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Artikel Asli/*Original Articles*

Levels of Non-Essential (Cd, Pb and Hg) Elements in Muscle Tissues of *Anguilla bicolor bicolor*, McClelland 1844 from Kedah and *Anguilla bengalensis bengalensis*, Gray 1831 from Perak and Human Consumption Risks

(Kepekatan Unsur Tidak Perlu (Cd, Pb dan Hg) dalam Tisu Otot *Anguilla bicolor bicolor*, McClelland 1844 dari Kedah dan *Anguilla bengalensis bengalensis*, Gray 1831 dari Perak serta Risiko ke Atas Konsumer Manusia)

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ABSTRACT

A study on heavy metals accumulation and human health risk assessment in the consumption of two tropical freshwater eel species (Anguilla bengalensis bengalensis) from the Sungai Perak at Kuala Kangsar, Perak and (Anguilla bicolor bicolor) from the Air Hitam irrigation canal, Kampung Kuala Sanglang, Kedah was carried out. Specimens were examined and analyzed for Pb and Cd concentrations using ICP-MS while the total Hg concentration was measured using a direct mercury analyzer (MA-3000). The range for the total concentrations (µg/g wet wt.) in Anguilla bicolor bicolor were 0.01-0.4 (Cd), 0.03-0.77 (Pb) and 0.36-0.94 (Hg) while for Anguilla bengalensis bengalensis, they were 0.76-1.23 (Cd), 0.01-0.10 (Pb) and 0.27-1.5 (Hg). Anguilla bengalensis bengalensis (Sungai Perak) showed a significant strong relationship between Hg/Pb (r = .771, P < 0.05) and Anguilla bicolor bicolor (Air Hitam irrigation canal) with Cd/Pb (r = -.895, P < 0.05) in muscle tissues and the results indicated Hg and Pb were introduced from point and non-point sources, therefore it is of concern. Interspatial comparison with the findings of previous local and international studies showed both species of freshwater eels accumulated Hg to high levels, exceeding the safe limits stipulated in the Malaysian Food Act of 1984, Food Regulations (1985) and USEPA (1997). However, the Target Hazard Quotient (THQ) and Hazard Index (HI) indicated that both freshwater eels from the studied sites are safe to consume as there is no risk posed from consumption based on the health risk assessment results as Pb, Cd and Hg concentrations were under the permissible limits of nutrient intake.

Keywords: Freshwater eels, metals, safety limits, THQ, HI

ABSTRAK

Kajian akumulasi logam berat beserta penilaian risiko kesihatan manusia ke atas pengambilan ikan tropis sidat spp. (Anguilla bengalensis bengalensis) dari Sg. Perak di Kuala Kangsar, Perak dan Anguilla bicolor bicolor dari tali air Air Hitam, Kedah telah dijalankan. Kepekatan Pb, Cd dan Hg dalam spesimen diperiksa dan dianalisa menggunakan ICP-MS dan direct mercury analyzer (MA-3000). Julat kepekatan logam berat (µg/g berat basah) dalam tisu Anguilla bicolor bicolor adalah di antara 0.01-0.4 (Cd), 0.03-0.77 (Pb) dan 0.36-0.94 (Hg), manakala kepekatan logam berat dalam tisu Anguilla bengalensis bengalensis adalah di antara 0.76-1.23 (Cd), 0.01-0.10 (Pb) and 0.27-1.5 (Hg). Selain itu, hubungan kuat di antara Hg/Pb (r = .771, P < 0.05) dalam tisu Anguilla bengalensis bengalensis (Sungai Perak) dan Cd/Pb (r = -.895, P < 0.05) dalam tisu Anguilla bicolor bicolor (terusan dan saliran Air Hitam) adalah dari sumber pencemaran yang pasti. Oleh itu, Hg dan Pb adalah membimbangkan. Perbandingan interspatial dalam kajian ini dengan kajian terdahulu serta peringkat internasional menunjukkan bioakumulasi Hg yang tinggi dalam ikan sidat tropis dan melebihi paras selamat seperti yang ditetapkan dalam Akta Makanan Malaysia 1984 dan Peraturan Makanan (1985) dan USEPA (1997). Walau begitu, Target Hazard Quotient (THQ) dan Hazard Index (HI) serta penilaian risiko kesihatan manusia menunjukkan ikan tropis sidat dari lokasi kajian adalah selamat untuk dimakan dan tidak berisiko serta kepekatan Pb, Cd dan Hg adalah di bawah paras pengambilan nutrien yang dibenarkan.

Kata kunci: Ikan tropis sidat, logam, paras had selamat, THQ, HI

INTRODUCTION

The level of trace elements in the ecosystem has increased as a result of anthropogenic activities. Aquatic ecosystems are primarily at risk as elevated volumes of industrial and urban effluents are being discharged into such environments. This not only results in diminished water quality but also a greater risk of exposure through consumption of contaminants in fish (Ong et al. 2015). In Asian countries, fish is an important source of protein, polyunsaturated fatty acids (PUFA), omega-3 (complex chain fatty acids) and docosahexaenoic acid (DHA) (Kusharto et al. 2014). The monitoring of element levels in the muscle tissue of fish is therefore of importance to evaluate the risk to human health associated with their consumption. Trace elements can be divided into essential and non-essential elements (Shazili et al. 2006). Essential trace elements are naturally present in all biological systems at low levels and produce no negative effects on exposure but at high concentrations, these elements could become toxic. In comparison, though non-essential elements are also naturally present in the ecosystem, even low levels can become toxic to many organisms (Aminah et al. 2013; Kalay & Canli 2000). Thus, fish and other aquatic organisms are continually being exposed to persistent trace metals discharged from water pollution (Ong et al. 2015) and this subsequently leads to accumulation in tissues. Under natural conditions, trace elements are present in low concentrations in the water and mostly in the sediment (Shazili et al. 2006). The absorption of trace elements in the sediment reduces their bioavailability in the water and thus high sediment trace element levels are not necessarily associated with increased bioaccumulation in fish. However, an increase in the concentrations of the elements can result in reactions manifesting within all levels of the food chain (Renata et al. 2016). *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* are tropical freshwater eels found in the rivers of northern Peninsular Malaysia. The species is catadromous because they naturally migrate from freshwater into seawater to spawn and eventually return to freshwater during the larval stage to grow (Arai et al. 2012; Arai 2016). Seven species or subspecies are found in the Western Pacific around Indonesia and Malaysia, e.g., *Anguilla celebesensis* Kaup 1856, *Anguilla interioris* Whitely 1938, *Anguilla bengalensis bengalensis* Gray 1831, *Anguilla marmorata* Quoy & Gaimard 1824, *Anguilla borneensis* Popta, 1924, *Anguilla bicolor bicolor* McClelland 1844 and *Anguilla bicolor pacifica* Schmidt 1928. Over the years, studies have shown that freshwater eel species (*ikan linang* or *ikan sidat tropis*) particularly, *Anguilla bicolor bicolor* has a high market value in South-East Asian countries (Arai et al. 2012; Le et al. 2009, 2010; Watanabe et al. 2009). However, in Malaysia, past studies are limited to tropical freshwater eel biology and ecology and there are only a small number of studies on metals accumulation in freshwater eels from Peninsular Malaysia being published (Arai et al. 2012; Khalil et al. 2017). In this investigation, the tropical freshwater eels from the states of Kedah and Perak were studied as their occurrence was mostly reported in the northern region of the Malay Peninsula (Arai et al. 2012; Khalil et al. 2017). The purpose of the study was to determine the concentrations of trace metals (Pb, Cd and Hg) in the muscles of tropical freshwater eel, *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* as well as to estimate the potential human health risks from consuming these species and the impact of metal pollution on population dynamics, stock assessment and for the conservation of these tropical freshwater eel species.

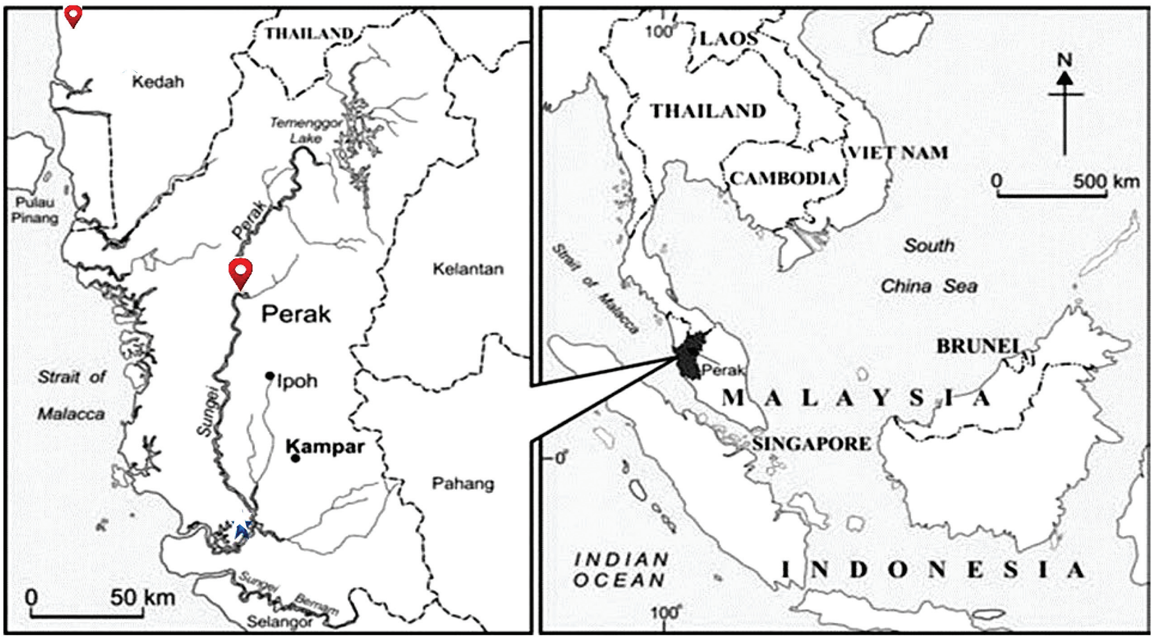

FIGURE 1. Map showing sampling sites in the Northern Peninsular Malaysia (The sampling site was labeled with a red mark indicator; Sungai Perak at Kuala Kangsar (midstream), Perak and Air Hitam irrigation canal at Kuala Sanglang (downstream), Kedah)

EXPERIMENTAL METHODS

STUDY DESIGN, SPECIMEN COLLECTION AND PREPARATION OF BIOLOGICAL SAMPLES FOR METAL ANALYSIS

Twelve wild *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* in total were collected using commercial nets, PVC traps or by hook and line with chicken intestines or small fish as baits. The eels were caught from the downstream of the Air Hitam irrigation canal, Kampung Kuala Sanglang, Kedah (06°14'42"N, 100°14'21"E) and from the midstream of Sungai Perak at Kuala Kangsar, Perak (04°48'57"N, 100°57'47"E). Samplings were done monthly from January until December 2014 except in June 2014 but later was replaced in June 2015.

External measurements of total length (TL) and total body weight (BW) and morphometric data of each freshwater eel (Ahmad et al. 2006; Han et al. 2003; Utoh et al. 2004) were carried out after landing the catch. Eels were transported to the laboratory in ice boxes. Approximately 3.0-5.0 g of muscle tissue from the dorsal side that is directly under the dorsal fin above the lateral line was removed and gonads were dissected using a ceramic knife. The gonads were later weighed and examined under the microscope to determine the gonadal somatic index (GSI) in order to categorize the eels as either immature (yellow eel) or mature (silver eel), and also the sex of the eels. The muscle tissue was freeze-dried (EYELA-freeze dryer FFD-550) and then ground to a fine powder using ceramic mortar and pestle which was then kept in a desiccator until further use.

Prior to metal analysis, all of the apparatus used were soaked in 10% HNO_3 (Suprapur, Merck Co. Germany) overnight and rinsed with deionized water, then oven-dried to eliminate potential contamination as part of the QA/QC program for laboratory analysis by Environmental Protection Agency (EPA 2011). For every sampling site, three samples were analyzed. Exactly 0.05 g of the muscle tissue powder samples were weighed and placed in Teflon vessels. Three mL of HNO_3 (Suprapur, Merck Co. Germany) and 1 mL H_2O_2 (Suprapur, Merck Co. Germany) were added. After overnight pre-digestion at room temperature, the Teflon vessels containing the samples were capped and heated at 120°C for 7 hours as described by (Le et al. 2009, 2010) with slight modifications. After cooling overnight, the contents of the vessel were transferred into polypropylene test tubes and diluted to 10 mL with Milli-Q water (82 Ωm).

DETERMINATION OF METALS

Concentrations of Pb and Cd in 0.05 g of muscle tissue were measured using an inductive coupled plasma mass spectrometer (ICP-MS; model 9000 Perkin Elmer Ltd). For total Hg analysis, 0.05 g of the untreated biological samples were directly measured using automated direct thermal decomposition mercury analyzer (MA 3000). The ICP-MS was calibrated with a multi-element standard solution (Perkin Elmer) in a range of low concentrations (0.1 to 100 µg/L). Perkin Elmer ELAN instrument was used with a Meinhart nebulizer and silica cyclonic spray chamber and continuous nebulization. The operating conditions are listed below: Nebulizer Gas flow rates: 0.95 L/min; Auxiliary Gas Flow: 1.2 L/min; Plasma Gas Flow: 15 L/min; Lens Voltage: 7.25 V; ICP RF Power: 1100 W; CeO/Ce = 0.031; Ba^{++}/Ba^{+} = 0.02.

VALIDATION OF METHODOLOGY

The accuracy of metal analysis data was checked using certified reference materials: CRM DORM-3 Fish Protein (n =10) and SRM 1947 Lake Michigan Fish Tissue (n =10). Blanks were similarly analyzed to ensure no contamination has occurred. The linear calibration curve was plotted with a correlation coefficient r = 0.9998 being achieved. All of the analyzed (certified) reference materials produced satisfactory recoveries from 96% to 99% (CRM DORM-3), see Table 1.

STATISTICAL ANALYSIS OF THE DATA

The statistical analysis was carried out using SPSS ver-22 (2016) by executing a Pearson correlation between metal concentrations in tissue. P values < 0.01, 0.05 indicate statistical significance in this study. Data were expressed as means ± standard dev. (±SD) of triplicate measurements.

CALCULATION OF HEALTH RISK ASSESSMENT ON TROPICAL FRESHWATER EEL CONSUMPTION

In human-health focused approach, contaminant concentrations are measured in fresh fish muscle tissues

TABLE 1. Metals concentration in certified reference materials (CRM DORM-3 Fish Protein and SRM 1947 Lake Michigan Fish Tissue)

Metal	(SRM 1947)			(CRM DORM-3)		
	Certified value (µg/g)	Measured value (µg/g)	Recovery (%)	Certified value (µg/g)	Measured value (µg/g)	Recovery (%)
Mercury	0.25±0.01	0.38±0.09	98			
Cadmium				0.29±0.02	0.29±0.1	99
Lead				0.4±0.05	0.37±0.1	96

and are compared with threshold values as defined by health agencies' regulatory guidelines relative to wet mass. Consumption of fish is for example considered safe by several agencies worldwide (Table 3) if the total metals concentration in food is < 1 mg/g wet mass (CCF 2013; EC 2006; Kinney et al. 2016). Thus, the use of dry mass concentrations collected as ecological tracers, are converted into wet mass concentrations using equation (1):

$$C_w = C_d \times \frac{100 - \%H}{100} \quad (1)$$

where C_d and C_w are the concentrations expressed relative to dry and wet mass of muscle tissues respectively, and with the percentage of humidity in wet muscle tissues (%*H*) classically ranging around 80% for a vast range of species (Alonso & Saborido 2012; EPA 2011; Fuad et al. 2014; Payne et al. 1999).

CALCULATION OF ESTIMATED DAILY INTAKE OF METALS (EDI)

EDI is measured in (µg/kg body wt. /day) (Song et al. 2009) as shown in equation (2):

$$\text{EDI} = \frac{C_{metal} \times W_{fish}}{BW} \quad (2)$$

where, C_{metal} is the metal concentration in freshwater eel muscle (µg/g, wet wt.), W_{fish} represents the daily consumption of eel/fish (in g/day), which is taken as 159 g/day for adults based on the Malaysian Adults Nutrition Survey (MAN 2014) and 16 g/day for children based on the Food and Drug Administration (FDA 1979) guidelines, BW is an average body weight (kg) of Malaysian adults which is taken as 70 kg (Aminah et al. 2013) and 16 kg for children (FDA 1979). The estimated daily intake of metals through the consumption of freshwater eel/person/ day was calculated and shown in Table 2.

TABLE 2. Parameters used in the calculation of health risk assessment according to USEPA (2011), FDA (1979) and Malaysian Adults Nutrition Survey (MAN 2014)

Symbol	Description	Unit	Value
C metal	metal concentration	µg/g wet wt.	Presented in Table 3
IR	Fish Intake rate of	kg/day	0.159 (adult), 0.016 (child)
ED	Exposure duration	years	30
R*f*D		µg/g/day	0.02 (Pb) , 0.001 (Cd), 0.0003 (Hg)
BW	Average Weight	kg	70 (adult), 16 (Child)

RISK FROM THE INTAKE OF METALS THROUGH INGESTION: TARGET HAZARD QUOTIENT (THQ)

According to the US Environmental Protection Agency (USEPA 2012), the average daily dose (ADD) in µg/kg/day body wt. and a reference dose of metal (R*f*D) in µg/g/day (USEPA 2012) is defined as the maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects. The THQ is used to determine the non-carcinogenic risk level due to pollutant exposure. To assess the health risk from metal-contaminated freshwater eel, the THQ was calculated as per USEPA Region III Risk-Based concentration Table (USEPA 2012) by using the following equation (4):

$$ADD = \frac{C \times IR \times ED}{BW} \quad (3)$$

and

$$THQ = \frac{ADD}{RfD} \quad (4)$$

where C is the trace metal mean concentration in freshwater eel (µg/g/wet wt.), IR is the freshwater eel intake rate (0.159 g/day for adult and 0.016 g/day for child), ED is the exposure duration (30 years or 10950 days) for non-cancer risk as used by USEPA, BW is an average body weight (70 kg for adults and 16 kg for children) and R*f*D is the reference dose of individual metal (0.02 µg/g/day/Pb, 0.001 µg/g/day/Cd and 0.0003 µg/g /day/Hg as per fresh wt. basis) as stated in the USEPA regional screening level summary table (USEPA 2012). According to the USEPA (2012), if the THQ ≤1, there is no known adverse effect from the exposure but if the THQ value ≥1, it means that the exposed population via the consumption of contaminated foods is likely to experience obvious deleterious effects and it is unsafe for human consumption. The higher the THQ value, the higher the probability of risk to the human body. In evaluating THQ on human health risk, there are two assumptions: - (a) the ingested dose of pollutant is equal to the absorbed dose (USEPA 1989) and (b) cooking has no effect on pollutants (Forti et al. 2011). The THQ-based assessment method, however, does not provide the probability of quantitative estimation on an exposed population experiencing from a toxic effect recovery (Chary et al. 2008). Thus, to assess the risk of multiple metals on freshwater eels, the hazard index (HI) was employed following USEPA (2012) as described in equation (5):

$$HI = THQ\,(Cd) + THQ(Pb) + THQ(Hg) \quad (5)$$

or

$$HI = \sum\nolimits_{i-1}^{n} THQi \quad (6)$$

From which, *THQi* is the target hazard quotient of each individual trace metal and *n* = 3.

RESULTS AND DISCUSSION

BIOACCUMULATION OF TRACE METALS IN MUSCLE OF TROPICAL FRESHWATER EEL

A total of 12 *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* were collected in this study.

Anguilla bicolor bicolor from the Air Hitam irrigation canal was found to have a total length and weight ranging from 58.3-68.4 cm and 362-824 g and *Anguilla bengalensis bengalensis* from Sungai Perak ranged from 88.5-129.5 cm and 1800-2143 g. The individual gonadal somatic index (GSI) value showed that 100% of *Anguilla bicolor bicolor* collected from the Air Hitam irrigation canal and 87.5% *Anguilla bengalensis bengalensis* from Sungai Perak were in the mature gonadal stage (silver) and were all females. The higher percentage rate of female eels might be related to spawning behavior (Arai 2016; Illiani et al. 2015; Khalil et al. 2017). We believe that both tropical freshwater eels sub species in Malaysia might have originated from the south-western coast of Sumatera which is their nearest spawning area as suggested from previous studies (Arai 2016; Khalil et al. 2017).

The mean (± standard dev.) and the range of concentrations of Pb, Cd and Hg in the muscle of *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* are shown in Table 3. Metals such as Pb, Cd and Hg have no biological role but are raising concerns among the scientific community as they have been found to accumulate to high levels in fish tissues (Arai et al. 2012; Khalil et al. 2017; Yap & Pang 2011). In general, between species, *Anguilla bengalensis bengalensis* showed higher accumulation rates (except for Cd) compared to *Anguilla bicolor bicolor*. The mean metal concentration accumulated in *Anguilla bicolor bicolor* from the Air Hitam irrigation canal showed a decreasing order of Hg > Pb > Cd in the muscle of freshwater eels. Pb presented a significant negative relationship with Cd ($r = -.895$, $P < 0.05$) indicating the latter was contributed either from a multisource of anthropogenic activities (nonpoint source) or from naturally occurring sources (Yap & Pang, 2011) (Table 4). From our observation, the Air Hitam irrigation canal is surrounded by rice farming/productions. Metals in the soil of the paddy fields may originate from the use of superphosphate fertilizers or wastewater as well from the natural geogenic composition. According to Page and Steinnes (1990); Hajar et al. (2014); Vikram et al. (2014),

TABLE 3. Mean metal concentrations in muscle of different *freshwater eel* spp. with comparison to recent studies and different international guideline standards

Country	Type	Muscle	Cd (µg/g wt.)	Pb (µg/g wt.)	Hg (µg/g wt.)	Reference
Malaysia						
Sg. Perak & Sg. Pinang	Dry		0.08 -0.44	2.39-13.2	N/A	Arai et al. (2012)
Sg. Pinang, Penang	Dry		N/A	6.98±3.79	N/A	Khalil et al. (2017)
Air Hitam irrigation canal, Kedah	Dry	*A. bicolor bicolor*	0.42±0.70 (0.04-1.55)	1.67±1.09 (0.1-2.94)	2.47±1.09 (1.38-3.61)	Present study
	Wet		0.11 (0.01-0.40)	0.43 *[f] (0.03-0.77)	0.65 *[a] (0.36-0.94)	
Sg. Kuala Kangsar, Perak	Dry	*A. bengalensis bengalensis*	1.25±0.22 (0.94-1.52)	0.04±0.03 (0.01-0.13)	0.89±0.41 (0.34-1.85)	Present study
	Wet		1.01 (0.76-1.23)	0.03 (0.01-0.10)	0.72 *[a] (0.27-1.5)	
France	Dry	*A. anguilla*	N/A	0.05-0.56	N/A	Neto et al. (2011)
Vietnam	Dry	*A. mamorata*	0.02-0.13	0.11-0.18	N/A	Le et al. (2009)
	Dry	*A. mamorata*	N/A	0.06±0.09	N/A	Le et al. (2012)
Morocco	Dry	*A. anguilla*	0.35-0.52	0.03-0.30	N/A	Morhit et al. (2009)
Turkey	Dry	*A. anguilla*	0.07±0.03	0.56±0.12	0.26±0.10	Ercument et al. (2008)
Spain	Dry	*A. anguilla*	0.003 -0.01	0.02-0.26	N/A	Bordajandi et al. (2003)
International guideline Standard	Wet	Fish muscle	*1*	*2*	*0.5*	MFR (1985)[a]
	Wet		*1.8*	*3*	-	ICES (2006)[b]
	Dry		*5*	*10*	-	ABIA (1991)[c]
	Dry		-	*6.67*	-	MPHT (1986)[d]
	Wet		*25*	*11.5*	-	USFDA (1993)[e]
	Wet		*2*	*0.3*	*1*	CODEX STAN 193-1995[f]
	Wet		-	-	*0.5*	USEPA (2012)[g]

[a]Malaysian Food Regulation Fourteen Schedule (MFR 1985); [b]European eel assessment working group report. (2006); [c]Brazilian Ministry of Health (1991); [d]Ministry of Public Health, Thailand (1986); [e]Food and Drug Administration of the United State (1993); [f]General Standard For Contaminants And Toxins In Food And Feed (1995); [g]United States Environmental Protection Agency (2012).

TABLE 4. Correlation matrices between Pb, Cd and Hg concentrations in muscles of (a) *Anguilla bengalensis bengalensis* from Sg. Perak at Kuala Kangsar, Perak and (b) *Anguilla bicolor bicolor* from Air Hitam irrigation canal, Kedah.

(a)

	Cd	Pb	Hg
Cd	1		
Pb	0.363	1	
Hg	-0.228	*.711**	1

(b)

	Cd	Pb	Hg
Cd	1		
Pb	*-.895**	1	
Hg	.499	-.060	1

Number of valid samples = 8 (*Anguilla bengalensis bengalensis*) and number of valid samples = 4 (*Anguilla bicolor bicolor*). *Correlation coefficient is significant with P value < 0.05 (2-tailed), **. Correlation coefficient is significant with P value < 0.01 (2-tailed); Bold: very strong correlation (> 0.8); ***Bold & Italic***: strong correlation (0.6 < x < 0.8; *Italic*: good correlation (0.4 < x < 0.6); and Regular: weak correlation (< 0.4)

it could be due to effects after the rain as fertilizer is the source of Se, Cd and Pb and leaches out into the water stream and bound to sediment or the metals might be contributed from the overland flow or from the storm-water runoff which often contains metals from the roadways.

Meanwhile, *Anguilla bengalensis bengalensis* of medium size (TL = 47.5-53.5 cm) from Sungai Perak showed the highest mean concentration of Cd (Table 3). This could be due to the silvering phase (growing stage) since the concentration of Cd is influenced by factors other than their environmental availability (Arai & Abdul Kadir 2017). For example, fish exposed to metals will synthesize metal-binding proteins (metallothionein) which not only bind Cd but also Zn and Cu (Ong et al. 2015). In this study, we also observed that the muscle of small size freshwater eels (TL = 40.0-46.0 cm) was seen to accumulate more and showed a significant positive relationship between Hg and Pb ($r = .771$, $P < 0.05$) in *Anguilla bengalensis bengalensis*. However, there was no significant correlation of Cd with Pb and Hg (Table 4). The higher Pb concentration in freshwater eels muscle could have originated from urban pollution (Mamun & Zainudin 2013). Although the means of Pb concentrations observed in both tropical freshwater eels in this study were within the reported range of *Anguilla* species from Malaysia, the concentrations were higher compared to previous studies (Arai et al. 2012; Khalil et al. 2017). Nevertheless, the data obtained in the present study is comparable or within the range of most international and local studies on freshwater eels from Malaysia (Table 3). Previous reports showed that the mean and range of Pb concentrations accumulated in tropical freshwater eels from various rivers in the Penang Island were between 2.39-13.2 and 6.98 ± 3.79 µg/g (dry wt.). Mercury concentrations in the muscle for both freshwater eel sub species were found to be higher than what was reported in Turkey by Ercument et al. (2008). Factors affecting the bioavailability of Hg are very complex, but an understanding of them is crucial if appropriate strategies for the management of contaminated rivers are to be developed. The factors which can influence the bioavailability of Hg to aquatic species include methylation rates, salinity and eutrophic status (Le et al. 2012). Concentrations of Pb reported in this study were 2-200 times higher and for Cd were 3.5 times higher than those reported for other freshwater eel species such as *Anguilla anguilla* from France, *Anguilla japonica* from Japan and *Anguilla mamorata* from Vietnam, (Table 3) and 3.4 times higher than what was reported by Arai et al. (2012). In this study, the lowest metal concentration was 0.42 Cd µg/g dry wt., 0.04 Pb µg/g dry wt. and 0.89 Hg µg/g dry wt. and the highest reached 1.25 Cd µg/g dry wt., 1.67 Pb µg/g dry wt. and 2.47 Hg µg/g dry wt. The mean concentration of Cd found in *Anguilla bengalensis bengalensis* from Sungai Perak was higher than in *Anguilla bicolor bicolor* from the Air Hitam irrigation canal while *Anguilla bicolor bicolor* showed higher accumulation of Pb and Hg. The present study is the first to report on the content of Hg in freshwater eel species found in northern Peninsular Malaysia. The concentrations of Hg in both freshwater eels species were high (0.65 µg/g wet wt. and 0.72 µg/g wet wt.) for *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* when compared to the studies reported by Ercument et al. (2008) on *Anguilla anguilla* from Turkey. Thus, Hg concentration in freshwater eel species from both Perak and Kedah especially is of concern. Previous studies have stated that the variability observed in the metals level of different species depended on ecological factors, metabolism (Canli & Kalay 1998), age, size (Orata & Birgen 2016) and length of the fish (Yousuf et al. 2000), feeding habits (Watanabe et al. 2004) and their habitat (Canli & Atli 2003). In the present study, the concentration of Pb in wet muscle were higher than allowed in the Codex STAN (1995) and Hg with respect to Malaysia Food and Regulations (MFR 1985) guidelines. Therefore, a human health risk assessment was carried out to estimate the risk posed by Pb, Cd, and Hg.

HEALTH RISK ASSESSMENT OF CONSUMING *ANGUILLA BENGALENSIS BENGALENSIS* AND *ANGUILLA BICOLOR BICOLOR*

According to USEPA (1989), the human health risk is based on several factors depending not only on ingested contaminant intake but with exposure occurrence and duration, with average body weight and oral reference dose (R*f*D). THQ is a ratio of metal concentrations content in the food item to its R*f*D, weighted by duration and frequency of exposure, intake amount and body weight (Harmanescu et al. 2011; New York State Department of

Health (NYSDOH 2007). Overall, the findings of the present study showed that the THQ obtained were several thousand times lower than their R*f*D, and the HI values observed in adults and children were ≤ 1 (Table 6) indicating there are no potential health risk exposed to the public (Islam et al. 2014; Zodape 2014) from *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* consumption. However, the EDI for Hg values from both target groups (adults and children) in consuming Anguilla *bicolor bicolor* from the Air Hitam irrigation canal were higher than their respective reference nutrient intake guidelines by the United States Environmental Protection Agency (USEPA 2009) of 0.6, though Pb and Cd were within the stipulated limits. Meanwhile, the EDI of the respective metals from *Anguilla bengalensis bengalensis* from Sungai Perak exceeded the limits of 0.8 for Cd and 0.6 for Hg. (Table 5) and (Table 6). Since toxicity due to metals may only be apparent after long term exposure to low environmental levels, it is important to monitor environmental contaminants in freshwater eels as Hg is listed as a potent carcinogen by USEPA (2012). This is to prevent excessive increase of metals concentration in the human food chain. To date, there are only two studies available with regards to freshwater eels exposure to metals in Malaysia. Hence, the present study provides additional information regarding tropical freshwater eels status from Malaysian waters.

TABLE 5. Mean metal concentration in muscles of *Anguilla bicolor bicolor* and *Anguilla bengalensis bengalensis* and EDI values for the individual (adult and child)

Study site	Age / Concentration	Metal Cd	Pb	Hg
Anguilla bicolor bicolor				
	(µg/g wet wt.)	0.11	0.43	0.65
Air Hitam irrigation canal, Kedah	EDI adult	0.25	0.98	1.48**
	EDI child	0.11	0.43	0.65**
Anguilla bengalensis bengalensis				
	(µg/g wet wt.)	1.01	0.3	0.72
Sg. Perak at Kuala Kangsar, Perak	EDI adult	2.29**	0.68	1.64**
	EDI child	1.01**	0.30	0.72**
Upper tolerable daily intake limit for both adult and child (USEPA 2009)				
	*EDI / RNI**	*0.8*	*3.57*	*0.6*

Reference nutrient intake (RNI*) is expressed as µg/day. Concentration; The mean values of heavy metal concentrations in tropical freshwater eel were in µg/g fresh. Estimated daily intake (EDI) for all metals is expressed as µg/kg body wt./day. **Value that exceeded the permissible limit set by USEPA (2009)

TABLE 6. The value of average daily doses (ADD), hazard quotient (THQ) and total hazard indices (HI) of Cd, Pb, and Hg

Site	Species	Age	µg/g/day (x 10^{-3}) Cd	Pb	Hg	HI	Overall Conclusion
Air Hitam irrigation canal, Kedah	*Anguilla bicolor bicolor*	ADD Adult	0.0250	0.0977	0.1476	0.522	No risk effect
		THQ Adult	0.025	0.005	0.492		
		ADD Child	0.0003	0.0430	0.0110	0.230	No risk effect
		THQ Child	0.217	0.002	0.011		
Sg. Perak at Kuala Kangsar, Perak	*Anguilla bengalensis bengalensis*	ADD Adult	0.2294	0.0681	0.1635	0.778	No risk effect
		THQ Adult	0.229	0.003	0.545		
		ADD Child	0.1010	0.0300	0.0720	0.343	
		THQ Child	0.101	0.002	0.240		No risk effect

CONCLUSION

The measurements of Cd, Pb and Hg concentrations in wild tropical freshwater eels, *Anguilla bicolor bicolor* from Air Hitam irrigation canal, Kedah and *Anguilla bengalensis bengalensis* from Sungai Perak indicate that these metals are of concern as some of the safety standards are exceeded. However, the Hazard Index (HI) calculation indicated that these metals as a whole do not pose a significant risk for consumption by adults and children. *Anguilla* has become a major target fish for human consumption and East Asian countries such as China, Philippines, Indonesia, and Malaysia are emerging as major exporters of freshwater eels. Thus, monitoring of the level of environmental contaminants in aquatic organisms is important in order to prevent future health concerns with respect to consumption of tropical freshwater eels.

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