ponds that could transport these metals into the catchment. Furthermore, the inflow of water from the catchment into Selangor River can make the conditions more adverse. During rainy season or monsoon season also could bring pollutants from nearby agricultural areas including metals into the catchment. Nonetheless, the concentrations of these metals were still below the permissible limit (Table 2, Fig. 3) suggested by the (MFA, 1983).

This study indicates that although still below hazardous level, non-essential metals namely, Pb and Sn and essential metal namely Zn were quite high and continues input could risk the catchment ecosystem. Continual accumulation in fish muscle could transports metals to human through diet. Local aborigines surround the lake catch fish for their daily diet.

CONCLUSION

The concentrations of heavy metals in the fish samples were quite variable and no patterns of distribution and behaviour were noted. However, high concentrations of some heavy metals measured in the fish tissues inhabiting that the Bestari Jaya catchment were related to a high influx of metals as a result of pollution from the dredged mined out ponds thereby increased bioavailability to the fish. The high levels also suggested that the fish were capable of concentrating the metals in their bodies from the aquatic environment. The ability of the fish samples to incorporate the elements into their tissue is another important factor to consider for further study. The data presented in this report indicates that the species have preference to retain some metals in their tissues than other metals. Although, no immediate health risk was estimated from consumption of the fish species, risk prevention on the consumption of fish should therefore focus on reducing the volume of heavy metals discharged from agriculture area as well as mined out ponds into the catchment.

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