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PRELIMINARY STUDY OF HEAVY METALS POLLUTION IN FRESHWATER FISHES OF SUNGAI SIMPANG EMPAT, PENANG.

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Abstract

A study was conducted to determine heavy metals content in different body parts of fish collected from five sampling stations in Sungai Simpang Empat, Penang from July to December, 2005. The liver, head, gills, muscles and bones of dried fish samples were extracted by the AOAC acid digestion method whilst iron (Fe), zinc (Zn), manganese (Mn), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni) and cadmium (Cd) were analyzed by atomic absorption spectrophotometry. Based on Interim National Water Quality Standard for Malaysia (INWQS), dissolved oxygen, pH, conductivity, ammonia, sulphate, nitrate and phosphate were within the recommended levels suggested for fish to survive except for turbidity levels in some stations, exceeding the recommended of 50 mg/L limit. Fe was accumulated at the highest levels while Cd was the lowest. Only Fe and Mn levels have exceeded the permissible limit of the Malaysian Food Act (1983) and Food Regulations (1985). In most of the fish, liver accumulated the highest concentration of all heavy metals compared to head, gills, muscles and bones. Snakeskin gouramy or Sepat Siam (Trichogaster pectoralis) and black tilapia (Oreochromis mossambicus) are good candidates for potential bioindicator for metals pollution in this study.

Keywords: Heavy metals, fish, Interim National Water Quality Standard for Malaysia, bioindicator

1. Introduction

Heavy metal discharges to the aquatic environment are of great concern, and have a great ecological significance due to their toxicity and accumulative behavior (Sivaperumal et al., 2007). Thus, it can both damage aquatic species diversity and ecosystem (Ozuni et al., 2010). Sources of heavy metals came from urban and industrial development (Tabari et al., 2010), agricultural development, in terms of sewage wastewater and commercial fertilizers and via natural mineralisation (Singh et al., 2006).

Many Malaysian rivers acting as a public water resource and supply are polluted and the physical water quality are degraded because of the presence of heavy metal contamination. There are many cases of watershed mismanagement in the country such as the watersheds of Sungai Langat, Sungai Skudai, Sungai Pendas (Azman et al., 2012) Sungai Damansara and Sungai Juru (Idriss et al., 2012).

Fish has been used as a biomonitor to assess the levels of heavy metal pollution (Kamaruzzaman et al., 2011). Apart from being a good source of protein, fish are also important sources for essential heavy metals. Fish are at the top of the food chain and may concentrate some amount of heavy metals from the water (Romeo et al., 1999). The gills may accumulate heavy metal from water whereas the liver represents storage of metals in the water (Romeo et al., 1999). If the edible part of the fish which contains high level of heavy metals is eaten, this may pose hazardous effect to the humans through consumption (Kamaruzzaman et al., 2010). Thus, this study has been carried out to document on freshwater fishes in Sg. Simpang Empat. This study determines the concentrations of heavy metals in different fish species and in different body parts of fish samples collected from Sungai Simpang Empat. In addition, the abundance of fish and water quality of the study river were also determined in contribution to existing record for Malaysian rivers.

2. Methods

2.1 Sampling Area

Samplings were conducted in Sg. Simpang Empat, between five sampling stations, from the upstream to the downstream area of the river. The locations of the five sampling stations in the map, which are St1 (N 5º 17’, E 100º 27’), St2 (N 5º 17’, E 100º 27’), St3 (N 5º 17’, E 100º 28’), St4 (N 5° 18’, E 100° 29’) and St5 (N 5° 18’, E 100° 29’) are as shown in Figure 1. Specification for the sampling stations selected were based on the presence of factories surrounding the sampling area, the possibility of effluents entering the water body, Indah Water, a sewage water treatment factory, a swamp and a cow’s farm. Samplings were carried out twice, in July and December 2005.

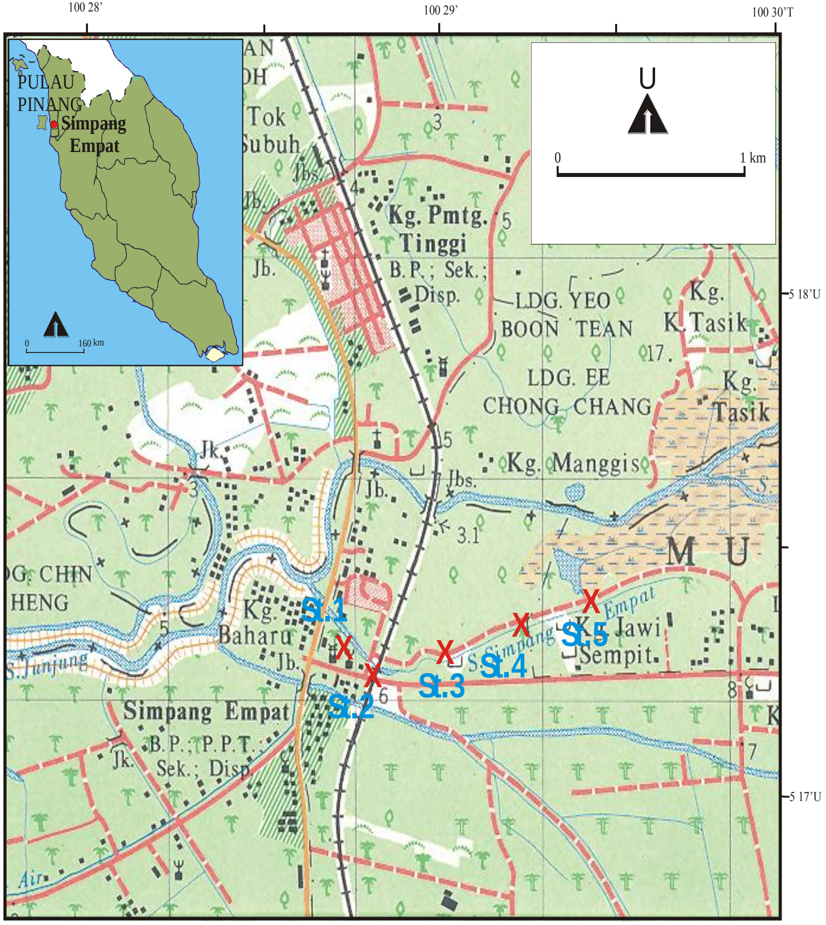

Figure 1.Five sampling stations in Sungai Simpang Empat, Penang

2.2 Sampling Method

An *in-situ* study was conducted on some physical and chemical aspects such as temperature, pH, conductivity, turbidity, dissolved oxygen and total dissolved solids. A multi-parameter equipment model YSI 551 was used to measure the pH, conductivity and total dissolved solids. A D.O meter model YSI 556 was used to measure dissolved oxygen. Turbidity, sulfate, nitrate, phosphate and ammonia nitrogen data were obtained by laboratory analyses using Hach DR 2010 Spectrophotometer.

Fish samples were caught using cast nets with a mesh size of approximately 2.5 cm. Captured fish were put into polyethylene bottles, labeled and the dates and location of samples were taken. Samples were transferred to the laboratory for identification, measurement and heavy metal analysis.

2.3 Species Identification and Measurement

All of the species collected was identified using key identification from Freshwater Fishes of Peninsular Malaysia by (Mohsin and Ambak, 1983). Assessment was done based on weight, body measurement, size, the physical appearance and according to colour.

2.4 Laboratory Method

Morphological aspects of the fish were measured in the laboratory. For each captured fish, the total length of the fish, the standard length and the body weight of the fish were recorded. Total length was measured from the end of the fish’s caudal fin to its head whereas standard length in centimeters was measured from the front of the caudal fin to its head. The body weight, measurements in gram was done using a digital electronic balance. The data were recorded in a data sheet for analysis process. After all of the measurement was completed, the species were put in the refrigerator at a temperature of -20ºC until analysis procedure was carried out.

For sample preparation, the whole body of fish was separated into specific parts. This was done by cutting the fish’s bodies into the head part, muscle, gill, liver and the vertebrae bones. The cutting procedure was done using a stainless steel knife to avoid metal contamination to the body parts. Then, drying method was carried out in Memmert oven (Model 854 Schwabach) at a temperature of 70ºC. Next, the dried samples were crushed and pounded into small pieces using the mortar until the samples have reached constant weight.

2.5 Digestion Method

For digestion, the method used is the Kjeldatherm method, using the Association of Official Analytical Chemists (AOAC) method. First, 5g of fish body parts samples were put into the PTFE beaker, then dried in the oven. Next, the samples in the PTFE beaker were mixed with 10 ml of nitric acid 69%. In prior, the Kjeldatherm, Gerhardt machine was already heated at a temperature of 200ºC until the green lamp has switched off. During the process of putting the PTFE beaker to the Gerhardt machine, the temperature was dropped to 20ºC.

After the placement of all PTFE beakers have been completed, the temperature was raised to 100ºC and was left to digest for 2 hours. After 2 hours, the temperature was dropped again to 20ºC. The samples were left to cool down and later, were added with 10 ml of hydrochloric acid 31%. The mixtures were left on the Gerhardt machine for another 2 hours for better and thorough effectiveness of digestion. The digestion process was conducted in order to break the solid structure of the samples into solutions.

The already digested samples were left to cool down. The result from the digested fish samples turned out to be a yellowish solution. This solution were then filtered using a filter paper of a 0.45 µm size and were put inside a conical flask with the top covered with parafilm. The filtered solutions were diluted with 50 ml of distilled water. Then, the extracted solutions were analyzed using the atomic absorption spectrophotometry (AAS) Perkin Elmer model for heavy metal evaluation.

2.6 Heavy Metal Analysis Method

Heavy metal contents in the samples were analyzed using the atomic absorption spectrophotometer (AAS) with air-acetylene ignition. Before the analysis can be carried out, first, the temperature must be ensured at room temperature level of 27 ºC. The procedure of using AAS and the process to make solutions for heavy metal analysis is provided from the AAS manual book.

2.7 Statistical Analysis

Data was analyzed using one way analysis of variance test (ANOVA) to see whether there is or there is no significant difference between fish body parts in this study. Correlation method was used to determine the relationship between two variables. Statistical computer analysis was calculated using SPSS.

3. Results and Discussions

3.1 Diversity and Abundance of Fish Species in the Study River

A total of 138 fishes were captured during the two days of sampling in July and December 2005, representing 8 families, namely Clariidae, Cichlidae, Anabantidae, Osphronemidae, Notopteridae, Channidae and Cyprinidae. The most abundant species was *Esomus malayensis* with a total of 67 individuals (46.85%), followed by *Trichogaster pectoralis* (26 individuals or 18.84%), *Oreochromis mossambicus* (24 individuals or 17.39%) and *Megalops cyprinoides* (3 individuals or 2.17%). The species *E.malayensis* which belongs to the family Cyprinidae is the most abundant species found in Sg. Simpang Empat. Fishes from the family Cyprinidae were found to be the dominant family captured from Perak River (Hashim et al., 2012). Usually, small-sized fish such as *E. malayensis* can be found in high numbers in smaller sized rivers such as Sg. Simpang Empat (Samat et al., 2003).

Station 1 which is located at the upstream of Sungai Simpang Empat had the highest abundance and diversity of fish species with 57 individuals collected (41.30% of total catch) comprising of *E. malayensis, Notopterus notopterus, Clarias batrachus, O. mossambicus, Anabas testudineus, T. pectoralis, Channa striatus* and *M. cyprinoides.* This followed by station 3 with 31 individuals (22.46%), station 4 with 20 individuals (14.49%), Station 2 with 18 individuals (13.04%) and lastly Station 5 with 12 individuals (8.69%). Fish captures were found to be higher in the upstream compared to the downstream of Sg. Simpang Empat. This might be influenced by less pollutant content in the upstream. Furthermore, the condition of the river such as floating garbage and debris would also influence the effectiveness of the fishing nets used, whereby the debris might be entangled with the net. A lot of rubbish was observed to be discarded in the downstream part of the river.

3.2 Physico-Chemical Parameters of Sungai Simpang Empat

The dissolved oxygen (DO) values were highest at station 5 (4.57 mg/L) and the lowest is 0.92 mg/L as shown in Table 1. The DO levels varied between the five sampling stations in this study, but overall, it was higher in the upstream compared to the downstream. This is a natural trend contributed by better mixing of water due to the rapid flow in the upstream (Samat et al., 2003). Suitable DO content for most of freshwater fishes is above 5.0 mg/L (Mallya and Thorarensen, 2007). However, some species such as *Oreochromis mossambicus* (tilapia fish) can withstand low DO levels because of their respiratory adaptations by irrigating the gills with the surface layer of water where oxygen exchange with the atmosphere occurs (Senguttuvan and Sivakumar, 2002). The results of DO obtained for this study ranged from 0.92 to 4.57 mg/L, which is in agreement with the minimal requirement of low DO level by many tolerant species captured. It is also within the Interim National Water Quality Standard for Malaysia (INWQS) recommended threshold level to support aquatic life (3.0-5.0 mg/L).

Table 1. Physical parameter readings for five sampling stations in Sg. Simpang Empat reported as mean ± SE, n = 3.

Stations	St 1	St 2	St 3	St 4	St 5
Temperature (° C)	28.10 ± 0.20	28.10± 0.10	28.90±0.15	30.50±0.30	32.30±0.05
Conductivity (uS/cm)	349.00 ± 4.3	349.00 ± 3.6	360.00 ± 2.9	1151.00 ±3.8	275.00 ± 1.5
Total Dissolved Solids (mg/L)	158.40 ± 2.1	158.40 ± 0.8	161.60 ± 2.0	484 ± 5.2	117.50 ± 1.8
Dissolved oxygen (mg/L)	3.51 ± 0.08	0.92 ± 0.15	1.70 ± 0.10	1.56± 0.03	4.57 ± 0.20
pH	5.90 ± 0.05	5.70 ± 0.07	5.69 ± 0.05	5.80 ± 0.10	6.03 ± 0.08
Turbidity (FAU)	29.50 ± 0.30	83.00 ± 0.15	151.00 ± 0.40	45.50 ± 0.30	31.00 ± 0.25

The pH was found highest at Station 5 and lowest at Station 3 with 6.03 and 5.69, respectively. In general, it is difficult to determine the safe value of pH for fish due to the presence of some pollutants such as ferric hydroxide, can easily change its reactions due to slight changes in the pH value. This change might give negative impact to fish although the pH value might still be within the safe range of pH 5-9 (Boyd, 1998). In this study, pH value is highest in station 5 (pH 6.03) and lowest in station 2 (5.69). All the pH values recorded in the study area were within the INWQS recommended 5-9 range. Thus, it can be concluded that Sg. Simpang Empat is safe for fish in terms of pH level. Although fish could die at pH below 5, some rivers in Malaysia with pH less than 5.0, or even 3.0, could still provide good habitats for certain well adapted fish species (Davis and Abdullah, 1989).

Conductivity values ranged from 275 µS/cm (at station 5) to 1151 µS/cm (at station 4). Electrolytic conductivity ranging from 275-1151 mS in Sg. Simpang Empat refers to the capacity of ions in a solution to carry electrical current and is the reciprocal of the solution resistivity. Current is carried by inorganic dissolved solids, for example chloride, nitrate, sulfate and phosphate anions and cations e.g. sodium, calcium, magnesium, iron and aluminium (Hamirdin and Nordin, 2002).

The highest values for TDS were from station 4 (484 mg/L) while the lowest was from and station 5 (117.4 mg/L). The value obtained for total dissolved solids (TDS) in this study is comparatively higher than reported in other studies of TDS in river water quality, for example in Sg. Jeluh, Kajang (Hamirdin and Nordin, 2002). Total dissolved solid ranged between 14-47 mg/L in Sg. Jeluh compared to Sg. Simpang Empat (TDS ranging from 117.4 to 484 mg/L). However, the range in Sg. Simpang Empat were reported to be within the recommended INWQS value, below 1000 mg/L.

The turbidity levels in Sg. Simpang Empat ranged between 29.5 FAU (at station 1) to 151 FAU (at station 3). Turbidity values were found to exceed 50 FAU, the acceptable standard for river water quality as set by INWQS. The observed turbidity level in Sg. Simpang Empat ranged between 29.5 to 151 FAU, were above the acceptable standard for physical water quality criteria in some stations. Water with high dissolved solids is generally of inferior palatability and may induce an unfavourable physiological reaction to the consumer (Hamirdin and Nordin, 2002). However, some species of fish are much more tolerant of muddy water than others and an increase in dissolved solids can lead to an increase in the number of the resistant fish as they are freed from competition with less tolerant species (Alabaster and Lloyd, 1982).

Ammonia was found to be highest at station 4 with 0.64 mg/L. The lowest reading was recorded at Station 5 with 0.14 mg/L (Table 2). Ammonia may be hazardous to fish, especially in high concentrations. Ammonia is a common pollutant in freshwater ecosystem and is frequently found associated with organic compounds or sometimes from industrial effluents. Rapid negative effects of ammonia to fish can be observed from ammonia concentrations exceeding 0.2 mg NH_3 /L (Alabaster and Lloyd, 1982). Ammonia level in Sg. Simpang Empat exceeded the safe permissible limit recommended by (Alabaster and Lloyd, 1982) except at station 5 (0.14 mg NH_3 /L). Station 4 was found to have the highest ammonia concentrations possibly due to proximity of a cattle farm which was situated on the left bank, thus increasing the possibility of the river water being polluted with cow dung. Continuously flowing water might reduce the effect of toxicity by ammonia, however water containing phosphates and nitrates might trigger algal blooms and could cause extremely toxic effects on fish.

Table 2. Nutrient parameter readings for sampling stations in Sg. Simpang Empat reported as mean ± SE, n= 3.

Stations	St1	St2	St3	St4	St5
Phosphate (mg/L)	0.30 ± 0.02	1.66 ± 0.20	1.07 ± 0.10	0.28 ± 0.05	0.02 ±0.01
Nitrate (mg/L)	0.50 ± 0.02	0.20 ± 0.01	0.20 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Sulfate (mg/L)	2.00 ± 0.08	7.00 ± 0.05	3.00 ± 0.08	4.00 ± 0.15	4.00 ± 0.10
Ammonia(mg/L)	0.55 ± 0.02	0.51± 0.01	0.43 ± 0.03	0.64± 0.05	0.14 ± 0.02

Phosphate was found to be highest in station 2 with 1.66 mg/L, while the lowest reading was from station 5 (0.02 mg/L). The highest nitrate readings were at Station 1 (0.5 mg/L) compared to the other four stations which ranged from 0.1 to 0.2 mg/L (Table 2). Station 2 gave the highest reading of sulfate with 7 mg/L, while station 1 was the lowest (2mg/L). Sg. Simpang Empat recorded lower levels of phosphate compared to Sg. Juru (12.9 mg/L) (Anhar, 1993). The low levels of nitrate (0.1-0.5 mg/L), sulfate (2.0-7.0 mg/L) and phosphate (0.02-1.65 mg/L) showed the unreproductive river condition and with limited nutrient content (Lelek, 1985). In comparing to classification of INWQS, the levels for sulfate and nitrate were still below the maximum acceptable limit of 200 and 7 mg/L, respectively.

3.3 Heavy Metals in Fish Samples

3.3.1 Iron (Fe)

The metal concentrations and the corresponding mean standard error (expressed as µg/g dry weight) were measured in the head, gill, liver, muscle and bones of eight species of fish from Sungai Simpang Empat and the results are summarized in Table 3. From eight fish species captured in Sg. Simpang Empat, the mean iron concentrations ranged from 1.32 ± 0.61 to 81.63 ± 4.49 µg/g. According to species, *T. pectoralis* accumulated the highest levels of Fe (38.95 ±1.13µg/g), followed by *O. mossambicus* and *E.malayensis* with 31.69 ± 0.46 and 15.80 ±0.03 µg/g, respectively. In most of the fish samples collected, Fe was found to accumulate at the highest levels in the liver and the lowest in the bones (Table 3). Fe concentrations in the liver of *T. pectoralis* and *O.mossambicus* are significantly different from the other fish species (Tukey, $p<0.05$). Fe levels in the different body parts have exceeded the maximum permissible limit of 0.5 µg/g set by the Malaysian Food Act 1983 and Food Regulations 1985. However, in terms of toxicity, Fe does not pose as a high risk threat since it is a non- critical heavy metal (Fernandes et al., 2008). The liver accumulates the highest levels of Fe compared to other tissues for most of the species collected. Fe is accumulated the highest in the liver of C. *gariepinus* (Osman et al., 2010). Previous study by Nath et al., (2001) found that Fe concentrations were higher in the liver compared to muscles of *Lates calcarifer*, possibly be due to high metal-enrichment factors in the liver compared to muscles (Usero et al., 2003). Furthermore, there is a greater tendency of the element to react with oxygen carboxylate, amino group, nitrogen or sulphur of the mercapto group in the metallothionein protein, whose concentration is highest in the liver (Al-Yousuf et al., 2000). The haempoietic function of the liver and the abundant blood supply in the gut would account for the accumulation of Fe in this particular tissue (Blasco et al., 1998).

3.3.2 Zinc (Zn)

Zn concentration was highest for *O. mossambicus* (2.88±0.02 µg/g), followed by *T. pectoralis (*2.72 ± 0.02 µg/g), while the lowest Zn level was in *M. cyprinoides* (1.60 ± 0.01 µg/g). Comparison of the different body parts of fish showed that the head of *O.mossambicus* and the gills of *T.pectoralis* were found to accumulate the highest Zn concentrations (Table 3). Significant differences were found for the gill of *T. pectoralis* compared to the other fish species (Tukey, $p< 0.05$). The gills are an uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organisms (Heath, 1987).

Zn exerts cytotoxic effects on fibroblastic cell lines of fishes in high concentrations (Velma et al., 2009). At a concentration of 25 µg/g, necrosis of the hepatic cells and veil-like film formation on the gills could occur, affecting respiration and blood circulation (Clearwater et al., 2002). However, only low concentrations were present for fish in this study. The presence of Zn concentration in the liver may be due to the detoxicating mechanisms and related to heavy metal in the food (Shoham-Frider et al., 2002). Low Zn concentrations in the muscles may be due to low levels of binding protein in muscles (Allen-Gill and Martynov, 1995). Zn concentrations have not exceeded the minimum allowable limit of 100 µg/g set by the Malaysian Food Act 1983 and Food Regulations 1985, indicating the fish is safe for consumption. The range of Zn levels recorded in this study is considered to be lower than in fishes from Sg. Juru

(8.60-56.26 µg/g) and Sg. Kelang (4.70-13.80µg/g) reported by Badri and Kirana (1993).

3.3.3 Manganese (Mn)

The highest Mn level was found in *T. pectoralis* (1.32 ± 0.02 µg/g), while the lowest was in *C. striatus* (0.23 ± 0.01 µg/g). *T. pectoralis* accumulated the highest levels of Mn and also other heavy metals, possibly due to its dietary habits and widely variable habitat. *T. pectoralis* being omnivores is both an exogenous invertebrate feeder and algae feeder (Vann et al., 2004). Significant differences were found for the liver of *M. cyprinoides* and the muscle of *A.testudineus* compared to other species (Tukey, $p< 0.05$). A study by Akan et al., (2012) also showed Mn accumulation in the liver of *Lates niloticus*. In this study, Mn concentrations in most of the fish species have exceeded the allowable limit of 0.3 µg/g set by Malaysian Food Act 1983 and Food Regulations 1985. Mn is especially stored in body parts which are rich in mitochondria, for example in the liver. The effect of excessively high Mn levels in fish may result in deformations of the vertebral column (Sivaperumal et al., 2007).

3.3.4 Lead (Pb)

The results showed that Pb levels in different body parts of fish were below the permissible limit of 2.0 µg/g set by the Malaysian Food Act 1983 and Food Regulations 1985. Pb concentrations were below 1.51 µg/g in all fish body parts (Table 3). In general, the head accumulated the highest Pb levels, followed by the gills (1.03µg/g and 0.57µg/g, respectively). Excessive Pb may cause learning disabilities, decrease survival and growth rates in vertebrates (Qiao-qiao et al., 2007). Pb are known as toxic metals, implying no known function in biochemical processes (Schlenk and Benson, 2001). Pb occurrence in Sg. Simpang Empat might be due to runoffs from the nearby busy road and emissions from heavy traffic (Banat et al., 1998). Less than 0.72 µg/g Pb were measured in the muscles of all fish species (Table 3). This result is consistent with a report by Chi et al., (2007) that little Pb accumulates in the muscle of marine and freshwater fishes.

3.3.5 Chromium (Cr)

Table 3 also shows chromium levels in different body parts of the fish species at Sungai Simpang Empat. There are significant differences in Cr levels in the head of *E.malayensis* and *A.testiduneus* with other species and the liver of *T.pectoralis* and other fish species (Tukey, $p<0.05$). Cr is transported by blood to tissues and organs which have different retention capacity (Valko et al., 2005). In this study Cr level was found to be highest in the head of *E.malayensis*, whereby Cr is thought to be stored linked to proteins and smaller peptides, such as glutathionine (Valko et al., 2005). According to species, Cr level was highest in *E. malayensis* (0.64 ±0.01 µg/g), followed by *T. pectoralis* (0.60 ±0.004 µg/g). Cr appears to accumulate differently in different species, for example bluegill (*Lepomis macrochirus*) can accumulate Cr quite well. Exposure to Cr may cause decreased sodium chloride and osmolality (Van der Putte et al., 1992). The source of Cr contamination may be from stainless steel waste products and other chemical industries from nearby Bukit Minyak Industrial Area, whereby small particles of Cr occur in wastewater and air emissions.

3.3.6 Copper (Cu)

Copper concentrations for all fish species in this study ranged from 0.05 ±0.01 µg/g to 0.51 ± 0.02 µg/g (Table 3). *O. mossambicus* accumulated the highest Cu levels, followed by *T. pectoralis* and *C.batrachus* (0.23 ± 0.01, 0.21 ± 0.01 and 0.20 ± 0.01 µg/g respectively). Different species may accumulate different Cu concentrations due to difference in species sensitivity, feeding behavior and toxic actions (Lloyd, 1992). In this study, *O. mossambicus* accumulated the highest Cu concentrations which associated with its feeding behavior. *O. mossambicus* belongs to the family Cichlidae which depend on food sources from aquatic plants distributed at the bottom of the river. Thus, they are prone towards ingestion of sediment, dissolved and undissolved particles. Its habit of searching for food by shoveling sediment increase heavy metal intake in this species compared to other species (Nyandoto 2010). The accumulation of Cu can be explained by its relation to low-molecular-weight proteins (metallothionein) which are concentrated in the hepatic tissues (Ayas and Kolankaya, 1996).

The liver of *M. cyprinoides* accumulated the highest level of Cu (0.51± 0.02 µg/g), followed by the liver of *T.pectoralis* and were significantly difference compared to other fish species (Tukey, $p<0.05$). The high levels of Cu in the liver can be associated with the binding of copper to metallothionein, which serves as a detoxification mechanism (Shoham-Frider et al., 2002). The results showed that Cu levels in fish were below the permissible limit of 30 µg/g set by the Malaysian Food Act 1983 and Food Regulations 1985. Fish muscle normally contains rather low concentrations of Cu (1.0 to 10.0 µg/g). In this study, Cu concentration in the muscle is low and this is consistent with the findings by Shoham-Frider et al., (2002) in the muscle of *O. Mossambicus* in Olifants River. The accumulation of Cu in the gills (Table 3) is due to the large surface area available for adsorption and the volumes of water passing over the gills. However, in certain situations where conditions of acute copper stress occur, the response to copper is the production of mucus, which can block the gills and result in rapid death. In such instances the Cu may be bound externally by the mucus, thus high Cu levels would not occur in the tissue (Heath, 1987).

3.3.7 Nickel (Ni) and cadmium (Cd)

Ni concentration in *E. malayensis* was 0.15 ± 0.005 µg/g, followed by *C.batrachus* at 0.07 ±0.003 µg/g. The head of *E.malayensis* accumulated the highest Ni concentration (0.37 ± 0.006 µg/g) compared to the other body parts (Table 3). Ni concentration is comparatively lower than other heavy metals in this study. Toxicity effect of Ni is less severe to fish compared to other heavy metals such as Cd, Pb, Cu and Zn. Ni contamination can result in lesser gas absorbance in gills and may be fatal to fish because of oxygen deficiency. Since Ni is easily absorbed by air particles, Ni usually is prone to cause cancer to respiratory organs such as the lung (Stoeppler and Optapczuk, 1992). Ni concentration of 0.7 µg/g is considered potentially lethal to fish (Lemly, 1993). None of the samples approached this level of concern in this study and below the safety limit 0.2 µg/g set by the Malaysian Food Act 1983 and Food Regulations 1985, except for the head of *E. malayensis*, slightly exceeding the limit.

Table 3. Mean Fe, Zn, Mn, Pb, Cr, Cu, Cd and Ni levels according to different body parts of fishes captured in Sungai Simpang Empat, Penang. The bold data is the heavy metal concentrations that exceeded the permissible limit set by the Malaysian Food Act 1983 and Food Regulations 1985. The blank spaces show that the metals are not detected. Data are presented as mean ± SE of µg/g, n = 12-15 and analysed with one way ANOVA and Tukey test.

Metals	Body parts	*Trichogaster pectoralis*	*Oreochromis mossambicus*	*Anabas testudinueus*	*Clarias striatus*	*Megalops cyprinoides*	*Notopterus notopterus*	*Esomus malayensis*	*Channa batrachus*
Fe	Head	**19.49 ± 0.81**	**22.27±2.29**	**12.61 ± 0.84**	**8.86 ± 0.28**	**24.79 ± 0.86**	**5.88 ± 0.48**	**16.19 ± 2.41**	**6.06 ± 1.89**
	Liver	**81.63 ± 4.49**	**69.19±2.50**	**12.61 ± 1.15**	**22.28 ± 2.09**	**1.32 ± 0.61**	**6.69±0.88**	**20.95 ±2.37**	**27.53±1.50**
	Muscle	**51.18 ± 4.21**	**24.34 ± 2.32**	**20.59 ± 5.73**	**4.42 ± 0.53**	**3.13 ±0.55**	**6.35 ± 1.24**	**10.26 ± 2.08**	**7.70 ± 0.53**
	Gill	**33.11 ± 1.97**	**32.74 ± 3.10**	**3.35 ± 0.677**	**7.64 ± 0.71**	**8.79 ±0.85**	**13.25 ± 0.82**	**-**	**9.88 ± 0.16**
	Bone	**9.31 ± 0.44**	**8.40 ± 1.91**	**11.53 ± 3.20**	**3.58 ± 0.81**	**7.45 ± 0.50**	**-**	**-**	**3.86 ± 0.25**
Zn	Head	3.42 ± 0.43	3.61 ± 0.11	2.46 ± 0.18	2.67 ± 0.12	2.89 ± 0.03	2.94 ± 0.30	3.33 ± 0.22	1.47 ± 0.02
	Liver	3.25 ± 0.39	2.83 ± 0.19	1.65 ± 0.17	1.73 ± 0.02	0.06 ± 0.01	1.33 ± 0.13	2.34 ± 0.07	1.88 ± 0.02
	Muscle	1.27 ± 0.19	2.9 ± 0.16	1.45 ± 0.02	1.80 ± 0.01	1.35 ± 0.19	2.63 ± 0.25	2.23 ± 0.07	1.45 ± 0.02
	Gill	3.61 ± 0.48	2.38 ± 0.23	1.92 ± 0.02	1.63 ± 0.03	1.84 ± 0.27	1.94 ± 0.19	-	1.33 ± 0.3
	Bone	2.06 ± 0.45	2.70 ± 0.04	1.52 ± 0.004	2.36 ± 0.11	1.84 ± 0.26	-	-	1.52± 0.004
Mn	Head	**1.24 ± 0.01**	**1.35 ± 0.08**	**1.60 ± 0.04**	**0.56 ± 0.12**	**0.51 ± 0.15**	**0.47 ± 0.04**	**1.23 ± 0.18**	**0.67 ± 0.08**
	Liver	**1.34 ± 0.02**	**2.37 ± 1.78**	0.13 ± 0.02	0.03 ± 0.02	**3.01 ± 1.21**	0.04 ± 0.01	0.13 ± 0.03	**0.86 ± 0.06**
	Muscle	**1.23 ± 0.01**	**0.83 ± 0.01**	**2.40 ± 0.21**	0.10 ± 0.05	0.003 ± 0.002	0.30 ± 0.04	0.17 ± 0.06	0.04 ± 0.01
	Gill	**1.96 ± 0.05**	**0.91 ± 0.04**	**0.86 ± 0.07**	0.06 ± 0.01	0.25 ± 0.12	0.29 ± 0.04	-	**0.86 ± 0.07**
	Bone	**0.83 ± 0.03**	**0.78 ± 0.04**	**0.70 ± 0.08**	0.24 ± 0.08	0.04 ± 0.02	-	-	0.12 ± 0.008
Pb	Head	1.18 ± 0.11	1.51 ± 0.09	1.34 ± 0.15	1.12 ± 0.09	1.03 ± 0.16	0.70 ± 0.03	0.97 ± 0.02	0.26 ± 0.06
	Liver	0.97 ± 0.03	0.52 ± 0.01	0.22 ± 0.05	0.49 ± 0.07	0.17 ± 0.03	0.27 ± 0.02	0.38 ± 0.03	0.56 ± 0.04
	Muscle	0.19 ± 0.05	0.72 ± 0.02	0.34 ± 0.11	0.51 ± 0.04	0.23 ± 0.05	0.50 ± 0.03	0.46 ± 0.02	0.30 ± 0.06
	Gill	1.03 ± 0.04	0.88 ± 0.01	0.27 ± 0.07	0.74 ± 0.09	0.37 ± 0.09	0.56 ± 0.02	-	0.72 ± 0.04

	Bone	0.87 ± 0.06	0.93 ± 0.01	0.38 ± 0.10	0.86 ± 0.04	0.48 ± 0.05	-	-	0.30 ± 0.04
Cr	Head	0.49 ± 0.05	0.29 ± 0.04	0.78 ± 0.04	0.35 ± 0.03	0.35 ± 0.03	0.64 ± 0.04	1.05 ± 0.09	0.35 ± 0.02
	Liver	1.03 ± 0.03	0.66 ± 0.05	0.42 ±0.03	0.41 ± 0.03	0.23 ±0.01	0.57 ± 0.02	0.43 ± 0.05	0.51 ± 0.02
	Muscle	0.56 ± 0.05	0.60 ± 0.06	0.70 ± 0.05	0.40 ± 0.02	0.22 ± 0.01	0.60 ± 0.02	0.44 ± 0.03	0.47 ± 0.02
	Gill	0.45 ± 0.05	0.42 ± 0.05	0.45 ± 0.02	0.34 ± 0.05	0.37 ± 0.03	0.58 ± 0.03	-	0.42 ± 0.02
	Bone	0.48 ± 0.05	0.41 ± 0.05	0.56 ± 0.04	0.38 ± 0.04	0.57 ± 0.03	-	-	0.26 ± 0.02
Cu	Head	0.13 ± 0.02	0.23 ± 0.04	0.34 ± 0.05	0.12 ± 0.01	0.18 ± 0.01	0.10 ± 0.03	0.20 ± 0.01	0.08 ± 0.01
	Liver	0.46 ± 0.05	0.38 ± 0.06	0.26 ± 0.03	0.26 ± 0.01	0.51 ± 0.02	0.14 ± 0.02	0.17 ± 0.01	0.24 ± 0.03
	Muscle	0.16 ± 0.03	0.20 ± 0.04	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.007	0.10 ± 0.008	0.28 ± 0.04
	Gill	0.16 ± 0.03	0.22 ± 0.02	0.08 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.15 ± 0.005	0.18 ± 0.009	0.32 ± 0.06
	Bone	0.11 ± 0.02	0.12 ± 0.02	0.18 ± 0.01	0.10 ± 0.01	0.04 ± 0.01	-	-	0.10 ± 0.01
Cd	Head	0.04 ± 0.001	-	0.004 ± 0.0004	0.009 ± 0.0002	0.002 ±0.00003	0.006 ± 0.0007	0.005 ± 0.0002	0.0006 ± 0.00005
	Liver	0.05 ± 0.003	0.005 ± 0.004	0.018 ± 0.002	0.007 ± 0.0008	0.03 ± 0.003	0.004 ± 0.0007	0.005 ± 0.0003	0.0003 ± 0.0002
	Muscle	0.002 ± 0.0005	0.002 ± 0.0004	0.004 ± 0.0007	0.002 ± 0.001	0.004 ± 0.0009	0.004 ± 0.0007	0.005 ± 0.0009	0.001 ± 0.0002
	Gill	0.002 ± 0.0006	0.012 ± 0.003	0.003 ± 0.0002	0.009 ± 0.004	0.006 ± 0.001	0.004 ± 0.0006	-	0.0001 ± 0.00003
	Bone	0.002 ± 0.001	0.05 ± 0.002	0.006 ± 0.002	0.004 ± 0.0002	0.004 ± 0.001	-	-	0.001 ± 0.0002
Ni	Head	0.05 ± 0.008	0.07 ± 0.007	0.04 ± 0.006	0.07 ± 0.005	0.04 ± 0.01	0.02 ± 0.003	0.37 ± 0.006	0.04 ± 0.005
	Liver	0.11 ± 0.007	0.06 ± 0.007	0.03 ± 0.004	0.05 ± 0.004	0.16 ± 0.003	0.06 ± 0.008	0.05 ± 0.005	0.16 ± 0.08
	Muscle	-	0.01 ± 0.003	0.16 ± 0.02	0.03 ± 0.002	0.02 ± 0.003	0.08 ± 0.04	0.04 ± 0.002	0.03 ± 0.003
	Gill	-	0.03 ± 0.003	0.03 ± 0.008	0.02 ± 0.009	0.02 ± 0.005	-	-	0.06 ± 0.009

Cadmium concentration was the lowest among eight heavy metals tested in various fish species and tissues. The highest concentration is not more than 0.05 µg/g (Table 3). Cd level was highest in *T.pectoralis*, followed by *O. mossambicus* (Table 3). Cd is a serious environmental contaminant that could be transported atmospherically. In fish, it can cause anemia and vertebral fractures, osmoregulatory problems, decreased digestive efficiency, hematological and biochemical effects, growth deficits, erratic swimming and mortality (Levit, 2010). However, since Cd concentrations in this study are below the maximum permissible limit of 1.00 mg/l set by Malaysian Food Act 1983 and Food Regulations 1985, it does not contribute as a major threat for fish collected from Sg. Simpang Empat. In body parts of the majority of fish species, the liver of *T. pectoralis* accumulated the highest concentrations of Cd. There are significant differences in cadmium levels in fish body parts (Tukey, $p<0.05$). Almost all body parts absorbed some Cd, but the highest amount was invariably found in the liver. Roughly, one-third of the body burden of Cd is stored in the liver (Levit, 2010) Once absorbed by the body, Cd tends to concentrate in the liver by a low molecular weight protein called *thionein*. This protein contains large number of sulfhydryl groups, which attract Cd as well as other heavy metals such as Zn and Cu (Levit, 2010). The muscles accumulated Cd at a range of 0.001-0.004 µg/g. This is comparable with findings by (Mushrifah et al., 1994) for fish from Taman Negara, in which Cd concentrations in fish muscles ranged from 0.00 to 0.21 µg/g and regarded as not polluted.

3.4 Correlation Analysis of Heavy Metals in Different Body Parts of Different Fish Species

The metal accumulation in different organs depends on their physiological role, behavior and feeding habits, as well as regulatory ability (Clearwater et al., 2002). Significant positive correlations were found between gills and liver, gills and muscle, gills and head and gills and bones of *T.pectoralis* and *O.mossambicus* for Mn, Cr, and Cu ($r> 0.7$, $p< 0.001$) as shown in Table 4. Four possible routes for a substance to enter a fish are through the gills, food, water and skin (Jezierska and Witeska, 2006). Metal uptake through the gills is by simple diffusion possibly through the pores (Jezierska and Witeska, 2006). Metal concentration in the gill could be due to the element complexion with the mucus such that it is impossible to completely remove from them from the lamellae (Romeo et al., 1999). Heavy metals might enter first through the gills, as these are the body parts associated with respiration, possibly metals entering through the water medium Kamaruzzaman et al., 2010).

Significant correlations were found between the liver and muscle, and the liver and the gill, liver and bones and liver and head of *T.pectoralis* for Mn, Cu, and Cr ($r > 0.7$, $p< 0.001$). The accumulation in the liver might be through the food source. Usually, food source of fishes such as nutrients, vascular plants, crustaceans and prawns entered the fish through the mouth, leading to the digestive tract system and eventually will accumulate in the liver (Romeo et al., 2009). Heavy metals accumulate mainly in the metabolic organs such as liver that stores metals to detoxicate by producing metallothioneins (Al-Yousuf et al., 2000). Eventually, metals in the liver will move to other body parts, particularly the head, bones and muscles for storage.

Metals concentration in muscles is of concern as these are the edible parts of the fish for human consumption. Heavy metal concentrations are relatively lower in the muscles compared to the gills and liver. The muscles are not known to be an active body part in accumulating heavy metals (Romeo et al., 1999). The possible directions of movement of a pollutant after it has been absorbed into the bloodstream of a fish is through the liver, kidneys and to the muscles as final storage (Jezierska and Witeska, 2006) thus explaining the correlations between the liver and muscle.

4. Conclusion

Sungai Simpang Empat can be categorized as not being highly polluted and is still within the safety limit for potable water usage. The physico-chemical parameters such as dissolved oxygen, conductivity, pH and turbidity were still at suitable levels for survival of different species of fish. The concentration of heavy metals in fish is in the following descending order: Fe followed by Zn, Mn, Pb, Cr, Cu, Ni and Cd respectively. Potentially toxic elements, namely Pb, Cd, Ni and Cu have not exceeded the allowable limit set by the Malaysian Food Act 1983 and Food Regulations 1985. However, Fe concentrations had exceeded the allowable limit of 0.5 µg/g for all of the fish captured in the study river, while Mn had exceeded the limit of 0.2 µg/g in some of the fish samples collected.

Most of the fish tend to accumulate metals at the highest levels in the liver, probably due to food intake and sediment accidently digested. Gill also accumulates metals since it is often exposed to pollutants through the respiratory organs. The head, bones and muscles might act as terminal storage organs for metals accumulation. However, heavy metal concentration in the muscles are low in most of these species, hence it is safe for human consumption. *T. pectoralis* and *O. mossambicus* were the two species which accumulated the highest level of metal concentrations in Sungai Simpang Empat, thus they are considered as good potential indicator species for the study river. Significant correlations were found among the various fish body parts of different fish species.

Various industries in Bukit Minyak Industrial Area and industrial effluents might be the potential source of heavy metal concentrations in Sungai Simpang Empat. Thus, efficient management through on-going monitoring should be practiced in the study area. In addition, future studies on heavy metals in this river may include analyzing the metal concentrations in the sediment and other components of biota such as in benthos and aquatic plants.

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Table 4. Correlation analysis of heavy metals in various body parts of fish (p<0.001 and p<0.05). Significant correlations mentioned in the text are shown in bold.

	n	Fe	Zn	Mn	Pb	Cr	Cu	Ni	Cd
T. pectoralis									
Head-liver	15			**0.915**	0.899	**0.983**	**0.972**	**0.952**	
Head-gill	12		0.962	**0.962**	**0.945**	**0.995**	**0.959**	**0.852**	
Head-muscle	15		0.885	0.922	0.964	0.914	0.679	0.937	
Head-bone	15		0.865	0.905	0.943	0.994	0.951		
Liver-muscle	15		0.552	**0.925**	0.888	**0.925**	**0.779**	**0.918**	
Liver-gill	12			**0.957**	**0.888**	**0.983**	**0.977**	0.943	0.687
Liver-bone	12		0.573	**0.915**	0.797	**0.983**	**0.962**	**0.906**	
Muscle-gill	12		0.871	**0.934**	**0.887**	**0.909**	**0.701**		
Muscle-bone	12		0.968	0.910	0.971	0.926	0.675	0.919	
Gill-bone	12		0.857	**0.955**	**0.816**	**0.991**	**0.978**	0.634	0.787
O.mossambicus									
Head-liver	15			**0.913**	0.812	**0.762**	**0.989**		
Head-gill	12	-0.998	0.843	0.865	0.700	0.874	0.755		
Head-muscle	15		0.752			0.579	0.729	0.695	
Head-bone	12	-0.998	0.691	0.721	0.643	0.851	0.899		
Liver -muscle	12		-0.610	0.591	0.537	0.715	0.797		
Liver -gill	15		-0.565	**0.945**		**0.918**	**0.785**	0.636	
Liver -bone	12		-0.688	0.830		0.945	0.938		
Muscle-gill	12		0.654	**0.713**		**0.713**	**0.972**		
Muscle-bone	12		0.636	0.693		0.736	0.829		
Gill-bone	12	1.000	0.897	**0.857**	0.547	**0.993**	**0.849**		
A.testudineus									
Head- liver	12		0.863		0.962	0.964	0.935	0.660	
Head-gill	12		0.734	0.745	0.876	0.972	0.809	0.874	
Head-muscle	12		0.981	0.882	0.984	0.990	0.912		
Head-bone	12		0.985	0.926	0.924	0.990		0.845	
Liver -muscle	12		0.940	0.561	0.981	0.956	0.627	0.763	
Liver -gill	12		0.585	0.560	0.919	0.957	0.842	0.704	
Liver -bone	12		0.932		0.932	0.974			
Muscle-gill	12		0.727	0.581	0.937	0.976	0.791		
Muscle-bone	12		0.997	0.926	0.965	0.983	0.786		
Gill-bone	12		0.717	0.670	0.982	0.977	0.520	0.811	

C.striatus								
Head- liver	12	0.873		0.973	0.694	0.718	0.845	
Head-gill	12	0.836	0.748	0.938	0.857		0.845	0.914
Head-muscle	12			0.829	0.745		0.781	
Head-bone	12	0.626	0.930	0.912	0.851			
Liver -muscle	12		0.691	0.851	0.685		1.000	
Liver -gill	12			0.947	0.841	0.815	0.899	
Gut-bone	12	0.782	0.810	0.931	0.788	0.744		
Muscle-gill	12		0.707	0.642	0.855			
Muscle-bone	12		0.627	0.950	0.889			
Gill-bone	12		0.844	0.786	0.983	0.838		
M.cyprinoides								
Head- liver	12		0.995	0.707	-0.711			
Head-gill	12		0.995	0.947		0.963		
Head-muscle	12			0.942		0.860		
Head-bone	12		0.925	0.928	0.984	0.920		
Liver -muscle	12	0.757		0.840			-0.816	
Liver -gill	12		0.996	0.776				
Liver -bone	12		0.933	0.842				
Muscle-gill	12			0.986		0.884		
Muscle-bone	12			0.924	-0.687	0.927		
Gill-bone	12	-0.713	0.927	0.928		0.970		
N.notopterus								
Head- Liver	12		0.885	0.779	0.876	0.956	0.633	
Head-gill	12	0.889	0.954	0.862	0.686	0.966		
Head-muscle	12	0.752	0.858	0.909	0.582	0.904		
Liver -muscle	12		0.639	0.587	0.835	0.935		
Liver -gill	12		0.774	0.623	0.780	0.853		
Muscle-gill	12		0.963	0.782	0.659	0.953		
E.malayensis								
Head- Liver	15	0.873	0.823	0.793		0.569		
Head-muscle	15		0.985			0.591		
Gut-muscle	15		0.860		0.926	0.917	0.824	

C.batrachus							
Head- Liver	12	0.981	0.958	0.752	0.852		
Head-gill	12	0.592	0.939	0.867	0.976		
Head-muscle	12	0.587		0.752	0.950		0.771
Head-bone	12		0.828		0.919		
Liver -muscle	12	0.610	0.612	0.859	0.708	0.936	
Liver -gill	12		0.883	0.872	0.850	0.948	
Liver -bone	12		0.881		0.926	0.900	
Muscle-gill	12	0.895		0.915	0.950	0.969	
Muscle-bone	12	0.862		0.790	0.825	0.876	0.910
Gill-bone	12	0.797	0.800		0.931	0.883	

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