Tropical Life Sciences Research, 25(2), 21–39, 2014

Determination of Heavy Metal Levels in Fishes from the Lower Reach of the Kelantan River, Kelantan, Malaysia

[1]Rohasliney Hashim*, [2]Tan Han Song, [2]Noor Zuhartini Md Muslim and [2]Tan Peck Yen

[1]Department of Environmental Management, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]School of Health Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

Abstrak: Satu kajian untuk menentukan tahap kandungan logam berat [kadmium (Cd), nikel (Ni) dan plumbum (Pb)] dalam tisu ikan telah dijalankan di Sungai Kelantan. Pensampelan ikan dilakukan pada musim kering dan basah menggunakan pukat. Enam famili, 11 genus dan 13 spesies daripada 78 ekor ikan dapat ditangkap. Tisu ikan tersebut dianalisa menggunakan relau grafit Spektrofotometer Serapan Atom (AAS). Kepekatan Cd dalam *Chitala chitala* (0.076 mg/kg) didapati melebihi nilai had kritikal European Commission (EC), World Health Organization (WHO) dan Food and Agriculture Organization (FAO). Kepekatan Cd dalam *Barbonymus gonionatus* dan *Tachysurus maculatus* pula didapati telah menghampiri nilai had yang ditetapkan. Kesemua spesies ikan yang diperolehi didapati tidak mengandungi kepekatan Ni yang melebihi had yang ditetapkan oleh WHO (1985) iaitu 0.5–0.6 mg/kg. *Osteochilus hasseltii* (0.169 mg/kg) dan *T. maculatus* (0.156 mg/kg) mempunyai nilai kepekatan Pb yang tinggi berbanding spesies lain. Tahap kandungan logam berat didapati lebih tinggi pada musim basah berbanding musim kering (p<0.05). Ikan omnivor telah dikesan dengan kepekatan tinggi Cd dan Ni, manakala ikan karnivor mempunyai kepekatan Pb tertinggi. Kepekatan Cd dan Pb dalam tisu ikan berkorelasi secara positif dengan berat ikan (p<0.05). Oleh itu, kajian ini menunjukkan bahawa spesies ikan yang ditangkap di Sungai Kelantan tercemar dengan logam berat. Walau bagaimanapun, kepekatan logam berat dalam tisu ikan tidak melebihi garis panduan EC, FAO, Malaysian Food Act (MFA) dan WHO kecuali *C. chitala, O. hasseltii and T. maculatus* yang telah menghampiri atau mencecah nilai had kritikal.

Kata kunci: Sungai, Logam Berat, Tisu Ikan, Kelantan, AAS

Abstract: This study aimed to assess the concentrations of cadmium (Cd), nickel (Ni) and lead (Pb) in the tissues of fish collected from the lower reach of the Kelantan River, Malaysia. Fishes were collected using gill nets during the dry and wet seasons. A total of 78 individual fish were caught and comprised 6 families, 11 genera and 13 species. The dorsal muscle was analysed using a graphite furnace Atomic Absorption Spectrometer (AAS). The mean concentration of Cd in *Chitala chitala* (0.076 mg/kg) was above the critical limit values of the European Commission (EC), World Health Organization (WHO) and Food and Agriculture Organization (FAO). The mean concentrations of Cd in *Barbonymus gonionatus* and *Tachysurus maculatus* were already at the level of concern, whereas the other species were approaching the limits of permissible levels. No fish samples were found to have a Ni level higher than the permissible limit of 0.5–0.6 mg/kg set by the WHO (1985). *Osteochilus hasseltii* (0.169 mg/kg) and *T. maculatus* (0.156 mg/kg) showed high Pb concentrations. The concentrations of heavy metals were found to be elevated in the wet season (p<0.05). Omnivorous fish were detected with elevated concentrations of Cd and Ni, whereas carnivorous fish had the highest concentration of Pb.

*Corresponding author: rohasliney_hashim@yahoo.com

The concentrations of Cd and Pb in fish tissues were positively correlated with fish weight (p<0.05). This study determined that the fish species caught in the Kelantan River were contaminated with non-essential metals (Cd, Ni and Pb). Nevertheless, the heavy metal concentration in the fish tissues, with the exception of *C. chitala, O. hasseltii and T. maculatus*, did not exceed the EC, FAO, Malaysian Food Act (MFA) or WHO guidelines.

Keywords: River, Heavy Metals, Fish Tissues, Kelantan, AAS

INTRODUCTION

After decades of rapid urbanisation, population growth and industrialisation, developing countries are now home to many of the world's most critical air, water and solid waste problems. Early studies have identified the rise in the pollution of particular heavy metals in freshwater systems around the world, particularly in rivers. The pollution has mainly been caused by industrial processes and industrial waste, typically from rubber and oil palm mills (Tariq *et al*. 1996). Despite the environmental controls that are being implemented by Malaysian governmental agencies, the contaminants from smaller, sometimes illegal industries are still extensive and represent the largest environmental problem (Tariq *et al*. 1996). In 2002, the Department of Environment (DOE) of Malaysia reported that industries such as metal finishing and electroplating, food and beverage, animal feed and textile did not achieve more than 65% compliance with the regulation for heavy metal contamination. Some of the industries were operating without an effluent treatment system (DOE 2009; Noor Syuhadah & Rohasliney 2011; Noor Syuhadah *et al.* 2014; personal observation).

Apart from that, the main source of heavy metal pollution also comes in the form of deforestation, domestic or animal farming sewage, sand mining and agriculture (DOE 2002). The DOE has reported that all analysed heavy metals in the water column in most rivers in Malaysia are within the Class IIB limits of the Malaysian Interim National Water Quality Standard (INWQS) in the order of lead (Pb) > zinc (Zn) > cadmium (Cd) > chromium (Cr) > arsenic (As) > mercury (Hg). Class IIB indicates that water can be used for recreational purposes with body contact. Although the Kelantan River system was classified as a Class I–III river based on the Malaysian INWQS (DOE 2009) [i.e., the water quality index (WQI) of the Kelantan River was 85 for 3 consecutive years from 2009 to 2011 with a river quality status of Class II], the DOE reported that the Kelantan River was categorised as a polluted river based on the Suspended Solid (SS) Index. The decrease in the SS Index was most likely due to the uncontrolled earthworks along the riverbank. In addition, Kadaruddin (2000) discovered Cd contamination in 53 rivers in Malaysia's river system, iron (Fe) in 44 rivers, Pb in 36 rivers, Hg and copper (Cu) in 24 rivers, and Cr and Zn in 4 rivers. Meanwhile, a previous study by Ahmad *et al.* (2009) found that heavy metals, such as Pb, Zn, Cu and Cd, were present at low concentrations in sediments of the Kelantan River.

The heavy metal contamination of aquatic ecosystems above the natural background load has drawn the attention of many researchers. Heavy metals may accumulate in aquatic species, enter the food chain and cause serious harm to human health when the contamination content and exposure are significant

(Goyer 1997; Papagiannis *et al*. 2004; Türkmen *et al*. 2005; Fernandes *et al*. 2007). Consequently, they have been listed by the US Environmental Agency (USEPA) based on their potential for human exposure and health risk (Birungi *et al.* 2007). The accumulation of heavy metals in fish is an important issue because many fish species are consumed as a source of protein by a large section of the population, especially those who live near rivers. The low saturated fat and sufficient omega fatty acids in fish are also important in supporting good human health. The levels of heavy metal accumulation in fish depend on the growth rate, metabolism, feeding pattern and ecological requirements of a given fish species (Yilmaz *et al*. 2005, 2010). Another factor is the differences in life history patterns among species (including trophic levels and geographical distribution of life stages), which influence their exposure to heavy metals (Allen-Gil & Martynov 1995).

Essential metals are important for the normal metabolism of fish, and non-essential metals may accumulate in their organs (Canli & Atli 2003). Essential metals include Fe, Cu, Zn and manganese (Mn), whereas non-essential metals are Hg, Pb, nickel (Ni) and Cd (Türkmen *et al*. 2005). Heath (1995) found that high concentrations of heavy metals affected the growth and development of fish during early life stages such as hatching, larval development and juvenile growth because they were more sensitive during these stages than during mature stages. Evidently, fish form the link for the transfer of toxic heavy metals from water to humans (Ashraf *et al.* 2010). The harmful effect of trace elements when consumed above the recommended limit can be toxic (acute, chronic or sub-chronic), and heavy metals can be neurotoxic, carcinogenic, mutagenic or teratogenic. The general symptoms of humans related to metal [e.g., Cd, Pb, As, Hg, Zn, Cu and aluminium (Al)] poisoning include vomiting, convulsions, paralysis, ataxia, hemoglobinuria, gastrointestinal disorder, diarrhoea, stomatitis, tremor, depression and pneumonia (McCluggage 1991).

This study aimed to assess the concentration of Cd, Ni and Pb in the tissues of the fish collected from the lower reach of the Kelantan River, Malaysia. The results were then compared to permissible limits from the Malaysia Food Act (MFA), European Commission (EC), US Food and Drug Administration (USFDA), Food and Agriculture Organization (FAO) and the World Health Organization (WHO) to detect whether the heavy metal contamination levels in fish of the Kelantan River exceed the safe consumption permissible limit.

MATERIALS AND METHODS

Site Description

The Kelantan River basin is located in the northeastern part of Peninsular Malaysia between 4° 40' and 6° 12' N and 101° 20' and 102° 20' E [Malaysian Meteorological Department (MMD) 2009]. The maximum length and breadth of the catchment are 150 km and 140 km, respectively. The river is approximately 248 km long and drains an area of 11,900 km^2, occupying more than 85% of the State of Kelantan (Ahmad *et al.* 2009). It divides into the Galas and Lebir rivers near Kuala Krai, approximately 100 km from the river mouth. Kelantan River is a unique river in southeast Asia because it is the only river known to flow northwards. The main river from source to river mouth has four names: it originates from the Betis River (first 30 km from the source), then to the Nenggiri River, followed by the Galas River before meeting the Lebir River to form the Kelantan River. The Kelantan River regularly has bank overflows during the months of November to February during the northeast monsoon season. The Kelantan River receives an annual rainfall range from 0 to 1750 mm in the dry season (March to May) and wet season (mid-October until mid-January) (MMD 2009; Tan & Rohasliney 2013). The river flows past several important towns: Kuala Krai, Tanah Merah, Tendong, Pasir Mas, Tumpat and Kota Bharu, the state capital, which lies near the mouth of the river.

The Kelantan River has been used heavily by the local people for domestic use, transportation, agriculture, plantation irrigation, small scale fishing and sand mining. The Kelantan River's water has been turbid since the early 1990s due to high levels of suspended solids and siltation. This has been caused by logging in the upstream areas (Lojing Highlands) (DOE 2009; Ambak & Mohd Zaidi 2010) and sand mining (Tan & Rohasliney 2013). During the sampling trip along the Kelantan River, heavy sand mining activities were observed. Pit excavation methods of sand mining were used along the river, which involves the extraction of sand and gravel from the riverbed by uncontrolled digging or diesel powered suction pumps. Ambak *et al*. (2010) reported that there were approximately 128 sand mining activities functioning along the Kelantan River from Kuala Krai to Tumpat. The detailed site description is shown in Table 1.

Table 1: Description of stream characteristics for each sampling station.

Site	Description
Station 1 Temangan	Main stream, large size stream approximately 300–600 m wide, fast flowing water; bottom substrate consists of gravel, sand, mud and silt; continuously turbid water.
Station 2 Tanah Merah	Main stream, large size stream approximately 300–500 m wide, fast flowing water; bottom substrate consists of gravel, sand, mud and silt; continuously turbid water; active sand mining activities.
Station 3 Tendong	Main stream, large size stream approximately 500–700 m wide; open water, fast flowing water; bottom substrate consists of gravel, sand, mud and silt; continuously turbid water; active sand mining activities.
Station 4 Kota Bharu	Main stream, large size stream approximately 300–500 m wide, with 4 m depth; fast flowing water; bottom substrate consists of gravel, sand, mud and silt; rock installed on the banks as a buffer; continuously turbid water; active sand mining activities; active development activities.

Sampling Design

Sampling was carried out during the dry season (July 2011) and wet season (February 2012). Four sampling stations were selected along the lower reaches of the Kelantan River: Station 1 (S1), Station 2 (S2), Station 3 (S3) and Station 4 (S4). S1 and S2 were chosen in between Temangan and Tanah Merah, whereas S3 and S4 were selected in between Tendong and Kota Bharu (Fig. 1). Five experimental gill nets (measuring 30 m length, 1.5 m depth with stretch mesh size of 3 and 4 inches) were set up and left for five consecutive days at each sampling station. Each net was inspected every day for five days from morning until afternoon. The net was set up along the river that covers the most river pools (areas immediately downstream from a dike or obstruction that formed a scour hole > 1.2 m deep). In total, 200 samples were collected (N = 5 fish nets × 5 days × 4 sampling stations × 2 seasons). All nets were set at least 500 m away from the operational sand mining site. All captured fish were labelled accordingly and placed in an ice chest before transport to the Environmental and Occupation Health Laboratory, School of Health Sciences, Universiti Sains Malaysia (USM). In the laboratory, fish samples were identified to species based on standard taxonomic keys (Mohsin & Ambak 1991; Kottelat *et. al*. 1993). Fish were also individually measured [total length (mm), weight (g)] and enumerated (collectively by species).

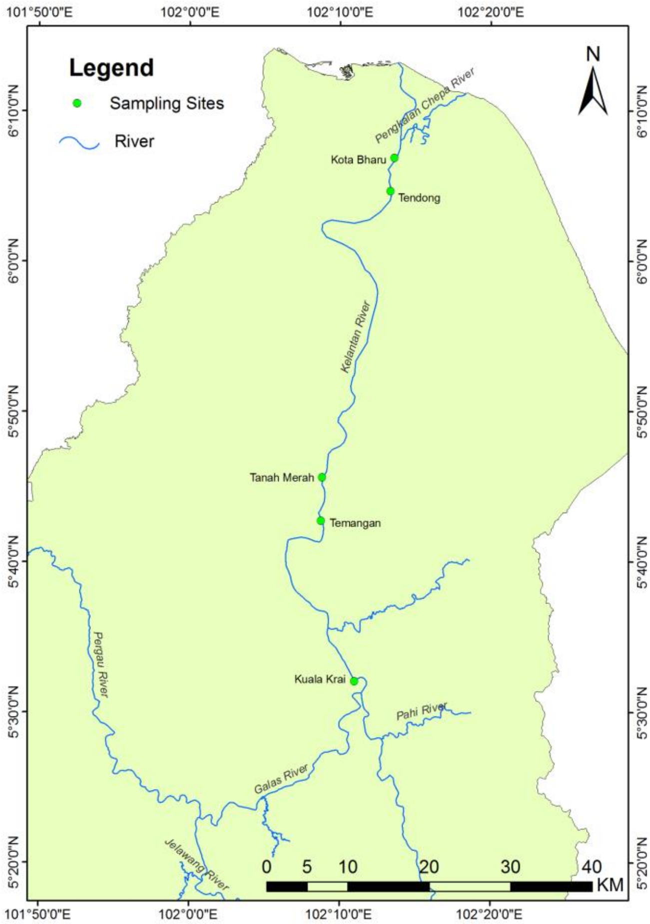


Figure 1: Location of sampling stations (adapted from JUPEM 2002).

Fish Tissue Preparation

Muscle tissue of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. Fish tissues were cut and oven dried at 110°C to a constant weight (Tüzen 2003). A wet digestion method was used based on the Analytical Methods for Atomic Absorption Spectrometry. Prior to use, all glassware was previously soaked in diluted nitric acid for 24 h and then rinsed with distilled deionised water.

The 5 g dry weight sample was put into a 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2SO_4. When the fish tissue stopped reacting with HNO_3 and H_2SO_4, the beaker was then placed on a hot plate and heated at 60°C for 30 min. After allowing the beaker to cool, 10 ml of HNO_3 was added and returned to the hot plate to be heated slowly to 120°C. The temperature was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding H_2O_2 until the sample was clear. The content of the beaker was transferred into a 50 ml volumetric flask and diluted to the mark with ultra-pure water. All the steps were performed in the fume hood. The above procedures in this section followed the guidelines from the Analytical Methods for Atomic Absorption Spectroscopy (Perkin Elmer 1996).

Analytical Measurement

Concentrations of heavy metals were then analysed using a graphite furnace Atomic Absorption Spectrometer (AAS, Analyst 800, Perkin Elmer, Massachusetts, USA) with high-purity argon. The results from the AAS were expressed as µg/g dry weight and converted to mg/kg in the results section. All reagents used were of analytical grade, and ultrapure water was used for the preparations of solutions. To acquire ultrapure water, water was purified using the LABCONCO water purification system from LABCONCO Instruments (Kansas City, USA) available in the analytical laboratory of the School of Health Sciences. To create pure and ultra-pure water for research laboratory applications, impurities that include particulates, colloids, ions, dissolved gases, dissolved organic solids, nucleases (RNase, DNase) and pyrogens need to be efficiently and effectively removed. Calibration standard solutions were made by stepwise dilution of the stock solution [Butanol dehyrogenase (BDH) spectrosol, Merck Ltd., England]. Fish samples were spiked with various concentrations of heavy metals for the recovery repeatability test and to verify the analytical methods. For each run, the spiked samples were analysed in triplicate. The effect of the sample matrix was studied by spiking an identified concentration of each heavy metal (Ni, Pb, Cd) into fish samples. Then, the spiked samples were digested. The content of heavy metals in the spiked samples was determined by using AAS. The percent recoveries were calculated by using the following formula: Recovery (%) = $[(x–y)/z] \times 100$, where x is the average concentration of heavy metal after spiking, y is the average concentration of heavy metal before spiking, and z is the concentration of spiked heavy metal. Acceptable results with recovery percentages of 77.3%–85.3%, 82.0%–83.0% and 78.0%–83.3% were obtained for Cd, Ni and Pb, respectively.

Statistical Analysis

Heavy metal concentrations were compared between the dry and wet seasons and among sampling stations and feeding habits. Seasonal comparison was analysed using a Mann-Whitney U-test. A Pearson correlation test was used to check the relationships between heavy metal concentrations and fish weight. All analysis was determined at significance levels of $p<0.05$. When necessary, data were $\log_{10}$ (x+1) transformed to normalise the distributions. All statistical analyses were computed using Statistical Package for Social Sciences (SPSS) version 16.0.

RESULTS AND DISCUSSION

A total of 78 fish were caught, comprising 6 families, 11 genera and 13 species. The captured fish species are listed in Table 2. The Cyprinidae family included *Barbonymus gonionotus, Barbonymus schwanenfeldii, Cyclocheilichthys apogon, Hampala macrolepidota, Puntioplites bulu* and *Osteochilus hasseltii*. We identified *Clarias gariepinus* from the Clariidae family, *Tachysurus maculatus* from the Ariidae family, *Hemibagrus wyckii* and *Hemibagrus nemurus* from the Bagridae family, *Chitala chitala* and *Notopterus notopterus* from the Notopteridae family, and lastly *Pangasius micronemus* from the Pangasiidae family. Inland fisheries are of high socio-economic and socio-cultural importance and provide a myriad of benefits to society. Approximately 31,530 (34.8%) fishermen in East Malaysia, 8% of who are inland fishermen that live along the Kelantan River, make their living via traditional fishing (Rohasliney 2010). It is estimated that river fish landings in Kelantan only contribute to approximately 3.5% to 4.1% of the total river fish production. This contribution has been considered as the optimum level, and it is expected to be in the region of 87,000 kg per year, with occasional fluctuations depending on the situation (Rohasliney 2010). In some locations along the Kelantan River, fish may be reserved by the fishermen for their own consumption, sold fresh, or preserved for local consumers, especially in the area of Temangan, Tanah Merah and Pasir Mas. Full-time fishermen depend annually on the sufficiently large quantities of commercially valuable fish to survive and typically migrate between a number of different habitats or river sections according to the season. However, part-time fishermen only practice fishing activities mainly during downtime from alternative activities. Additionally, many local people fish to supplement the family diet during slack periods in their daily schedule or seasonal calendar. By contrast, recreational fishermen often fish for leisure entertainment. Sport and recreational fishing were observed in several locations along the river at Kota Bharu. From personal communication with several of the anglers, the captured fish are either brought home (for human and/or livestock consumption) or discarded into the river. This demonstrates that dependence of the local population on the need for protein from freshwater fish still exists in the state of Kelantan.

Table 2: Distribution of fish caught from all sampling stations (S1, S2, S3 and S4) during the dry (July 2011) and wet seasons (February 2012).

Species name (n)	Common name	S1	S2	S3	S4
Barbonymus gonionotus (9)	*Lampam jawa*	+	–	+	+
Barbonymus schwanenfeldii (22)	*Lampam sungai*	+	+	+	+
Chitala chitala (1)	*Belida*	–	–	–	+
Clarias gariepinus (2)	*Keli*	–	–	–	+
Cyclocheilichthys apogon (3)	*Temperas*	+	–	–	–
Hampala macrolepidota (5)	*Sebarau*	+	+	–	+
Hemibagrus nemurus (3)	*Baung*	+	+	+	–
Hemibagrus wyckii (2)	*Baung*	+	–	–	+
Notopterus notopterus (6)	*Selat*	+	+	+	+
Osteochilus hasseltii (1)	*Terbul*	+	–	–	–
Pangasius micronemus (4)	*Patin*	+	–	–	+
Puntioplites bulu (13)	*Tengalan*	+	+	+	+
Tachysurus maculatus (7)	*Ikan duri*	–	–	+	+

Note: + Present; – Not present; n = number of individual fish caught

In many developed countries, the limits of heavy metal concentration in fish have been set to safeguard public health. Malaysia, for example, has set maximum limits of contamination for Cd and Pb based on permissible limits recommended by the Malaysian Food Act (MFA 1983). In this study, however, the heavy metal permissible levels were also compared with others such as the WHO (1985), USFDA (1993), EC (2001) and FAO (2003) (Table 3). Apparently, there is no information about the maximum permissible Ni limits in fish tissues in the MFA, EC, FAO and USFDA standards. Although there is no precise description of Ni toxicity to fish reported in the literature, especially in the Kelantan River, it does not indicate that the presence of Ni in the water is not harmful to fish species. *C. chitala* had the highest mean concentration of Cd (0.076 mg/kg,) which was above the critical limit set by the EC (2001), WHO (1985) and FAO (2003). The study found that the mean concentration of Cd in *B. gonionatus* and *T. maculatus* had already reached the level of concern, whereas other species were approaching the acceptable limits (Table 3). Cadmium can be accumulated with metallothioneins; an uptake of 3.0–330.0 mg/day is deadly and 1.5–9.0 mg/day is lethal to humans (Bowen 1979). Cadmium affects the kidneys and causes symptoms of chronic toxicity, such as the impairment of kidney function, poor reproductive capacity, hypertension, tumours and hepatic dysfunction (Waalkes 2000). Generally, the mean concentration of Ni found in all fish species was below the permissible limit of 0.5–0.6 mg/kg set by the WHO (1985) (Table 3). However, the mean concentration of Ni obtained from *B. schwanenfeldii, C. gariepinus, C. apogon* and *N. notopterus* indicated that serious attention should be given accordingly because the mean concentration of Ni in these four species was approaching the limits of the permissible level. The highest mean concentration of Pb was detected in *O. hasseltii* and *T. maculatus*, with values of 0.169 mg/kg and 0.156 mg/kg, respectively. Meanwhile, the mean concentrations of Pb in *C. apogon*, *H. nemurus* and *B. schwanenfeldii* were slightly below the limits of the permissible level with 0.137, 0.103 and 0.100 mg/kg, respectively. Pb is a non-essential element and can be toxic to humans when ingested or inhaled in high doses (Salem *et al.* 2000). Trace metals such as Pb will interfere with essential nutrients of similar characteristics such as calcium (Ca) and Zn. Pb also causes renal failure and liver damage in humans (Salem *et al.* 2000). In fish, Pb causes decreases in survival, growth, development, behaviour and metabolism, in addition to an increase in the formation of mucus (Eisler 1988).

Table 3: Mean concentrations of heavy metals in fish species from the Kelantan River during the dry (July 2011) and wet seasons (February 2012) and the permissible limit in fish.

Species	Mean heavy metal concentration (mg/kg d.w.)		
	Cd	Ni	Pb
Barbonymus gonionatus	0.050±0.035	0.061±0.076	0.072±0.115
Barbonymus schwanenfeldii	0.030±0.032	0.100±0.156	0.100±0.125
Chitala chitala	0.076	0.262	0.070
Clarias gariepinus	0.013±0.09	0.056±0.079	0.081±0.118
Cyclocheilichthys apogon	0.044±0.014	0.084±0.084	0.137±0.069
Hampala macrolepidota	0.023±0.019	0.024±0.037	0.042±0.055
Hemibagrus nemurus	0.023±0.019	0.056±0.069	0.103±0.074
Hemibragus wyckii	0.029±0.035	0.090±0.112	0.062±0.062
Notopterus notopterus	0.030±0.029	0.183±0.265	0.075±0.061
Osteochilus hasseltii	0.024	0.053	0.169
Pangasius micronemus	0.027±0.018	0.048±0.033	0.022±0.011
Puntioplites bulu	0.038±0.032	0.079±0.107	0.069±0.071
Tachysurus maculatus	0.053±0.054	0.097±0.113	0.156±0.157
Permissible limit in fish			
EC (2001)	0.05–0.10	–	0.20–0.40
USFDA (1993)	–	–	0.5
WHO (1985)	2.0	0.5-0.6	2.0
FAO (2003)	0.05	–	0.20
MFA (1993)	1	–	2
National Water Quality Standard for Malaysia	Mean heavy metal concentration (mg/l)		
Class I	Natural levels or absent		
Class IIA/IIB	0.01	0.05	0.05
Class III	0.01[a](0.001)	0.90[a]	0.02[a](0.010)

(*continued on next page*)

Table 3: (*continued*)

	Mean heavy metal concentration (mg/l)		
	Cd	Ni	Pb
Class IV	0.01	0.20	5.00
Class V	Levels above IV		
Water classes and uses to water class			
Water class	Uses		
Class I	Conservation of natural environment Water supply I – Practically no treatment necessary Fishery I – Very sensitive aquatic species		
Class IIA	Water supply II – Conventional treatment required		
Class IIB	Fishery II – Sensitive aquatic species Recreational use with body contact		
Class III	Water supply III – Extensive treatment required Fishery III – Common, of economic value and tolerant species; livestock drinking		
Class IV	Irrigation		
Class V	None of the above		

Note: [a]At hardness 50 mg/l $CaCO_3$

In this study, metal concentrations were found to be elevated during the wet season (Fig. 2). Fish samples collected during the wet season had significantly higher concentrations of Cd, Ni and Pb (p<0.05) compared to the dry season (Table 4). Exposure to higher concentrations of heavy metals in the water during the wet season was the result of increased surface runoff. Heavy rainfall leads to farm draining. Large amounts of pesticides containing metal compounds are brought to the surface via runoff from the farms to the river and highly contribute to agricultural pollution, especially chemical fertilisers containing Ni and Pb. However, several previous studies found that mean heavy metal concentrations in fish were higher in the dry season (Fufeyin 1998; Idodo-Umeh 2000; Oguzie 2003; Obasohan & Eguavoen 2008). This is because higher concentrations of metals in fish during the dry season were due to high temperatures, which increased the activity, ventilation, metabolic rate and feeding sessions (Nussey *et al*. 2000). The low heavy metal concentrations in the wet season were due to the dilution of metal levels associated with heavy rains (Obasohan & Eguavoen 2008).

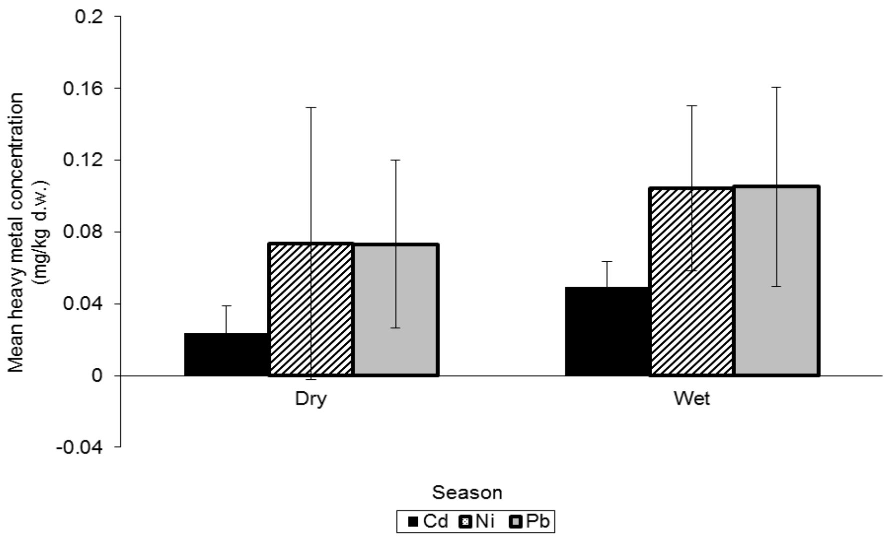


Figure 2: Mean concentration of heavy metals in fish tissue at all sampling stations (S1, S2, S3 and S4) from the dry (July 2011) and wet seasons (February 2012).
Note: n = 78 fish

Table 4: Comparison of heavy metal concentrations among fish collected in different seasons, feeding habits and sampling stations.

Parameter		Heavy metal concentration (mg/kg d.w.) Mean (Standard Deviation)		
		Cd	Ni	Pb
Season	Dry	0.023 (0.030)	0.073 (0.152)	0.073 (0.093)
	Wet	0.049 (0.028)	0.105 (0.092)	0.105 (0.111)
	p value	*<0.001	*<0.001	*0.039
Feeding habit	Herbivore	0.030 (0.031)	0.041 (0.042)	0.043 (0.043)
	Omnivore	0.038 (0.029)	0.149 (0.190)	0.103 (0.116)
	Carnivore	0.039 (0.038)	0.080 (0.093)	0.137 (0.122)
	p value	0.37	0.277	*0.002
Sampling stations	S1	0.049 (0.035)	0.066 (0.066)	0.087 (0.069)
	S2	0.015 (0.015)	0.010 (0.025)	0.068 (0.124)
	S3	0.025 (0.023)	0.112 (0.175)	0.119 (0.119)
	S4	0.028 (0.031)	0.128 (0.167)	0.070 (0.121)
	p value	*0.002	*0.017	0.056

Note: *$p<0.05$

The mean concentration of Cd found in all fish species at sampling station S1 (0.049±0.035 mg/kg) was significantly higher ($p<0.05$) among the four sampling stations (Table 4). Sampling station S1 also had more agricultural activity along the riverbank, and thus, the use of fertiliser and fuel at this agricultural site most likely contributed to the increased level of Cd in fish that were caught at this location. S3 accumulated the highest mean concentration of Pb (0.119±0.119 mg/kg) in fish tissues, but the difference when compared to the other stations was not significant (*p>0.05*) (Fig. 3). Such trace levels of heavy metal concentration could be the result of oil spills from boats that are used for regular transportation. Furthermore, the use of fuel for sand mining activities also contributed to the release of heavy metals such as Pb and Ni, especially if the fuel was accidentally released into the river, as observed at S3. The mean concentration of Ni found at S4 (0.128±0.0167 mg/kg) was significantly higher (*p<0.05*) among the four sampling stations (Fig. 3). The lowest number of fish was caught at S2 due to heavy sand mining activities. This indicated a lack of uniformity in the fish distribution and the possible bio-availability of metals at the corresponding sampling station. Disturbed biological resources due to sand mining have seriously affected the distribution of benthic fish.

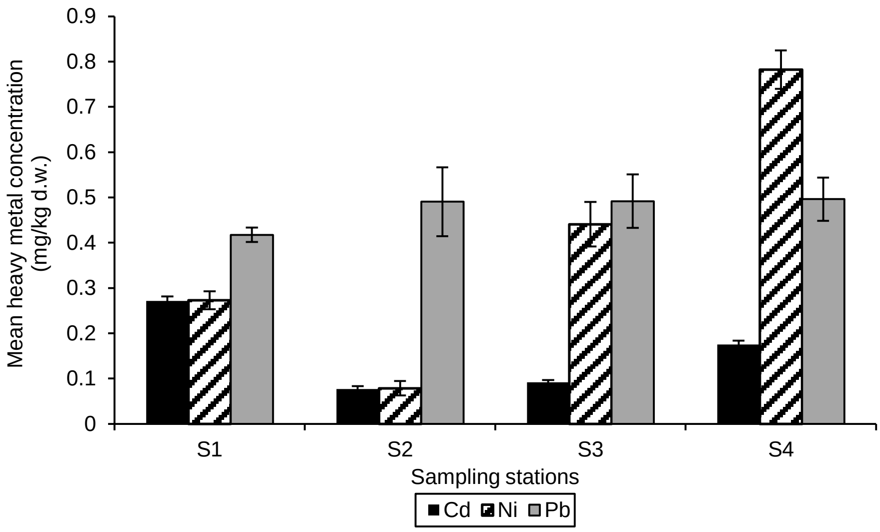
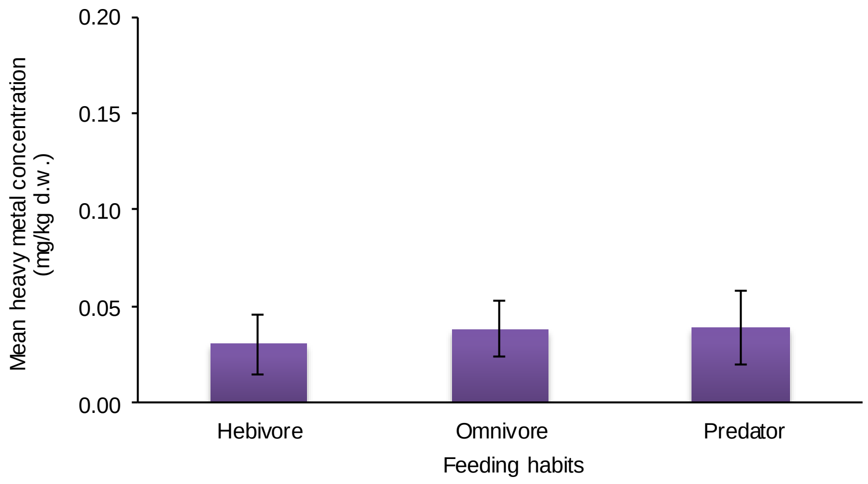


Figure 3: Mean concentration of heavy metals in fish tissue at the four sampling stations along the Kelantan River (S1, S2, S3 and S4) from the dry (July 2011) and wet seasons (February 2012).
Note: n = 78 fish

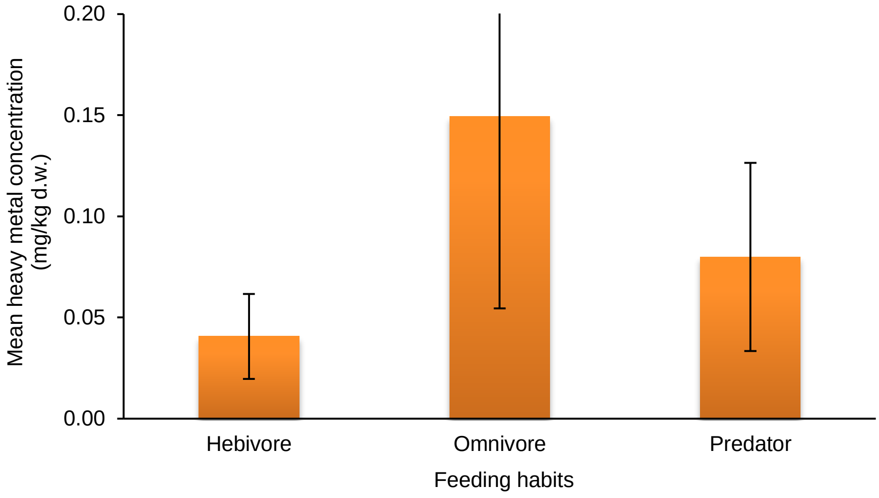
Out of the 78 fish caught, 26% were carnivorous fish species that mainly feed on small fish, insects, shrimp and crustaceans. Herbivorous and omnivorous fish occupied 41% and 33%, respectively. The feeding habits of herbivorous, carnivorous and omnivorous fish were significantly different only for Pb (*p<0.05*) (Table 4). The Pb concentration was found to be significantly higher in the carnivorous fish species (*p*<0.05) (Fig. 4). Thus, this study suggested that predatory fish, which are at a higher trophic level, accumulate more heavy metals compared with non-predatory fish (Kidwell *et al.* 1995; Voigt 2004; Weber *et al*. 2013). This result would have been more significant if fish with different feeding behaviours were equally distributed among the study sites. Carnivorous fish, which mainly eat fingerlings, shrimp and zooplankton, are known to be active swimmers. These activities are known to accumulate high levels of heavy metals in the body (Karadede *et al.* 2004). Concentrations of Cd and Ni were observed in omnivorous fish, but the differences were not significant (Table 4). The diet of omnivorous fish includes aquatic insects, shrimps, small fish, crustacean, algae, plankton and detritus. The accumulation of Cd and Ni in omnivorous fish may have been due to diversity of food intake [Fig. 4(a) and 4(b)]. In addition, most omnivorous fish caught in this study were bottom dwellers; thus, they may also obtain contaminants bound to sediments. Three herbivorous fish were caught during this sampling period, namely *B. gonionotus*, *B. schwanenfeldii* and *O. hasseltii*. The accumulation of Cd, Ni, and Pb in herbivorous fish tissues was low compared to fish at other trophic levels. This trend may be related to their feeding behaviour and habitat. Herbivorous fish, which are primary consumers, eat aquatic macrophytes, submerged land plants and filamentous algae (Ambak *et al*. 2010). By being at a lower trophic level, herbivores do not have the variety of diet items found in carnivores and omnivores. Thus, biomagnification in herbivorous fish is not as large as for secondary consumers.



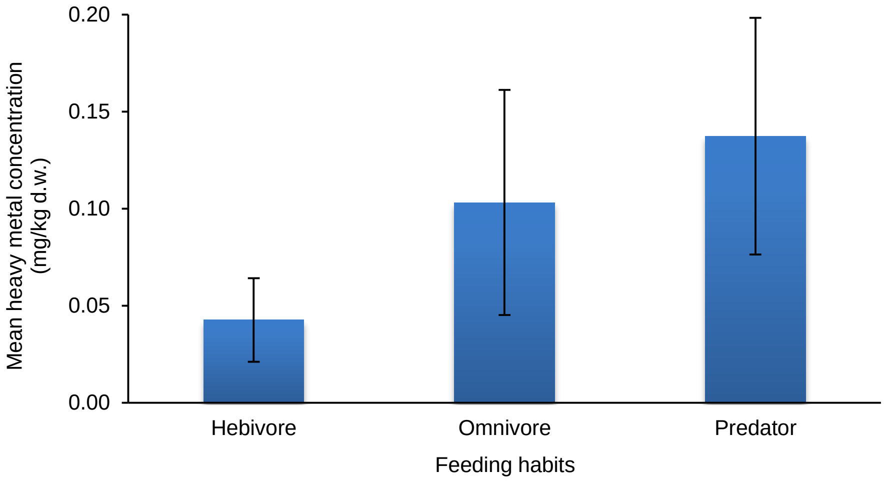
(a)

Figure 4: Mean concentration of (a) Cd, (b) Ni and (c) Pb in fish tissue at all sampling stations from fish with different feeding habits for one hydrological cycle *(continued on next page).*

Note: n = 78 fish



(b)



(c)

Figure 4: *(continued)*

The weight of the fish was correlated with Cd (p<0.01) and Pb (p<0.01) (Table 5). Meanwhile, Ni showed a positive correlation with Cd (p<0.01) and Pb (p<0.05). The fish ranged in size from 18.7–67.4 cm in total length and 67–1800 g in weight (Table 6). Length and weight play an important role in the accumulation of heavy metals. Small fish are enriched with accumulated heavy metals from the aquatic microfauna and microflora that constitute their diet (Obasohan *et al.* 2006). Mohsin and Ambak (1991) found that maturity, which was measured by fish length, influenced the accumulation of heavy metals. In addition, Ahmad and Suhaimi-Othman (2010) found that mature fish accumulated higher metals compared to juvenile and premature fish. This is because fish with a constant growth rate that inhabit continuous polluted habitats stabilise the accumulation of heavy metals (Ahmad & Suhaimi-Othman 2010). In addition, the range of overall fish caught in this study showed considerably large variations in fish size, thus indicating that the gill net sampling was conducted efficiently. The selection of mesh size also contributed to the minimum and maximum length of the captured fish. Furthermore, fish weight was correlated with the levels of Cd and Pb.

Table 5: Correlation matrix for fish weight (g) and heavy metal concentration (mg/kg) in all sampling stations.

Parameter		Weight	Cd	Ni	Pb
Weight	Pearson Correlation	1			
	Sig. (2-tailed)				
Cd	Pearson Correlation	0.373**	1		
	Sig. (2-tailed)	0.001			
Ni	Pearson Correlation	0.114	0.372**	1	
	Sig. (2-tailed)	0.319	0.001		
Pb	Pearson Correlation	0.369**	0.272*	0.290*	1
	Sig. (2-tailed)	0.001	0.016	0.010	

Notes: *Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Table 6: List of fish caught from all sampling stations (S1, S2, S3 and S4) during the dry (July 2011) and wet seasons (February 2012).

Species name	Common name	Feeding habit	Length (cm)	Weight (g)	[a]Maturity size (cm)	Status
Barbonymus gonionotus	*Lampam jawa*	Herbivore	13.5–24.5	67–422	25–30	Premature
Barbonymus schwanenfeldii	*Lampam sungai*	Herbivore	14.0–24.4	94–332	6–20	Mature
Chitala chitala	*Belida*	Carnivore	28.9	259	75–120	Premature
Clarias gariepinus	*Keli*	Omnivore	28.0–32.0	258–478	30	Mature
Cyclocheilichthys apogon	*Temperas*	Carnivore	15.3–19.6	94–182	7–20	Mature
Hampala macrolepidota	*Sebarau*	Carnivore	21.0–31.3	222–602	20–35	Mature
Hemibagrus nemurus	*Baung*	Carnivore	25.9–34.9	306–636	21–35	Mature
Hemibagrus wyckii	*Baung*	Carnivore	24.9–61.5	246–1800	20–35	Mature
Notopterus notopterus	*Selat*	Carnivore	21.2–28.5	87–210	20	Mature

(*continued on next page*)

Table 6: (*continued*)

Species name	Common name	Feeding habit	Length (cm)	Weight (g)	[a]Maturity size (cm)	Status
Osteochilus hasseltii	*Terbul*	Herbivore	18.4	182	10–20	Mature
Pangasius micronemus	*Patin*	Omnivore	21.2–48.0	106–1553	21–35	Mature
Puntioplites bulu	*Tengalan*	Omnivore	15.5–25.4	120–508	30–50	Premature
Tachysurus maculatus	*Ikan duri*	Omnivore	17.4–29.8	92–314	12–24	Mature

Note: [a]Maturity size: Sourced from Mohsin and Ambak (1983; 1991).

CONCLUSION

Overall, the total mean heavy metal concentration of all fish species in this study revealed an order of Pb>Ni>Cd. These results were concordant with the surrounding waters of the Kelantan River reported by the DOE (2002). Therefore, the results in this study demonstrated that fish species caught in the Kelantan River were contaminated with heavy metals. Nevertheless, the heavy metal concentration in the fish tissues, with the exception of *C. chitala, O. hasseltii* and *T. maculatus*, did not exceed the MFA, WHO, USFDA, EC or FAO guidelines. The regular monitoring of heavy metal concentrations in fish tissue is necessary. Although the concentrations of heavy metals in the fish and water column were detected in low concentrations, the potential for metal toxicity danger may become more severe in the future depending upon the extent of industrial and domestic wastewater influx into the Kelantan River due to human activities in the adjacent areas. To develop a healthy freshwater fishing industry and to prevent heavy metal risks to human health in the Kelantan River, the water standards and concentrations of heavy metals in the water column and fish should be monitored regularly.

ACKNOWLEDGEMENT

We are appreciative of Wan Mohd Amzar, Siti Zulfarina, Md. Khairul Azuan and Mohd Kamarizan for their help in the field and laboratory. We also thank Wan Mohd Sahnusi Wan Alias and Rosliza Haron for assisting with the AAS. Our thanks to the Environmental and Occupational Health Laboratory and Analytical Laboratory of School of Health Sciences, USM Health Campus, for the laboratory facilities that supported the study. Special gratitude goes to the DOE (Kelantan) for providing information and data. Finally, cordial appreciation also goes to all the staff from the research offices of the School of Health Sciences. Last but not least, we thank Dr. Ho Yuek Ming from the Department of Environmental Management, Faculty of Environmental Studies, Universiti Putra Malaysia for proofreading this article. This work was supported by USM under the short-term grant 304/PPSK/61311022.

REFERENCES

Ahmad A K, Mushrifah I and Othman M S. (2009). Water quality and heavy metal concentrations in sediment of Sungai Kelantan, Kelantan Malaysia: A baseline study. *Sains Malaysiana* 38(4): 435–442.

Ahmad A K and Shuhaimi-Othman M. (2010). Heavy metal concentrations in sediments and fishes from Lake Chini, Pahang, Malaysia. *Journal of Biological Sciences* 10(2): 93–100.

Allen-Gil S M and Martynov V G. (1995). Heavy metal burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia. *The Science of the Total Environment* 160/161: 653–659.

Ambak M A, Isa M M, Zakaria M Z and Ghaffar M A. (2010). *Fishes of Malaysia*. Kuala Terengganu: Universiti Malaysia Terengganu (UMT).

Ambak M A and Mohd Zaidi Z. (2010). Freshwater fish diversity in Sungai Kelantan. *Journal of Sustainability Science and Management* 5(1): 13–20.

Ashraf M A, Maah M J, Yusoff I, Wajid A and Mahmood K. (2010). Sand mining effects, causes, and concerns: A case study from Bestari Jaya, Selangor, Peninsular Malaysia. *Scientific Research and Essays* 6(6): 1216–1231.

Birungi Z, Masola B, Zaranyika M F, Naigaga I and Marshall B. (2007). Active biomonitoring of trace heavy metals using fish (*Oreochromis niloticus*) as bioindicator species. The case of Nakivubo wetland along Lake Victoria. *Physics and Chemistry of the Earth* Parts A/B/C 32(15–18): 1350–1358.

Bowen H J M. (1979). *Environmental chemistry of the elements*. London: Academic Press.

Canli M and Atli G. (2002). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental Pollution* 121(2003): 129–136.

Department of Environment (DOE). (2009). *Kelantan Environmental Quality Report 2009*. Kelantan: Department of Environment.

____. (2002). *Malaysia Environmental Quality Report 2002*. Kuala Lumpur: Ministry of Natural Resources and the Environment.

European Commission (EC). (2001). Commission Regulation 466/2001/EC of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Community* 77: 1–13.

Eisler R. (1988). Lead hazards to fish, wildlife, and invertebrates: A synoptic review. *US Fish and Wildlife Service Report* 85: 1–14.

Fernandes C, Fontaínhas-Fernandes A, Peixoto F and Salgado M A. (2007). Bioaccumulation of heavy metals in *Liza saliens* from the Esmoriz–Paramos coastal lagoon, Portugal. *Ecotoxicology and Environmental Safety* 66(3): 426–431.

Food and Agriculture Organization (FAO). (2003). *Heavy Metal Regulations – Faolex. Legal Notice no. 66/2003.* http://faolex.fao.org/docs/pdf/eri42405.pdf (accessed on 21 April 2012).

Fufeyin T P. (1998). Heavy metal levels in some dominant fish of Ikpoba Reservoir in Benim City, Nigeria. *Environmental Review* 2: 61–69.

Goyer A R. (1997). Toxic metals and essential metal interactions. *Annual review of Nutrition* 17: 37–50.

Heath A G. (1995). *Water pollution and fish physiology*. Florida: CRC Press.

Idodo-Umeh G. (2000). *Freshwater fishes of Nigeria (Taxonomy, ecological notes, diet and utilization)*. Benin City, Nigeria: Idodo-Umeh Publishers.

Jabatan Ukur dan Pemetaan Malaysia (JUPEM). (2002). *eMap peta elektronik Kelantan.* Kuala Lumpur: JUPEM.

Kadaruddin A. (2000). Pencemaran air dan pengurusannya di Malaysia: Cabaran alaf baru. In J Jamaluddin (ed.). *Pengurusan persekitaran di Malaysia: Isu dan cabaran*. Kuala Lumpur: Akam Print Malaysia, 104–126.

Karadede H, Oymak S A and Ünlü E. (2004). Heavy metals in mullet, *Liza abu*, and catfish, *Silurus triostegus*, from the Ataturk Dam Lake (Euphrates) *Turkey. Environment International* 30: 183–188.

Kidwell J M, Philips L J and Birchard G F. (1995). Comparative analyses if contaminant levels in bottom feeding and predatory fish using the national contaminant biomonitoring program data. *Bulletin of Environmental Contamination and Toxicology* 54(6): 919–923.

Kottelat M, Whitten A J, Kartikasari S N and Wirjoatmodjo S. (1993). Freshwater fishes of Western Indonesia and Sulawesi. Jakarta: Periplus Editions Ltd.

Malaysian Food Act (MFA). (1983). *Malaysian food and drug*. Kuala Lumpur: MDC Publishers Printer Sdn. Bhd.

Malaysian Meteorological Department (MMD). (2009). *Annual Report 2009*. Kuala Lumpur: MMD, Ministry of Science, Technology and Innovation.

McCluggage D. (1991). *Heavy metal poisoning*. Colorado, USA: NCS Magazine.

Mohsin A K M and Ambak M A. (1991). *Ikan air tawar di Semenanjung Malaysia*. Kuala Lumpur: Dewan Bahasa dan Pustaka.

____. (1983). *Freshwater fishes of Peninsular Malaysia*. Serdang, Selangor: Universiti Putra Malaysia.

Noor Syuhadah S and Rohasliney H. (2011). A preliminary study on batik effluents in Kelantan state: A water quality perspective. In P S Sandhu and N Delcoure (eds.). *International Conference on Management, Social Sciences, Biology & Pharmaceutical Sciences (ICMSSBPS) 2011*. First Hotel, Bangkok, Thailand, 23–24 December 2011. Bangkok: Planetary Scientific Research Centre.

Noor Syuhadah S, Rohasliney H and Noor Zuhartini M M. (2014). Heavy metal analysis of batik industry wastewater, plant and soil sample: A comparison study of FAAS and HACH colorimeter Analytical capabilities. In A Z Aris, T H Tengku Ismail, R Harun, A M Abdullah and M Y Ishak (eds.). *From Sources to Solution. Proceedings of the International Conference on Environmental Forensics 2013*. Marriot Hotel, Putrajaya, Kuala Lumpur, 12–13 November 2013. London: Springer, 285–289.

Nussey G, Van Vuren J H J and Du Preez H H. (2000). Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga S. Africa. *Water SA* 26(2): 269–284.

Obasohan E E, Oronsaye J A O and Obano E E. (2006). Heavy metal concentrations in *Malapterurus electricus* and *Chrysichthys nigrodigitatus* from Ogba River in Benin City, Nigeria. *African Journal of Biotechnology* 5(10): 974–982.

Obasohan E E and Eguavoen O I. (2008). Seasonal variations of bioaccumulation of heavy metals in a freshwater fish (*Erpetoichthys calabaricus*) from Ogba River, Benim City, Nigeria. *African Journal of General Agriculture* 4: 153–163.

Oguzie F A. (2003). Heavy metals in fish, water and effluents of lower Ikpoba River in Benim City, Nigeria. *Pakistan Journal of Science and Industrial Research* 46(3): 156–160.

Papagiannis I, Kagalou I, Leonardos J, Petridis D and Kalfakakou V. (2004). Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environment International* 30(3): 357–362.

Perkin Elmer. (1996). *Analytical methods for atomic absorption spectroscopy*. USA: The Perkin-Elmer Corporation.

Rohasliney H. (2010). Status of river fisheries in Kelantan, Peninsular Malaysia, Malaysia. *World Academy of Science, Engineering and Technology* 65: 829–834.

Salem H M, Eweida E A and Farag A. (2000). Heavy metals in drinking water and their environmental impact on human health. *The International Conference for Environmental Hazard Mitigation ICEHM 2000*. Cairo University, Egypt, 9–12 September 2000. Egypt: Cairo University, 542–556.

Tan P Y and Rohasliney H. (2013). Status of water quality subject to sand mining in Kelantan River, Kelantan. *Journal of Tropical Life Sciences Research* 24(1): 19–34.

Tariq J, Ashraf M and Afzal M. (1996). Pollution status of the Indus River, Pakistan, through heavy metal and macronutrient contents of fish, sediment and water. *Water Research* 30(6): 1337–1344.

Türkmen A, Türkmen M, Tepe Y and Akyurt İ. (2005). Heavy metals in three commercially valuable fish species from İskenderun Bay, Northern East Mediterranean Sea, Turkey. *Food Chemistry* 91(1): 167–172.

Tüzen M. (2003). Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chemistry* 80(1): 119–123.

US Food and Drug Administration (USFDA). (1993). *Food and drug administration. Guidance document for arsenic in shellfish*. Washington DC: DHHS/PHS/FDA/CFSAN/Office of Seafood.

Voigt H R. (2004). Concentration of mercury and cadmium and the condition of some coastal Baltic fishes. *Environmentalica Femnica* 21: 1–26.

Waalkes M P. (2000). Cadmium carcinogenesis in review. *Journal of Inorganic Biochemistry* 79(1): 241–244.

Weber P, Behr E R, Knorr C D L, Vendruscolo D S, Flores E M M, Dressler V L and Baldisserotto B. (2013). Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchemical Journal* 106(1): 61–66.

World Health Organization (WHO). (1985). *Guidelines for drinking water quality (ii): Health criteria and supporting information*. Geneva: WHO, 130.

Yilmaz A B, Sangün M K, Yağlıoğlu D and Turan C. (2010). Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from İskenderun Bay, Turkey. *Food Chemistry* 123(2): 410–415.

Yilmaz F, Nozdemir N, Demirak A and Tuna A L. (2005). Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Food Chemistry* 100(2007): 830–835.