RESEARCH NOTE

HEAVY METAL CONCENTRATIONS IN A TROPICAL EEL *Anguilla bicolor bicolor* IN PENINSULAR MALAYSIA, MALAYSIA

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Heavy metals are omnipresent in hydrosystems as a consequence of the daily anthropogenic activities and the persistence of some compounds used in the past. The detection and evaluation of the influence of hazardous chemicals is of growing concern because of the potential threat to wildlife and human health by inducing a wide range of adverse effects. Biological indicators such as bivalves (oysters, mussels), crustaceans or fish are commonly used to evaluate the chemical state of aquatic environments. A number of studies supported the use of freshwater eels *Anguilla spp*. as reliable bioindicators of environmental changes and contaminant concentrations in the eel tissue are suitable indicators reflecting the environmental exposure to pollutants (Le *et al*., 2009, 2010a, b). The EIFAC/ ICES Working Group on Eels (WG Eel, 2006) and the Scientific, Technical, and Economic Committee for Fisheries (STECF, 2006) have recommended that the Water Framework Directive should use the anguillid eel as a sentinel species for monitoring the chemical status of surface waters with respect to hazardous substances.

Freshwater eels are widely consumed in many countries including Malaysia. The major markets for the eel export are Asian countries such as China, Japan and Taiwan and a small number of European countries including Italy, Germany and The Netherlands. The market value of the anguillid eels depends on the species and varies between different countries. Currently prices range from USD 3-15/kg which rank the eel among the most precious species of food fish.

In Peninsular Malaysia, there is a species *Anguilla bicolor bicolor* (Arai *et al*., 2011, 2012). Recently this species was considered to be a major target species for the eel trading market. However, little attention has been given to the natural populations and resource management of eels in Malaysia. In Malaysia, a lot of information has been reported in the literature using mussels as biomonitor of heavy metals (e.g. Yap *et al*., 2003, 2006, 2009) but not for eels. Therefore, the objective of this study was to gain basic information of heavy metals such as cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), nickel (Ni) and Zinc (Zn) in a tropical eel *A. bicolor bicolor* collected in the Peninsular Malaysia. This is the first study to assess the heavy metal levels in freshwater eels in Malaysia.

A total of 30 specimens were collected by local fishermen mainly in Kurau River in Bukit Merah (7 specimens) and Pinang River in Penang Island (23 specimens) of the northwestern peninsular (4°59'-5°23 N, 100°12-100°40'E) during November 2008 and August 2010. The eels were collected by angling and bamboo trap at night. The total length (TL) and body weight (BW) were measured. The samples were brought to the laboratory on the same day and kept in a freezer (-20°C) until metal analysis. For metal analysis, muscle tissues were dissected out, put in clean polyethylene bags, and stored at -20°C. The muscles were dried in an oven at 60°C for 72 hours until a constant dry weight (dw) by using an air-circulating oven. About 1.0 g of the tissue parts were weighed and digested in 10 mL of concentrated nitric acid (AnalaR grade, BDH 69%). They were put into a digestion block first at 40°C for 1 hour and then the temperature was increased to 140°C for at least 3 hours (Ismail & Ramli, 1997). Later, the digested samples were diluted to 40mL with double distilled water (DDW). The samples were then filtered through Whatman No. 1 filter-papers and the filtrates were stored in 4°C until metal determination. After filtration, the prepared samples were determined for Cd, Cu, Fe, Ni, Pb and

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Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 880. The data are presented in µg/g dry weight basis. Multiple-level calibration standards were analysed to generate calibration curves against which sample concentrations were calculated. Standard solutions were prepared from 1000 mg/l stock solution of each metal (MERCK Titrisol). All apparatus were acid-washed (5% of nitric acid) by dipped for 24 h then rinsed with distilled water before used. All solutions used were prepared using double de-ionized water (USF Maxima, 18.2 MÙ cm^{-1}). Procedural blanks were analyzed once for every ten samples to check for sample accuracy. A quality control sample was routinely run through during the period of metal analysis. The limits of detection (µg/g) achieved in this study were calculated as Cd: 0.003, Cu: 0.001, Fe: 0.001, Ni: 0.005, Pb: 0.006 and Zn: 0.002. Differences between data were analyzed using the Mann-Whitney *U*-test (Sokal & Rohlf, 1995). Significance of the correlation coefficient and regression slope were tested by Fisher's Z-transformation and an analysis of covariance (ANCOVA) (Sokal & Rohlf, 1995).

The TL and BW of *Anguilla bicolor bicolor* eels ranged from 293 to 686 mm and from 38.9 to 645 g, respectively. In the relationship between each metal level and both TL and BW, no relationship was observed between those levels and both TL and BW (ANCOVA, $p > 0.05$) in this eel species. The levels of Cd, Cu, Fe, Ni, Pb and Zn in muscles of the freshwater eel are given in Table 1. There were no significant difference between samples from Bukit Merah and Penang in Cd, Cu, Ni, Pb and Zn (Mann-Whitney *U*-test, $p>0.05$) but a significant difference was found in Fe between those sites (Mann-Whitney *U*-test, $p<0.005$). The ranges of concentrations of metals (µg/g dry weight) were Cd: 0.08-0.44, Cu: 0.84-2.07, Fe: 11.0-36.6, Ni: 1.88-4.09, Pb: 2.39-13.2 and Zn: 39.2-113. The mean concentrations of heavy metals in the muscle of *Anguilla bicolor bicolor* are followed an increasing sequence of Cd < Cu < Ni < Pb < Fe < Zn.

Fish has been considered good indicators for heavy metal contamination in aquatic systems because they occupy different trophic levels with different sizes and ages (Burger *et al*., 2002). Meanwhile, fish are widely consumed in many parts of the world by humans, and polluted fish may endanger human health. The freshwater eel genus *Anguilla* has been also a major target species for human consumption especially in East Asia. The annual eel consumption in Japan is 120,000 to 130,000 tonnes with per capita consumption of about 5 eels a year (Kato & Kobayashi, 2003). Eighteen percent of the eel consumed in Japan is produced in the country while the remainder is imported from China, Taiwan, and Malaysia (Kato & Kobayashi, 2003). In the present study, levels of heavy metals such as Cd, Cu, Ni and Zn in the muscles of *Anguilla bicolor bicolor* were found to be below the acceptable limits of heavy metal pollution in fishes and shell fish (FAO/WHO, 1984), although some Pb levels were higher than the world standard (10 µg/g). This shows that the levels of Cd, Cu, Ni and Zn in the eel habitats are still tolerable, while there is a need for constant monitoring of the level of Pb. The Pb level in *A. bicolor bicolor* found in the present study was 2-200 times higher than the other anguillid eel species such as *A. anguilla* from France, *A. japonica* from Japan and *A. marmorata* from Vietnam (Table 2). The Pb level in muscles found in tilapia fish *Oreochromis niloticus* collected in Bangi area, Selangor of Peninsular Malaysia was 0.10-0.18 µg/g dry wt, respectively (Taweel *et al.*, 2011), and the level was much less than those found in *A. bicolor bicolor*. Anguillid eel has common ecological niche and the eel lives with tilapia fish sympatrically in freshwater ecosystem, although their lives such as migration, habitat and feeding are different. Thus, the difference found in Pb level in the eel muscles could be due to regional difference in Pb level. Further study should be carried out the systematical monitoring to reveal the reason of high Pb accumulation in *A. bicolor bicolor*. The other metal levels such as Cd, Cu, Ni and Zn found in *A. bicolor bicolor* were in the range of the other eel species collected various sites (Table 2). However, it should be noted that heavy metals are toxic or poisonous even at low concentrations. It suggests that heavy metal pollution of water bodies should be reduced to the barest minimum. This is to minimize fish food contamination that will in turn reduce clinical poisoning in human who consume the freshwater eel as well as other fish species.

There was no relationship between each biological character such as TL and BW and each metal level in *Anguilla bicolor bicolor*. Such failure to detect bioaccumulation of metal with growth was

Table 1. Heavy metal concentrations (µg/g dry weight) in muscle of the freshwater eel *Anguilla bicolor bicolor* collected in Peninsular Malaysia

Location	Number of samples	Cd		Cu		Fe		Ni		Pb		Zn	
		mean±SD	range	mean±SD	range	mean±SD	range	mean±SD	range	mean±SD	range	mean±SD	range
Bukit Merah	7	0.19±0.12	0.08-0.40	1.12±0.24	0.88-1.40	16.4±3.85	11.3-23.6	2.54±0.68	1.88-3.68	9.33±3.10	4.35-13.2	65.9±10.6	53.0-87.3
Penang	23	0.26±0.09	0.08-0.44	1.22±0.34	0.84-2.07	23.8±5.66	11.0-36.6	3.04±0.52	2.11-4.09	7.18±2.75	2.39-12.2	75.3±18.9	39.2-113

Table 2. Heavy metal concentrations (µg/g dry weight) in muscle of the freshwater eels *Anguilla* spp.

Country	Species	Cd	Cu	Fe	Ni	Pb	Zn	Source
France	*Anguilla anguilla*	NA	0.70-1.33	NA	NA	0.05-0.56	38.3-90.6	Neto *et al.,* 2011
Japan	*Anguilla japonica*	0.32-1.32	32.5-175	NA	NA	0.25-1.10	122-416	Le *et al.,* 2010a
Malaysia	*Anguilla bicolor bicolor*	0.08-0.44	0.84-2.07	11.0-36.6	1.88-4.09	2.39-13.2	39.2-113	This study
Vietnam	*Anguilla marmorata*	0.02-0.13	1.21-2.19	NA	NA	0.11-0.18	42.3-82.1	Le *et al.,* 2009

NA: data not available

found in the Japanese eel *A. japonica* (Le *et al.,* 2010a). The sizes of aquatic animals including fish have been shown to play an important role in metal contents of tissues. However, this evidence was consistent generally for mercury in different groups of aquatic animals (Canli & Atli, 2003). Heavy metals like copper, zinc and iron are essential for fish metabolism while some others such as mercury, cadmium and lead have 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. Studies from the field and laboratory experiments showed that accumulation of heavy metals in a tissue is mainly dependent upon water concentrations of metals and exposure period, although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation (Canli & Atli, 2003). Thus, such various factors would suggest less correlation between growth and metal accumulation in the freshwater eel. However, when using the eel as a biological indicator to reveal the pollutants in aquatic systems, we do not need to consider the size effect in our interpretation of the results.

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