ASSESSMENT OF METAL VARIATIONS IN WATER, SEDIMENT, AND FISH SPECIES (*PANGASIUS SP*. AND *HEMIBAGRUS SP*.) FROM SELANGOR RIVER, PAHANG RIVER AND NEARBY AQUACULTURE PONDS

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ASSESSMENT OF METAL VARIATIONS IN WATER, SEDIMENT, AND FISH SPECIES (*PANGASIUS SP.* AND *HEMIBAGRUS SP.*) FROM SELANGOR RIVER, PAHANG RIVER AND NEARBY AQUACULTURE PONDS

ABSTRACT

This study aims to evaluate the spatial distributions of metal elements in water, sediments, and fish species (*Pangasius* sp. and *Hemibagrus* sp.) sampled from Selangor River, Pahang River and nearby aquaculture ponds, and to assess the safety of the fish consumption. The concentrations of Na, Mg, K, Ca, Cr, Fe, Ni, Cu, Zn, As, Se, Cd, and Pb in those samples were determined using the microwave-assisted digestion-inductively coupled plasma-mass spectrometric method which has been verified with a series of certified reference materials. The natural clustering tendency corresponding to metal variability was explored using principal component analysis and hierarchical cluster analysis. The results revealed that the metal variability in the waterbody and sediment samples mostly originated from human activities around the sampling sites, in addition to natural variations. Such variations were also reflected on the metal accumulation pattern in the sampled fish, although the variations between different tissues were mainly subjected to their metabolic activities. Based on the findings, *Hemibagrus* sp. was suggested as a potential bioindicator for hazardous metal pollution. In risk and safety assessment, the metal concentrations in the edible muscle of the fish samples were found to be below the established limits, although target hazard quotient of As and Se in wild *Hemibagrus* sp. from Selangor River approached unity.

Keywords : patern recognition, principal component analysis, risk assessment, fish

PENILAIAN KE ATAS VARIASI LOGAM DALAM AIR, SEDIMEN, DAN SPESIS IKAN (*PANGASIUS* SP. DAN *HEMIBAGRUS* SP.) DARI SUNGAI SELANGOR, SUNGAI PAHANG DAN KOLAM TERNAKAN BERDEKATAN

ABSTRAK

Kajian ini bertujuan untuk menilai penyebaran elemen logam dalam air, sedimen dan spesis ikan (*Pangasius* sp. dan *Hemibagrus* sp.) dari persampelan Sungai Selangor, Sungai Pahang dan kolam akuakultur berdekatan, dan untuk menilai tahap keselamatan penggunaan ikan. Kepekatan bagi Na, Mg, K, Ca, Cr, Fe, Ni, Cu, Zn, As, Se, Cd dan Pb dalam sampel-sampel tersebut ditentukan dengan kaedah percernaan dengan bantuan mikro-plasma gandingan teraruh-jirim spektrometri di mana telah di sah betulkan dengan siri bahan rujukan. Kecenderungan kluster yang sepadan dengan kepelbagaian logam diterokai menggunakan analisis komponen utama dan analisis kluster hierarki. Hasil dapatan kajian menunjukkan bahawa kepelbagaian logam dalam jasad air, dan sedimen kebanyakannya berasal dari aktiviti manusia di sekitar kawasan persampelan, di samping variasi semulajadi. Variasi sedemikian juga menggambarkan corak akumulasi logam dalam sampel ikan, walaupun perbezaan variasi antara tisu adalah disebabkan oleh aktiviti metabolik ikan. Berdasarkan hasil kajian, *Hemibagrus* sp. telah dicadangkan sebagai bioindikator yang berpotensi untuk pencemaran logam berbahaya. Dalam penilaian risiko dan keselamatan, kepekatan logam dalam tisu otot sampel ikan di dapati berada di bawah had yang ditetapkan, walaupun sasaran bahaya dari As dan Se menunjukkan *Hemibagrus* sp. dari Sungai Selangor menghampiri nilaian 1.

Kata Kunci : corak taburan, analisis komponen utama, analisis risiko, ikan

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percent
ºC	:	Degree Celsius
µm	:	Micrometers
ABS	:	Absorption into blood stream
ADI	:	Acceptable daily intake
ANOVA	:	Analysis of variance
As	:	Arsenic
AT	:	Average time
BCF	:	Bioconcentration factor
BCR-146R	:	Sewage sludge certified reference material
BSAF	:	Bio-sediment accumulation factor
BW	:	Body weight
Ca	:	Calcium
Cd	:	Cadmium
CDI	:	Chronic daily intake
CF	:	Condition factor
C_{fish}	:	Concentration of the metal elements in the muscle of fish
cm	:	Centimeter
C_m	:	Metal concentration in water
CP	:	Aquaculture pond
Cr	:	Chromium
CRM	:	Certified reference material
C_s	:	Mean concentration of metal in the sediment
Cu	:	Copper

C_{water}	:	Concentration of the metal elements in water
Cx	:	Mean concentration of metal in the muscle tissue
DO	:	Dissolved oxygen
DOLT-4	:	Dogfish liver certified reference material
DORM-3	:	Dogfish muscle certified reference material
ED	:	Exposure duration
EDI	:	Estimated daily intake
EF	:	Enrichment factor
EF	:	Exposure frequency
Fe	:	Iron
FIR	:	Fish ingestion rate
g	:	Gram
$g\ L^{-1}$	:	Gram per litre
g/mm^3	:	Gram per cubic meter
HCA	:	Hierarchical cluster analysis
HNO_3	:	Nitric acid
H_2O_2	:	Hydrogen peroxide
HCI	:	Hydrochloric acid
HRI	:	Health risk index
HSI	:	Hepatosomatic index
I_{AD}	:	Administered dose
ICP-MS	:	Inductively coupled plasma-mass spectrometry
I_{geo}	:	Geoaccumulation index
I_{ID}	:	Intake dose
ISTD	:	Internal standard
I_w	:	Average daily intake of water

K	:	Potassium
kg	:	Kilogram
MAD	:	Microwave assisted digestion
MDL	:	Method detection limit
Mg	:	Magnesium
mg kg·day^{-1}	:	Milligram per kilogram per day
mg kg^{-1}	:	Milligram per kilogram
mg L^{-1}	:	Milligrams per litre
mg/kg bw/day	:	Milligram per kilogram of body weight per day
min	:	Minute
mL	:	Milliliter
n	:	The number of sample
Na	:	Sodium
Ni	:	Nickel
NWQS	:	National water quality standards
ORS	:	Octopole reaction system
Pb	:	Lead
PC	:	Principal component
PCA	:	Principal component analysis
PEC	:	Probable effect concentration
PMTDI	:	Provisional maximum tolerable daily intake
PWTI	:	Provisional tolerable weekly intake
RF	:	Radio frequency
RfD	:	Reference dose
RR	:	Retention rate
RSD	:	Relative standard Deviation

Se	:	Selenium
SLRS-4	:	River water certified reference material
SQGs	:	Numerical sediment quality quidelines
TA	:	Average time of exposure
TDS	:	Total dissolved solid
TEC	:	Threshold effect concentration
TH	:	Target hazard
THQ	:	Target hazard quotient
UPW	:	Ultra pure water
W_b	:	Average body weight
WL	:	Liver weight
WT	:	Body weight
Zn	:	Zinc
µg kg-1	:	Microgram per kilogram
µS cm^{-1}	:	Micro-Siemens per centimeter
C	:	Concentration at exposure point
CR	:	Contact rate

CHAPTER 1: INTRODUCTION

1.1 Environmental Quality

Indicators of environmental quality encompass a number of environmental aspects such as water, soil, air and biotic life. As an example, one of the fundamental human needs is access to clean water and food (Streimikiene, 2015). Freshwater play a vital role for human needs, therefore its quality is a matter of global concern (Priscoli, 1998). For example, rivers perform a variety ecological function like a water transport, agricultural purposes, aquaculture, domestic used and tourism. Based on different research and policy objectives, various ecological functions of freshwater especially rivers have been studied and explored, which include hydrology (Jacobson & Jacobson, 2013; Ikem & Adisa, 2011), water quality (Ocampo-Duque *et al.*, 2013; Zhang *et al.*, 2012), sediment quality (Nilin *et al.*, 2013; Yang *et al.*, 2012), vegetation study (Cui *et al.*, 2013) and animal dynamics research (Ayllón *et al.*, 2012).

However, the changes in water quality have been reported due to industrial activities, urban development and agricultural waste, and also, (Islam *et al.*, 2015; Li *et al.*, 2011) through the natural processes such as volcanism and bedrock weathering (Kumar *et al.*, 2017; Meng *et al.*, 2016). Apart from that, all the mentioned activities can put a greater pressure and impair the quality of the water resources due to release of trace elements into the fluvial aquatic system (Misaghi *et al.*, 2017).

Furthermore, anthropogenic activities could change the hydrodynamic conditions of rivers, thus inducing sediment re-suspension or suspended sediment deposition. This will further affect the characteristics, concentrations and fluxes of suspended sediments in the water column (Dai & Liu, 2013; Meybeck *et al.*, 2004).

Introduction of organic pollutants from wastewater can also cause accumulation in river sediment and excessive degradation of the organic matter will consume dissolved oxygen at a higher rate which can result in hypoxia of the sediment (Liu *et al.*, 2017). Rivers are essential in the transport of water, sediment, and nutrients from terrestrial to marine ecosystem. However, concentrations and characteristics of suspended sediments in rivers not only influence the fates of pollutants and heavy metals but also affect their transport from land to water column (Dong *et al.*, 2013; Xia *et al.*, 2013; Wölz *et al.*, 2010). Information on the quality of aquatic environment by knowing their physical, chemical, biological and ecological characteristics are very useful for water use. Therefore, it is important to undertake monitoring to ensure that water resources and their quality stay within the acceptable limits.

1.1.1 Metal Pollutions

Metal pollution in river systems is a common environmental problem due to rapid industrialization, rapid demographic growth and economic evolution (Staley *et al.*, 2015; Hudson-Edwards *et al.*, 2001). Trace elements can be strongly accumulated and biomagnified in the water, sediment and aquatic food chain, while under certain conditions they are able to accumulate up to hazardous levels (Monferran *et al.*, 2016; Yi *et al.*, 2011). Enrichment of trace elements in water columns can result water being unsuitable for drinking as well as for industrial and agricultural purposes (Zhang *et al.*, 2009; Nazeer *et al.*, 2014). Additionally, these elements can be absorbed by aquatic organisms through the food chain, which lead to severe health risk (Pekey *et al.*, 2004). It has been revealed that considerable intake of metals can lead to mental-problems and cancers (Li *et al.*, 2011).

Pollution of trace elements in water system has been identified as an environmental problem due to potential threats to human health and aquatic ecosystem (Nan *et al.*, 2016; Farahat & Linderholm, 2015; Giri & Singh, 2013). Thereby, it is important to understand the distribution, sources, and health risk of these elements in order to protect water resources and control water pollution (Islam *et al.*, 2014; Xiao *et al.*, 2014). Moreover, the concentration of metals in sediments and biota can reach to a greater magnitude than in the overlying water due to the geochemical and biochemical processes (Bryan & Langston, 1992). Due to bioaccumulation and biomagnification process, measuring only the level of metal concentrations in water column and sediments does not provide adequate information to address the actual threats to biota and human as well (Maceda-Veiga *et al.*, 2013). Therefore, monitoring the total concentrations of metals in water, sediment and biota are important in environmental assessments.

1.2 Background of Sampling Site

In Malaysia, increasing metal pollution has been discovered in freshwater fishes; however, data and publications on metal concentrations in freshwater fish are still limited. It has been indicated that studies on metal pollution in fishes started in early 1979 by Babji *et al.*, (1979) in West Malaysia. Data from several researches have demonstrated the distribution trend of metals in the fishes increased yearly (Tukimat *et al.*, 2006; Tukimat *et al.*, 2002). However, based on the wide range of availability of fish species, not many researchers had measured the concentration of metal for comparison between two habitats (aquaculture and wild-caught species) for the same species.

The difference between cultured and wild-caught fish are always a subject of argument. For an example, some researchers have reported their differences in term of chemical composition and nutritional value (Dincer *et al.*, 2010; Yildiz *et al.*, 2008;

Grigorakis *et al.*, 2002). Studies have confirmed that fatty acid compositions and diet intake have been identified as the main cause for the differentiation between cultured and wild-caught fish species (Grigorakis *et al.*, 2002; Chen *et al.*, 1995; Van Vliet *et al.*, 1990). Previous research has established that cultured fish can be categorized by higher fat and *n*-6 fatty acid contents compared to the wild-caught due to the presence of *n*-6 fatty acid in the fish feed formulations (Busetto *et al.*, 2008). However, there are also many factors that contribute to the variations such as seasonal and biological differences (species, size, dark/white muscle, age, sex and sexual maturity), food source, environmental conditions (water chemistry, salinity, temperature and contaminants), production technologies and catching area (Zeynali *et al.*, 2009; Alasalvar *et al.*, 2002). Therefore, to close the gap in scarcity of data, a research on the metal accumulation in cultured and wild-caught fish species is needed.

1.2.1 Water

Water quality is expressed by its physical, chemical, and microbiological characteristics (Rajeshwari & Saraswathi, 2009). Traditional approach that is usually applied in assessing water quality is based on comparison of experimental parameters with the existing guidelines. This methodology allows the identification of contamination sources and can help in checking legal compliance. However, it does not give an overall view of water quality in aquatic system (Debels *et al.*, 2005). Therefore, appropriate approaches are needed to describe the characteristics of water quality in aquatic environments.

A good water quality assessment method should not only provide the water quality status, but also reflect the spatial and temporal alteration of water quality condition (Li *et al.*, 2016). Good water quality in fish ponds is advantageous to the fish by serving plankton as foods and buffering harmful matters, while decrease in water quality can easily result in lowering fish production. For these ponds however, resulting effluents can potentially be harmful to the environment, although in certain cases, these effluents have been environmentally neglected (Xu & Boyd, 2016). Therefore, proper assessment of pond water quality is essential to improve fish yield and at the same time protect the ecosystem.

1.2.2 Sediments

Sediments consist of inorganic and organic particles with complicated characteristics of physical, chemical and biological aspects (Kumar Sarkar *et al.*, 2004). Sediments can act as an adsorptive sink with metal which concentrations are many times greater than in the water column. Therefore, sediments are classified as an appropriate matrix to evaluate and monitor level of contamination in the aquatic environment (Kalantzi *et al.*, 2013; Cukrov *et al.*, 2011; Giarratano & Amin, 2010).

Most research on sediments analysis gives several advantages, notably on providing the time averaged values for contaminants richness (Mendiguchía *et al.*, 2006). However, measuring metals concentrations either in sediment and water does not provide full information on the risk posed by metal bioaccumulation and bioamagnification (Maceda-Veiga *et al.*, 2013; Ricart *et al.* 2010). Therefore, determination of metals in fish can provide more useful information to monitor metals in the aquatic environment.

1.2.3 Fish

Monitoring contaminants in fish can give concise information on the water quality status than only monitoring of water column and sediments (Tekle-Giorgis *et al.*, 2016). Previous research has established that fish are able to accumulate metals many times higher in concentration than that present in water column and sediment (Olaifa *et al.*, 2004). Fishes are able to accumulate contaminants from their diet and surrounding water, then, deposit them in their organs and skeletal tissues (Alasalvar *et al.*, 2002). Information on the metal concentration in biotic species is important because they can create serious environmental health hazards (Sfakianakis *et al.*, 2015).

The evaluation of metals in the food-chain is also used as an indicator to know the metals transfer to the human body through the fish consumption (Arun Kumar & Achyuthan, 2005). According to Paquin *et al.* (2003), they suggested that possible ways of metals accumulation in fishes can be studied through direct intake of food and water on the polluted environment. Metals entering the fish through gills are able to accumulate in fish tissues and the excessive amount can build up to a toxic level (Arun Kumar & Achyuthan, 2005).

1.2.3.1 *Pangasius* sp.

Pangasius is a freshwater predatory fish, which belongs to the Pangasiid family. This fish species is categorised as an omnivorous species based on low protein (12.6 – 15.6%) and fat (1.3-3.0%), but high content of water (80-85%) (Orban *et al.*, 2008). *Pangasius* sp. is also referred to as sutchi catfish and striped catfish and is an economically valuable freshwater aquaculture fishery product in South-East Asia.

In European countries, this fish species is mainly marketed as skinned and boneless frozen fillets (Karl *et al.*, 2010). The success in the cultivation of this species is explained well by their capability to be reproduced in captivity, fast growing and tolerance at low concentration of oxygen (Andrieu *et al.*, 2015). Due to rising popularity and intake of this species, it is important to evaluate the metal concentration in order to understand the risk assessment from its consumption better.

1.2.3.2 *Hemibagrus* sp.

Tropical bagrid catfish (*Hemibagrus* sp.), known as omnivorous freshwater fish (Deng *et al.*, 2011) forms a significant part of Malaysian reservoir fisheries (Khan *et al.*, 1990). However, this species also can be found in the rivers of Indonesia, and other Asian countries like Thailand and Vietnam. This species is categorised as the largest bagrid catfish in Asia and can reach 80 kg in wild habitat (Ng & Rainboth, 1999), where their body length could increase up to 3.25 cm in 6 weeks and the growth rate can be up to 10.23 % per day (Amornsakun *et al.*, 2000).

Due to its taste, high protein content and non-bony flesh, this species is commercially important for aquaculture sector (Muchlisin *et al.*, 2009). Information on metal concentration levels related to this species in Malaysia is still limited although they are one of the important freshwater fisheries sector (Mohsin & Ambak, 1983). This present study will provide data about the *Hemibagrus* sp. and ascertain the risks in consuming it.

1.3 Bioaccumulation, Bioconcentration and Biomagnification of Metals

Contaminants present in the aquatic environments can accumulate in the tissues of organisms via various route and mechanisms to the extent that they create toxic effects. Therefore, it is of great concern to know the possibility of these contaminants to accumulate in the body of the two fish species (Palaniappan & Karthikeyan, 2009). There are many different ways of determine the definition of bioaccumulation. Normally, it is defined as the simple uptake of substances from the environment or the ability of substances to accumulate overtime or retention of the substance (Meador, 2006). For the fish species, they are able to bioaccumulate metals ingested with food and bioconcentrate metals from water (Maule *et al.*, 2007). Bioconcentration is known as the increase in the concentration level of a certain substance in the organism tissue relative to the concentration of the substance in the water column in which they are exposed (USEPA, 2000).

In aquatic systems, accumulation of metals in fish suggests that this biotic species can be an important and useful indicator for metal pollution due to their response sensitivity to the changes in aquatic environment (Aas *et al.*, 2001; Mondon *et al.*, 2001). Apart from that, fish can be an early warning indicator of aquatic pollution because they are significant to human as a protein source (Murtala *et al.*, 2012). The metal accumulation in fish normally depends on the physico-chemical parameters of water quality and ecological factors. Moreover, studies using several fish species with different habitats have revealed that physiological conditions, trophic position, age, body size, feeding behaviour, sex and spawning capability will also influence the bioaccumulation pattern of metals (Farkas *et al.*, 2003).

Among many attributes available to characterize the routes of metal distribution, the biological concentration factor (BCF) has proven very important as far as the characteristics and distribution of metals in aquatic systems is concerned (Barron, 1990). Bioaccumulation factor (BAF) refers to the ratio of the internal concentrations due to exposures (field or laboratory studies), that is, to the amount of substances taken up by an organism from water (bioconcentration) and ingestion (diet and inhalation) (Shenker *et al.*, 2011; USEPA, 2010). In other words, bioconcentration and bioaccumulation are known as processes by which the concentration of elements overcome their concentrations in the surrounding environment (Grisoni *et al.*, 2015). Bioconcentration occur mainly through exposure via non-dietary routes like respiratory and dermal contact, while bioaccumulation refers to all potential routes. The combination between these two processes within the food chain will created the biomagnification process, for example the level of element concentration in the food chain will increase with increasing trophic level (Gobas & Morrison, 2000).

1.3.1 Bioavailability of Metals

Environmental availability point out to the ability of an element to interact with other environmental factors, then undergo processes of transport and final fate. Environmental availability is a focused on the existing environmental conditions and changing with the alteration of environmental conditions. However, metal bioavailability concepts refer to the metals that are bio-accessible and being absorbed by an organism that has the potential for distribution, elimination and bioaccumulation processes (USEPA, 2003). The level of metal concentrations in an organism is due to many factors such as the metal concentration in water, the physicochemical form of metal, the organism membrane permeability, the fat quantity in the organism, the degree of contamination, the characteristics of the physical and biological environment and the organism physiological state (Papafilippaki *et al.*, 2008).

1.3.1.1 Factors affecting the bioavailability and bioaccumulation of metals

(a) ***Temperature***

Temperature plays an important role in influencing and affecting the pyhsico-chemical quality and ecology of water systems. It does not only alter the physical characteristics of water but also change the rate and types of chemical reaction. Apart from that, increase in temperature will cause the decline of concentration of dissolved oxygen in water system (Dallas & Day, 2004). In polluted water, the changes in temperature depend on the type and concentration of the metal (King *et al.*, 2003). Therefore, a higher level of temperature can increase the toxicity effect of metal thus may cause organism sensitivity to increase. In addition, higher temperature can cause metals to be more soluble and concentrate in fish tissue (USEPA, 2003).

(b) ***pH***

pH can be an important indicator to assess water quality and pollution level in the aquatic environment (Jonnalagadda & Mhere, 2001). Although in human health pH has no direct effects, pH can influence water quality parameters, such as changing the ionic solubility and pathogen survival, which can directly affect human health (Khan *et al.*, 2013). Moreover, changes of water quality due to pH may ultimately increase level of stress among aquatic organisms (Dallas & Day, 2004). While in soil, bioavailability of metals increase with decreasing pH. The increase in the availability of metals is influenced by lower adsorption and precipitation in alkaline and neutral state of environments (Morel, 1997).

(c) ***Total dissolved solid (TDS)***

Total dissolved solid (TDS) is a measure of the amount of material which can be dissolved in the water and can usually fit a filter of up to 0.45 µm. TDS is often used as an indicator to assess the total dissolved (ionized) pollutants in water systems. Normally, TDS can measure the presence of chemicals such as bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), phosphate (PO_4^{-3}), and other ions which have the ability to conduct electricity. Increase in the concentration level of TDS will increase the ability of water to transmit electrical current. Therefore, TDS conduction ability is able to affect the solubility of certain compound in water systems (Wetzel, 2001).

1.3.2 Bioindicators

Assessment of environmental exposure related to contaminants can be observed by using indicator species such as fishes. These biomarker species are able to accumulate chemicals in their tissues from the surrounding environment, therefore become an important tool for biomonitoring devices (Cunha *et al.*, 2017). Fish has been particularly identified as a biological indicator for assessing aquatic condition with associated sediment and water quality. Normally, fish have been used in studies related to the evaluation of biological and biochemical response towards environmental contaminants (Hauser-Davis *et al.*, 2012).

Fish is useful in assessing water-borne disease and sediment-deposited toxins because it can provide early warning alarm of the potential danger of the new contaminants in the aquatic systems. Furthermore, fish is known as a good indicator for studies related to biochemistry, since they live in a variety of habitats and able to comply with the changes in environmental quality (Hauser-Davis *et al.*, 2012).

For monitoring purposes, concentration of metals are identified in the liver, muscle and gill tissues of fishes. This is because metals are accumulated at a varying level of concentrations in different organs and tissues of fishes (Vieira *et al.*, 2011; Ciardullo *et al.*, 2008; Rashed, 2001). However, metals are usually found in higher concentration in the liver than other tissues (Atli *et al.*, 2016). Liver and muscle tissues are suitable for investigation of temporal trends due to the fact that these tissues can accumulate metals at low concentrations between-year variability (Boalt *et al.*, 2014). Although muscle is not an active tissue in accumulating metals, but it had been reported that concentrations of metals in this tissue in polluted region exceeded acceptable levels. For this reason, it is important to determine concentration of metals in fish for evaluating human health status (Uysal *et al.*, 2008).

1.4 Health Risk Assessment

To address the importance of human health risk, an extensive study on concentrations of metal in fish, water and sediment had been conducted. With regard to adverse effects of metal exposure on human health, it is important to examine human health effects due to metal exposure. Therefore, the USEPA (2000) has developed methods of risk assessment associated with human health for carcinogenic and non-carcinogenic chemicals due to fish consumption. Despite the growing public appreciation in metal exposure by humans, there are still limited research evaluating human health risk with respect to fish consumption and exposure to sediment and water column. Therefore, Håkanson (1984), has proposed a method to assess the human health risk posed by metals in the sediment.

Previous studies have shown that for many years, level of metals in the water, sediment and fishes has exceeded safe limits in some regions of the world (Yabe *et al.*, 2010). Because of that, risk assessment of metals exposure via dietary intake is an important issue. Data from several researchers have confirmed the contamination in the fish from the metal released into the aquatic systems (Copat *et al.*, 2013; Copat *et al.*, 2012; Domingo *et al.*, 2007), but to date, the identification of metals concentration in fish by comparing their origins has been poorly analysed. For this reason, the present study can provide a baseline view for analysing human health risk from two habitats of fish species. Additionally, the result of metal levels in the water column, sediment and fish species are useful for controlling pollution and risk management.

1.5 Research Objective

The main objective of this research is to identify and assess the potential health risks in consuming wild-caught freshwater fishes compared to aquaculture fish species. This can be achieved in the following manner:

i. Determine the concentration of metals (Na, Mg, Ca, Cr, Fe, Ni, Cu, Zn, As, Cd, and Pb) in muscle, liver and gills of *Pangasius* sp. and *Hemibagrus* sp.
ii. Determine the metals distribution in water and sediment samples of freshwater and aquaculture habitats where fish samples are taken.
iii. Determine the accumulation pattern between wild-caught and aquaculture fish species.

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CHAPTER 2: LITERATURE REVIEW

2.1 Aquatic Ecosystem

Water is a chemical compound that serves a fundamental environmental importance to human life and to the environmental cycle. As a profitable natural resource, water can be divided into marine, estuarine, freshwater and groundwater (Frankowski *et al.*, 2009). The surface water percentage of earth found as lakes/river is about 3% and covers about 4.2 million km^2 (Dowing *et al.*, 2006). Malaysia uses about 99% of water supply for domestic use from surface water, while groundwater supply contributes only about 1% of demand (Azrina *et al.*, 2011). Apart from that, the riverine ecosystem in Malaysia is of particular concern since river water contribute 98% of the country's water needs (Azhar, 2000). However, most of the surface water is often overlooked and not sufficiently studied (Céréghino *et al.*, 2008; Scheffer, 2006).

Aquaculture ponds are artificial water bodies normally with a maximum depth of 8 metres, usually offering the chances for aquatic life to colonize this entire area (Oertli *et al.*, 2002). Literally, these ecosystems have a relationship between direct and indirect services of complex and diverse array. Direct services include water supply and usage from communities and harvesting of aquatic products such as fish and seafood. While indirect benefits are obtained from environmental functions such as flood water retention, groundwater recharge, nutrient cycle, and water characteristics association with biotic influences (Mitsch, 1993). Consequently, these dynamic services usually rely on geomorphology, climate, plant cover and nutrient flow. In contrast to this, the modification and degradation normally depends on the industrial development, agriculture activities, urbanization and population density (Hussain, 2010).

2.1.1 Threats to Aquatic Ecosystem

Water pollution have always been one of the main issues in the environmental crises correlated with the rapid economic development, rising of anthropogenic activities and population growth with insecure policies of water management (Rui *et al.*, 2015; Varis, 2001). Besides common water pollution, there are also sudden tragedy usually involving the discharge of contaminants into water over very short periods such as via road traffic accidents (Hou *et al.*, 2013), oil spill (Duarte *et al.*, 2013), explosions from chemical factory (Zhang *et al.*, 2011), leakage from contaminated storage, and natural disaster like earthquakes and heavy rain. Due to these events, water quality will be affected, reducing the effectiveness of water resources usage, influencing socio-economics life, and destroying water ecosystems (Rui *et al.*, 2015).

2.1.2 Water Quality

Water quality can be described as its chemical, biological, and physical characteristics taking into consideration the intended use with a set of guidelines and standards (Boyacioglu, 2007). Thus, water quality assessment can be described as the estimation of the biological, chemical, and physical properties of water to characterize its quality, its health effects and its usage (Pesce & Wunderlin, 2000).

Malaysia's urban environment has been classified as one of the least polluted area in Asia. However, by the year 2020 Malaysia have set their goal of being and industrial country and surely, related rapid economic development will result in an increase in water pollution and deterioration of water quality (Borhan & Ahmed, 2012). These days, waste from animals and domestic effluents are among the major contributor of organic pollution sources in the aquatic ecosystems. The extensive usage of pesticide might result in detrimental effects on aquatic life and contribute to water pollution (Abdullah, 1995). Apart from that, metals can also be introduced to the aquatic ecosystem by natural and anthropogenic sources and may be distributed between the aqueous phase and bed sediments which can lead to water pollution (Sin *et al.*, 2001). These activities can degrade the quality of surface and groundwater, and limit their usage as consumption, agricultural and recreation purposes (Fergusson, 1990). Moreover, metal residues can accumulate in aquatic life which may enter into human body through food chain and cause human health problems (Deniseger *et al.*, 1990).

2.2 General Concept of Heavy Metals

Generally, metals in the environment can be considered to occur naturally, created or destroyed by chemical or biological processes. On the other hand, their concentrations may differ significantly due to geological processes and anthropogenic activities such as industry and domestic activities. Economic activities such as mining, agriculture and manufacturing can be categorized as common sources of metal in the environment (Samecka-cymerman & Kempers, 2004).

In natural systems, metals can originate from rocks, ore minerals, volcanoes, and discharge during weathering process (Szyczewski *et al.*, 2009). In addition, metals have reached worldwide attention and received extensive attention due their toxicity effects, ubiquity, persistency ability, non-biodegradability and ability to accumulate in human tissues (Liu *et al.,* 2016; Xu *et al.¸2016;* Diagomanolin *et al.,* 2004). However in the aquatic system, these elements occur in a form of chemical species, like free hydrated ions, inorganic and organic complexes or absorbed on bio surfaces (Tessier & Turner, 1996).

Heavy metals are potentially hazardous to aquatic ecosystem due to their toxicity and accumulative potential, thus may affect human and animal health (Tchounwou *et al.,* 2012). Therefore, heavy metals can be classified as inhibitors of life cycle, although their inhibiting factors lean on several factors such as level of existence, degree of oxidation and capacity to form complexes (Szyczewski *et al.*, 2009).

Generally, metals can be divided into two categories, essential and non-essential. Essential metals which refer to micronutrient such as Cr, Co, Cu, Mn, Fe, Se, and Zn, are important for biological and biochemical functions in organisms, which include redox reactions and formation of pigments and enzymes (Babula *et al.*, 2009). Opposite to non-essential metals, such as As, Cd and Pb have no known biological function and may exert their toxicity effects by competing with essential elements for placement in membrane proteins or active enzymes (Torres *et al.*, 2008). However, essential elements also can cause detrimental effects to aquatic life and ecosystems as well if they are in high levels of concentration (Nagajyoti, 2010).

2.2.1 Profile Summaries of Hazardous Metals

2.2.1.1 Arsenic (As)

Arsenic can be present and widely distributed in the aquatic environment in the form of metalloids as a result of natural or anthropogenic processes (Azizur Rahman *et al.*, 2012). These elements are mostly mobilized under natural conditions such as processes of weathering, volcanic emissions, geochemical reactions and biological activities (Mohan & Pittman Jr, 2007). Meanwhile, load of this element in water and aquatic sediment are due to anthropogenic activities such mining and smelting processes, combustion of fossil fuels, wastes from industrial and agricultural activities such as application of arsenic additives in pest control and disease prevention (Nachman *et al.*, 2005). Previous studies have indicated that most rivers and lakes are being contaminated with arsenic because of anthropogenic activities (Inam *et al.*, 2011; Casiot *et al.*, 2005; Wong *et al.*¸1999).

In the aquatic environment, arsenic is also introduced primarily through runoff and leaching pathway (Balzer *et al.*, 2013; De Gieter *et al.*, 2005). Apart from that, there are several factors that give impact and determine the form of As present in the aquatic system, like redox conditions, salinity (Kitts *et al.*, 1994), turbidity (Sánchez-Rodas *et al.*, 2005), microbial activity (Oremland & Stolz, 2003) and the behaviour of phytoplankton and zooplankton communities (Caumette *et al.*, 2014). Conversely, aquatic microorganism (phytoplankton and zooplankton) can create organic forms of As such as monomethyllarsonic acid (MMA), and dimethylarsinic acid (DMA) from biomethylation processes with the aim to mitigate As pollution stress (Franco *et al.*, 2015).

Dissolved organic arsenic can be degraded into inorganic forms when released from organisms into the water column (Azizur Rahman *et al.*¸2012; Anderson Bruland, 1991). In many natural waters, the primary inorganic arsenic species can be found in the forms of arsenate $H_2AsO_4^-$: As (+V) and arsenite H_3AsO_3 : As (+III). These arsenate forms are able to bind to iron oxide solids under oxidizing conditions which cause As to precipitate out of the water column. The result of this precipitation is to stimulate the accumulation of arsenic in aquatic ecosystems over the time (Aggett & O'Brien, 1985; Ferguson *et al.*, 1972).

The continuing mobilization of arsenic in contaminated river systems can create the toxicity and cause human health problems (Ferguson *et al.*, 1972). This is because As is carcinogenic on living organism due to its high toxicity (Ng *et al.*, 2003). Its fate, pathways and biological effects such as toxicity, bioaccumulation and bioavailability depend strongly on its form (Anderson & Bruland, 1991). Arsenic can be transferred to human through the food chain via fish consumption and irrigation of crops using contaminated water (Azizur Rahman *et al.*, 2012; Arain *et al.*, 2009). According to the United States and Drug Administration (USFDA, 1993), about 90% of arsenic is exposed to human via oral route through intake of contaminated fish and seafood. Furthermore, epidemiological studies have shown that chronic exposure to inorganic arsenic can cause, even at low dose, adverse health effects, primarily cancers (Chiou *et al.*, 2005; Yang *et al.*, 2005; Yoshida *et al.*, 2004). Based on these reasons, arsenic should be included in water monitoring programmes.

2.2.1.2 Cadmium (Cd)

Cadmium (Cd), a non-essential element, is very rare in nature. Normally, their compounds are bound with the ores of other metals in a small quantities such zinc, lead, mercury and copper (Cambier *et al.*, 2010; Ensafi *et al.*, 2006). Moreover, cadmium is known as a strong metallic nephrotoxin when treated as a xenobiotoc has a high anthropogenic enrichment factor of about 89% (Walker *et al.*, 2006). Cadmium is categorized as one of the hazardous metals (Wang *et al.*, 2010), due to its biological half-life of about 10-30 years (Žemberyová *et al.*, 2007). Therefore, most world and international organizations give attention to this element in their environmental assessment (Xiao *et al.*, 2007).

Various field studies have proved that pollution by cadmium in the aquatic environment due to direct and indirect inputs is strongly associated with industrial processes such as smelting, mining, refining, batteries, plastics, and agricultural practices (Cambier *et al.*, 2010). Natural sources of cadmium can be found from sedimentary rocks, volcanic activity, petroleum and coal sources. World of Health Organization (1992), have reported that about 2600 tons per year of cadmium sources are from nature and approximately 14,640 tons per year are obtained from anthropogenic origins. This element has a potential to accumulate in the environment through leaching into ground water and surface water via landfill, while introduction of cadmium to the atmosphere had been demonstrated through the incinerator smokestack emissions. Due to its high toxicity effects even at low concentration and ability to accumulate in the human body (Ensafi *et al.*, 2006), USEPA (2000), have set a reference dose for cadmium through ingestion of water of 0.5 ppb while 1 ppb was set for food ingestion.

Cadmium is widely distributed in the aquatic system due to its high solubility (Segovia-Zavala *et al.*, 2004). Due to that, bioaccumulation and biomagnification can occur through food chain, thus, fish have a tendency to accumulate this element (Silva *et al.*, 2016). Intake of cadmium through fish consumption can result in disruption of endocrine and various physiological effects (Georgescu *et al.*, 2011). Cadmium can bring about the production of reactive oxygen species (Romeo *et al.*, 2000), disturbance in ion balance and alterations in the acid-base balance (Couture& Rajender, 2003). In aquaculture fish, concentration of cadmium is not only subjected to its availability in their environment but also to the composition of fish feed (Martins *et al.*, 2011).

Based on ATSDR (2008), it was determined that long-term intake of cadmium through dietary pathway is of most concern in humans (Lindén *et al.*, 2003). Due to its common existence, this element can be found in environmental samples and can be taken up through respiratory and digestive system (Nordberg *et al.*, 2007). Breathing cadmium can severely damage the lungs and may cause death. Apart from that, ingestion of high levels of this contaminant can cause vomiting, diarrhea, stomach irritation (ATSDR, 2008) and deleterious effects such carcinogenesis and nephrotoxicity (WHO, 1992). These include the itai-itai disease, the well-known scenario of cadmium poisoning which disturbs kidney function and causes ostemomalacia. Furthermore, high concentration of cadmium was also found in the rice, and affected people with elevated proteinuria concentration due to cadmium exposure (WHO, 1992). With this outbreak and incidence, monitoring and assessment programmes are important as precautions against cadmium risks.

2.2.1.3 Lead (Pb)

Lead is a non-essential element that occurs naturally (Arain *et al.*, 2008; Karve & Rajgor, 2007) and usually incorporate with other element that are scattered in deposits (Al-Saleh *et al.*, 2009). This contaminant is mostly introduced to aquatic systems by anthropogenic activities such as battery industry, paint production and leaded gasoline (Monteiro *et al.*, 2011). Although this element can is naturally flexible, resistant to corrosion and have low melting point, they can cause toxicity effects to human, damage the hemopoietic, nervous and cardiovascular systems, reproductive systems and urinary tract (Wang *et al.*, 2010). Previous studies have demonstrated that lead does not give any benefit to human health (Shah *et al.*, 2011; Al-Saleh *et al.*, 2005; Karita *et al.*, 2005). It was revealed tht exposure to lead can cause numerous neurological disease, behavioural deformities, retardation of judgment and brain problems (Baysal *et al.*, 2010).

As a result of accumulation, lead can exist in fish tissue and consequently open to human consumption. Several literatures have documented about bioaccumulation of lead in fish species and is open to human consumption (Maceda-Viega *et al.*, 2013; Mendil *et al.*, 2010; Ureña *et al.*, 2007). The concentration of lead might differ depending to the type of species and geographical origin where in some cases it was found that the level exceeded the permissible limits (Qiu *et al.*, 2011). A study conducted by Herreros *et al.*, (2008), had revealed that lead was found in the muscle and organs of fish at high concentrations. Due to this, Joint FAO/WHO Expert Committee and Food Additives recommended a provisional tolerable weekly intake (PWTI) of 25 µg kg^{-1} $week^{-1}$ while 3-4 µg kg^{-1} body $weight^{-1}$ by infants and children are recommended for daily intakes (JECFA, 2003).

In a study done by Pizzol *et al.* (2010), for the long term exposure, it was indicated that the ingestion route accounts for about 99% of the total intake. Furthermore, Qiu *et al.* (2011), also suggested that potential human health risk might exist due to elevated levels of lead in fish tissues. The major factor behind the high lead levels may be related to feeds and sediment depositions, as high concentrations were detected in those samples due to lead exposure. Indeed, the chosen fish species can be used in the biomonitoring programmes of the environmental quality and indication of exposures (Nordberg *et al.*, 2007).

2.2.1.4 Selenium (Se)

Selenium is an essential trace element widely distributed in the environment. Significant amounts of selenium are usually found in sediments which are easily drained into aquatic environments from the processes of run-off and weathering (Navarro-Alarcon & Cabrera-Vique, 2008). The presence of Se in aquatic food chains had been documented by several studies (Sampaio da Silva *et al.*, 2013; Lemly, 1999). It has been proven that the presence of Se in these processes is due to the association of microorganism with the detritus of sediments (Hamilton & Lemly, 1999). The tendency of bioaccumulation in the food chain can lead to potential toxicological impact and undesirable changes in aquatic communities (Schmitt & Brumbaugh, 1990).

The deficiency of selenium can result in muscle pathology, reproduction failure and death (Kalisinska *et al.*, 2017). However, long term exposure to selenium within a moderate concentration can cause anorexia, dermatitis, fatigue and hepatic degeneration (ATSDR, 2003). According to WHO (1986), certain population in most countries considered fish as an important source of selenium in their dietary intake. Similar with other elements, biomagnification and bioaccumulation processes of selenium in fish are mainly by dietary intakes (Lemly, 1997). Thus, it is important to determine and measure the benefit-risk balance related to selenium content in fish tissue (Elia *et al.*, 2011).

2.2.1.5 Other metals

Other metals such as zinc, chromium, copper, nickel and iron are generally classified as essential metals for life and human needs. Numerous studies have shown that their nutritional value is important in supporting biochemical processes (Islam *et al.*, 2015; Qin *et al.*, 2015; Palaniappan & Karthikeyan, 2009). For instance, these elements are required by living organism in a small amount for metabolic functions and oxygen transport (Viarengo *et al.*, 1990). Nevertheless, essential elements can create deleterious effects if intake exceeds the concentration requirements of the organism and its detoxification capability (Correia *et al.*, 2002).

Copper is known to cause health problems when consumed via dietary exposure (Lushchak, 2011), while deficiency of zinc element have been reported to result in lipid peroxidation (Stohs & Bagchi, 1995). Chromium compounds which are frequently encountered as environmental pollutants have been observed to cause mutagenic and carcinogenic effects in the biological systems (Parlak *et al.*, 1999). Nickel as a ubiquitous element can easily be found in the water, air and soil. Once released to the environment, this element is able to be more mobile with many ligands compare to others metals (Magyarosy *et al.*, 2002). Apart from that, various health effects have been reported due to nickel exposure such allery, dermatitis and organ system-toxicity (Palaniappan & Karthikeyan, 2009). Although these essential elements provide health benefits, numerous studies have also reported health effects due to excessive exposure (Abdel Ghani, 2015; Monroy *et al.*, 2014; Wei *et al.*, 2014; Zeng *et al.*, 2012,Mendil *et al.*, 2010; Uysal *et al.*, 2008)

2.3 Metal Distribution and Accumulation in the Environment

2.3.1 Metals in Water

The evaluation of persistent metal content in natural or contaminated waters is important due to deleterious effects at different levels (Niu *et al.*, 2015). The presence of metals in aquatic ecosystems can be natural via slow leaching from soil or rock, and are normally found at low levels with no serious human health effects (Chang & Wang, 2000). Even so, anthropogenic activities are able to modify the natural concentrations of metals in water, thus posing serious risks to life and the ecosystem. Therefore, World Health Organization (WHO), the US Environmental Protection Agency (EPA) and the European Union have set the priority list of metals to be monitored (USEPA, 2009).

Various health effects have been reported due to metal exposure in the aquatic ecosystem including abnormal development of foetus, procreation failures and immune system deficiencies (Chang & Wang, 2000). Presence of metals in the water system shows different bioavailabilities depending on their state whether they are in the dissolved phase or bound to suspended matter. Metal bioavailability in this system can also be influenced by water chemistry characteristics such as dissolved and suspended organic carbon, hardness, alkalinity and pH (Niyogi & Woods, 2004). Hence, analysis of metal deposition in water is a convenient tool for evaluating the status of pollution in various ecosystems, which can reflect the level of metal contamination (Su *et al.*, 2013; Haloi & Sarma, 2012; Li *et al.*, 2012).

2.3.2 Metals in Sediment

Generally, sediments are recognized as the main sink for many contaminants in aquatic systems and a potential source of dissolved and particulate-bound contaminants to overlying waters, thus may be considered to have an adverse impact to aquatic life

(Segura *et al.*, 2006). Transportation of sediments along the upstream-downstream river gradient has been identified as one of the main pathways of metal load in these ecosystems. It is noted that sediments are more suitable for monitoring purposes of the long-term deposition due to the fact that concentration of metals in sediments is less variable than in water (Tsakovski *et al.*, 2012; MacDonald *et al.*, 2000).

Sediment-bound metals have the ability to accumulate and be adsorbed by fine grained particles that finally shift into the depositional area (Mendiguchia *et al.*, 2006). Although sediment-adsorbed pollutants are not readily available for aquatic life, changes in environmental conditions such chemical and physical water characteristics may contribute to the release of metal elements back into overlying water, thus making sediment as the crucial indicator for monitoring environmental pollution sources (Li *et al.*, 2000; Li *et al.*, 2014). Furthermore, metal toxicity is closely related to the bioavailability of metals in sediment, depending on the type of chemicals and their total concentrations (Wang *et al.*, 2013; Yu *et al.*, 2010).

Sediment pollution by metals has been regarded as one of the critical environmental issues and received extensive consideration because of their toxicity effects, bioaccumulation potential and hard degradation (Todd *et al.*, 2010). Agricultural practices and industrial activities have been indicated as the main reason for presence of metals in aquatic ecosystems (Islam *et al.*, 2014; Deepulal *et al.*, 2012, Gao *et al.*, 2006; Dassenakis *et al.*, 2003). Previous studies have reported that concentration of metals in sediment could significantly degrade the quality of river system (Besser *et al.*, 2009; Snodgrass *et al.*, 2008; Zheng *et al.*, 2008). It has been proven that metals distribution in sediment which location adjacent to settlement areas is suitable to human health risks evaluation associated with water pollution (Zheng *et al.*, 2008; Ruiz *et al.*, 2006). Thus, evaluation of metal concentration in sediment can provide information about the risk posed to human health.

2.3.3 Metals in fish

Metal elements accumulate in fish via direct absorption from water through their gills and skin, and by ingestion of food or non-food particles (Weber *et al.*, 2013; Jargensen, 1994). Therefore, metal concentrations in ambient water, sediment and fish food are important factors that influence the metal concentration in fish tissues (Maceda-Veiga *et al.*, 2012; Vicenta-Martorell *et al.*, 2009; Canli & Atli, 2003). These elements enter the fish's blood stream and are carried to targeted tissues or organs. Then, they are bio-transformed in the liver and excreted or further accumulated through the food chain (Weber *et al.*, 2013). In addition, concentration of these elements in fish can represent a potential risk to human as well (Adam *et al.*, 1992). The risks to humans are of most concern when metals concentrations of the consumed contaminated fish exceed their allowable daily intake levels (Ahmad *et al.*, 2010).

Fish is an important part of human diet due to its nutritional values and benefit health and is also a good bioindicator in assessing metal contamination in the aquatic environment (Zhao *et al.*, 2012; Morgano *et al.*, 2011). Concentrations of metals in fish tissues are directly or indirectly dominated by abiotic and biotic factors (Weber *et al.*, 2013). Environmental factors such as pH, temperature, and alkalinity (Wagner & Boman, 2003), are the factors that influence the rate of metal bioaccumulation. Apart from that, this rate also depends on the pollutant type, sampling location and species-specific physiological and ecological characteristics such feeding habits, age/life span, size and trophic level (Rejomon, 2010).

Fish located at the top of trophic levels usually accumulate fewer metals than those positioned at high level in the food chain (Cui *et al.*, 2011). In environmental assessment, liver, gills and muscle are the most targeted organs and used due to their roles in metal bioaccumulation and their potential in passing metals to the human diet (Yilmaz *et al.*, 2010; Agusa *et al.*, 2005; Al-Yousuf *et al.*, 2000). Muscle is the most important tissue in human health assessment associated with metal pollution because it is the main edible part of fish (Yi *et al.*, 2011). From the perspective of health issues, it is important to have a permissible limit in consuming fish in order to protect human health (Qin *et al.*, 2015).

2.4 Fish as Bioindicator

The aquatic ecosystems is possibly the most endangered part of the Earth's biosphere due to fact that it is the last target of contamination deposition (Abel, 1996), hence, determination of pollutant levels of the ecosystem is imperative (Svihlikova *et al.*, 2015; Stahl *et al.*, 2014). The relationship between man-made activities and result of ecological effects may be clearly explained through sensitive approaches such as employing aquatic organism as a means of direct measurement of pollutants (Gerber *et al.*, 2017).

There are various tools for describing the quality of this environment such as biomonitoring which uses various aquatic biota such as plants, planktonic/benthic communities of invertebrates and fish (Grabicova *et al.*, 2015; Net *et al.*, 2015). However, determination of ecological response through water quality (physical and chemical parameters) of the water column may not be an adequate substitute (Gerber *et al.*, 2017), thus, by using fish in environmental monitoring, levels of contaminants in the fish species can be regarded as representative of the study area. The benefits of using fish as bioindicator in aquatic assessment are well reported (Schlacher *et al.*, 2007; Whitfield & Elliot, 2002).

Fish is a suitable bioindicators for environmental monitoring because its position in the food chain and it has the ability to concentrate pollutants in their tissues (Zhao *et al.*, 2012; Bervoets, 2003; Agarwal *et al.*, 2007). Moreover, fish have slow turnover rate of metal accumulation in their tissues due to their life span, therefore, they can integrate pollution over a long period of time, thus, providing more time to overview what is happening in the aquatic environment (Smit *et al.*, 2009). Furthermore, fish is excellent as an ecological indicator of environmental degradation, because fishes are abundant, readily identified and sensitive to contaminants (Gratwicke & Speight, 2005). Due to this reason, fish has been widely documented as indicators for human health risk assessment (Mora *et al.*, 2008; Das & Chakrabarty, 2007; Mora & Robertson, 2005).

Fish is well suited for biomonitoring programs for assessing water quality of interest areas; this is because it is able to survice in the closed area (Aguilar-Betancourt *et al.*, 2016). Apart from that, fish can be found in a wide variety of habitats and trophic positions, thus it is able to provide indicator of anthropogenic impact studies (Gerber *et al.*, 2017). In addition, understanding the pollutant levels in the fishes can help in ascertaining the risk of consuming fish as part of human diet. The risks to human are noticeable when these contaminated fishes have metals concentrations beyond the levels of recommended daily intake (Ahmad *et al.*, 2010).

2.4.1 Candidate Species

2.4.1.1 *Pangasius* sp.

Pangasius sp. is a freshwater predatory fish, known commonly as 'ikan patin', belonging to the Pangasiidae family (Figure 2.1). This freshwater fish species has low protein level (12.6-15.6 %) and fat (1.3-3.0%), but high content of water (80-85%) (Orban *et al.*, 2008). Pangasiidae are grouped under large catfishes where their adults can achieve the size of about 20 cm to 3 m (Roberts, 1991). Normally, the fish belongs to this family have features of shark head with maxillary barbels and mandibular barbels, posterior nostril and anterior nostril and lying behind or slightly medial to it (Muhamad & Mohamad, 2012).

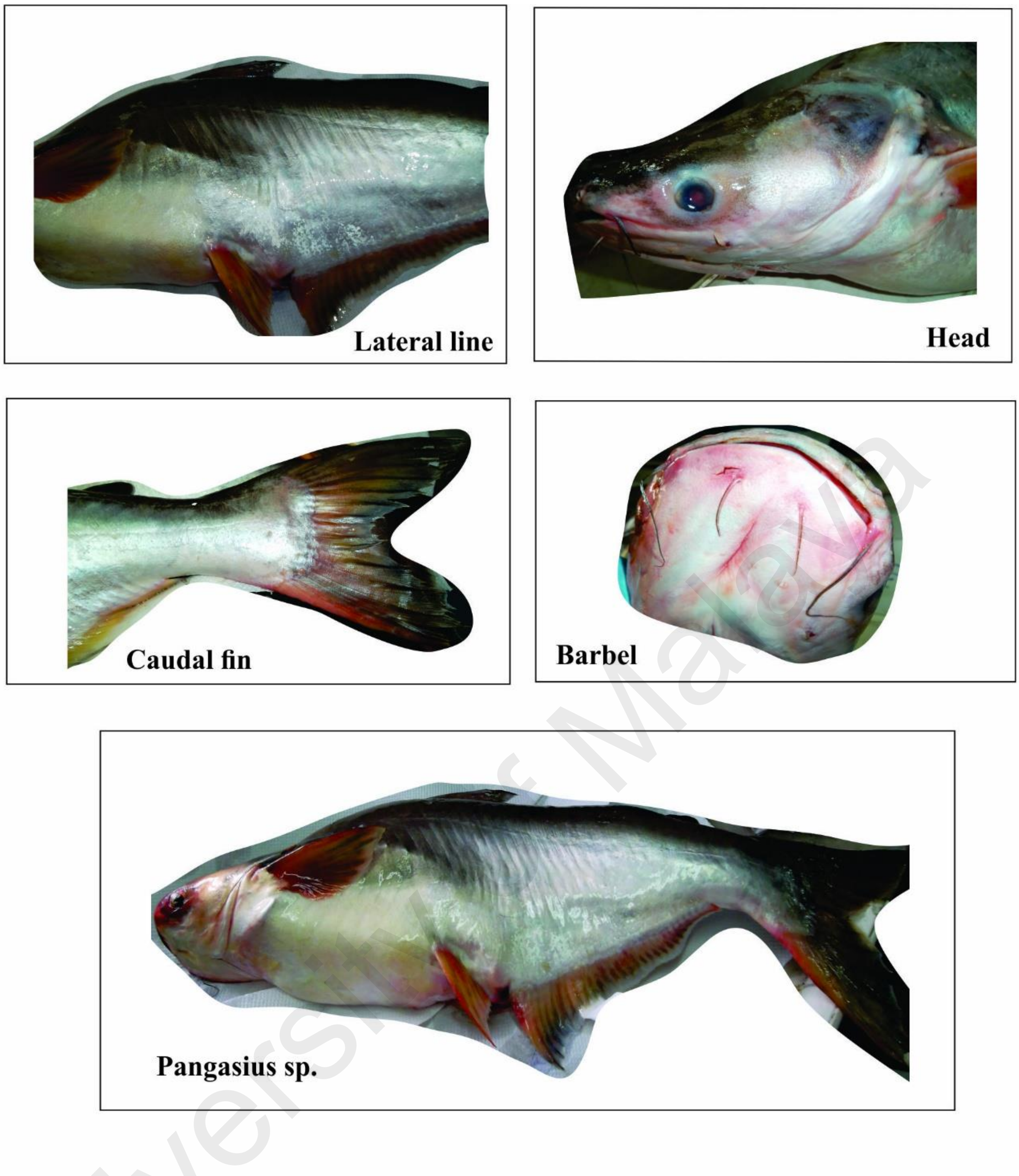


Figure 2.1: Basic *Pangasius* sp. anatomy

This omnivorous species is of great importance to aquaculture fisheries food supply in Southeast Asia and is one of the favourite freshwater fish due to their taste with white and tender flesh (Ambak, 2010; Hung *et al.*, 2004). *Pangasius* sp. naturally inhabits the rivers of India, Indonesia, Malaysia, Cambodia and Thailand and is a cultured species in Southeast Asian countries (Debnath *et al.*, 2006). In Malaysia, this species is considered as one of the favourite and most popular freshwater fish for human consumption (Muhammad & Mohamad, 2012; Abbas *et al.*, 2006).

In actual fact, this species is uniquely aquacultured due to its ability to reproduce in captivity and fast growing (Phuong & Oanh, 2010; Phan *et al.*, 2009). A study carried out by Ferrantelli (2012) concluded that *Pangasius* sp. has a potential to be a bioindicator in environmental assessment due to its competency in tolerating low levels of oxygen and poor water quality and has a high demand for human consumption. Hence, it is important to assess the temporal trends in human exposure to metal pollutants via this fish species.

2.4.1.2 *Hemibagrus* sp.

Hemibagrus sp. is an omnivorous, europhagus feeder and indigenous freshwater fish that can normally be found in the lakes and rivers of Southeast Asia (Figure 2.2) (Usmani *et al.*, 2003; Khan, 1987). This tropical bagrid catfish belongs under the Bagridae family and is known as 'ikan Baung' by the locals. This catfish species is widely distributed in most of the inland water bodies and forms an abundant population in Malaysian reservoir fisheries (Khan *et al.*, 1995).

In 1763, for the first time Gronow used the name of *Mystus* which has been proven by Scopoli in 1977. However, in 1856, Dumerill replaced the name of *Mystus* with *Macrones* which is still used in most studies as a reference. Other names that are also used referring to this species are; *Aoria, Hemibagrus, Hypselobagrus* and *Aspidobagrus* (Mohsin & Ambak, 1983).

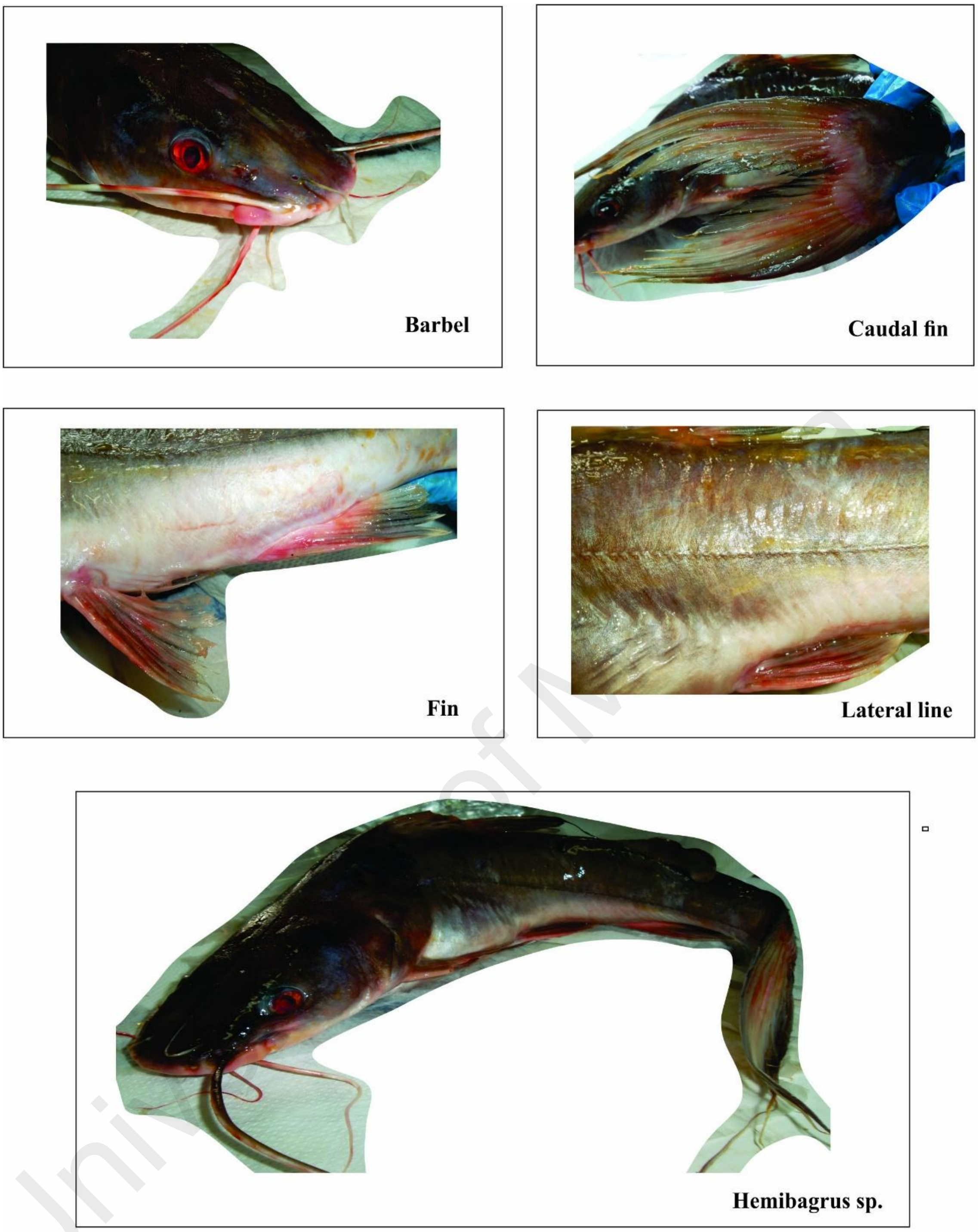


Figure 2.2: Basic *Hemibagrus* sp. anatomy

The distribution of *Hemibagrus* sp. is also reported in brackish water (Inger, 1995). As a bottom feeder, this species feed on a wide range of food such small teleosts, crustaceans, benthic invertebrates and detritus (Khan, 1987). In Southeast Asia, this species is known to be a popular freshwater fish, therefore, they are cultured in large quantities to fulfil the human demands in freshwater cage system in the river (Molnar *et al.*, 2006). Apart from that, this freshwater fish has the potential to be cultivated due to their good market value because of their taste, non-bony meat and high protein content (Chew & Zulkafli, 2012; Amornsakun *et al.*, 1998).

However, there are several reasons for the lack of cultivation of this fish, namely difficulty in spawning it artificially, sensitivity to changes in water quality and easily stressed. However, in natural habitat, they spawn from May to July and September to November which is twice a year (Muchlisin *et al.*, 2004). With all the reasons stated, therefore, *Hemibagrus* sp. might be used as a bioindicator for metal pollution in river systems.

2.5 Risk Assessment

2.5.1 Risk Concepts

In the risk assessment process, risk perception has become a main focus of environmental concerns. Historically, there have been a lot of changes in the approach of risk assessment that led to the identification of risk assessment objective to identify and measure the impact of available contaminants from the study area under a variety of conditions. Yet, earlier efforts in this process does not lean on scientific principles related to the interested contaminants (National Research Council, 1983). Therefore, it is important to differentiate the definition of hazard and risk; hazard is defined as an unpleasant adverse consequence because of the interested pollutant. Hazard can happen due to; i) ingestion, dermal contact or inhalation of pollutants; ii) poor of water quality; and iii) exposure to highly contaminated water and/or food (National Research Council, 1983). The main objective of risk assessment is to evaluate and identify the acceptable level of each pollutant, which does not give any risk to the surrounding environment (National Research Council, 1983).

There is no widely accepted definition of risk, however, it can be assumed as the likelihood of an adverse effect or threat to environments/human due to hazardous situational exposure (Asante-Duah, 1998). Risk could be defined as the probability of a likelihood of an unpleasant adverse threat effect multiplied by the degree of hazard due to the threat (Equation 2.1) (National Research Council, 1983).

$$\text{Risk} = \text{Probability x severity of consquences} \qquad (2.1)$$

A better understanding of risk category could help in evaluating the hazards and threats due to contaminants. There are three types of risks; i) background risk, which refers to the exposed risk to the people before any remedial action is taken to reduce their effect; ii) incremental risk, which is due to the sources of threat/risk, and iii) total risk, which is the total of the previous risks.

Figure 2.3 and Figure 2.4 illustrate the process involved in risk assessment and risk management. Based on Figure 2.4, all the elements have to be analysed in detail with consideration on their variation and levels of hazard. Due to this reason, the international scientific community have made some efforts and paid attention in forming a procedure with the aims to protect human life against the potential contaminants risks (Dorne & Fink-Gremmels, 2013). As an example, Global Environment Monitoring System-Food Contamination and Assessment Programme (GEMS/Food) was set up by WHO in order to analyse the occurrence and exposure effects of contaminants in food and their significant health risks (Dorne & Fink-Gremmels, 2013).

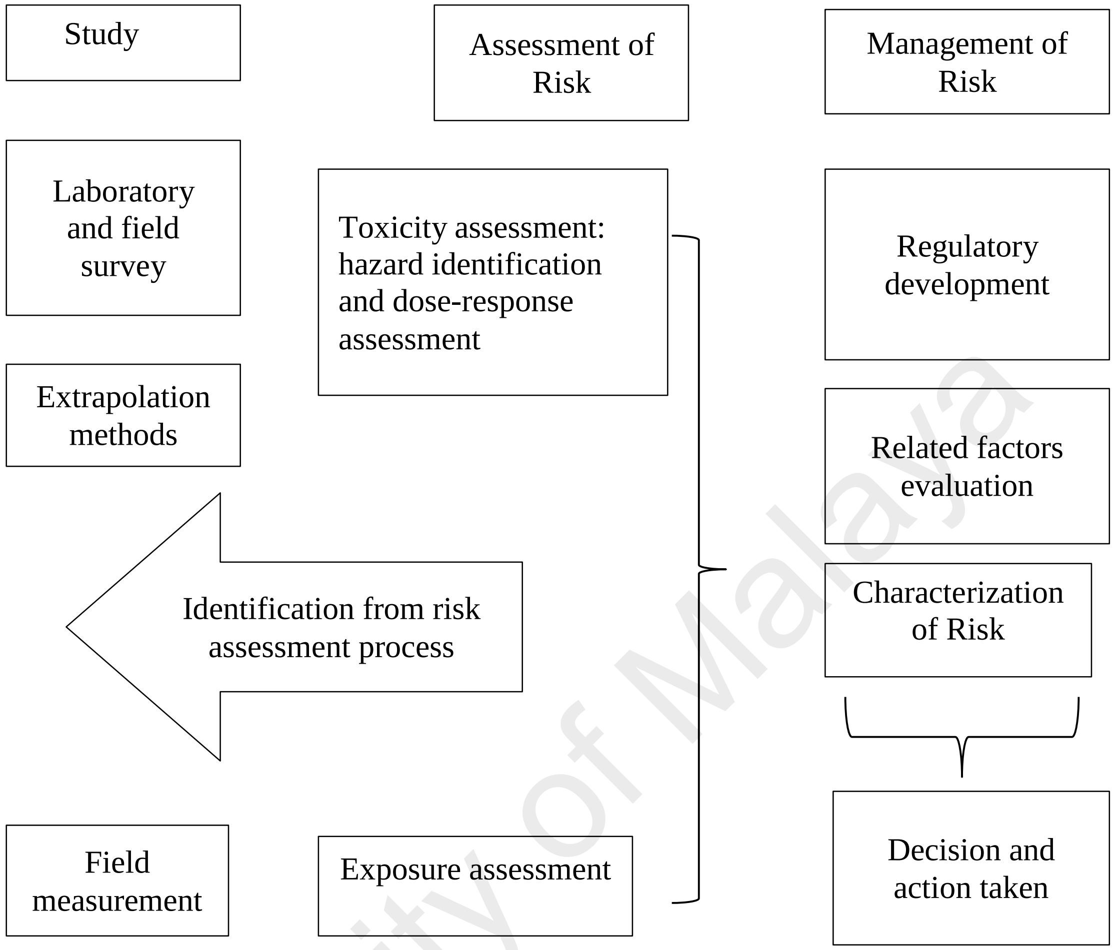


Figure 2.3: Risk assessment and risk management elements (NAS, 1994)

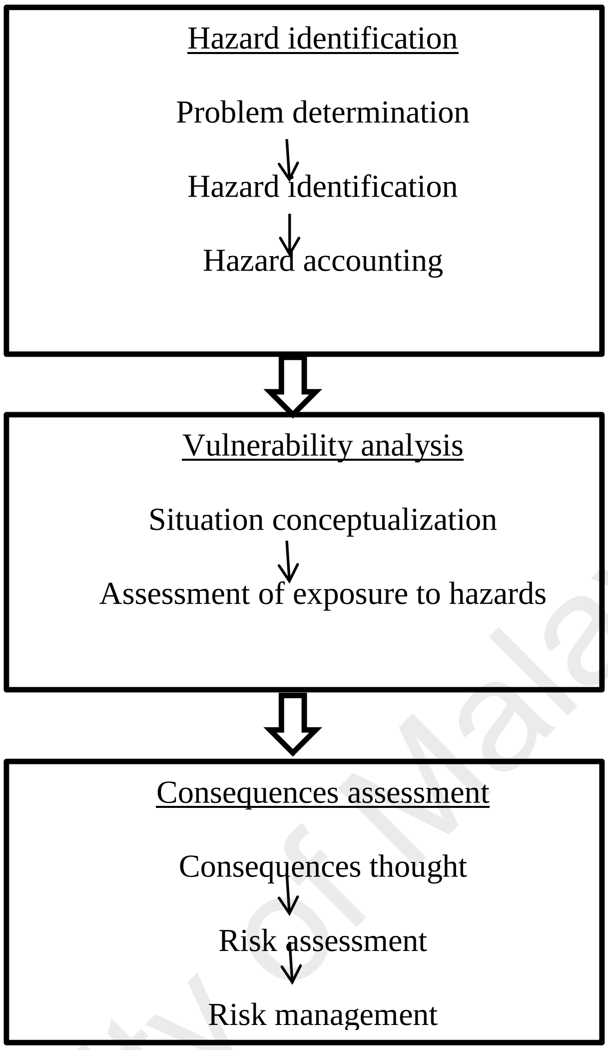


Figure 2.4: Basic steps of hazard and risk analysis (Asante-Duah, 1998)

2.5.2 Human health risk assessment

Human health risk assessment have been given more attention based on the numerous studies and international bodies that deal with food safety, such as Joint Expert Committee on Food Additives (JEFCA) of the Food and Agriculture Organisation (FAO)/World Health Organization (WHO), the US Environmental Protection Agency (EPA), the US Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and etc. (Dorne *et al.*, 2009). Human health risk assessment can be defined as the process of characterizing and analyzing the potential adverse health effects due to exposure to environmental hazards and contaminants (Asante-Duah, 1998).

When discussing about contaminants exposure, human health risks assessment aims to protect human health from the potential risks associated with the contaminants by deriving their safe and acceptable levels, which is based on guidance values of distinct approaches for genotoxic carcinogens or threshold toxicant (WHO, 2009). In general, human health risk assessment process can be divided into four steps - identification of hazard, data collection and evaluation, followed by the hazard assessment together with toxicity testing assessment and finally, risk characterization (Figure 2.5).

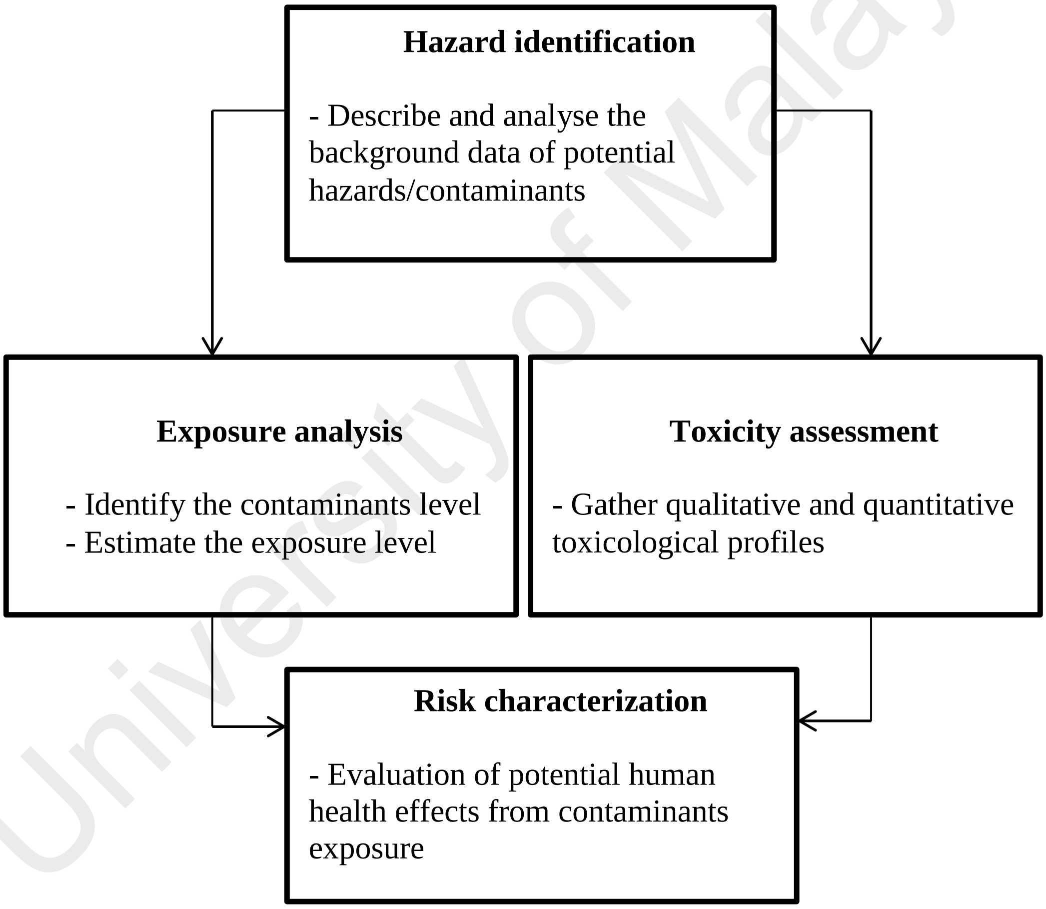


Figure 2.5 : Human health risk assessment process (Asante-Duah, 1998)

2.5.2.1 Hazard identification

Hazard identification is the first step involved in the risk assessment which covers the assessment of the likelihood due to the exposure of certain chemicals under defined exposure condition that can cause threat to human health. This step consists of a review of chemical, biological and exposure effect on the potential pollutants to pose specific risk. Furthermore, this step also require evaluation and collection data on the types of health effects of potential pollutants/agents under specific exposure conditions like chronic, acute, airborne or food borne (USEPA, 1985).

For a certain potential pollutants, the characterisation of hazard step point to evaluate all the available data associated with the toxicokinetics including their absorption, distribution, metabolism, and excretion potential. In addition, mode of action and toxicity profiles (target organ and toxicological endpoint) of pollutants also needs to be considered. In a certain cases, human epidemiological data also evaluated in this step particularly for contaminants such as Aflatoxin B_1, heavy metals and metalloids (Dorne, 2010; EFSA, 2009). Therefore, identification of identified hazards together with their human health effects is a conclusive process in order to evaluate the resulting health risk credibly (Wells, 1996).

2.5.2.2 Exposure assessment and analysis

The process of exposure assessment combines the patterns of human consumption of contaminated food and the occurrence of contaminants concentration in the food categories using validated analytical techniques. Moreover, a multiple of exposure/intake episode also taken into a consideration, thereby, special subgroups such as children, vegetarian that may be at high dietary exposure can be taken into account (Kroes *et al.*, 2002). Generally, the process of exposure and analysis involved gathering all the available and potential data of chemical substances that likely human consume (USEPA, 2000).

For quantitative exposure assessment, combination approaches either using probabilistic or deterministic approach will be used for data on food consumption and chemical occurrence (Kroes *et al.*, 2002). Apart of that, this quantitative method cans gives numerical estimates of the rates at which contaminants/chemical are absorbed by the targeted receptors using a combination of logical statement and mathematical equation. This method also requires valid and reliable analytical method to ensure equivalence and consistency data are acquired. This means, some laboratory procedures are needed including sample handling and analysis should display and transform into a sufficient technical merit, sensitivity, data quality, and cost efficiency to a certain extent (USEPA, 2000).

2.5.2.3 Toxicity assessment

Generally, toxicity assessment is focused on compiling toxicological profiles for the potential chemicals that can be used as a basis for evaluating and estimate the safe threshold doses. Basically, it's involved data of quantitative evaluation of toxicity information and characterization of exposure-response relationship that focused on health effects on the targeted subject due to contaminants exposure. These data usually depend on the extrapolated results of animal studies which correlated to human health assessment. Previous research have concluded that common practice in evaluating the assessment is by adopting the data of toxicological from the Integrated Risk Information System (IRIS) (United States Environmental Protection Agency, USEPA) and Agency for Toxic Substances and Disease Registry (ATSDR) under United States Department of Health and Human Services (USDHHS) (Mohmand *et al.*, 2015; USEPA, 2000).

2.5.2.4 Risk characterisation

Risk characterisation is the final step in risk assessment and has been describe as the qualitative and/or quantitative estimation, include uncertainties of the occurrence probability and severity of the potential adverse effects to the targeted population based on hazard identification, hazard characterisation and exposure assessment. For genotoxic carcinogens, the margin of exposure (MOE) is derived from dividing the point of departure (benchmark dose lower limit (BMDL) of animal carcinogenicity data) with the human exposure (EFSA, 2009). When using the data of animal that derives from benchmark dose (BMD) and BMDL, MOE values more than 10,000 are considered as of low concern for risk management to take an action. Whereas, for low MOE values, they require intervention strategies like mitigation measures to reduce the exposure level (EFSA, 2005).

2.5.3 Human health risk characterization associated with fish consumption

Human health risk characterization consists of evaluation of hazardous impacts to the affected population due to contaminants exposure. In general, the associated risks are quantitatively derived from combination of exposure and toxicological profiles. Basically, contaminants can be divided into two classifications, non-carcinogenic and carcinogenic risk. For carcinogenic risk, their exposure can cause both carcinogen and non-carcinogen effects to the affected target. With the aims to protect human health, USEPA have suggested the lower one for the maximum allowable of fish consumption rate (USEPA, 2000).

The hazard identification in human health risk assessment of associated chemicals have been overviewed by EPA of their characteristics, which involve (USEPA, 1989):

i. High persistence in the aquatic system
ii. Potential to accumulate in tissue

iii. Identify the sources contaminants in interest areas

iv. Potential to create toxicity effects to humans

United States Environmental Protection Agency (USEPA) (2000), have set hazard quotient (HQ), hazard index (HI) and carcinogenic risk (CR) to determine the non-carcinogenic hazards and the potential risks that arise due to exposure of potential contaminants. While the non-carcinogenic risk is normally estimated by using the concept of systemic toxicity by referring to the reference dose (*RfD*). Reference dose is the maximum level of an interest chemical that human beings can tolerate without experiencing any chronic effects (USEPA, 2000).

2.6 Quantitative Risk Assessment (QRA)

The objective of quantitative assessments is to predict the future impact due to the potential of chemicals being released. It involves numerical methods to assess the existing or/and future risk according to exposures of identified routes. Furthermore, this assessment includes estimation of characteristics, pathways, exposure and toxicity effects of the contaminants. This assessment also consists of analysis and assessment of contaminant discharge, uptake rates and toxicity assessment (USEPA, 2000).

The third stage of the quantitative risk assessment is included in the evaluation of the affected population. This stage includes two quantitative aspects, which are the number of the involved population and the estimation of exposure and uptake rates. For the uptake rates, there are three types; administered dose, meaning the amount of ingested contaminants, inhaled or contacted by the skin; intake dose, meaning the amount body absorbed the contaminants; and target dose, meaning the amount of contaminants that reach the target site. There are a variety of methods that can be used to measure each of the above-mentioned doses and some of them can be measured by calculating the intake of target dose from administered dose.

For example, the United States of Environmental Protection Agency have stated the following formula to measure administered dose (USEPA, 1989);

$$I_{AD} = \frac{C \text{ x } CR \text{ x } EF \text{ x } ED}{BW \text{ x } AT} \qquad (2.2)$$

where ,

I_{AD} = administered dose (mg kg^{-1} of body weight.day)

C = concentration of contaminant at exposure point (mg m^{-3})

CR = contact rate (m^3d^{-1})

EF = frequency (day $year^{-1}$) & ED = exposure duration (year)

BW = body weight (kg) & AT = average time (days)

Intake dose from the administered dose can be calculated by using the following equation;

$$I_{ID} = I_{AD} \text{ x } RR \text{ x } ABS \qquad (2.3)$$

where,

I_{ID} = intake dose of contaminant (mg kg^{-1} of body weight day)

RR = retention rate (decimal fraction)

ABS = absorption into blood stream (decimal fraction)

It is important to pay an attention to risk assessment as an iterative method built upon several assumptions. The sensitivity of the degree of risk assessment may require researchers to review all the assumptions and refine them to enhance this procedure to make them clearer and give better understanding.

Consequently, additional info or sampling procedure may need to clear confusing and ambiguous data with the objectives of producing more reliable and better assessment (Burmaster & Harris, 1993).

CHAPTER 3: METHODOLOGY

In this chapter, the methodology used in this research is discussed. Figure 3.1 summarizes the methodology used. Two sites were selected as the sampling sites for the collection of water samples, sediment samples, and freshwater fish samples. Verification of method were carried out by verify with their respective certified standard reference as mentioned in each subtopic below. Certified standard reference materials were used to validate the method. The analyses were carried out by using inductively-coupled plasma spectrometry (ICP-MS). For the risk and safety assessment, the samples of water, sediment and fishes were analysed using their respective indices. Hierarchical cluster analysis and principal component analysis were performed on all experimental data. Detailed explanation of Figure 3.1 is described in each subtopic below.

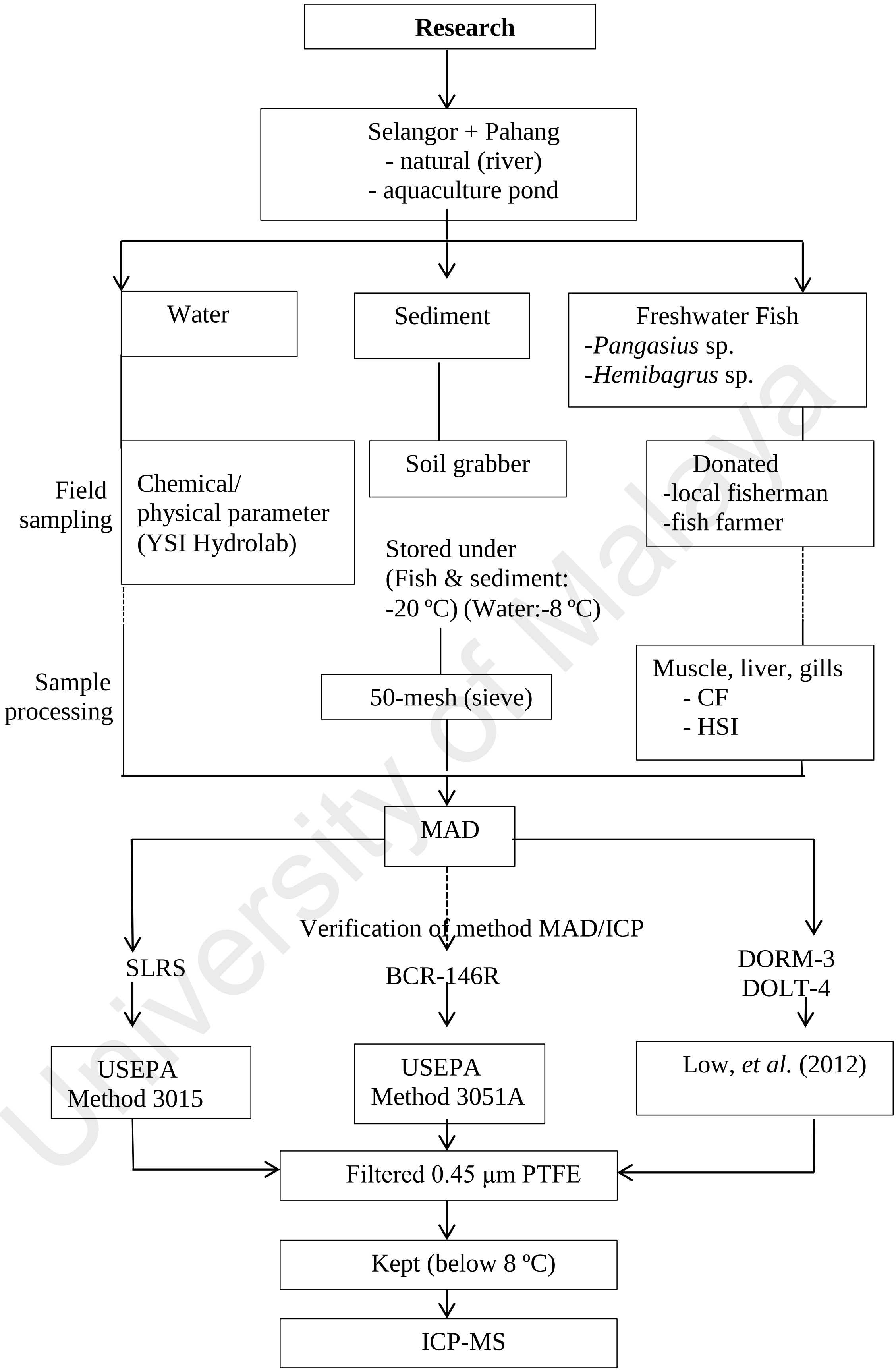
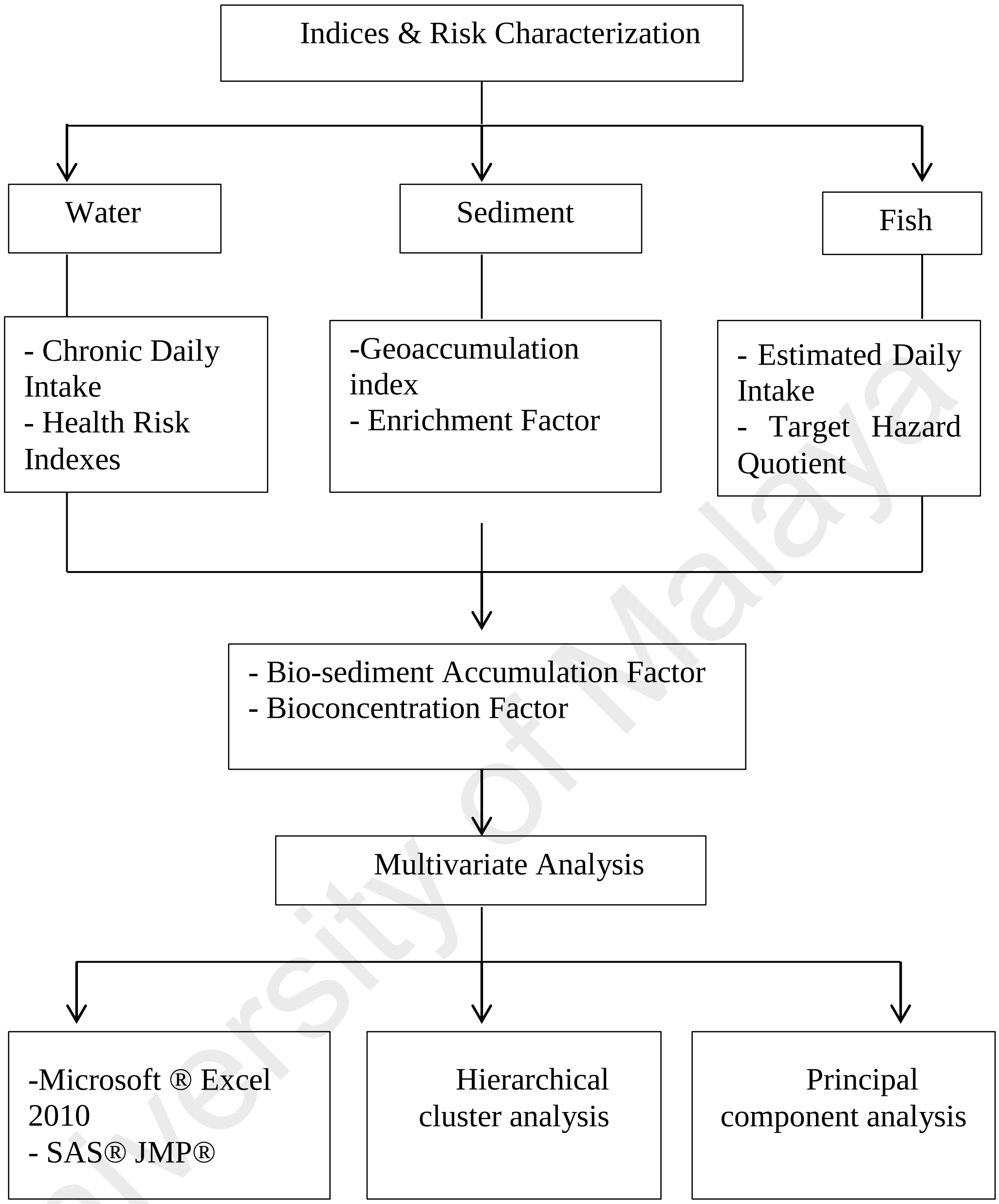


Figure 3.1: Research Methodology Flowchart

Figure 3.1, continued



3.1 Sampling sites

Briefly, Selangor River is located in a naturally vegetated areas an originates at an attitude of 1500 m. As a major river in Selangor, this river flows in a south-westerly direction before discharging into the Straits of Malacca. The catchment area of this river is about 1700 km^2 and can serve raw water supply needs for agricultural activities, such as Taman Agroteknologi Pertanian Bestari which generate their fish pond activities based on water from the Selangor River (Table 3.1). However, this farmed pond uses mechanical aeration to supplement natural dissolved oxygen (Figure 3.2). Nevertheless, the river is exposed to contaminants, particularly from the discharge of agricultural and industrial wastewater (Leong *et al.*, 2007).

The longest river in Peninsular Malaysia, Pahang River is located in the Main Range of Titiwangsa with a length of 459 km. This river is located in the Pahang River Basin as the main channel that is responsible in draining water during the wet season particularly from flood tragedies (Lun *et al.*, 2011; Jaafar *et al.*, 2010). History shows that Pahang River had been reported to have a decrease number of fish species as a result of floods. Improper implementation of hydrology practices and fast growing infrastructural and industrial activities are the main reasons of the flood events (Nasly *et al.*, 2013). Due to this reason, aquaculture fish ponds located at Kampung Paloh Hinai, Pekan, Pahang was chosen as the sampling site. This pond also depends to mechanical aeration to increase the supply of dissolved oxygen (Figure 3.3)

Table 3.1: Sampling sites

	Selangor		Pahang	
	Aquaculture pond	**River**	**Aquaculture pond**	**River**
Location	N 03º 25.0' E 101º 21.2' Taman Agroteknologi Pertanian Bestari Jaya, Selangor	N 03º 21.9' E 101º 18.7'	N 03º 31.4' E103º 10.1' Kampung Paloh Hinai, Pekan, Pahang	N 03º 30.5' E103º 05.1'
Pond Practices	Mechanical aeration		Mechanical aeration	

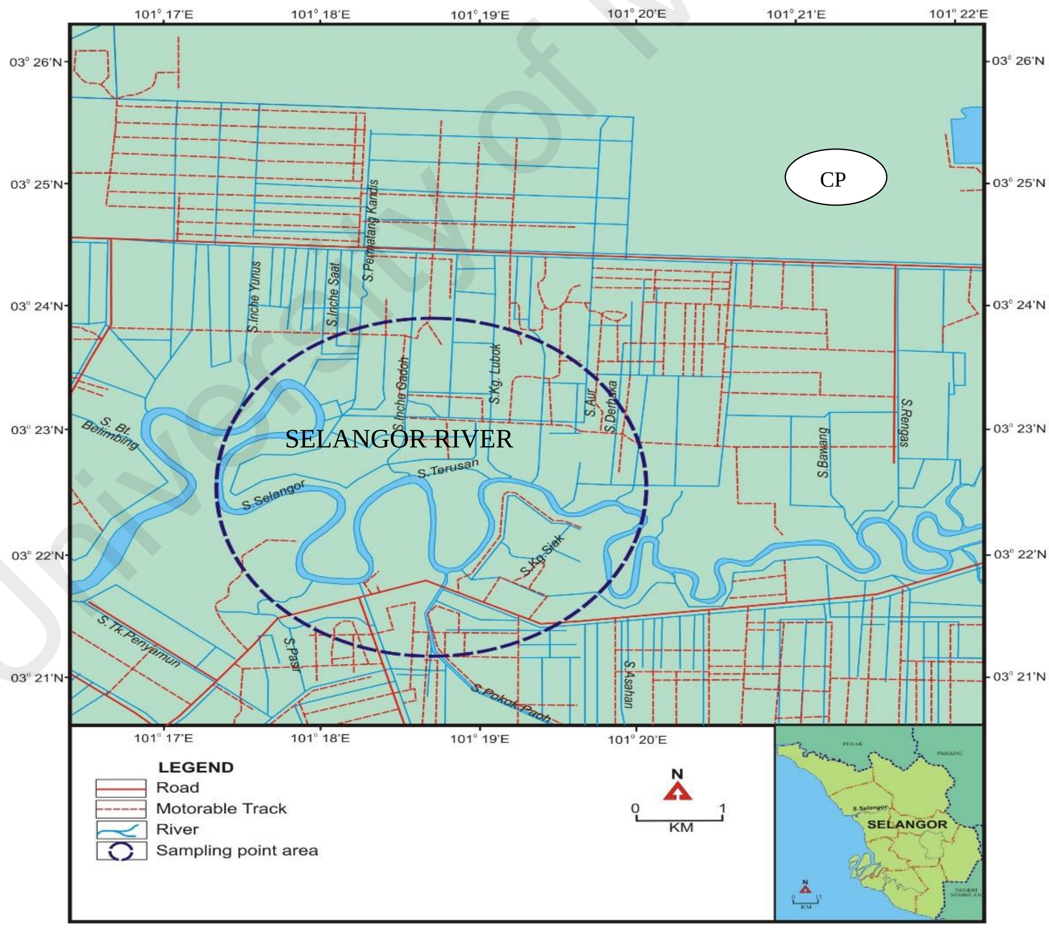


Figure 3.2: The map showing the sampling site in Selangor River and aquaculture pond (CP)

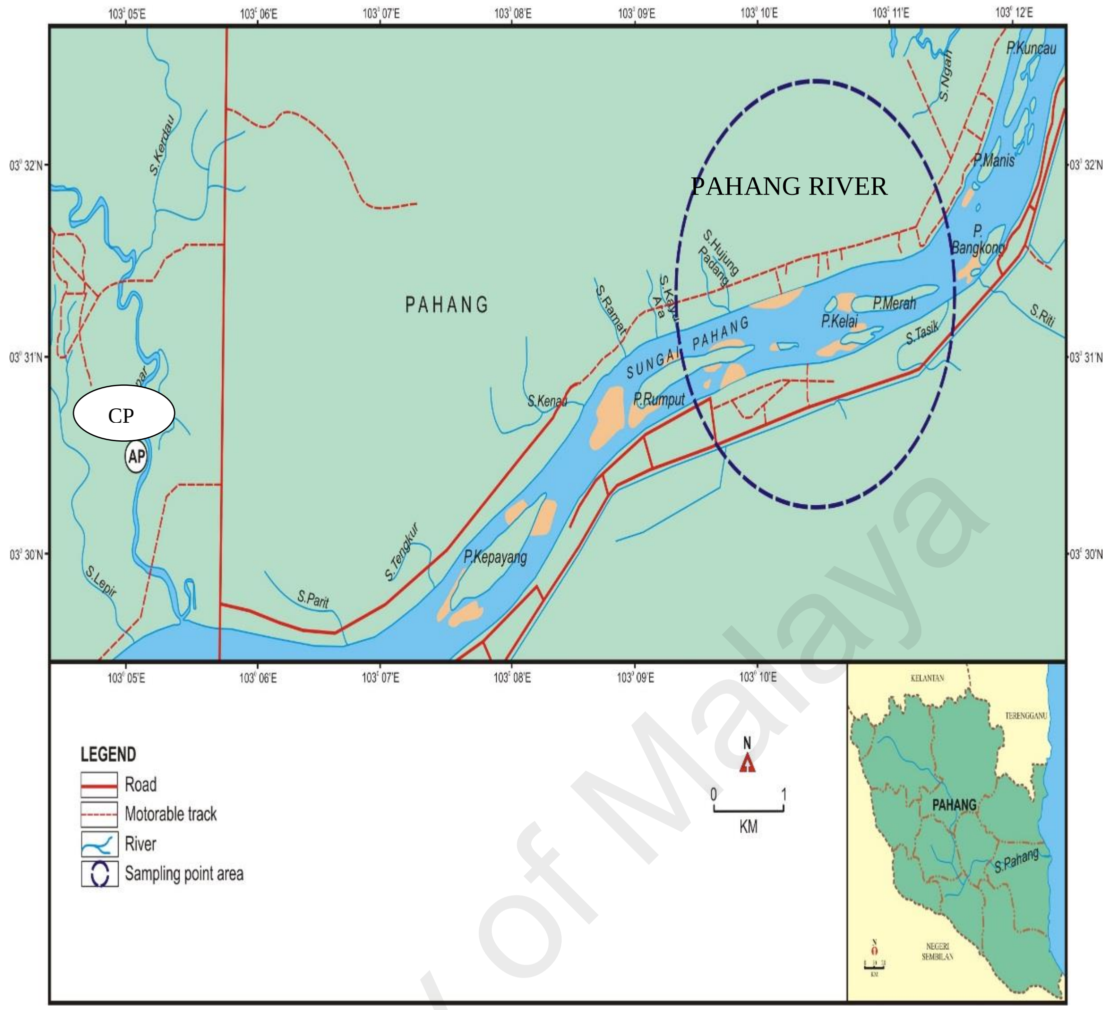


Figure 3.3: The map showing the sampling site in Pahang River and aquaculture pond (CP)

3.2 Reagents and Standard Materials

3.2.1 Acids and reagents

For the preparation of the sample solution, Ultra Pure Water (UPW) with resistivity more than 18 MΩcm^{-1} obtained from ELGA®*PURELAB®UHQII* (UK) system was used. 65% (v/v) HNO_3, 30% (v/v) H_2O_2, and 30% (v/v) HCI solutions of Suprapur® quality from Merck (Germany) were used.

3.2.2 Calibrations standards

Standards solutions of inductively coupled plasma (ICP) multi-element stock consists of 1000 mg L^{-1} Fe, Ca,K, Mg and Na while 10.0 mg L^{-1} of As, Cd, Cr, Cu, Ni, Pb, Se and Zn in 5% (v/v) HNO_3 obtained from Agilent Technologies (USA). Fresh calibration standards were prepared by an appropriate dilution of the ICP multi-element stock standard solution (Agilent Tecnology, USA).

Gaseous used

Purity of argon plasma gas and helium collision gas used was higher than 99.999%.

3.2.3 Certified Reference Materials

Certified reference materials (CRMs) for trace metals were used to demonstrate the validity of methods used. They were dogfish muscle (DORM-3), dogfish liver (DOLT-4) and river water (SLRS-4) certified reference materials (CRMs) for trace metals from National Research Institute, Canada. Sewage sludge (BCR-146R) CRM used was from the Institute for Reference Materials and Measurement, Belgium.

All the CRMs were stored under a desiccator prior to use. Sample preparation of certified reference materials dogfish muscle (DORM-3) and dogfish liver (DOLT-4) was prior to microwave assisted digestion (MAD) procedure of fish sample, while river water (SLRS-4) and sewage sludge (BCR-146R) were analysed by appropriate water and sediment MAD methods, respectively.

3.3 Cleaning Glassware

In order to avoid cross-contamination and reduce possible decontamination activities, all apparatuses were cleaned and soaked overnight in dilute 10% (v/v) HNO_3. They were rinsed thoroughly with the UPW and dried prior to use.

3.4 Sample Collection and Preparation

3.4.1 Field Sampling

3.4.1.1 Sampling of fish

Field study and sampling were conducted during June to September 2012 (Table 3.2). The *Pangasius* sp. and *Hemibagrus* sp. were donated by local fisherman and fish farmer, which were wild-caught from Selangor and Pahang River and aquaculture pond from Taman Agroteknologi Pertanian Bestari Jaya, Kuala Selangor, Selangor, and Kampung Paloh Hinai, Pekan, Pahang. The fish specimens were wrapped individually in low-density polyethylene sampling bag, kept in ice-box and transported to the laboratory on the same day.

The total fish length and weight were recorded before storing under -20 ºC until dissection can be performed. The length was measured as the distance from the tip of snout to the tip of the caudal fin (Alex *et al.*, 2012) (Figure 3.4).

Table 3.2: Sampling timeline period

2012	June	July	August	September
Selangor				
Pahang				

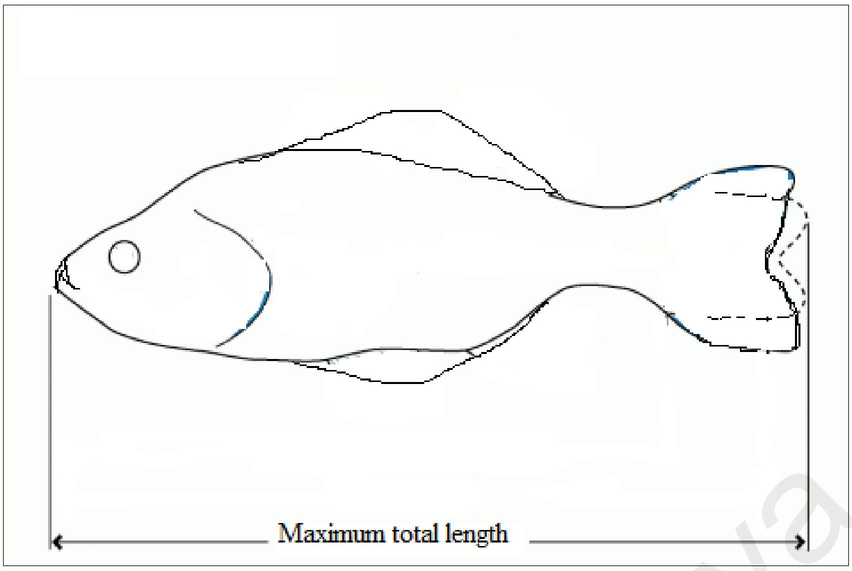


Figure 3.4: Measuring fish length

(Anderson & Gutreuter, 1983)

3.4.1.2 Sampling of sediment

Surficial sediments were collected from the bottom of the rivers and aquaculture ponds using a soil grabber (Figure 3.5). Bulk samples were placed into a low-density polyethylene sampling bag, labelled and returned to the laboratory and stored under -20 ºC.

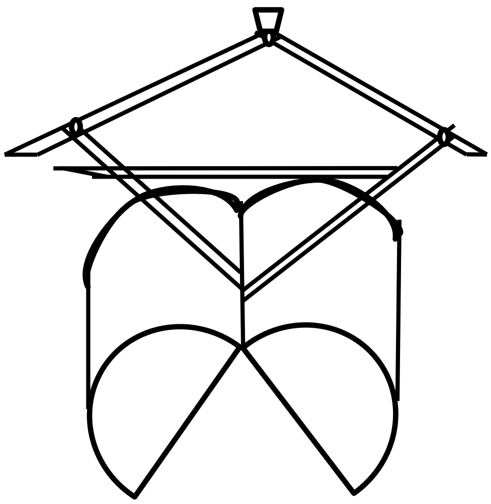


Figure 3.5: Illustration of a soil grabber

3.4.1.3 Sampling of water

The basic physical and chemical water quality parameters include temperature, conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), and pH were measured in-situ using a YSI Hydrolab multiparameter unit (YSI). For water sampling, the sampling bottles (high density polyethylene bottle) were rinsed twice with the water sample before collection of surface water. The closed-sampler was submersed and the bottle was opened to fill-in the sample and recapped sub-surface (USEPA, 1999). The samples were stored under 8 ˚C for further elemental analysis.

3.4.2 Sample pre-treatment

3.4.2.1 Processing of fish samples

After fish samples were partially thawed, tissues of muscle, gills, and liver had been removed using dissecting kits (stainless steel) and were cleaned several times with UPW. Each dissected sample was ground using octagonal agate mortar and pestle as to improve sample homogeneity. Next, these tissue samples were freeze-dried (CHRiST, Germany) and further homogenized with an octagonal agate mortar and pestle. Dried samples were kept in amber jars under the desiccator before undergoing microwave assisted digestion (MAD).

3.4.2.2 Processing of sediment samples

All foreign matters (stones, detritus) were removed from the sample before freeze-dried. The dried samples were ground and then passed through a 50-mesh sieve. The homogenized samples were kept then in amber jars under desiccator for the further analysis.

3.5 Microwave Assisted Digestion (MAD)

3.5.1 Digestion of fish sample

Tissue samples were analysed according to the method described by Low *et al.* (2012) in which microwave assisted digestion (MAD) was carried out in CEM Mar Xpress Microwave Accelerated Reaction System (CEM, Corporation, Matthews, NC, USA). About 0.25 g of dried samples was weighed directly into each 55 mL self-regulating control PFA® vessel and digested with a reagent consists of 2.50 mL of HNO_3, 0.50 mL of 30% (v/v) HCI and 7.00 mL of UPW. The microwave temperature was ramped to 185 ºC in 10.5 min. and held for 14.5 min. under microwave power of 800 W. After digestion, sample was cooled and filtered through 0.45 µm PTFE membrane before being transferred to a polypropylene volumetric flask (Class A) and the volume was made up to the mark with UPW. Analysis of certified reference material dogfish muscle (DORM-3) and dogfish liver (DOLT-4) also follow the same procedure.

3.5.2 Digestion of water sample

Water sample were treated according to USEPA Method 3015 (USEPA, 1994) using a Mar Xpress Microwave Accelerated Reaction System (CEM Corporation, USA). About 9.00 mL of water sample and 1.00 mL of 65% (v/v) HNO_3 were pipetted into a precleaned vessel for MAD. The temperature was brought to 160 ºC in 10 minutes and gradually risen to 170 ºC during the second 10 minutes. The sample was cooled down, filtered through 0.45 µm PTFE membrane into 25.0 mL volumetric flask and made up to the mark with UPW.

3.5.3 Extraction of sediment sample

Fine powdering of sediment sample was performed according to USEPA Method 3051A (USEPA, 2007). Approximately 0.50 g of sample was accurately weighed into a precleaned digestion vessel and 3.00 mL of 65% (v/v) HNO_3, 2.00 mL of 30% (v/v) H_2O_2 and 5.00 mL of UPW were added. The temperature of the mixture was risen to 175 ºC in 5.5 minutes and remained for 4.5 minutes in the microwave oven. After the sample was cooled to room temperature, the digestate was filtered through 0.45 μm PTFE membrane directly into a polypropylene volumetric flask (Class A). The residue was washed thoroughly with UPW, as the wash collected and filtered into the same volumetric flask before bought up to mark of 50.0 mL.

3.5.4 Storage of the Digestate Samples

All the digestate solutions were stored into polyethylene centrifugal tubes below 8 ºC and were analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) within 5 days (Low *et al.*, 2011).

3.6 Inductively Coupled Plasma-Mass Spectrometry

3.6.1 ICP-MS Setting

Studied elements concentrations were analyzed by Agilent 7500ce ICP-MS system which is equipped with an octopole reaction system (ORS). ICP-MS operating conditions are summarized in Table 3.3 and Table 3.4 (Low *et al.*, 2011).

Table 3.3: ICP-MS operating conditions

Tuning parameter	
Plasma RF power :	1500
Reflected power :	< 15 L min $^{-1}$
Plasma gas flow :	15 L min $^{-1}$
Auxiliary gas flow :	1.0 L min $^{-1}$
Sampling depth :	7.0 – 9.0 mm
Carrier gas flow :	0.8 – 1.0 L min $^{-1}$
Makeup gas flow :	0.1 – 0.3 L min $^{-1}$
Peristaltic pump :	0.1 rps
Collision gas type :	None/He/H_2
Collision gas flow :	0 – 5.0 L min $^{-1}$
Spray chamber temperature :	2 ºC
Sampler & skimmer cones :	Ni

Table 3.4: ICP-MS analytical conditions

Analyte	Isotope	ISTD	ORS	Possible interferences	Integration time (s)	MDL (µg kg^{-1})
Cr	52	^{45}Sc	He	$^{40}Ar^{12}C^+$,$^{35}CI^{16}OH^+$	1.5	2
Fe	56	^{45}Sc	H_2	$^{40}Ar^{16}O^+$, $^{40}Ca^{16}O^+$	0.3	30
Cu	63	^{72}Ge	He	$^{40}Ar^{23}Na^+$, $^{12}C^{16}O^{35}CI^+$, $^{12}C^{14}N^{37}CI^+$	1.5	9
Zn	66	^{72}Ge	He	$^{48}Ca^{18}O^+$	0.3	49
As	75	^{72}Ge	He	$^{40}Ar^{35}CI^+$, $^{40}Ca^{35}CI^+$	1.5	11
Se	78	^{72}Ge	H_2	$^{40}Ar^{38}Ar^+$	1.5	1
Cd	111	^{103}Rh	Normal		0.3	1
Pb	*	^{209}Bi	Normal		0.3	6

* Pb = ^{206}Pb + ^{207}Pb + ^{208}Pb

3.6.2 ICP-MS Tuning

Tuning the ICP-MS parameters is indispensable to address the sensitivity and matrix tolerability for efficient experiments. The ICP-MS operating conditions were optimized daily with the help of a tuning solution from Agilent Technologies (USA) consisting of 1 μg L^{-1} each of Li, Co, Y, Ce and TI in 2% HNO_3 which was spiked with 0.5% HCI in order to cover the entire mass region of interest including the chloride interferences.

3.7 Data Analysis

All calculations and statistical analyses were performed with Microsoft® EXCEL® 2010 and SAS® JMP® version 9 software package. The whole ICP-MS data matrix is composed of 520 samples (20 samples of each sample form each habitat) and 13 variables (concentrations of 13 elements). Analysis of variance (ANOVA) was carried out to assess the significant differences of studied samples between the two habitats. For unsupervised study, HCA and PCA were applied with training set with the aim of obtaining an overview of the clustering and multivariate distribution pattern of the studied elements.

HCA converts the original ICP-MS data into one-dimensional dendograms based on the Ward algorithm and Euclidean distance (Low *et al.*, 2009). Principal component analysis (PCA) was used to ascertain source of pollution (natural and anthropogenic) in the sediment sample. Varimax rotation was applied to minimize the number or variables with high loading on each factor (varimax factor) and facilitate interpretation of results (Low *et al.*, 2011).

In order to eliminate variance difference between variables, PCA was performed on the correlation matrix of the original variables. The original dataset of fish samples composed a 360 x 12 matrix (360 tissue samples by 12 elements). PCA linearly transforms the training set into a product of two matrices, one which contained information about the metal concentrations (loadings) and the other provides information of the samples (scores). Each PC value is associated with an eigenvalue, where PC1 had the largest eigenvalue and carries the most variance of the original data compared to other PCs, which then decreases in the order of variation. The score-loadings biplots improve the interpretability of the ICP-MS results (Low *et al.*, 2009).

3.8 Indices and Risk Characterization

3.8.1 Water

3.8.1.1 Chronic daily intake of water

Human health risks associated with the ingestion of metals through water were assessed in this study. Metals can enter into human body through several pathways including food intake, dermal contact and inhalation (Muhammad *et al.*, 2011). The chronic daily intake (CDI) [mg/(kg·day)] of a metal species through water ingestion was calculated by equation (Shah *et al.*, 2012; Muhammad *et al.*, 2011);

$$\mathrm{CDI} = \frac{\mathrm{Cm\ x\ Iw}}{\mathrm{Wb}} \tag{3.1}$$

where Cm (mg L^{-1}) is the metal concentration in water, Iw (L day^{-1}) is the average daily intake of water (2 L day^{-1}) (USEPA, 2011), and Wb (kg) is the average body weights (64 kg) respectively (Muhammad *et al.*, 2011; Jan *et al.*, 2010; Khan *et al.*, 2010).

3.8.1.2 Health risk indexes of metals

To estimate the chronic health risk associated with a particular metal species via daily intake of water, health risk index (HRI) was calculated by using the equation below (Shah *et al.*, 2012; Muhammad *et al.*, 2010);

$$HRI = \frac{CDI}{RfD} \quad (3.2)$$

where the oral toxicity reference dose [(RfD, mg (kg.·day)$^{-1}$] values are given in Table 3.5. Values of HRI less than one is considered to be safe for the consumers, while HRI > 1 may cause potential health risk (Kumar *et al.*, 2016; Khan *et al.*, 2008).

Table 3.5: Oral toxicity reference dose (*RfD*)

(USEPA, 2000; 1997)

Metal	*RfD* [mg(kg·day)$^{-1}$]
[a] Cr	1.5
Fe	7×10^{-1}
Cu	4×10^{-2}
Zn	3×10^{-1}
[b] As	3×10^{-4}
Se	5×10^{-3}
Cd	1×10^{-3}
Pb	4×10^{-3}

[a] Cr^{3+} (assuming all Cr are Cr^{3+})
[b] Inorganic As (assuming 10% of the total As are inorganic As)

3.8.2 Sediment

Background values play an important role in the interpretation and analysis of geochemical data. Previous studies used average shale values or average crustal abundance data as a reference baseline (Islam *et al.*, 2015; Sakan *et al.*, 2009; Rubio *et al.*, 2000). In this work, sediment contaminations of the study area were assessed and evaluated by using four different indices.

3.8.2.1 Geoaccumulation Index (I_{geo})

The geoaccumulation index can be used to assess the degree of contamination from the metals in sediments (Saleem *et al.*, 2015; Santos Bermejo *et al.*, 2003). It was calculated by using the equation (Müller, 1969);

$$I_{geo} = \log_2 {}^{(\text{sediment})}\!/_{1.5 \text{ x reference sample}} \tag{3.3}$$

where (sediment) is the measured concentration of metal (mg kg^{-1}) in the sediment sample, reference sample is refer to geochemical background value (mg kg^{-1}). While factor 1.5 was introduced to reduce the effect of possible variations in the background values which might be associated with lithologic variation in the sediments and to discover small anthropogenic activities (Al-haidare *et al.*, 2010). According to the crustal abundance data of the previous studies (Kabata-Pendias & Mukherjee, 2007; Krauskopf & Bird, 1995), the reference samples were Cr : 100, Fe : 50 000, Ni : 75, Cu : 55, Zn : 70, As : 1.8, Cd : 0.2, and Pb : 13 mg kg^{-1}. According to Müller (1969), the corresponding relationship between the Igeo and the pollution level was interpreted as reported in Table 3.6.

3.8.2.2 Enrichment Factor (EF)

The Enrichment Factor (EF) is widely used as a tool to differentiate natural and anthropogenic sources and to reflect the status of environmental contamination, based on the use of a normalization element in order to lighten the variations produced by heterogeneous sediments (Zahra *et al.*, 2014; Zhang *et al.*, 2007).

The EF for metal concentrations in sediments was calculated as (Varol, 2011);

$$EF = \frac{X/\mathrm{Fe\ (sediment)}}{X/\mathrm{Fe\ (background)}} \tag{3.4}$$

where X is the studied metal and x/Fe is the ratio of the concentration of element X to iron. Iron (Fe) was used as the reference material and element of normalization to the textural characteristics of sediments. In this study, iron was used as the reference element for geochemical normalization because of the following reasons: 1) Fe is associated with fine solid surfaces; 2) their geochemistry is likely the same to other elements and 3) their natural concentration tends to be uniform (Bhuiyan *et al.*, 2010). The background concentrations of Cr, Fe, Ni, Cu, Zn, As, Cd and Pb were obtained from Krauskopf & Bird (1995) and Turekian & Wedepohl (1961) while EF values were interpreted as in Table 3.6.

Table 3.6 : Classes of Igeo and EF in relation to enrichment and pollution levels

I_{geo} value	I_{geo} class	Pollution level	EF classes	Enrichment level
≤ 0	0	Unpolluted	EF = < 1	No enrichment
0 -1	1	Unpolluted to moderately polluted	EF = 1-3	Minor enrichment
1 -2	2	Moderately polluted	EF = 3-5	Moderate enrichment
2 - 3	3	Moderately to strongly polluted	EF = 5-10	Moderately severe enrichment
3 - 4	4	Strongly polluted	EF = 25-50	Very severe enrichment
4 - 5	5	Strongly to very strongly polluted	EF > 50	Extremely enrichment
> 5	6	Very strongly polluted		

I_{geo} : (Abrahim & Parker, 2008; Müller, 1969)
EF : (Ghrefat *et al.*, 2011; Sakan *et al.*, 2009)

3.8.3 Fish

3.8.3.1 Morphometric data of Fish

The condition factors (CFs) used to quantify the condition of fish which include the degree of maturity and nourishment (Williams, 2000). CFs can be estimated by the following equation;

$$CF = \frac{100000m}{l} \qquad (3.7)$$

where *m* is the weight of the studied fish in grams, and *l* is the length of the fish in millimetres.

3.8.3.2 Hepatosomatic index (HSI)

Another metric commonly used to evaluate fish condition, HSI can provide information about metabolic activity in the liver, and is calculated as;

$$HSI = \frac{WL}{WT} \qquad (3.8)$$

where HSI is defined as the ratio of liver weight (WL) to body weight (WT) that normally provides the status of energy reserve in a fish (Ureña *et al.*, 2007).

3.8.3.3 Estimated daily intake (EDI)

Daily intake was estimated based on the basis metal concentration levels in the fish muscle. Several methods have been proposed for the estimation of potential risk to human health due to metal exposure. Thus, dietary exposure was calculated based on the total concentration of exposed metal in the fish muscle and daily fish consumption rates.

Apart of that, estimated daily intake was compared with the current provisional maximum tolerable daily intake (PMTDI) and acceptable daily intake (ADI) previously founded by the Joint FAO/WHO Expert Committee on Food Additive online database (FAO, 2006; JECFA, 2000; 1982). If the EDI exceeds this threshold, it might cause potential non-carcinogenic effects. The EDI of metal from fish consumption per meal was calculated according to the equation used by Wei *et al.* (2014);

$$\text{EDI} : \frac{(\text{C} \times \text{FIR} \times \text{ED} \times \text{EF})}{\text{WAB} \times \text{TA}} \text{ x } 10^{-3} \qquad (3.9)$$

where C is the average content of metal in fish (mg kg^{-1} wet weight)

FIR is the fish ingestion rate, 71 g day^{-1} $person^{-1}$

ED is the exposure duration; 70 years, average lifetime

EF is the exposure frequency; 365 day $year^{-1}$ (Agusa *et al.*, 2007; FAO, 2009; 2008; 2005)

WAB is the average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Lim *et al.*, 2000)

TA is the average time of exposure; 365 day $year^{-1}$ $\times$ ED (Wei *et al.*, 2014)

3.8.3.4 Target Hazard Quotient (THQ)

In the present study, the methodology of estimation target hazard (TH) will provide indication of human health risk due to metal exposure. The equation was used to estimate the risk as follows (Waheed *et al.*, 2013);

$$\text{THQ} : \frac{(\text{C} \times \text{FIR} \times \text{ED} \times \text{EF})}{\text{WAB} \times \text{TA}} \ \text{x}\ 10^{-3} \qquad (3.10)$$

where EF is the exposure frequency ; 365 days year^{-1}

ED is the exposure duration; 70 years (Agusa *et al.*, 2007; FAO, 2009; 2008; 2005)

FIR is the fish ingestion rate; fish : 71 g day^{-1} person^{-1} (FAO, 2008)

C is the metal concentration in muscle of studied fish; edible fish part (mg kg^{-1};wet weight)

RfD is the oral reference dose (USEPA, 2000; 1997)

WAB is the average body weight; 64 kg, the reference weight were derived from numerous local Malaysia studies (Lim *et al.*, 2000)

TA is the average time of exposure; 365 day year^{-1} $\times$ ED (Wei *et al.*, 2014)

As a conclusion, there is no potential risk found related to the studied metals if their hazard quotients are less than one (HQ<1) (Khan *et al*., 2008).

3.8.4 Bioconcentration factor (BCF)

For the evaluation of the fish's ability to accumulate metal elements from the ambient, the bioconcentration factor was used. It was calculated as (Islam *et al.,* 2015);

$$BCF = \frac{Cfish}{Cwater} \tag{3.11}$$

where C_{fish} is the concentration of the metal elements in the muscle of fish; C_{water} is the concentration of the metal element in water environment.

If BCF >1, it indicates that the fish has a potential to accumulate the metal but is generally not considered to be significant unless the BCF exceeds 100 or more (USEPA, 1991).

3.8.5 Bio-sediment accumulation factor (BSAFs)

In order to estimate the proportion in which metal occurs in the organism in the associated sediment, bio-sediment accumulation factor (BSAF) was calculated for selected studied elements in the muscle tissue according to formula below (Szefer *et al.*, 1999);

$$BSAF : \frac{Cs}{Cx} \tag{3.12}$$

where, Cx and Cs are the mean concentration of metal in the muscle tissue and in the associated sediment, respectively.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Microwave Assisted Digestion Method Verification

The analytical performance of the microwave assisted digestion/extraction of fish, water and sediment were checked with the respective certified reference materials (CRMs), i.e DORM-3 dogfish muscle, DOLT-4 dogfish liver, SLRS-4 riverine water from the National Research Council Canada (NRCC) and BCR-14R powdered sewage sludge from Institute for Reference Materials and Measurements (Belgium).

As can be seen in Tables 4.1 - 4.4, there are good agreements between the ICP-MS measurements and the certified values. In all cases, the recoveries were found to be in the range of 78% - 115% with relative standard deviation < 5%, within the traditionally accepted results for the certified values; which means that the results of the present study can be considered to be accurate with respect to the true concentrations in the samples (Mziray & Kimirei, 2016).

Table 4.1: Analysis of DORM-3 dogfish muscle certified reference material

		DORM-3		
	Certified / mg kg^{-1}	Found / mg kg^{-1}	Recovery / %	RSD / %
Cr	1.9 ± 0.2	1.9 ± 0.2	102	0.1
Fe	347 ± 20	365 ± 23	105	0.08
Ni	1.3 ± 0.2	1.2 ± 0.1	92	0.2
Cu	15.5 ± 0.6	15.8 ± 0.2	102	0.02
Zn	53 ± 3	55 ± 3	104	0.06
As	6.9 ± 0.3	7.3 ± 0.1	106	0.02
Se		-		
Pb	0.40 ± 0.05	0.44 ± 0.02	110	0.1

95% confidence interval (*n*=7)
Note : Se not certified

Table 4.2: Analysis of DOLT-4 dogfish liver certified reference material

DOLT-4				
	Certified / mg kg^{-1}	Found / mg kg^{-1}	Recovery / %	RSD / %
Cr		-		
Fe	1833 ± 75	1817 ± 43	99	0.03
Ni	1.0 ± 0.1	0.9 ± 0.1	90	0.1
Cu	31 ± 1	32 ± 2	102	0.08
Zn	116 ± 6	118 ± 7	102	0.08
As	9.7 ± 0.6	9.6 ± 0.3	99	0.04
Se	8 ± 1	9.2 ± 0.7	115	0.1
Pb	0.16 ± 0.04	0.16 ± 0.04	100	1.0

95% confidence interval (*n*=7)
Note : Cr not certified

Table 4.3: Analysis of SLRS-4 riverine water certified reference material

SLRS-4				
	Certified / µg L^{-1}	Found / µg L^{-1}	Recovery / %	RSD / %
Cr	0.33 ± 0.02	0.3 ± 0.1	91	4.2
Fe	103 ± 5	106 ± 3	103	3.2
Ni	0.67 ± 0.08	0.58 ± 0.5	87	1.1
Cu	1.81 ± 0.08	1.8 ± 0.1	99	0.8
As	0.68 ± 0.06	0.66 ± 0.2	97	0.3
Zn		-		
Pb		-		

95% confidence interval (*n*=7)
Note : Pb and Zn not certified

Table 4.4: Analysis of BCR-14R powdered sewage sludge certified reference material

	BCR-14R			
	Certified / mg kg^{-1}	Found / mg kg^{-1}	Recovery / %	RSD / %
Cr	196 ± 7	152 ± 9	78	0.08
Fe		-		
Ni	70 ± 5	63 ± 10	90	0.2
Cu	838 ± 16	814 ± 32	97	0.05
As		-		
Zn	3060 ± 60	3010 ± 57	98	0.03
Pb	609 ± 14	569 ± 13	93	0.03

95% confidence interval (*n*=7)
Note : Fe and As not certified

4.2 The Water Quality

Water quality is a reflection of the source environment and degree of anthropogenic activities which highly influence the uses of a water-body. The appraisals of water quality are displayed on Table 4.5.

Table 4.5: The physico-chemical quality of water at each sampling site

	Selangor		**Pahang**	
	Aquaculture pond	**Selangor River**	**Aquaculture Pond**	**Pahang River**
Location	N 03º 25.0' E 101º 21.2'	N 03º 21.9' E 101º 18.7'	N 03º 31.4' E103º 10.1'	N 03º 30.5' E103º 05.1'
Temperature (ºC)	32 ± 1	30 ± 1	27 ± 1	25 ± 1
Conductivity (µS cm^{-1})	524 ± 1	203 ± 43	45 ± 1	44 ± 1
TDS (g L^{-1})	0.31 ± 0.02	0.12 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
Salinity	0.20 ± 0.02	0.10 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
DO (%)	7.7 ± 0.4	3.0 ± 0.3	5.2 ± 0.1	3.0 ± 0.9
pH	6.8 ± 0.1	5.5 ± 0.4	6.2 ± 0.4	6.0 ± 0.3

95% confidence interval (*n*=20)

There were noticeable discrepancies observed between the physico-chemical properties plausibly owing to spatial variability between sampling sites and the water management program.

4.2.1 Physico-chemical characteristics

Among the water quality assessment, the measurements of physico-chemical conditions have been regarded as one of the common practices that address the water status, productivity and sustainability. In this study, the physico-chemical parameters comprise temperature, conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), and pH measurement collected at each sampling sites summarized in Table 4.5. Each parameter has its own role to play; moreover, the aggregate effect is the summation of the interaction of all the parameters.

4.2.1.1 Water temperature

Water temperature is a crucial factor in water quality assessment as all the water chemistry, biochemical and geochemical processes are functions of temperature. It can interact with several other parameters and alter the physicochemical properties of a water-body (Rice & Jastram, 2015; Luce *et al.*, 2014; Xin & Kinouchi, 2013).

Table 4.5 shows a deviation of about ± 1 ºC in all the temperatures measured. The variations in water temperature are related to the temporal variability during sampling period (10 am – 1 pm). The mean water temperature observed at the Selangor sampling sites was significantly higher than those at Pahang. Nevertheless, the mean value was within the acceptable condition outlined by World Health Organization (2011). Water temperature can vary due to the length of a river and human activities which include urban and industrial effluent release, agricultural harvesting and urban development (Perry &

Vanderklein, 1996). Due to this, water temperature was found to be significantly higher in Selangor sampling sites because of the water pollution in this river basin as reported by Sharif *et al.* (2015).

Another noteworthy observation is the mean temperature at aquaculture sites which was always greater than the rivers. This might be caused by specific aquaculture practices and management, which recommend temperature range between 26 – 32 ºC to support fish growth performance. It is suggested that warm temperature of pond waters should be managed to maintain faster growth of fish (Boyd, 1990).

4.2.1.2 Conductivity

The water conductivity is affected by the water temperature. In this regard, the temperature can alter the viscosity of water and the solubility of salts which affect both the mobility and the concentration of dissolve ions that determining the electrical potential in a conductivity measurement.

For comparison of conductivity measurements at different temperature, the reading was corrected to 25 ºC. In Table 4.5, much higher conductivity readings were found on the sampling sites in Selangor compared to Pahang. This was most likely due the runoffs and/or discharges from the palm oil, rubber plantations, and aquaculture farms nearby the study area of Selangor (Ali *et al.*, 2009; Leong *et al.*, 2007).

The statistics also indicates the conductivity measured in Selangor aquaculture pond sites to be significantly higher than Selangor River. The deviation was subjected to the effect of total dissolved solids (TDS) as a consequence of high organic residue from the scheduled feeding activity implemented by the pond management.

Moreover, high water temperature favours the degradation of organic residues thus increasing the level of conductivity in water (Mishra *et al.*, 2008; Sreenivaso Rao & Ramamohana Rao, 2001).

4.2.1.3 Total disssolved solids

Total dissolved solids (TDS) dependent on concentration of soluble particles, which is in turn dependent of temperature. Higher TDS readings were found in the Selangor sampling sited which most likely of anthropogenic origin (Sharif *et al.*, 2015). Besides this, the sedimentation of feed residues, unused agrochemicals and excreta also contribute towards the increase of TDS in aquaculture pond (Mishra *et al.*, 2008).

4.2.1.4 Salinity

Salinity is the measurement of dissolved salts in the water system. Salinity is associated with evaporation and precipitation processes which are important to study because of their impact on density; where warm water is less dense and salty water is heavier. Most of the aquatic systems with high level of salinity are due to dissolved chemicals from rocks and soil and human activities as well. Thus, higher readings of salinity in the Selangor sampling sites are most likely due to anthropogenic factors and natural depositions (Mishra *et al.*, 2008; Pillsbury, 1981).

4.2.1.5 Dissolved oxygen

Dissolved oxygen (DO) is an important environmental parameter which indicates the ecological health status of the aquatic ecosystem as well as a parameter which is important to monitor in order to protect aquatic life (Chang, 2005). Basically, the solubility of oxygen decreases as temperature increases, however, inverse trend was revealed in the data. Higher percentage of DO was recorded in the aquaculture ponds where the aeration system available helped in sustaining the concentration of DO (Boyd, 1997). Although DO levels were much lower in the rivers, the levels were still above the minimum requirement to support aquatic life (USEPA, 2000).

4.2.1.6 pH

Water pH can affect both chemical and biological processes in the water-body. Values of pH below than 4.5 and above 9.5 are lethal to aquatic organism, furthermore, extreme changes of pH values also alter the reproduction and biological processes. Moreover, highly acidic waters had been reported to affect the solubility of certain minerals and metals in the water (Perry & Vanderklein, 1996).

The river waters were less influenced by a higher degree by biological activity, therefore, showed lower pH than aquaculture sites (Table 4.5). In order to control the pH in aquaculture ponds, several treatments are commonly introduced such as agricultural limestone, gypsum, calcium chloride, alum, and sulphuric acid. Such water management practices definitely alter the metal concentrations in the aquaculture ponds (Mishra *et al.*, 2008).

4.2.2 Metal Concentrations in Surface Water

The concentration of 13 trace elements in water samples are summarized in Table 4.6. The mean metal concentrations in water differed significantly between the studied site for all the elements ($p < 0.05$) with the exception of Cr and Cd (which remained below the detection limits). Variations in the metal concentrations can be related to the human activities surrounding them.

Although the aquaculture ponds heavily relies respectively on Selangor River and Pahang River as their main sources, there are still variance between some of their physical and chemical characteristics. These are due to the practices and water management program related to fish farming.

For instance, high density and substantial feeds increase the load of metals in pond water body (Simões *et al.*, 2008), moreover, the usage of chemical additives to enhance the water quality and/or control biological problems also contribute to the increased metal levels (Boyd, 1995). Consequently, the metal concentrations in the water samples from the aquaculture ponds are generally higher than the river.

One of the significant observations are the mean concentrations of Na, Mg, K, and Ca that were found to be higher in the water body of aquaculture ponds compared to the riverine sources regardless of the geographical location of the study area ($p < 0.001$) (Figure 4.1). This pattern is mostly due to the application of gypsum and lime which are used to control the alkalinity, hardness, turbidity, nitrite level, bacteria and abundance of phytoplankton in an objective for better production (Boyd, 1990).

Introduction of such soluble salts into the aquaculture practices not only shifted the levels of Na, Mg, K, and Ca concentrations, but also alters the physicochemical conditions of water body such as conductivity, TDS, DO and pH (Mishra *et al.*, 2008) (as seen in Table 4.5 and Table 4.6).

Table 4.6: Mean concentrations of metals in the water body

	Selangor		Pahang	
(mg L^{-1})	Aquaculture pond	Selangor River	Aquaculture pond	Pahang River
Na	24.9 ± 0.4	6.5 ± 0.1	10.0 ± 0.1	2.5 ± 0.02
Mg	8.3 ± 0.1	0.2 ± 0.02	1.7 ± 0.02	0.9 ± 0.02
K	22.9 ± 0.2	2.0 ± 0.1	4.5 ± 0.2	3.3 ± 0.1
Ca	21 ± 1.0	3.1 ± 0.2	2.8 ± 0.2	1.8 ± 0.2
Cr	< 0.007	< 0.007	< 0.007	< 0.007
Fe	< 0.5	1.0 ± 0.1	4.03 ± 0.1	5.4 ± 0.1
Ni	< 0.01	< 0.01	< 0.01	< 0.01
Cu	< 0.008	< 0.008	< 0.008	< 0.008
Zn	< 0.09	< 0.09	< 0.09	< 0.09
As	< 0.002	< 0.002	< 0.002	< 0.002
Se	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cd	< 0.0003	< 0.0003	< 0.0003	< 0.0003
Pb	< 0.001	< 0.001	< 0.001	< 0.001

95 % confidence interval, n = 20
Note : Cr, Ni, Cu, Zn, As, S, Cd, Pb : below the LoD

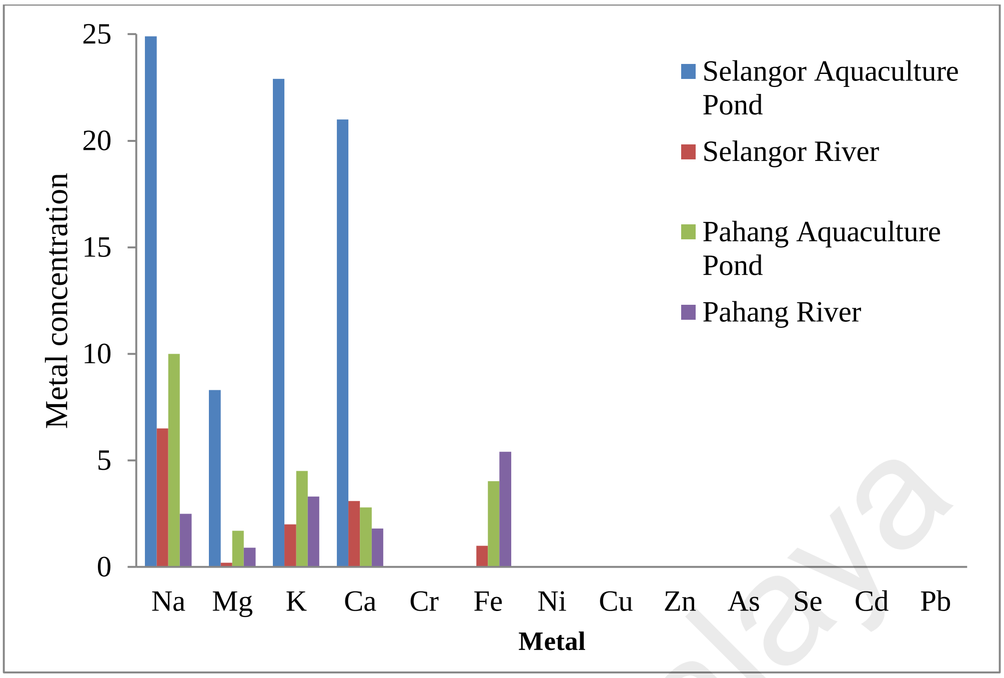


Figure 4.1: Distribution of metal concentration in water samples
Note : Cr, Ni, Cu, Zn, As, Se, Cd, Pb : below the LoD

According to the literature, the domestic and industrial wastes are commonly contribute to anthropogenic inputs of Cr, Fe, Cu, Zn, Se, As, Cd, and Pb in the water body (Morais *et al.*, 2016). For instance, the metals in the aquaculture ponds were plausibly contributed from residues of metal enriched feeds (Table 4.7) (Low *et al.*, 2016). In this regard, relatively higher metal content was expected from Selangor River due to the burden from discharge of sewage, industrial wastewater and agricultural runoffs (Leong *et al.*, 2007), while agricultural and mining activities are the suspected sources of metal inputs into Pahang River (Kamaruzzaman *et al.*, 2011; Shuhaimi-Othman *et al.*, 2008). However, the findings from surface water quality assessment did not fully reveal the situation because part of the metal inputs could be absorbed onto sediment particles or up taken and accumulated in aquatic organisms (Rios-Arana *et al.*, 2003).

Table 4.7: Selected metal concentrations in the feeding pellets

Mean concentration of metal / mg L^{-1}	Selangor	Pahang
As	1.6 ± 0.1	1.4 ± 0.03
Cd	0.22 ± 0.01	0.23 ± 0.01
Pb	0.15 ± 0.001	0.12 ± 0.01

95% confidence interval, n = 20

4.2.3 Water Quality Criteria

The National Water Quality Standards (NWQS) provides a general guideline for river water quality assessment and management in Malaysia (Hasan *et al.*, 2015). Based on the water NWQS quality criteria specified in Table 4.8, a minimum of Class III water supply is necessary to sustain the aquatic species in aquaculture fishery (Hasan *et al.*, 2015). Alternatively, USEPA's aquatic life ambient water quality criteria can also be adopted (USEPA, 1999).

The USEPA recommended values refer to the highest concentration of metal or the desired conditions which are not expected to pose a significant risk to the majority of aquatic species. The results shown in Table 4.6 indicate that the mean concentration of Cr, Ni, Cu, Zn, Se, Cd and Pb were well below those benchmark levels, except for Fe. Elevated level of Fe recorded at Pahang sampling sites has been linked with the consequences of bauxite mining activities in Pahang and could well be correlated to the rainfall intensity (Kamaruzzaman *et al.*, 2011; Suhaimi-Othman *et al.* 2008).

Table 4.8: Water Quality Criteria and Standards

	NWQS (DOE, 2007)	Drinking Water Quality Standards		
Concentration of metal / mg L^{-1}	Class III[#]	Malaysia (MOHM, 1995)	WHO (2006)	USEPA (2009)
Na	-	200	-	-
Mg	-	150	-	-
K	-	-	-	-
Ca	-	-	-	-
Cr	-	0.05	0.05	0.1
Fe	1	1.0	-	0.3
Ni	0.9*	-	0.07	-
Cu	-	1.0	2	1.3
Zn	0.4*	3	3	5
As	0.4(0.05)	0.01	0.01	0.01
Se	0.25(0.04)	0.01	0.01	0.05
Cd	0.01*(0.001)	0.003	0.003	0.005
Pb	-	0.01	0.01	0.015

[#] : Maximum (unbracketed) and 24-hour average (bracketed) concentrations
* : At hardness 50 mg L^{-1} $CaCO_3$
- : Not reported

In the context of health concern, the measurements obtained were compared to parametric values documented in Malaysian Raw Water Quality Criteria (MOHM, 1995), The World Health Organization (WHO)'s Drinking Water Standard (WHO, 2006) and National Drinking Water Regulations were established by the United State Environmental Protection Agency (USEPA, 2009). These references provide convenient compendium guidance limits to safeguard the community. Although Table 4.6 demonstrates variability in surface water quality across sampling locations, all targeted elements are well within those defined limits.

4.2.4 Principal Component Analysis of Water Samples

Principal Component Analysis (PCA) is a tool to observe the variability of multivariate data by converting all the possible correlated variables into a set of linearly independent variables, which are called the principal components (PCs). This standard approach has been often used to identify environmental pollution due to major pollutants, influential factors or identified sources (Liu *et al.* 2008). In order to explore the latent patterns in multi-element data from ICP-MS analysis, PCA was used to examine the interrelation among the water samples and metal content (Low *et al.*, 2016).

PCA decomposed the data matrix (80 samples < 20 samples of each sampling site > and 13 metal variables) into a product of two matrices which contain information about the water samples (scores) and the metal (loadings) (Low *et al.*, 2016). The first principal component (PC1) accounts for the largest amount of variation in the data set, while subsequent components describe progressively decreasing amounts (Astorga-España *et al.*, 2014).

To ease interpretation of PCA, the Kaiser Criterion and scree test are most widely used as guidelines to extract the significant principal components (PCs) (Catell, 1996; Kaiser, 1960). Kaiser Criterion (1960) drops the PCs that explain less than a single unit of the observed variable's variation, whereas scree test retains only those PCs above the point of inflection on a scree plot (Figure 4.2) (Catell, 1996). Hence, the following results and discussion are focused on the variations described by PC1 (45%) and PC2 (12%) only as shown in Figure 4.3.

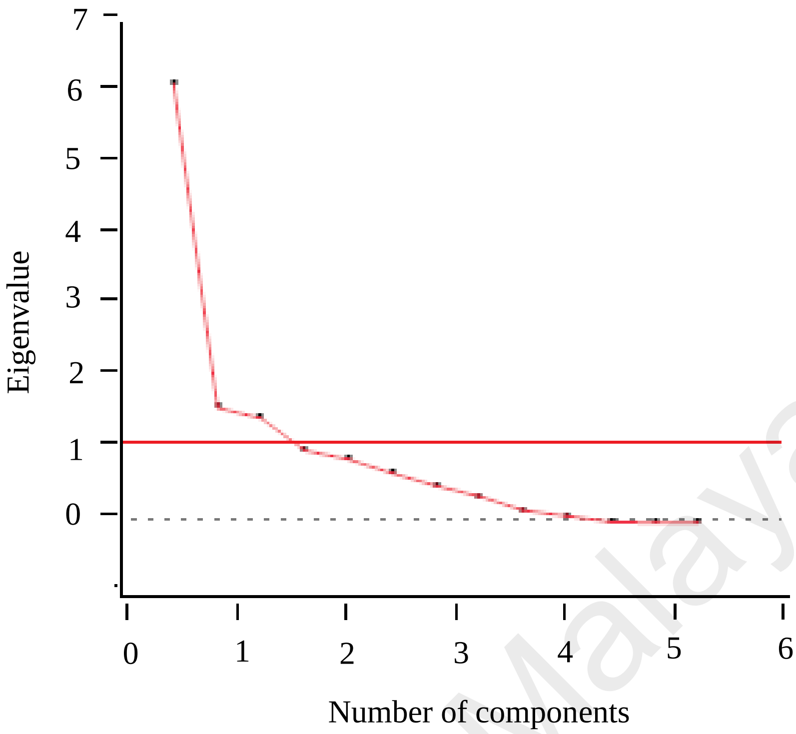


Figure 4.2: Scree graph for PCA with 13 elemental variables of water samples

As shown in the biplot by the first two PCs (Figure 4.3), the water samples are grouped into three major clusters that could be linked with their origins. For instance, Cluster I comprises the PC scores of Pahang water samples (river and aquaculture pond), whereas water samples from Selangor River and Selangor aquaculture pond make up Cluster II and Cluster III respectively. Such classification demonstrates the reasonable association between elemental variability and the identity of water sources, although there was non-linear separation between water samples from Pahang River and Pahang aquaculture pond.

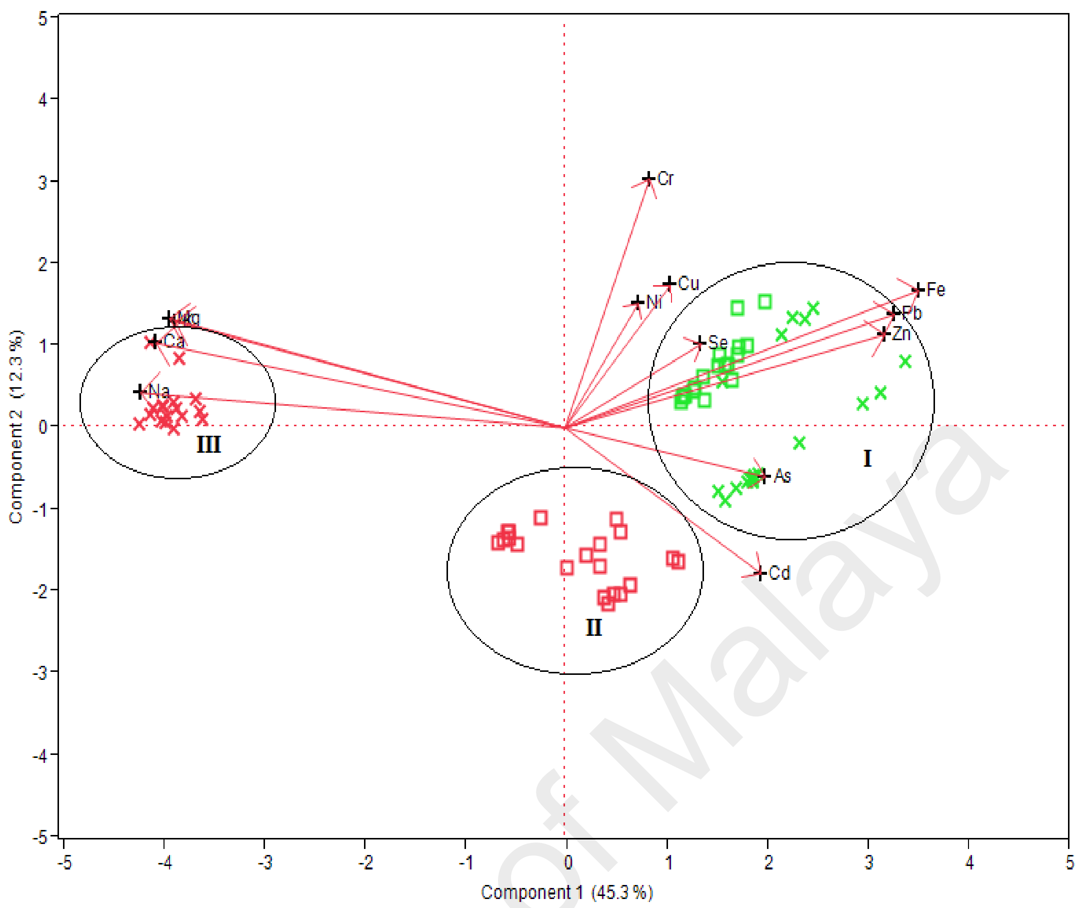


Figure 4.3: PCA bi-plot for water samples (*n* = 80). The red colour denoted to water samples from Selangor and green colour denotes water samples from Pahang (□ ; river, x ; aquaculture pond)

Basically, Cluster I (Pahang water samples) can be characterized with high loadings of Cr, Cu, Fe, Ni, Pb, Se, and Zn. This was possibly due to the results of mining and plantation agriculture (Kamaruzzaman *et al.*, 2011). From Figure 4.3, it is also noted that the Cd loadings mainly correspond to the Cluster II (water samples from Selangor River). The input of Cd is arguably related to the utilization of phosphate fertilizer and pesticide from palm oil plantation and rubber estate along the Selangor River (Fu *et al.*, 2014). The observations are compatible with the findings reported at Sungai Gombok, Selangor (with 0.0012 mg Cd L^{-1} (Ismail *et al.*, 2013)). The negative loadings of Ca, K, Mg, and Na on PC2 in Figure 4.3 are strongly associated with Cluster III. Such pattern is consistent with the measurement displayed in Table 4.6, where the highest concentrations of Ca, K, and Na were observed on the water samples from Selangor aquaculture pond. Again, the utilization of various salts in aquaculture water quality management is evident (Boyd, 1990).

In order to have a better insight about Cluster I, the water samples from both Pahang River and aquaculture pond of Pahang underwent further PCA. The biplot is shown in Figure 4.4 with two distinct clusters where the first two PCs accounted about 86% of the total variation. In this case, the Cluster I(a) is made up of the scores of water from aquaculture pond, whereas the samples from Pahang River are grouped in Cluster I(b). These clusters are partitioned by the axis of PC2, and can be simply discriminated based on their members' scores along PC1.

In other words, on PC1 the positive loadings of Mg, K, Na, and Ca are associated with Cluster I(a), whereas the negative loadings of Fe is for Cluster I(b). The revealed trends agree with the argument discussed in the previous section (Table 4.6), which show positive loadings of Mg, K, Na, and Ca in the aquaculture pond indicating the practices that have been applied (Boyd, 1990).

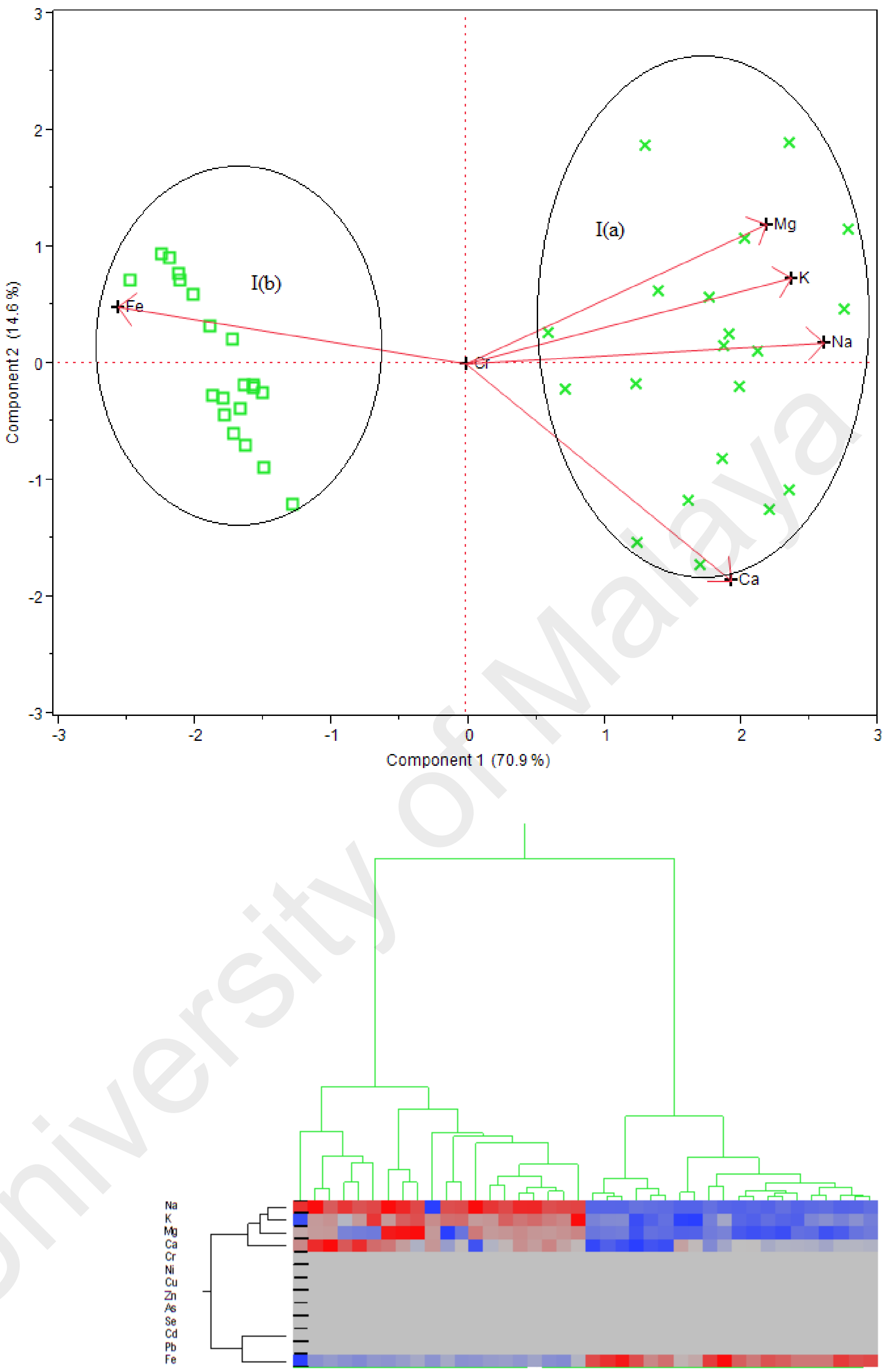


Figure 4.4: Biplots and dendograms using subset comprising water samples from Pahang (n = 40). (□ ; river, x ; aquaculture pond)

Note : Cr, Ni, Cu, Zn, As, Se, Cd, Pb : below the LoD

A finding from the dendogram analysis is identified by two distinct clusters based on sample origins (Figure 4.4). The observed clustering tendency shows that the variance is due to the variability in metal concentration at each sampling site. The two-way dendogram shows that the water sample from the aquaculture pond was the main depository for Mg, K, Na, and Ca. Concentration of these elements are significantly related to the pond management and practices (Boyd, 1995). Whereas, level of Fe concentration in Pahang River can be associated with bauxite mining activities (Kamaruzzaman *et al.*, 2011; Suhaimi-Othman *et al.* 2008).

4.2.5 Human Health Risk Assessment

4.2.5.1 Chronic daily intake of metals

From the perspective of health, it is important to evaluate and study the metal concentrations present in water column (Frisbie *et al.*, 2002). The discharge of metals into water systems may affect the environment including the ecological community and living organisms in the receiving water. Moreover, metal elements able to contaminate aquatic ecosystems resulting in declined quality of drinking and water irrigation (Muhammad *et al.*, 2011). Through inter-state raw water transfer project, Pahang River became important in the water supply sector because of its role in supplying water to Selangor residents (Abidin, 2004). Deterioration of water quality in Pahang River basin not only affects the water supply in Pahang but also the other areas including Selangor State and Federal Territory of Kuala Lumpur (Tan & Mokhtar, 2009). Therefore, several methods have been proposed for the estimation of the potential risks to human health due to water contamination.

The USEPA describes chronic daily intake (CDI) as ‘exposure to contaminant which expressed as a mass of substance contacted per unit of body weight per unit time averaged of time’ (McKone & Daniels, 1991). The CDI is also known as the ‘lifetime average daily dose’; which describes the probability of getting cancer over human lifetime (Crockett, 1996). Through this study, the CDI of selected metal elements through water ingestion were assessed (Shah *et al.*, 2012) and are summarize in Table 4.9, focusing only on water sample from the river. This is because Pahang River and Selangor River play an important role as water supply for domestic uses (Fulazzaky, 2010; Abidin, 2004).

The CDIs of metals were found to be in the order of Fe > Zn > Cu > Cr > As > Pb > Se > Cd for both river with the highest Fe recorded at Pahang River. There are many activities along the Pahang River that can enhance the exposure of Fe. In recent years, the Pahang River estuary has been heavily impacted by discharges from municipal and industrial overflows in addition to bauxite mining (Kamaruzzaman *et al.*, 2011; Suhaimi-Othman *et al.* 2008). Nevertheless, the selected metals were within their respective RfD limit set up by the United States Environmental Protection Agency (Astorga-España *et al.*, 1999)

4.2.5.2 Health risk indexes of metals

The preliminary health risk can be estimated from Health Risk Index (HRI) (Shah *et al.*, 2012; Muhammad *et al.*, 2010). The calculations based on the calculated CDI are summarized in Table 4.9. HRI for Pahang River was found to be higher than Selangor River. It is believed that surrounding activities could well had impacted the concentration level of Fe in Pahang River (Kamaruzzaman *et al.*, 2011; Suhaimi-Othman *et al.* 2008).

Table 4.9: Chronic Daily intakes (CDIs, mg kg·day^{-1}) and Health Risk Indexes (HRIs) of metals through water ingestion

		Selangor River		Pahang River	
Metal	RfD (mg kg·day^{-1})	CDI (mg kg·day^{-1})	HRI	CDI (mg kg·day^{-1})	HRI
Cr	1.5	2.2×10^{-4}	1.5×10^{-4}	2.2×10^{-4}	1.5×10^{-4}
Fe	0.7	3.1×10^{-2}	4.5×10^{-2}	1.7×10^{-1}	2.4×10^{-1}
Cu	4×10^{-2}	2.5×10^{-4}	6.3×10^{-3}	2.5×10^{-4}	6.3×10^{-3}
Zn	3×10^{-1}	2.8×10^{-3}	9.4×10^{-3}	2.8×10^{-3}	9.4×10^{-3}
As	3×10^{-3}	6.3×10^{-5}	2.0×10^{-2}	6.3×10^{-5}	2.0×10^{-2}
Se	5×10^{-3}	3.1×10^{-6}	6.3×10^{-4}	3.1×10^{-6}	6.3×10^{-4}
Cd	1×10^{-3}	9.4×10^{-6}	9.4×10^{-3}	9.4×10^{-6}	9.4×10^{-3}
Pb	4×10^{-3}	3.1×10^{-5}	7.8×10^{-3}	3.1×10^{-5}	7.8×10^{-3}

As can be seen in Table 4.9, the data demonstrates that the HRI values in this study were within safe limit, with values less than one (HRI < 1). This suggests that there are no health risks in both sampling sites (Muhammad *et al.*, 2011).

4.3 Metal Concentrations in the Sediments

The sediment samples collected from both Pahang and Selangor sites were analysed for elements Na, Mg, K, Ca, Cr, Fe, Ni, Cu, Zn, As, Se, Cd and Pb. The 95 % intervals of the mean concentrations according to the dry weight of sediment samples are reported in Table 4.10.

4.3.1 Variations in the Metal Concentrations

Table 4.10 shows that the mean concentrations varied significantly across the sampling sites ($p < 0.001$; except Ni; $p < 0.05$). The elemental variations in the sediment samples are mainly from the deviation in the ratio of their organic and inorganic factions (Pinto, 2009). Part of the variations is inherited from the parent rocks, and addition deviations could be attributed by anthropogenic impacts from the surrounding (Bereswill *et al.*, 2013; Uysal, *et al.*, 2009; Demirak *et al.*, 2006).

To a certain extent, the metal elements associated with surface sediment fractions can also reflect the quality of the ambient, as these metals can be remobilized and redistributed between water and sediment under favourable conditions (Klavins *et al.*, 2009). However, from Table 4.10 contrasting trend is seen for certain elements where elevated levels of Mg, K, and Ca were recorded in the sediments of Selangor River instead of the Bestari Jaya aquaculture pond; similarly, greater concentration of Mg was found in the sediments from Pahang River rather than the aquaculture site.

Table 4.10: Mean concentrations of metals in the sediment layer for both sampling sites

Conc. of metals (mg kg^{-1})	Selangor		Pahang		Sediment Quality Guidelines (SQGs)	
	Aquaculture pond	**River**	**Aquaculture pond**	**River**	TEC*	PEC*
Na	$(1.2 \pm 0.3) \times 10^3$	$(0.3 \pm 0.1) \times 10^3$	$(0.13 \pm 0.01) \times 10^3$	$(0.04 \pm 0.01) \times 10^3$	-	-
Mg	$(0.66 \pm 0.03) \times 10^2$	$(16 \pm 1) \times 10^2$	$(5.0 \pm 0.1) \times 10^2$	$(10.0 \pm 0.2) \times 10^2$	-	-
K	$(0.16 \pm 0.02) \times 10^3$	$(1.5 \pm 0.2) \times 10^3$	$(1.3 \pm 0.1) \times 10^3$	$(1.2 \pm 0.1) \times 10^3$	-	-
Ca	$(1.3 \pm 0.1) \times 10^2$	$(12.0 \pm 0.02) \times 10^2$	$(8.0 \pm 0.4) \times 10^2$	$(5.0 \pm 0.1) \times 10^2$	-	-
Cr	0.8 ± 0.1	24 ± 1	25 ± 1	12 ± 1	43.4	111
Fe	$(0.040 \pm 0.003) \times 10^4$	$(2.7 \pm 0.1) \times 10^4$	$(25 \pm 1) \times 10^4$	$(2.0 \pm 0.3) \times 10^4$	-	-
Ni	0.22 ± 0.03	7.9 ± 0.3	4.0 ± 0.2	5.3 ± 0.3	22.7	48.6
Cu	2.3 ± 0.1	19.0 ± 0.2	21.0 ± 0.7	9.0 ± 0.2	31.6	149
Zn	2.8 ± 0.3	140 ± 3	53 ± 2	40 ± 1	121	459
As	0.4 ± 0.1	51 ± 1	12 ± 1	11 ± 1	9.79	33

Table 4.10, continued

Conc. of metals (mg kg^{-1})	**Selangor**		**Pahang**		**Sediment Quality Guidelines (SQGs)**	
	Aquaculture pond	**River**	**Aquaculture pond**	**River**	TEC*	PEC*
Cd	0.01 ± 0.01	0.2 ± 0.1	0.10 ± 0.01	0.10 ± 0.01	0.99	4.98
Pb	5.3 ± 0.3	41 ± 1	31 ± 1	28 ± 1	35.8	128

All the reported concentrations are 95 % intervals for the means (n = 20) based on dry weight
- : Not reported
TEC : Threshold effect concentration ; PEC : Probable effect concentration
* : (MacDonald *et al.*, 2000)

These observations suggest that the metal concentrations retained in the sediment fractions are basically independent of the current inputs (i.e. the additives used in water quality management), it most likely reflects the long-term metals deposition in the aquatic system where the sediment serves as a sink of metal residues (Alloway, 2013).

In addition to the natural variation associated with the background concentrations, the elevated levels of heavy metals found on the sediments can plausibly be linked to the long-term anthropogenic activities nearby the river basins (Marchand *et al.*, 2012; Nannoni *et al.*, 2011). In fact, it has been reported that the nearby industrial estates were the major contributors of the metal loads in Selangor River, with an estimated mineral discharge exceeding 1 tonne per day (Mohamad Ali *et al.*, 2010; DID, 2007; Rashid, 2000). Meanwhile, according to Department of Environment's annual report (DOE, 2004), Pahang River basin became polluted due to waste water discharging from the rubber and oil palm estates. Moreover, the findings from Kusin *et al.* (2016) have highlighted metal contamination in the sediments from Pahang River caused by bauxite mining activities.

4.3.2 Assessment of Metal Contamination in Sediments

In this study, metal concentrations in sediment samples were compared with numerical sediment quality guidelines (SQGs), such as consensus-based TEC (Threshold effect concentration) and PEC (Probable effect concentration) values (Table 4.10). These guidelines are used to assess the possible risks arising from metal contamination in sediment samples (MacDonald *et al.*, 2000). The SQGs are recognized as perfect threshold to assess the adverse effects of metal contamination in sediment for aquatic life and human health as well. Malaysian researchers, Gharilbreza *et al.* (2013), Sultan & Shazili (2009), and Ling (2007) have conducted studies to evaluate the level of metals contamination in the sediment by using SQG.

Classification of the published SQGs (consensus-based TEC and PEC) have been calculated to identify contaminant concentrations which may give harmful effects on sediment-dwelling organism. The organisms are important as food sources for aquatic life especially fish (MacDonald, 2000). Thus, pollution in the sediment has a strong association with metal concentrations in fish species. This study showed that sediment quality indices can clearly assess the pollution status in the sediment samples (Gharilbreza *et al.*, 2013). It has been found that most metals are below the SQGs (consensus-based TEC) except for Zn, As, and Pb (Figure 4.5). The highest concentration of Zn was recorded in Selangor River with 140 ± 3 mg kg^{-1} which exceeded SQGs (TEC : 121 mg kg^{-1}). Sediment samples are predicted to be toxic if the measured concentration of a studied element exceeds the corresponding TEC and PEC quotients (MacDonald, 2000).

It is scientifically proven that the sources of Zn, As and Pb in Selangor River are due to pollutants from industrial, domestic wastewater and agricultural activities. While the industrial estates are known to release a major portion of metals in this river. It has been reported that the respective metal loads of 181.4 and 912 kg day^{-1} are from pollutants (such as Zn, Pb, Ni, As and Fe) from 11 of industrial estates (DID, 2007). Contamination in sediments that exceeds the guidelines may need management plans which include controlling the source of the pollution and removing the polluted sediment.

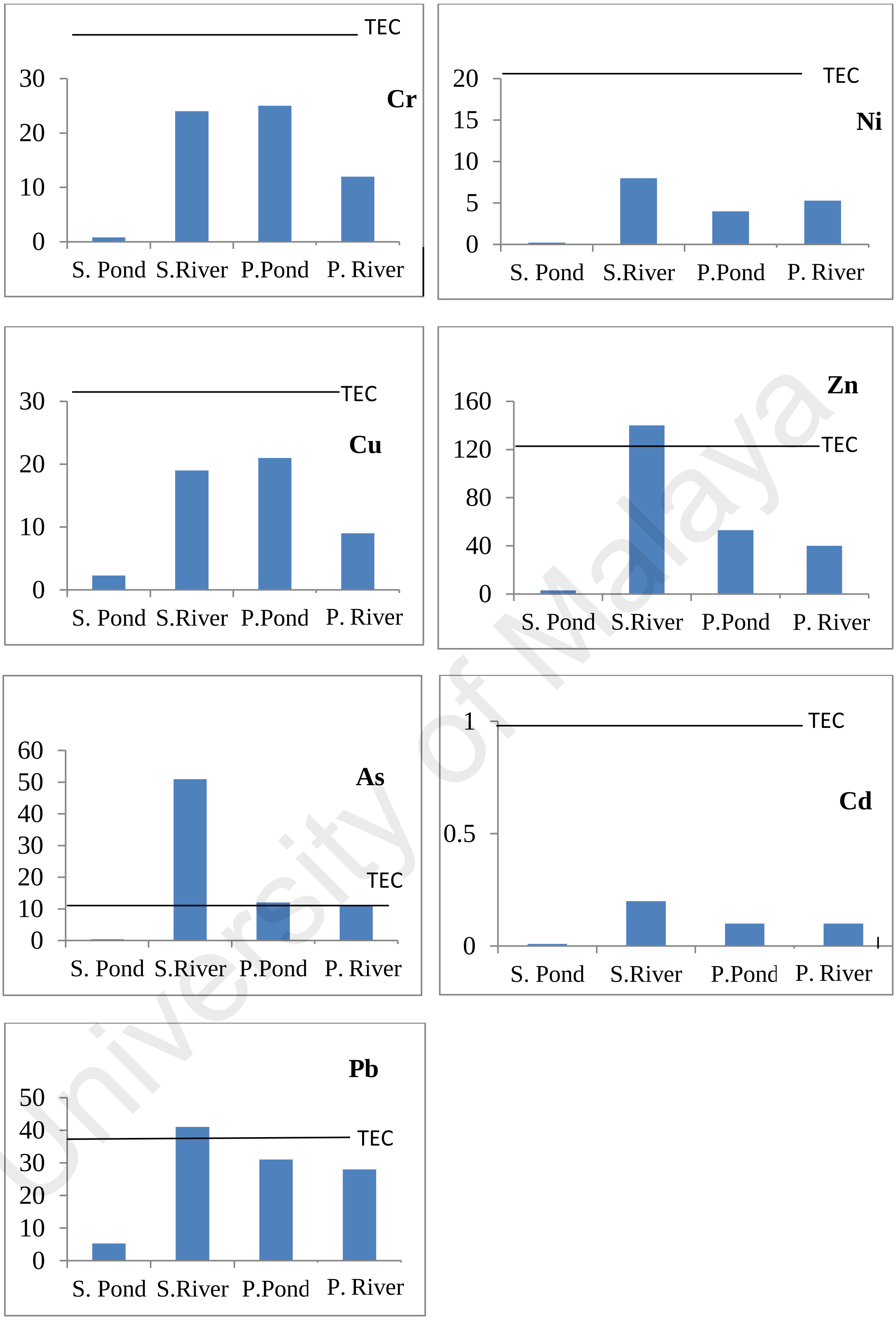


Figure 4.5: Heavy metal concentrations (mg kg^{-1}) in the sediment samples and TEC levels. The S denotes sample from Selangor, P denotes sample from Pahang

Table 4.11: Classess of Geoaccumulation Index (I_{geo})

I_{geo} value	I_{geo} class	Pollution level
≤ 0	0	Unpolluted
0 -1	1	Unpolluted to moderately polluted
1 -2	2	Moderately polluted
2 - 3	3	Moderately to strongly polluted
3 - 4	4	Strongly polluted
4 - 5	5	Strongly to very strongly polluted
> 5	6	Very strongly polluted

Ref : (Abrahim, 2008; Müller, 1969)

Table 4.12: Geoaccumulation Index (I_{geo}) (mg kg^{-1}) of metals for sediments of all studied sites

	Selangor				Pahang			
	Aquaculture Pond		River		Aquaculture Pond		River	
Metal	I_{geo}	**Class**	I_{geo}	**Class**	I_{geo}	**Class**	I_{geo}	**Class**
Cr	-7.46	0	-2.66	0	-2.59	0	-3.68	0
Fe	-7.61	0	-1.47	0	-1.61	0	-2.32	0
Ni	-9.00	0	-3.84	0	-4.89	0	-4.41	0
Cu	-5.18	0	-2.11	0	-1.99	0	-3.20	0
Zn	-5.23	0	0.43	1	-0.99	0	-1.41	0
As	-2.69	0	4.25	4	2.17	2	1.97	2
Cd	-4.81	0	-0.39	0	-2.04	0	-1.41	0
Pb	-1.87	0	1.08	1	0.67	1	0.52	1

4.3.2.1 Geoaccumulation Index

By considering the effect of geochemical background concentrations in the assessment of metal contamination in sediment, geoaccumulation index (I_{geo}) basically outlines 7 descriptive classes as shown in Table 4.11. The I_{geo} values and their respective classes for Cr, Fe, Ni, Cu, Zn, As, Cd and Pb for each site are listed in Table 4.12. The results revealed that the surface sediments can be classified as 'unpolluted' with respect to most of the targeted elements; except for Zn, As, and Pb at certain sites. This totally agrees with the findings noticed in sediment quality guidelines of the previous section.

Among the elements, As showed the highest accumulation in sediments which suggests a category of 'heavily polluted' for Selangor River, 'moderately polluted' for Pahang River and Pahang aquaculture pond. The aforementioned sites were also found to contain similar pollution level of Pb which is classified as 'moderately polluted'. The mean I_{geo} value for Zn in Selangor River was 0.43 (moderately polluted). These imply the existence of considerable contamination via anthropogenic activity, particularly the industrial and agricultural impacts on Selangor River (Mohamad Ali *et al.*, 2010; DID, 2007; Rashid, 2000). This trend is mainly consistent with the trend shown in Table 4.10. Hence, this analysis reveals that the sediments of the Selangor River are contaminated with Zn, As, Pb and Pahang River suffers contaminationof As and Pb according to the I_{geo} values. Mining activities in general have been known to generate environmental impacts at Pahang River such as degradation of sediment quality and detrimental impacts on water and aquatic life (Kusin *et al.*, 2016).

4.3.2.2 Enrichment Factor

The enrichment factor (EF) is an acceptable measurement of geochemical trends and normally used for making comparison between areas. A value of unity represent that there is no enrichment or depletion relative to the Earth's crust from the sample (Hasan *et al.*, 2013).

With help of the support classification of Ghrefat (2011), and Sakan *et al. (*2009), the enrichment factor values were interpreted (Table 4.13). Enrichment factor is applied to differentiate the metal sources in the sediment, where EF values between 0.05 and 1.5 indicate that the metal is due to natural processes or entirely from crustal material. The EF values above 1.5 for metals imply the influence of anthropogenic activities (Selvaraj *et al.*, 2004; Zhang & Liu, 2000).

In this study, the EF values of Zn, As, and Pb are > 1.5 in the sediment of Selangor River, suggesting that it is affected by anthropogenic impact. Principal sources of these elements can be mainly associated with municipal and domestic waste, and agricultural runoff (Leong *et al.*, 2007). The EF for Pb recorded very severe enrichment from Selangor River, possibly due to domestic and industrial discharges along the river (Ma *et al.*, 2013).

The result also reveals that the sediment in Pahang River are moderately enriched by As and Pb metals. This trend is found consistent with the I_{geo} value. The high EF values for As and Pb suggests that the input was due to anthropogenic activities. This trend can be explained as due to bauxite mining activities and palm oil plantation surrounding the sampling sites (Amin *et al.*, 2009).

Table 4.13: Enrichment factors (EFs) of metals for sediments of all studied sites

Metal	Selangor				Pahang			
	Aquaculture Pond / EF value	**EF level**	**River / EF Value**	**EF level**	**Aquaculture Pond / EF value**	**EF level**	**River / EF value**	**EF level**
Cr	1.16	Minor	0.46	No enrichment	0.53	No enrichment	0.41	No enrichment
Ni	0.39	No enrichment	0.20	No enrichment	0.11	No enrichment	0.24	No enrichment
Cu	6.21	Moderately severe enrichment	0.74	No enrichment	0.89	No enrichment	0.62	No enrichment
Zn	3.61	Moderate	2.59	Minor	1.06	Minor	1.31	Minor
As	3.94	Moderate	6.89	Moderately severe enrichment	1.79	Minor	2.54	Moderate
Cd	4.39	Moderate	1.33	Minor	0.47	No enrichment	1.18	Minor
Pb	32.65	Very severe enrichment	3.58	Moderate	2.97	Moderate	4.38	Moderate

High metal enrichment in sediment fraction is likely to increase metal availability, and therefore stimulates their high accumulation in the aquatic life. When the environmental conditions change, the adsorbed metals can desorb from surface sediments and may biologically affect aquatic organism (Segura *et al.,* 2006). Therefore, the interaction between metals that deposit in the sediment layers and aquatic life could threaten biotic organism and humans as these metals are able to accumulate in the food chain.

4.3.3 Hierarchical Cluster Analysis of Sediment Samples

Hierarchical Cluster Analysis (HCA) is one of the multivariate approaches with a purpose to identify natural groupings or clusters amongst samples in the data set, by applying the concept of minimization of the within-cluster variance and maximization of the between-cluster variance (Kaufman & Rousseeuw, 1990). In this study, HCA was used by divisive category, where the whole data were clustered and sequentially divided into a dendogram.

This dendogram groups the data set with the most similar pair of samples (Wunderlin *et al.*, 2001). A low distance between the groups in the dendogram showed that the two samples have high similarity, whereas a large distance indicate dissimilarity (Huang *et al.*, 2014).

To provide an overview and quantify the similarity of the samples, they were sorted via HCA to find out the relationship between variables. The dendogram identifies three distinct clusters by elements irrespective of their sampling sites (Figure 4.6). Cluster 1 contains Na, Mg, K, and Ca which can mostly originate from additive applied in the aquaculture pond water management. These metals are usually related to the application of lime, gypsum and fertilizers to greatly increase fish yield in aquaculture ponds (Boyd, 1990).

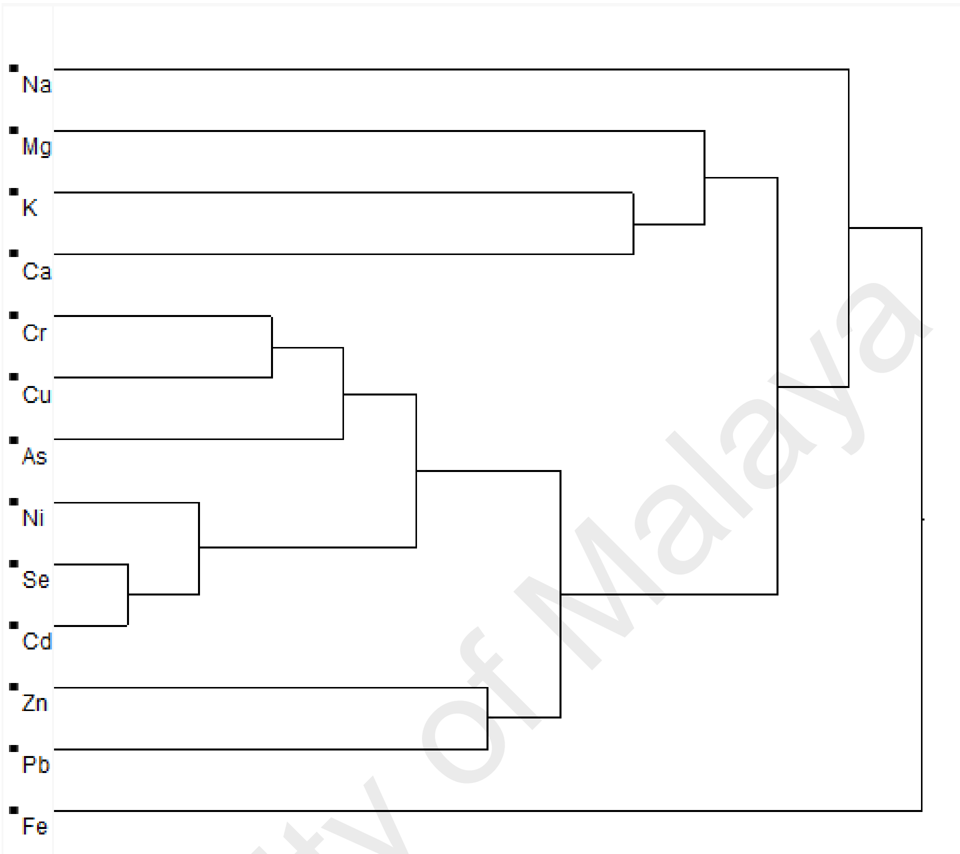


Figure 4.6: Dendogram of hierarchical cluster analysis for metals in the sediment of study area

Elements in Cluster 2 (Cr, Cu, As, Ni, Se, Cd, Zn, Pb) have similar behaviour indicating that they are derived from anthropogenic sources (domestic, industrial, agricultural effluents and runoffs). The existence of Fe in Cluster 3 probably originated from natural sources, because Fe is mainly derived from lithogenic sources (Pejman *et al.*, 2015). Therefore, this evidence can support the trend in metal concentration of the enrichment factor analysis in the previous section. In brief, there are two sources of pollution in the sediments due to metal loadings - either through natural processes or anthropogenic activities.

4.4 Metal Concentrations in the *Pangasius* sp. and *Hemibagrus* sp.

There has been growing awareness about the anthropogenic impacts on the freshwater aquatic ecosystem (Ayni *et al.*, 2011). Metals are recognized as one of the most important contaminants in aquatic ecosystem due to their ability to accumulate in organism and their assimilation ability (Copat *et al.*, 2013). If accumulation of metals is found in the sediment and water column, then these metals will be introduced to fish through the feed web (Galay *et al.*, 2001). Besides addressing the quality of freshwater supplies, the main concern is the metal bioaccumulation and biomagnification potential in fishes that may bring about human health risks via food chain.

The metal concentrations recorded in fish species not only depend on the bioavailability and bioaccessibility of the elements, but may also be subject to several factors such as species, age, size, feeding habits, environmental condition, season, location, duration of exposure, and homeostatic regulation activity etc (Dhaneesh *et al.*, 2012; Morgano *et al.*, 2011; Dural *et al.*, 2007; Fernandes *et al.*, 2007; Sankar *et al.*, 2006; Karadede *et al.*, 2004; Karadede & Ünlü, 2000). This means that there can be vast variations in the metal accumulation patterns of fishes even if they are from the same area (Mendil *et al.*, 2010; Canli & Atli, 2003; Topcuoglu *et al.*, 2002).

4.4.1 Relationship between Metals Levels in Fish, Water Body and Sediments

Metals that enter a water body would be adsorbed on sediments, and are subsequently transferred to other media as a result of exchange between water body, sediment, and biota either through chemical or biological processes (Yi *et al.*, 2011). The results show that the metal levels found in the environmental samples can be ranked as follow: sediment > fish > water body. The concentrations recorded in sediments were two to three orders higher than those in the water body while concentrations in fish were higher than the water body by about one/two orders. These are consistent with the findings from a number of other studies (Sunjog *et al.*, 2016; Monroy *et al.*, 2014; Wang *et al.*, 2014).

In general, low metal concentrations are found in a water body due to high volume dilution, deposition in sediments and uptakes by animals and plants (Yi *et al.*, 2008). The metal uptakes in fish primarily result from surface contact with the water, by breathing and via the food chain, while the sediment is the major sink. In some cases, the metals in the sediments layer can also enter the food chain due to feeding habits of benthic animals (Yi *et al.*, 2011).

4.4.1.1 Metal distribution associated with environmental data

Knowledge on the distribution and accumulation processes of metals in fish tissues could help researchers to understand the processes involved in the uptake and excretion of metals and pollution sources in the aquatic system. It was observed that the concentrations of metals accumulated by fishes are not only depending on the water quality but also influenced by seasonal factors, habitat status and diet intake (Kamaruzzaman *et al.*, 2011). Due to this reason, it is important to study those factors so that risk to human health due to consumption of contaminated fish can be prevented. As shown in the score plot of PC1 (x – axis) and PC2 (y – axis), the samples can be classified into three separate groups (Figure 4.7 and 4.8).

The classification of the groups based on their metals accumulation clearly shows that the discrimination of samples using PCA is an effective way for differentiating metals distribution. It is noted that sediment samples are mapped on the positive region of PC 1, where samples at the right-hand side of the scores plot are associated with higher concentrations of metals compared to those at the left-hand side. In general, the results indicate that the total metal accumulation is of the order of: sediment > fish > water based on the positive and negative scores of the respective region.

Basically, Cluster I in Figure 4.7 and Figure 4.8, can both be characterized with high loadings of Pb, Cr and Fe. This is possibly due to mining and agricultural waste (Kusin *et al.*, 2016; Mohamad Ali *et al.,* 2010). The negative loadings of Na and K on PC1 in Figures 4.7 and 4.8 are strongly associated with Cluster III which are related to the sampling location of fishes. Such pattern is consistent with the trends displayed in Table 4.6, where the highest concentrations of Na and K were due to difference in water management practice of the different sites (Boyd, 1990). The levels of Na and K in the water sample most likely reflect the current input situation in the aquatic system (Boyd, 1990). In general, the results indicate that the total metal level is ordered as: sediment > fish > water based on positive and negative scores of the respective region. Such pattern is also consistent with the measurements noted in Table 4.6 and Table 4.10, where the highest concentration of most elements were observed on sediment samples.

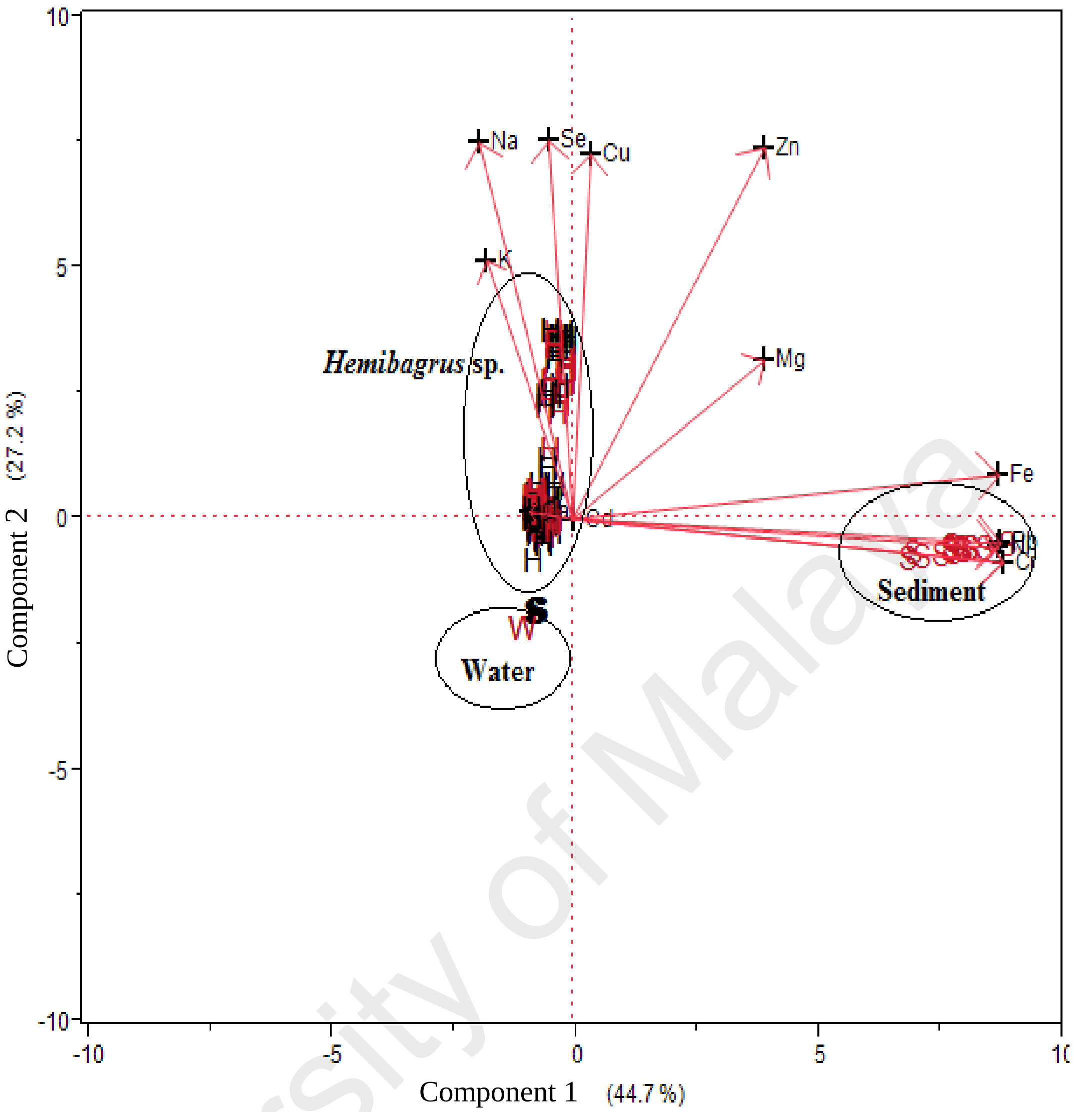


Figure 4.7: PC2 versus PC1 score-loading biplot for *Hemibagrus* sp. and their associated environmental data. The red symbol denotes samples from Selangor and black symbol denotes samples from Pahang (S : sediment; W : water; H : *Hemibagrus* sp.)

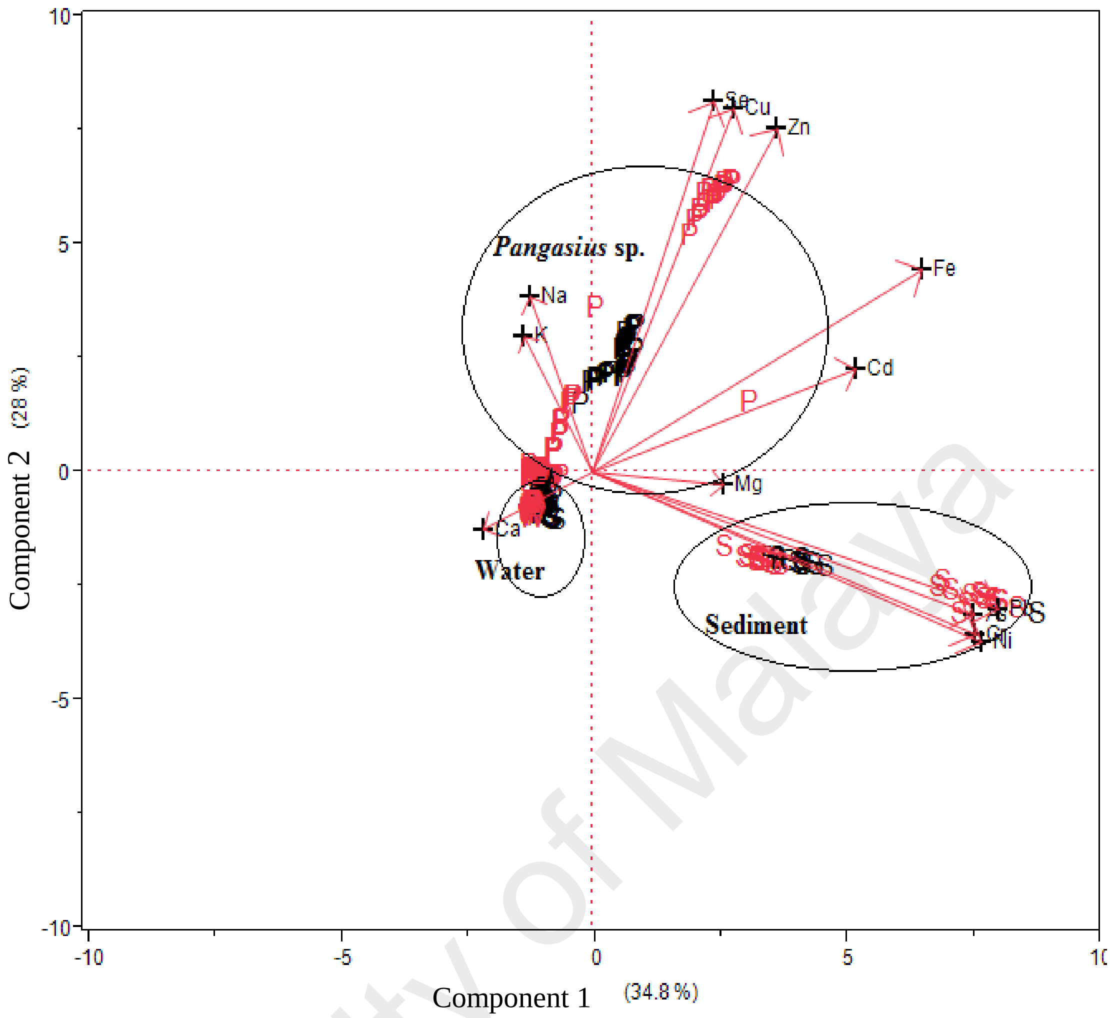


Figure 4.8: PC2 versus PC1 score-loading biplot for *Pangasius* sp. and their associated environmental data. The red symbol denotes samples from Selangor and black symbol denotes samples from Pahang (S : sediment; W : water; P : *Pangasius* sp.)

Sediments are suitable for monitoring long-term metal deposition in the aquatic system due to its capability to concentrate metals and generally concentration of metals in sediment is less variable than in water body (Alloway, 2013; MacDonald *et al.*, 2000). However, measuring metal concentrations either in water and sediment does not provide information on the risk posed by metal bioaccumulation (Maceda-Veiga *et al.*, 2013; Ricart *et al.*, 2010), therefore by measuring metal levels in the fish the risk of consuming the fish due to metal deposition in the environment can be studied.

4.4.2 Bioaccumulation of Metal Substances

Metal bioaccumulation refers to the build-up of metal concentrations in the tissues of a fish when their uptake rates are greater than that which are lost. The process often takes place simultaneously via progressive transport from water and ingestion of food, sediment particles etc. Thus, metal species with high degree of toxicity and persistence may induce chronic risk even if their concentrations are not very high.

4.4.2.1 Bioconcentration factors

Bioconcentration factor (BCF) is used to evaluate metal bioaccumulation potential in fish and can also be used for site survey (USEPA, 1991). The metal bioconcentration factors (BCFs) derived from water and dietary exposure are listed in Table 4.14. Generally, the BCFs of Zn, Se, and Cu are higher compared to other elements, which suggest the uptake tendency of these biologically essential elements (Qiu, 2015). In some cases, although the magnitude of BCFs were found to be greater than 1000, this does not necessarily indicate a hazard condition. This is because the regulation/homeostasis process in freshwater fishes is able to invert the BCF values and exposure concentrations. In other words, an unpolluted system is expected to demonstrate higher metal BCFs for fish. Likewise, low BCFs observed is due to significant levels of elements found in the water body.

When comparing within a site, the BCFs for *Hemibagrus* sp. are generally higher than those for *Pangasius* sp (Figure 4.9) and this can be due to the higher ability of the former to enrich metals from the water column. Furthermore, this omnivorous species is enriching metals from the water environment through ingestion (Islam *et al.,* 2015). However, the BCF values are not consistent between different fish species.

Table 4.14: Bioconcentration factors (BCFs) for muscle of *Pangasius* sp. and *Hemibagrus* sp. for selected metals.

	Bioconcentration factor (BCF)					
	Selangor				Pahang	
	Pangasius sp.		*Hemibagrus* sp.		*Pangasius* sp.	
	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught
Cr	49	51	45	43	1	1
Fe	33	15	121	14	5	3
Ni	2	1	31	8	1	1
Cu	86	4	144	100	177	110
Zn	256	234	366	252	257	221
As	25.	80	125	1134	10	23
Se	9700	20900	16143	32136	15737	14099
Cd	10	10	10	10	10	404
Pb	3	3	230	3	3	3

Note : Cr, Ni, Cu, Zn, As, Se, Cd, Pb : below the LoD

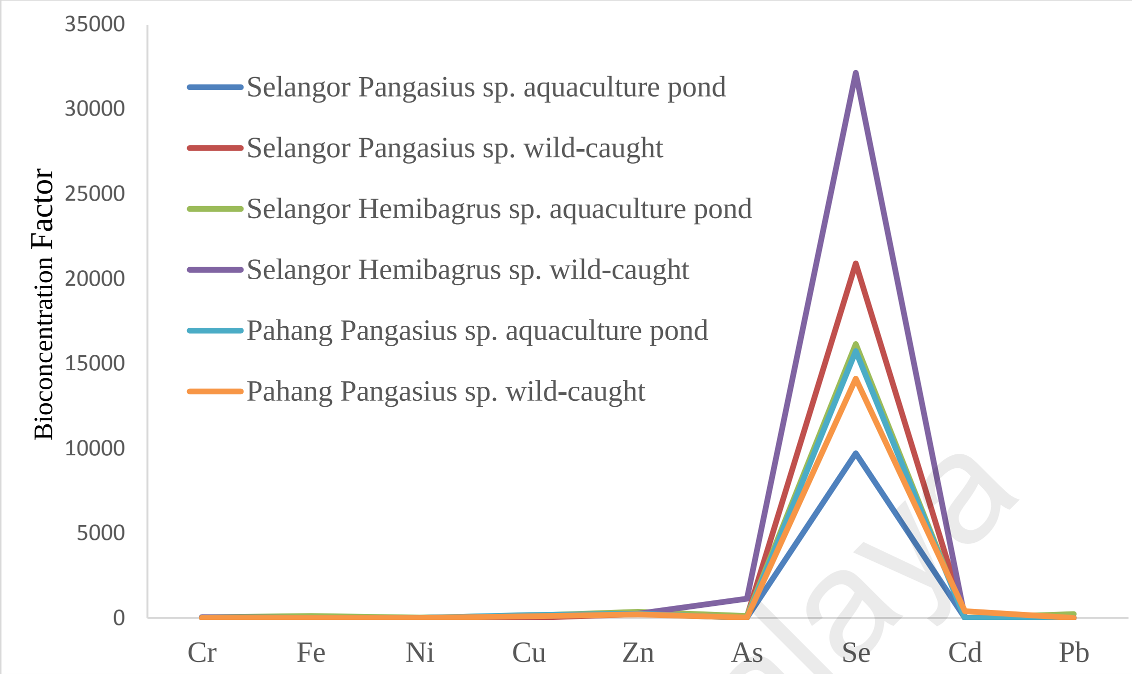


Figure 4.9: Bioconcentration factors for muscle of *Pangasius* sp. and *Hemibagrus* sp.
Note : Cr, Ni, Cu, Zn, As, Se, Cd, Pb : below the LoD

This is due to the reason that fishes are more broadly distributed and might migrate between river areas in response to different environmental changes of the water system (Abal *et al.*, 2005). Variation in metal bioaccumulation capability can be linked to the physiological factors and natural behaviour of the fish (Tao *et al.*, 2012). This observation suggests that *Hemibagrus* sp. Is a more sensitive bioindicator in environmental monitoring and assessment of metal pollution.

Generally, *Hemibagrus* sp. has a faster growth rate than *Pangasius* sp. which indicates high food intake, thus making it a suitable species as a bioindicator (Baras *et al.*, 2013). Moreover, throughout its geographical range, most notably in Southeast Asia, *Hemibagrus* sp. has been a valuable candidate as bioindicator, because of its large size (more than 1 m in length). To this effect, this species has been cultured for food, mostly in Peninsular Malaysia and Thailand, where previous studies have been conducted which points to variouos ways to increase the efficiency of its production (Ng & Kottelat, 2013; Abidin *et al.*, 2006 ; Ng *et al.*, 2001).

Basically, if the elements are not detected in the water body but detected in the fish species, the values of BCF are extremely high (Table 4.14). Selenium shows a higher value of BCF due to the fact that its concentration is lower value in the water body. Selenium is required for normal growth, biochemical and physiological functions in fish (Khan *et al.*, 2017). Therefore, fish has the tendency to retain Se in its body due to its metabolism requirements. Moreover, concentration of Se have been found to increase linearly with fish consumption (Berr *et al.*, 2009). Thus, these observations are compatible with the findings displayed in Figure 4.9.

4.4.2.2 Biota-sediment accumulation factors

Metal biota-sediment accumulation factors (BSAFs) are useful in outlining the bioaccumulation of sediment metals in fish under non-equilibrium conditions which incorporates the influences of biomagnification (Qiu *et al.*, 2011). Similar to BCF, BSAF is defined as the ratio between metal levels in the fish and the sediments where a magnitude above or below 1-2 is considered reasonable (Szefer *et al.*, 1997).

Table 4.15 shows that BSAFs depend strongly upon the metal availability at sampling sites as in BCFs. The accumulation sequences are Se > Zn > As > Cr > Cu > Ni > Fe > Cd, > Pb for *Pangasius* sp. and Se > Zn > Ni > As > Cu > Cr > Fe > Cd > Pb for *Hemibagrus sp.*. The lowest BSAF value for Pb may be able to be explained by the significantly higher level of this element in the sediment (Szefer *et al.*, 1999). Similar to BCF, the BSAF value for a given metal is generally different depending on the sampling site of the fish. This observation suggests that there are many factors that influence the metal bioaccumulation such as sediment and water column chemical disequilibrium, chemical bioavailability and dietary uptake (Wong *et al.*, 2011; Burkhard *et al.*, 2004).

Again, fish species from aquaculture ponds show high BSAF values of Zn and Se (Figure 4.10). The high BSAF values may be explained by the significantly lower levels of the elements in the sediment. Therefore, high accumulation of metals does not reflect its actual concentration in the ambient sediment as a substrata (Szefer *et al.*, 1999). Several investigators have also reported that differences in chemical composition of tissues between wild and aquaculture fish are due to variability in background conditions and dietary habits (Idris *et al.*, 2017; Martinez *et al.*, 2010; Dincer *et al.*, 2010; Grigorakis *et al.*, 2002)

Table 4.15: Biota-sediment accumulation factor values for selected metals (in respect to sediment extract) in the *Pangasius* sp. and *Hemibagrus* sp.

	Biota-sediment accumulation factor (BSAF)					
	Selangor				Pahang	
	Pangasius sp.		*Hemibagrus* sp.		*Pangasius* sp.	
	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught
Cr	0.4	1.5×10^{-2}	0.4	0.01	4.0×10^{-5}	8.6×10^{-5}
Fe	4.3×10^{-2}	5.4×10^{-4}	0.2	5.1×10^{-4}	9.0×10^{-4}	1.1×10^{-3}
Ni	0.1	9.0×10^{-4}	1.4	1.0×10^{-2}	1.2×10^{-3}	1.3×10^{-3}
Cu	0.3	1.5×10^{-3}	0.5	4.2×10^{-2}	7.0×10^{-2}	1.0×10^{-2}
Zn	8.2	0.2	11.8	0.2	0.4	0.5
As	0.1	3.1×10^{-3}	0.6	4.0×10^{-2}	1.3×10^{-3}	4.0×10^{-3}
Se	8.7	2.2	14.5	3.4	1.3	1.0
Cd	0.3	1.3×10^{-2}	0.3	1.3×10^{-2}	4.0×10^{-2}	1.1
Pb	5.6×10^{-4}	7.3×10^{-5}	4.0×10^{-3}	7.3×10^{-5}	10.0×10^{-5}	1.1×10^{-4}

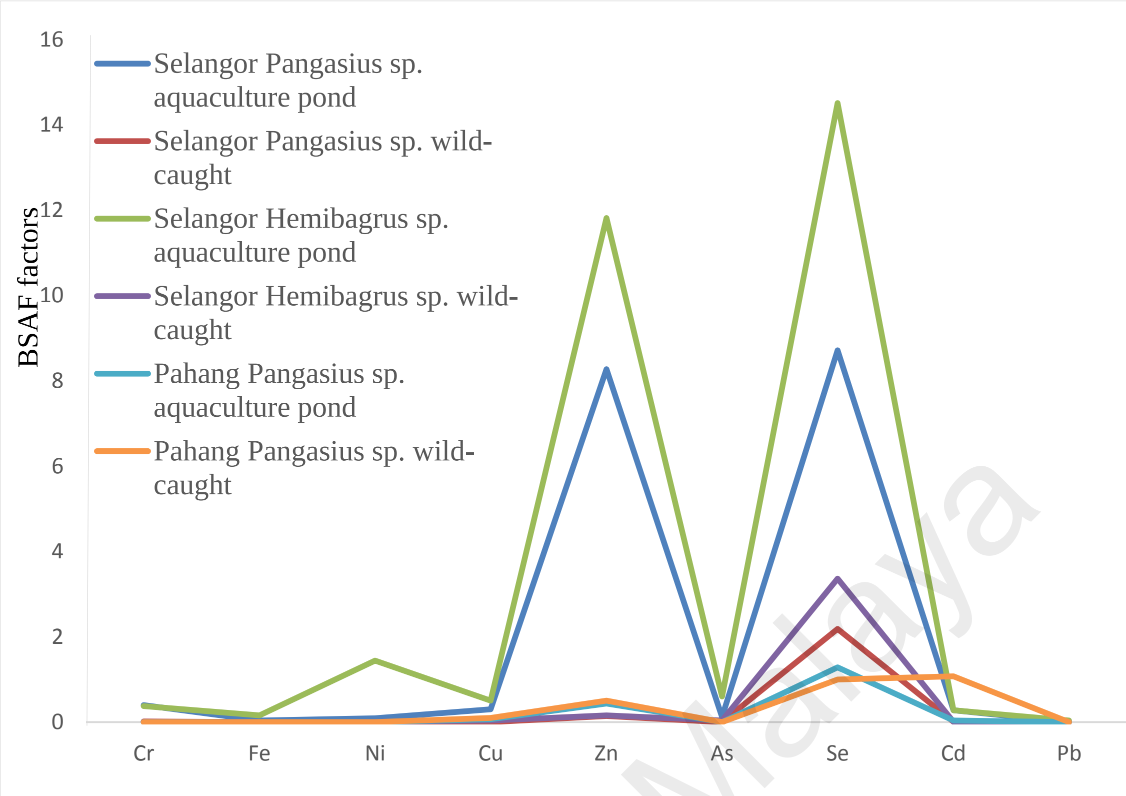


Figure 4.10 : Biota-sediment accumulation factors of *Pangasius* sp. and *Hemibagrus* sp.

4.4.3 The Morphometry of Fish

Many studies reported that to a certain extent the concentrations of accumulated contaminations in a fish could be related to its weight, length, and age (Hajeb *et al.*, 2009; De Marco *et al.*, 2006; Agusa *et al.*, 2005). However, the fish weight is highly dependent on the composition of muscle tissues especially lipid percentage that might demolish such relationship. In a study conducted by Boalt *et al.* (2014), it was revealed that the concentrations of Cd and Hg in the whole fish (herring and perch) increase with its age and total length.

In order to overcome the potential variability associated with size variation in this work, an effort was taken to sample fish with comparable size and their morphometric statistics are summarized in Table 4.16 and Table 4.17. Effort in sampling fishes of similar size is also meant to minimize the possible effect due to size and age variation on metal accumulation. However, exact similarity is difficult to achieve. There were still discrepancies in the weight and/or length observed between samples harvested from aquaculture ponds and from rivers.

However, for freshwater fishes, the size variation rarely shows significant correlation with the content of metals, since the internal metal concentrations are always regulated biologically (Yi & Zhang, 2012). Moreover, there was not much difference found in the condition factor (CF) and hepatosomatic index (HSI) of the fish. These suggest that the physiological status of both the fish species samples are actually comparable for the investigation of their potential as a bioindicator of hazardous metals pollution.

Basically, CF is regarded as a general index of fish health (Ureña *et al.*, 2007; Eimers *et al.*, 2001), that is expressed as length-weight factor (Khallaf, 2003), while HSI reflects the status of fish liver and is associated to the contaminant exposure (Dethloff, 2000). Both indices depend greatly upon the fish species, age, sex, maturity and health (Vosyliene, 1999). Table 4.16 demonstrates that CF and HSI for *Pangasius* sp. and *Hemibagrus* sp. in Selangor sites does not differ significantly, which confirms the claim that high accumulation rate in *Hemibagrus* sp. is not due to this factor, but is more likely associated with its living nature, such as feeding habits, food quality, and ambient metals concentrations (Vicente-Martorell *et al.*, 2009).

Table 4.17 displays the possible classification according to CF values as suggested by Barnham and Baxter (2003); the higher the CF value, the better is the fish condition. Due to the nature of the fish species, much deviation in CF is observed between *Pangasius* sp. and *Hemibagrus* sp. samples even when they were collected from the same origin.

Table 4.16: Biometric information related to *Pangasius* sp. and *Hemibagrus* sp.

	Selangor				Pahang	
	Pangasius sp.		*Hemibagrus* sp.		*Pangasius* sp.	
	Aquaculture Pond	River	Aquaculture Pond	River	Aquaculture Pond	River
Length (cm)	68 ± 1	77 ± 6	40 ± 1	44 ± 6	48 ± 2	45 ± 3
Weight (kg)	3.3 ± 0.3	3.7 ± 0.7	0.5 ± 0.1	0.6 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
Condition Factor (g/mm^3)	1.1 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.9 ± 0.1	0.8 ± 0.1
Hepatosomatic Index	1.1 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.3

Mean ± 95 % confidence interval, n = 20

Table 4.17: Condition factor (Barnham & Baxter, 2003)

CF	Comments
1.60	Excellent condition, trophy class fish
1.40	A good, well-proportioned fish.
1.20	A fair fish, acceptable to many anglers.
1.00	A poor fish, long and thin.
0.80	Extremely poor fish, resembling a barracouta; big head and narrow, thin body.

Besides that, aquaculture practices could also contribute to the variation in CF. A study carried out by Ureña *et al.* (2007), demonstrated that there was significant difference between the CF values of *Anguilla anguilla* from aquaculture pond and the wild-caughts of commercial size. A similar finding is captured in this study where the aquaculture produce attains a slightly higher mean CF values compared to wild-caught.

4.4.4 Metal Distribution in Fish Tissues

Tables 4.18 – 4.20 indicate the levels of metals in the muscle, liver and gill tissue of *Pangasius* sp. and *Hemibagrus* sp. expressed in mg kg^{-1} on dry weight basis. The concentrations measured varied over a wide range but there is a general trend independent of the fish species; where the levels of metals in liver > gills > muscle due to the physiological roles of the tissues and the metabolism (Jayaprakash *et al.*, 2015).

Such trends have been extensively documented in other fishes as well (Mziray & Kimirei, 2016; Pannetier *et al.*, 2016; Fallah *et al.*, 2011; Jarić *et al.*, 2011; Brucka-Jastrzębska *et al.*, 2009), which suggested that liver and gills as active metabolic organs which can accumulate higher amounts of metals (Dural *et al.*, 2007); whereas muscle has a low fat affinity to absorb and retain the metals (Uluturhan & Kucuksezgin, 2007). It was reported that accumulation of metals in fish tissues generally depends on the fish physiological role although the fish species may come from the same area (Uysal *et al.*, 2008). Studies done by Dural *et al.*, (2007) and Dhaneesh *et al.* (2012), proved that concentrations of metals in fish tissue/organs were different due to variations in feeding habits and behaviour.

Table 4.18: Metal concentrations in freeze dried *Pangasius* sp.

	Selangor					
	Aquaculture Pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Na	$(2.6 \pm 0.1) \times 10^3$	$(4.3 \pm 0.2) \times 10^3$	$(4.7 \pm 0.4) \times 10^3$	$(5.0 \pm 0.3) \times 10^3$	$(7.9 \pm 0.6) \times 10^3$	$(17.4 \pm 0.4) \times 10^3$
Mg	$(1.5 \pm 0.4) \times 10^3$	$(0.6 \pm 0.2) \times 10^3$	$(0.7 \pm 0.04) \times 10^3$	$(1.3 \pm 0.03) \times 10^3$	$(0.6 \pm 0.2) \times 10^3$	$(0.8 \pm 0.04) \times 10^3$
K	$(19 \pm 0.4) \times 10^3$	$(10.0 \pm 0.4) \times 10^3$	$(3.4 \pm 0.2) \times 10^3$	$(21 \pm 0.03) \times 10^3$	$(8.3 \pm 0.2) \times 10^3$	$(2.8 \pm 0.6) \times 10^3$
Ca	$(0.4 \pm 0.01) \times 10^3$	$(0.2 \pm 0.01) \times 10^3$	$(29 \pm 0.03) \times 10^3$	$(0.4 \pm 0.01) \times 10^3$	$(0.6 \pm 0.2) \times 10^3$	$(32 \pm 0.02) \times 10^3$
Cr	0.30 ± 0.03	0.03 ± 0.04	0.4 ± 0.03	0.4 ± 0.02	0.4 ± 0.02	0.6 ± 0.03
Fe	17 ± 0.1	30812 ± 25	263 ± 9.0	15 ± 1.2	41672 ± 23	261 ± 7
Ni	0.02 ± 0.01	0.01 ± 0.01	0.6 ± 0.1	< MDL	0.1 ± 0.1	0.8 ± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg $^{-1}$), n = 20
Method Detection Limits (MDLs) : Ni : 0.007 mg kg $^{-1}$

Table 4.18, continued

	Selangor					
	Aquaculture Pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Cu	0.7 ± 0.1	115 ± 4	1.8 ± 0.3	< MDL	404 ± 12	1.7 ± 0.1
Zn	23 ± 0.1	321 ± 3.0	48 ± 3	21 ± 0.7	443 ± 23	38 ± 2
As	0.05 ± 0.01	0.01 ± 0.01	0.4 ± 0.1	0.10 ± 0.02	1.0 ±0 .03	0.8 ± 0.1
Se	1.0 ± 0.1	11.3 ± 0.4	0.5 ± 0.1	2.0 ± 0.1	26 ± 0.4	1.1 ± 0.1
Cd	< MDL	< MDL	< MDL	< MDL	< MDL	<LoD
Pb	< MDL	3.2 ± 0.1	0.6 ± 0.1	< MDL	3.0 ± 0.1	0.7 ± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg^{-1}), n = 20
Method Detection Limits (MDLs) : Cr : 0.01 mg kg^{-1}, Ni : 0.007 mg kg^{-1}, Cu : 0.03 mg kg^{-1}, As : 0.02 mg kg^{-1}, Cd : 0.003 mg kg^{-1}, Pb : 0.003 mg kg^{-1}

Table 4.19: Metal concentrations in freeze dried *Pangasius* sp.

	Pahang					
	Aquaculture pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Na	(2.2 ± 0.2) x10^3	(4.1 ± 0.2) x10^3	(3.2 ± 0.2) x 10^3	(2.1 ± 0.1) x10^3	(4.6 ± 0.4) x 10^3	(3.3±0.3) x10^3
Mg	(1.3 ± 0.1) x 10^3	(6.5 ± 0.2) x 10^3	(0.7 ± 0.1) x 10^3	(1.3 ± 0.1) x 10^3	(0.6 ± 0.1) x 10^2	(0.9 ± 0.1) x10^3
K	(17 ± 0.2) x 10^3	(90 ± 0.2) x 10^3	(3.2 ± 0.2) x 10^3	(20 ± 0.1) x 10^3	(10 ± 0.1) x10^3	(3.3 ± 0.3) x10^3
Ca	(0.3 ± 0.1) x 10^3	(0.2 ± 0.3) x 10^3	(21 ± 0.4) x 10^3	(0.4 ± 0.2) x 10^3	(0.1 ± 0.5) x 10^3	(38 ± 3.0) x 10^3
Cr	< MDL	< MDL	0.05 ± 0.0	< MDL	0.1 ± 0.02	0.2 ± 0.03
Fe	22 ± 2.2	2347.3 ± 7.3	409.9 ± 4.2	17±1.6	1503.4 ± 2.0	102.7 ± 8.5
Ni	< MDL	< MDL	0.1 ± 0.02	< MDL	< MDL	0.1± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg $^{-1}$), *n* = 20
Method Detection Limits (MDLs) : Cr : 0.01 mg kg $^{-1}$, Ni : 0.007 mg kg $^{-1}$,

Table 4.19, continued

	Pahang					
	Aquaculture Pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Cu	1.4 ± 0.2	205.8 ± 1.7	1.8 ± 0.1	0.9 ± 0.2	71 ± 14	1.0 ± 0.1
Zn	23 ± 3.0	215.7 ± 1.2	49 ± 2.6	20 ± 1.2	189.9 ± 1.6	47 ± 3.0
As	< MDL	0.1 ± 0.02	< MDL	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02
Se	1.6 ± 0.2	15 ± 1.3	1.0 ± 0.1	1.4 ± 0.2	8.0 ± 3.0	0.8 ± 0.1
Cd	< MDL	0.1 0.02	< MDL	0.1 ± 0.01	< MDL	< MDL
Pb	< MDL	5.5 ± 1.1	5.3 ± 1.0	< MDL	0.7 ± 0.1	0.6 ± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg^{-1}), n = 20
Method Detection Limits (MDLs) : As : 0.02 mg kg^{-1}, Cd : 0.003 mg kg^{-1}, Pb : 0.003 mg kg^{-1}

Table 4.20: Metal concentrations in freeze dried *Hemibagrus* sp.

	Selangor					
	Aquaculture pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Na	(2.2 ± 0.1) x10^3	(5.2 ± 0.3) x10^3	(4.2 ± 0.5) x 10^3	(1.7 ± 0.1) x10^3	(4.2 ± 0.3) x10^3	(3.6 ± 0.2) x 10^3
Mg	(1.2 ± 0.1) x 10^3	(0.66 ± 0.05) x 10^3	(1.4 ± 0.1) x 10^3	(1.3 ± 0.1) x 10^3	(0.58 ± 0.03) x 10^3	(1.1 ± 0.1) x 10^3
K	(16 ± 1) x 10^3	(9.8 ± 0.6) x 10^3	(4.6 ± 0.5) x 10^3	(18.4 ± 0.4) x 10^3	(9.6 ± 0.4) x 10^3	(4.2 ± 0.2) x 10^3
Ca	(0.34 ± 0.02) x 10^3	(0.43 ± 0.04) x 10^3	(58 ± 5) x 10^3	(0.4 ± 0.02) x 10^3	(0.21 ± 0.03) x 10^3	(41 ± 2) x 10^3
Cr	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Fe	61 ± 4	4463 ± 6.8	107.1 ± 1.4	14 ± 1	4363 ± 2.3	130.0 ± 1.2
Ni	0.30 ± 0.02	0.1 ± 0.1	1.2 ± 0.1	0.10 ± 0.01	0.30 ± 0.02	1.1 ± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg^{-1}), *n* = 20

Table 4.20, continued

	Selangor					
	Aquaculture Pond			Wild-caught		
	Muscle	Liver	Gills	Muscle	Liver	Gills
Cu	1.2 ± 0.2	143.1 ± 1.6	2.9 ± 0.4	0.8 ± 0.1	79 ± 7	1.6 ± 0.1
Zn	33 ± 4	177.8 ± 2.2	56 ± 4	23 ± 1	147.6 ± 9.8	50 ± 3
As	0.3 ± 0.03	0.1 ± 0.01	0.6 ± 0.1	2.3 ± 0.5	0.4 ± 0.1	0.9 ± 0.1
Se	1.6 ± 0.1	4.1 ± 0.4	0.8 ± 0.1	3.2 ± 0.3	7.9 ± 0.7	1.8 ± 0.2
Cd	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Pb	0.2 ± 0.1	1.2 ± 0.3	0.20 ± 0.03	< MDL	5.0 ± 0.6	0.9 ± 0.1

All reported values are 95 % confidence interval referred to dry base (mg kg^{-1}), n = 20
Method Detection Limits (MDLs) : Cd : 0.003 mg kg^{-1}, Pb : 0.003 mg kg^{-1}

4.4.4.1 Metals concentrations in the liver

Most of the metal concentrations were found highest in the liver of the fish samples regardless of their geographical origins. In particular, the mean concentrations of bioactive elements such as As, Cd, Pb, Fe, Cu, and Zn were very significantly higher ($p < 0.001$) than the mean concentrations recorded in gills and muscle tissues. This is mainly due to the biological function of liver, as the main site for the storage and biotransformation of toxics (Waheed *et al.*, 2013). Higher accumulation ratios of these metals are associated with the binding tendency of the elements to oxygen carboxylate, amino groups, nitrogen and/ or sulphur of the mercapto group in the metallothionein protein (Uysal *et al.*, 2008).

On the other hand, the deviation in the concentrations found between the aquaculture and wild-caught samples are mainly subjected to the aquaculture practices at the ponds and degree of contamination in the rivers (Low *et al.*, 2016). This means that the elemental accumulation patterns observed in the liver tissues of both species could be used as reasonable candidates of bioindicators. Such potential will be explored and discussed in later sections of this thesis.

4.4.4.2 Metal concentrations in the gills

Gills is another potential tissue to be explored as they are sensitive respiratory organ that has close contact with the surrounding water and resuspended sediment particles. Basically, the gills have ion regulatory membranes in the presence of mucous layer which induce metal ion interaction and accumulation through the high volume of water filtered (Pereira *et al.*, 2010; Bebianno *et al.*, 2004; Pringle *et al.*, 1968). Therefore, the metal concentrations found in the gills are expected to have a direct relationship with the degree of contamination and the exposure of fish to the contaminated media (Tekin-Özan & Aktan, 2012; Karadede & Ünlü, 2000).

In this work, it is found that the gill tissues contain the highest concentrations of Ca, Cr, and Ni compared to other tissues. The gills are expected to contain higher amount of Ca (Uysal, 2011), because Ca is a major component of the gill arches, furthermore, gill epithelium has been demonstrated to be the main site of Ca uptake from the water in order to sustain the Ca requirement of the fish (Pinto *et al.*, 2010).

4.4.4.3 Metals concentrations in the muscle

Similar with previous research (Djikanović *et al.*, 2016; Authman *et al.*, 2012; Uysal, 2011), this study also found that muscle tissue generally exhibits lower metal concentrations compared to other tissues except for Mg and K. Mg and K play vital roles in enzyme co-factors, structural components of cell membranes and extracellular fluids. They are the macro-elements which are needed for fish survival where the dietary requirements for fish are 1 to 3 g kg^{-1} and 0.5 g kg^{-1} respectively. According to the National Research Council, fish are able to extract Mg from the environment, whereas about 40% of the body, Mg is distributed throughout the organs and muscle tissues (National Research Council, 1977).

Hazardous metals are likely to be accumulated in the internal organs, however, in this study, As was detected in the muscle tissues and Cd detected in some wild-caught *Pangasius* sp. samples. It is believed that the significant amount of As found in the fish samples particularly *Hemibagrus* sp., could be linked with industrial pollution and intensive agricultural activities along the Selangor River (Fang *et al.*, 2014; Ma *et al.*, 2013; Wang *et al.*, 2013; Mohamad Ali *et al.*, 2010).

This is deduced based on the high concentration of As (approximately 51 mg kg^{-1}) observed in the sediment samples of the river. Such elements are toxic and persistent in humans (Adel *et al.*, 2016; Qin *et al.*, 2015), therefore their accumulation in fish muscle can threaten human health if consumed. However, As concentration in the sediment fractions shows insignificant correlation ($p > 0.05$), with the As concentration in muscle tissues. This indicates that the As concentration in the aquatic environment may not necessarily have high As bioavailability to the fish species. This suggests that the As bioaccumulation is very much dependent on other factors, such as metal speciation, multiple routes of exposure (diet) and geochemical effects (Yap & Norhaidah, 2011).

4.4.4.4 Metals distribution pattern in the tissues

To provide an overview of metals distribution in tissues, PCA is used to transform the original data matrix which is composed of 360 samples and 13 metal variables (20 samples of each tissues from each sampling site) into a product of two matrices. From the PCA model (Figure 4.11), the first two PCs accounted for about 59% of the total variability of the associated elemental concentrations in all tissues of fishes. Figure 4.11 shows that three major distinct clusters are revealed by the score-loading biplot. The identities of these clusters are perfectly matched with the types of sample tissues, regardless of the fish origins.

Figure 4.11 demonstrates that Cluster I is made up of the PC scores associated with elemental variations that contributed to the liver tissues. Cluster II are composed of the scores of muscle, while scores of gills are grouped in Cluster III. The variations among the studied tissues clearly indicate that the variations in elemental content caused by the variance in the different tissues are greater than those caused by the habitat. In other words, variation in the elemental accumulation pattern is strongly dependent on the type of studied tissues (Low *et al.*, 2011).

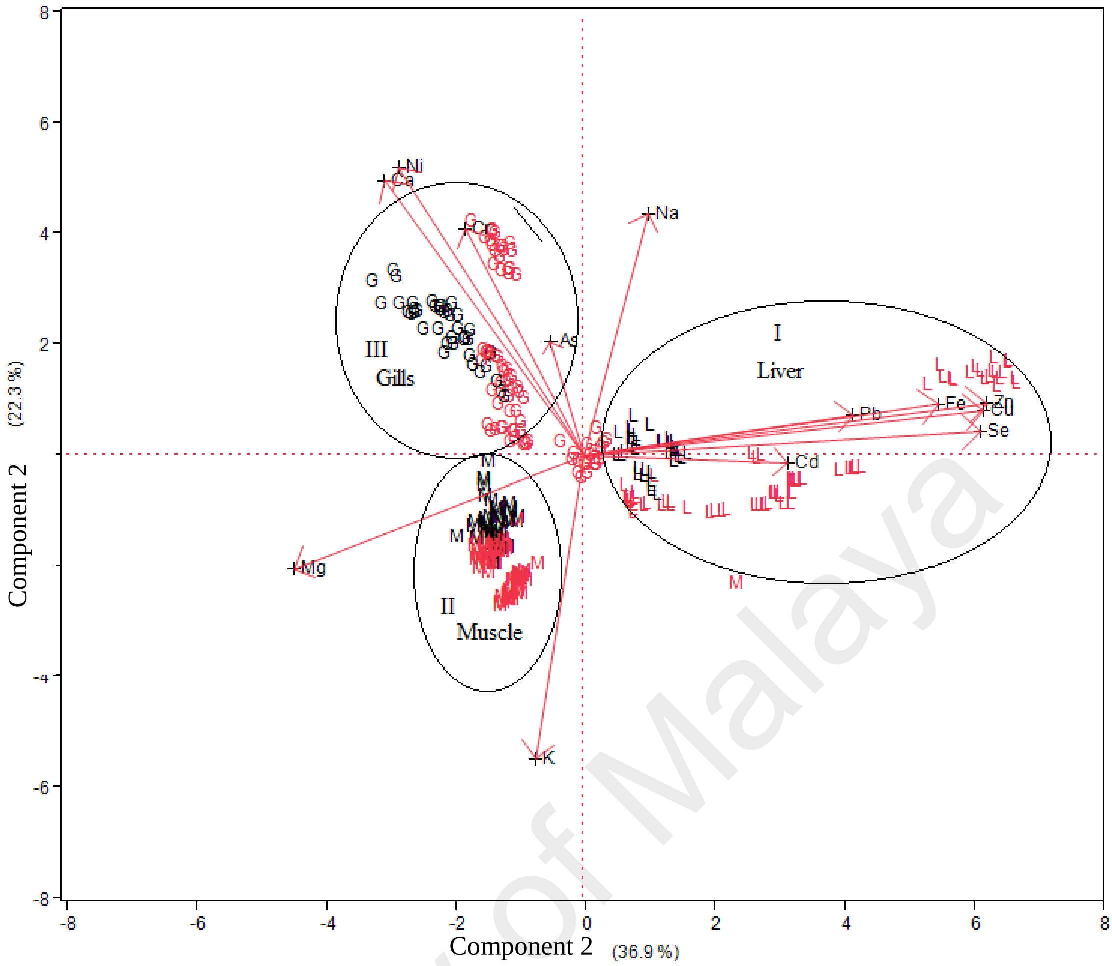


Figure 4.11: PCA bi-plot for fish samples (*n* = 360). The red symbol denoted to *Hemibagrus* sp., black symbol denotes to *Pangasius* sp. Letters M, L, G refer to different tissues (muscle, liver and gills)

It is noticed that liver sample are dispersed at the upper-right region with positive loadings of Cd, Pb, Fe, Zn, Cu, Se, and Na. This can be interpreted as the liver tissues that normally contain higher levels of elements, making them stand out more from those at on the left-hand side of the score plot.

In other words, samples at the right-hand side of the scores plot contain higher levels of metal concentration compared to those at the left-hand side. The result suggests that the total elemental accumulation is in the order of liver > gills > muscle. This trend is also consistent with the previous research done by Low *et al.* (2009). Based on the findings, the accumulation of pattern of studied elements should be able to conclude the identities of unknown *Pangasius* sp. and *Hemibagrus* sp. tissues.

The findings are also found consistent with the trends in the Tables 4.18 – 4.20. Fe was found higher in liver tissue due to their physiological role in blood synthesis (Wagner & Boman, 2003), while higher concentration of Cu has been reported because 95% of the accumulation are transferred into the liver in the form of tetrahedral metalloenzymes complex forms (Al-Yousuf *et al.*, 2000). The scores of muscle in cluster II is loaded with Mg and K, suggesting that the muscle tissues exhibit lower metal concentration than other tissues, except for Mg and K elements. In addition, the scores of muscle tissues were compactly grouped and do not disperse as other tissues. One reason for this situation might be due to the fact that muscle tissue has a weak accumulating potential (Uysal *et al.*, 2003). Referring to the bi-plot, all gills samples are located at the upper-left quadrant, which is associated with significant postitive loadings of As, Cr, Ca, and Ni. Concentrations of metals in the gills are directly dependent on the ambient conditions. Therefore, it is noteworthy to indicate the habitat of fishes based on metal concentration in the gills (Low *et al.*, 2016).

Since the BCF and BSAF values of *Hemibagrus* sp. shows more variation with respect to metal accumulation, an effort was made to overview metal concentration between two habitats via Hierarchical Cluster Analysis (HCA). Analysis was made based on similarity shared across the concentration patterns as shown in Figure 4.12. The observed clustering indicate that the variance originates from the variability in metal level in different tissues whereas variation caused by difference in sampling sites is masked. Different tissues are able to accumulate different targeted metals due to tissue-specific association where this factor becomes important in the selection of fish tissues for metal pollution assessment (Low *et al.*, 2016).

Similar to PCA analysis (Figure 4.11), the two-way dendogram (Figure 4.12) shows that the liver tissue of *Hemibagrus* sp. is the main depository of Fe, Cu, Zn, Se and Pb. (Karadede & Unlu, 2000). Due to metabolic activities, concentrations of Ca, Ni and Cr were accumulated in the gills while Mg, As and K in the muscle tissue. The variations in metal accumulation in tissues could be related to dissolve metal ion exchange through the lipophilic membrane, and combination of metallothionein protein with the metals in the different tissues (Malik *et al.*, 2014).

Several studies have been reported on the differences in metals concentrations of fish tissues between aquaculture and wild fish (Idris *et al.*, 2017; Martinez *et al.*, 2010). In this work, concentrations of Ni, As, Se and Pb were found higher in the liver of *Hemibagrus* sp. from Selangor River, while concentrations of Ca and Na were predominant for aquaculture pond (Figure 4.13a). Basically, the concentrations found in the fish reflect human activities at the sampling sites.

The agricultural and industrial effluents are the sources of metals deposition in the Selangor River (Daniel & Kawasaki, 2016; Mohamad Ali *et al.*, 2010; Sarkar & Datta, 2004), while concentration of Na, Ca, Mg and Cu observed in the livers of aquaculture fish can be associated with the extensive usage of limestone for pond management and/or the constituents of feeding diet (Stephens & Ingram, 2006; McCarthy & Shugart, 1990).

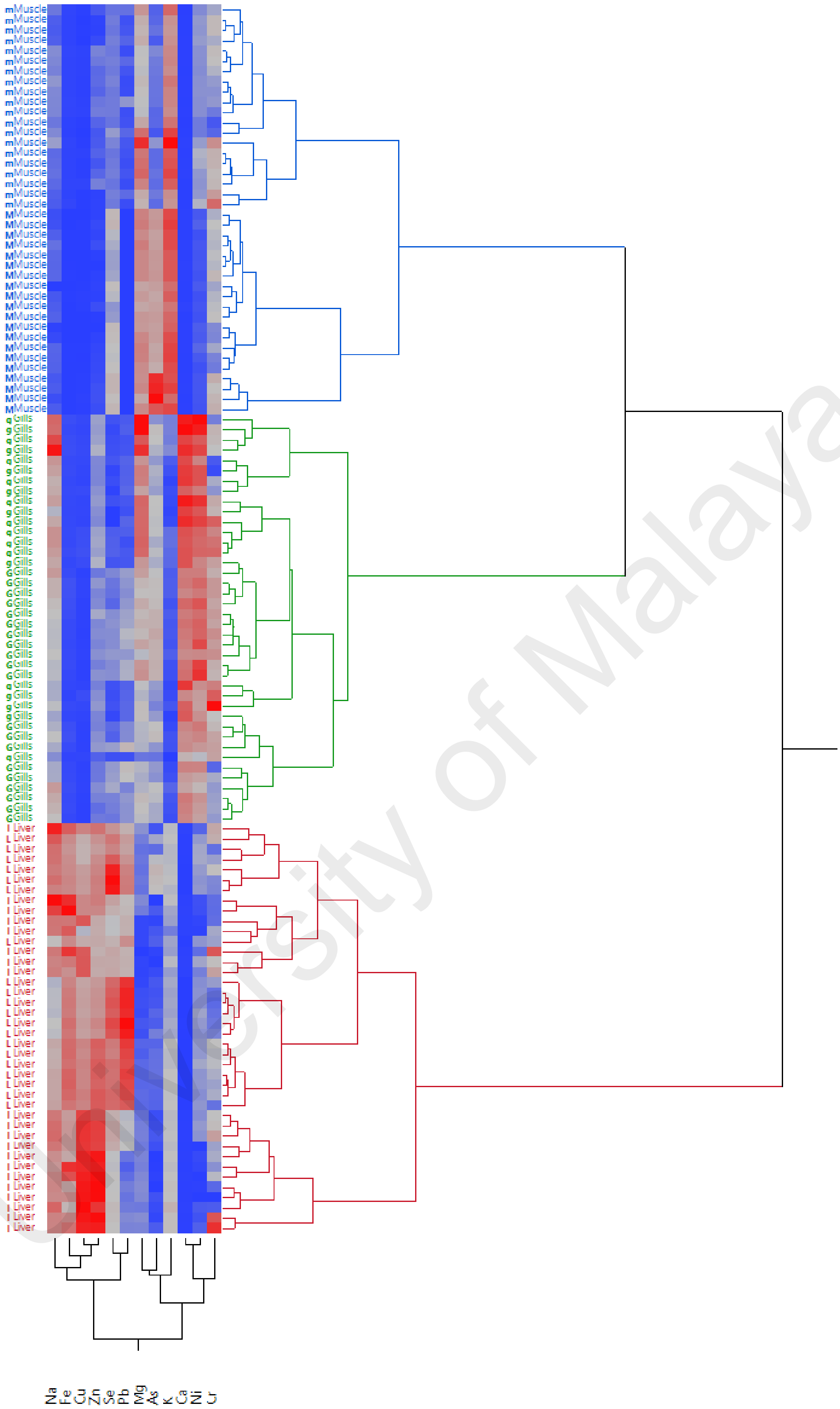


Figure 4.12: Dendogram of *Hemibagrus* sp. tissue sample (muscle, liver, gills) from aquaculture pond and Selangor River.

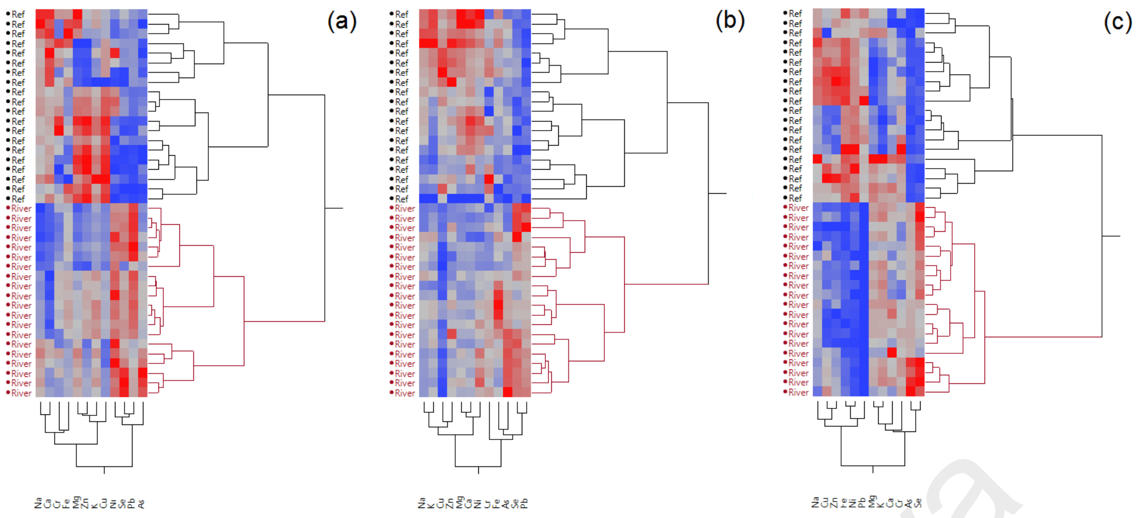


Figure 4.13: Dendogram of *Hemibagrus* sp. (a) liver (b) gills and (c) muscle sample from Selangor River and aquaculture pond

Therefore, this trend suggests that feeding habits, food quality and metal concentrations in the water column play an important role in influencing metal accumulation in the fish tissues (Vicente-Martorell *et al.*, 2009). Figures 4.13b and c shows that the pattern of metals distributions in the gills and muscle samples are similar to the liver, but at low level of metals concentrations. Gills are the first organ to be in contact with water and sediment particles; therefore they can be relevant in monitoring metal ions (Pereira *et al.*, 2010; Bebianno *et al.*, 2004). Thus, the degree of metal pollution and the exposure duration of fish to the metal have a strong relationship with the concentrations of metals in the gills (Tekin-Ozan & Aktan, 2012; Karadede & Unlu, 2000).

In general, *Hemibagrus* sp. from Selangor River showed elevated concentrations of hazardous metals such as As, Se, and Pb. Those concentrations found in the fish tissues can reflect the degree of metal pollution observed on the sediment column. On the other hand, aquaculture fish was found to have higher mineral contents of Na, Cu, Zn and Fe, where usually these elements correlate with feeding habits and metal levels in the water body (Yildiz, 2008). The high variation of these elements in the aquaculture fish could be the reason why only these elements are significantly ($p < 0.05$) influenced by the metal levels in the water and feeding pellets. This suggests that *Hemibagrus* sp. could be used as a bioindicator in aquatic ecosystem metal pollution monitoring and assessment (Idris *et al.*, 2017).

4.4.5 Chronic Human Health Risk Assessment

Since *Pangasius* sp. and *Hemibagrus* sp. are common protein supply for the Malaysian community, the metal exposure via fish consumption is of great concern. For comparison purposes, the metal concentrations in the edible muscle tissues are reported in wet weight basis in Table 4.21 and Table 4.22 together with established limits and literature findings.

At a glance, most of the elemental concentrations found in the muscles of aquaculture samples are lower than that of wild-caught samples which suggests dominant environmental input. In terms of metal accumulation pattern, the results show that the macro-element found in the muscles exhibitd the following order : K > Na > Mg > Ca regardless of the sampling sites and fish species, whereas the accumulation order of the trace elements varies. The similarity in the accumulation pattern of macro-elements is attributed to the fish regulation processes while the variations in trace metals can be associated with the environmental conditions. This corroborates the potential of those fishes as bioindicators of trace metal pollution.

Table 4.21: Total metal concentration in muscle tissues of *Pangasius* sp. (wet weight basis)

Metal conc. / mg kg $^{-1}$	This study				Previous studies					
	Selangor		Pahang		Sarawak, Malaysia (Mok *et al.*, 2012)	Bangladesh (Ahmed *et al.*, 2015)	Thailand (Busamongkol *et al.*, 2014)	Legal limits		
	Aquaculture pond	River	Aquaculture pond	River	Aquaculture pond	Aquaculture pond	River	MFA	FAO	USEPA
Na	2022.72 ± 12.5	3715.65 ± 11.6	1773.59 ± 12.8	1671.15 ± 10.6	-	-	-	-	-	-
Mg	1120.31 ± 4.2	970.29 ± 5.6	1014.67 ± 11.6	1049.66 ± 8.9	-	-	-	-	-	-
K	14458.89 ± 6.2	15798.31 ± 6.4	13652.99 ± 4.5	14083.27 ± 6.7	-	-	-	-	-	-
Ca	273.88 ± 3.1	259.09 ± 2.4	207.6 ± 4.7	279.72 ± 6.4	-	-	-	-	-	-
Cr	0.26 ± 0.01	0.27 ± 0.01	0.0008	0.0008	-	1.349	8.51	-	-	-
Fe	12.73 ± 2.4	10.79 ± 1.6	17.48 ± 2.6	13.63 ± 1.8	-	-	19.58	-	-	950
Ni	0.002 ± 0.001	0.0052	0.006	0.006	-	0.012	-	-	-	-

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported
Note : Malaysian Food Act 1983 ; Food and Agricultural Organization, 2008; USEPA, 2000

Table 4.21, continued

Metal conc. / mg kg $^{-1}$	This study				Previous study					
	Selangor		Pahang		Sarawak, Malaysia (Mok *et al.*, 2012)	Bangladesh (Ahmed *et al.*, 2015)	Thailand (Busamongkol *et al.*, 2014)	Legal limits		
	Aquaculture pond	River	Aquaculture pond	River	Aquaculture pond	Aquaculture pond	River	MFA	FAO	USEPA
Cu	0.53 ± 0.01	0.022	1.13 ± 0.1	0.696 ± 0.4	24.54	0.658	-	30	30	54
Zn	17.68 ± 1.2	15.67 ± 0.6	18.39 ± 0.4	15.81 ± 1.6	53.51	3.201	26.8	100	40	410
As	0.038 ± 0.01	0.12 ± 0.01	0.016	0.039 ± 0.01	0.001	0.077	0.19	1	-	0.41[a]
Se	0.74 ± 0.1	1.55 ± 1.2	1.25 ± 0.2	1.12 ± 0.2	-	1.04	0.5	-	-	6.8
Cd	0.0023	0.0022	0.0024	0.09 ± 0.1	0.17	0.001	< 13	1	-	1.4
Pb	0.0023	0.0022	0.0024	0.0024	1.2	0.017	-	1	0.5	-

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported
Note : Malaysian Food Act 1983; Food and Agricultural Organization, 2008; USEPA, 2000

Table 4.22: Total metal concentration in muscle tissues of *Hemibagrus* sp. (wet weight basis)

	This study		Previous study				
Metal conc. / mg kg $^{-1}$	Selangor		Sg. Galas, Kelantan (Baharom *et al.*, 2015)	Yantgze River, China Yi *et al.*, 2011	Legal limits		
	Aquaculture pond	River	River	River	MFA	FAO	USEPA
Na	1732.74 ± 1.2	1422.74 ± 2.5	-	-	-	-	-
Mg	992.38 ± 3.1	1113.71 ± 12.1	-	-	-	-	-
K	13180.58 ± 3.5	15332.89 ± 5.4	-	-	-	-	-
Ca	278.58 ± 2.1	308.33 ± 2.5	-	-	-	-	-
Cr	0.26 ± 0.5	0.26 ± 0.3	-	0.017	-	-	-
Fe	48.75 ± 0.3	11.62 ± 1.2	-	-	-	-	950
Ni	0.25 ± 0.1	0.069 ± 0.01	00.58	1.718	-	-	-

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported
Note : Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

Table 4.22, continued

Metal conc. / mg kg $^{-1}$	This study: Selangor		Previous study: Sg. Galas, Kelantan (Baharom *et al.*, 2015)	Previous study: Yantgze River, China Yi *et al.*, 2011	Legal limits		
	Aquaculture pond	River	River	River	MFA	FAO	USEPA
Cu	0.93 ± 0.01	0.67 ± 0.1	0.027	-	30	30	54
Zn	26.51 ± 0.2	18.91 ± 1.4	0.136	7.58	100	40	410
As	0.20 ± 0.01	1.9 ± 0.1	-	0.025	1	-	0.41[a]
Se	1.61 ± 0.2	2.67 ± 2.1	-	-	-	-	6.8
Cd	0.0024	0.0025	-	0.11	1	-	1.4
Pb	0.18 ± 0.01	0.0025	0.011	0.184	1	0.5	-

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported
Note : Malaysian Food Act,1983; Food and Agricultural Organization, 2008; USEPA, 2000

Table 4.21 shows that metals concentrations in *Pangasius* sp. muscle from aquaculture pond in Sarawak (Mok *et al.*, 2012) were higher than this study while a study done at Bangladesh showed a vice versa trend (Ahmed *et al.*, 2015). Table 4.22 also shows the same pattern of metal concentration for *Hemibagrus* sp. The trend suggests that environmental input, habitat, catching place, time and life style of fish species can influence metal accumulation in fishes (Morgano *et al.,* 2011; Karadede & Ünlü, 2000).

Table 4.22 shows that the mean concentrations of the studied elements are far below the permissible limits except for the total As found in *Hemibagrus* sp. sampled from Selangor River. This could be an alarming finding in the view of general public if the total As is perceived as hazardous. In fact, the toxicity of As depends upon its chemical species where the arsenobetaine (organic As) that is found mostly in fish is innocuous compared to toxic inorganic As that accounts for less than 10% of total As (Low *et al.*, 2015). With this in mind, the estimated mean concentration of inorganic As in those samples is only about 0.19 ± 0.01 mg kg^{-1}, which is well below the limits. However, the human health risk associated with the fish ingestion is yet to be concluded at this stage because the chronic risk is not only dependent on the concentration and toxicity of As alone.

Concentration of metals in the edible portion of fish tissues can give an overview of environmental pollution and risk for human health. It is known that fish accumulate different level of metal concentration based on their trophic levels, habitat, life span, as well as chemical characteristics of specific metals (Teffer *et al.*, 2014; Lopez *et al.*, 2013). Studies of metal accumulation in fish have largely been conducted in terms of food safety purposes (Gu *et al.*, 2015). Thus, it is important to consider both metal intakes and doses that lead to adverse human effect due to ingestion of metals (Low *et al.*, 2015).

4.4.6 Estimation of Potential Health Risk

To evaluate the health risk of consumption of contaminated fish species to Malaysians, the intake rates of the toxic elements were estimated. The estimation was made based on the wet weight basis of the concentration of trace elements in the fish muscle.

4.4.6.1 Estimated Daily Intake (EDI)

Daily metal intake (EDI) was estimated based on the average metals concentrations in fish muscle and the daily fish consumption rates. Current metal intakes were compared with the respective provisional tolerable weekly intake (PWTIs). An international regulation does not set concentration threshold in consumption of fish species for studied metals, but the World Health Organization suggests the use of PTWI as shown in Table 4.23. PWTIs were establishes by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (1993).

The estimated daily intakes of metals (EDIs) of Cr, Fe, Ni, Cu, Zn, As, Se, Cd and Pb (mg kg^{-1} per day body weight) are illustrated in Table 4.23. The results show that aquaculture fish is generally less susceptible to environmental pollution but consumer preference is usually in favour of wild-caught fishes, as its safety has been presumed. Hence, a qualitative estimation of the daily intakes of metals which reflect the metal exposures through fish consumption must be carried out.

EDI of studied elements was calculated on the basis of the concentrations measured in fish muscle and daily fish consumption rate (71 g day^{-1} per person) (FAO, 2008), where the average Malaysian body weight was assumed to be 64 kg (Lim *et al.*, 2000).

Table 4.23: The estimated daily intakes (EDI) of metals (mg/kg bw /day) through consumption of freshwater fish species by adult people (assuming 64 kg person)

	Estimated Daily Intake (EDI)							
	Selangor				Pahang		PTWI	RfD
	Pangasius sp.		*Hemibagrus*sp.		Pangasius sp.			
	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught	Aquculture pond	Wild-caught		
Cr	2.8×10^{-4}	3.0×10^{-4}	2.8×10^{-4}	2.8×10^{-1}	8.7×10^{-7}	8.7×10^{-7}	-	1.5
Fe	0.012	0.014	0.054	0.012	0.02	0.02	-	7×10^{-1}
Ni	1.7×10^{-5}	0.5×10^{-5}	28×10^{-5}	7.7×10^{-5}	6.2×10^{-6}	6.1×10^{-6}	-	-
Cu	6.0×10^{-4}	0.2×10^{-4}	10.3×10^{-4}	7.4×10^{-4}	12.5×10^{-4}	7.7×10^{-4}	-	4×10^{-2}
Zn	0.02	0.017	0.03	0.02	0.02	0.017	-	3×10^{-1}
As[a]	0.4×10^{-5}	1.3×10^{-5}	2.2×10^{-5}	20.1×10^{-5}	0.2×10^{-5}	0.4×10^{-5}	0.015	3×10^{-4}
Se	0.8×10^{-3}	1.7×10^{-3}	1.8×10^{-3}	3.0×10^{-3}	1.4×10^{-3}	1.2×10^{-3}	-	5×10^{-3}
Cd	2.5×10^{-6}	2.4×10^{-6}	2.7×10^{-6}	2.8×10^{-6}	2.7×10^{-6}	1.1×10^{-4}	0.007	1×10^{-3}
Pb	2.5×10^{-6}	2.4×10^{-6}	2.0×10^{-4}	2.8×10^{-6}	2.7×10^{-6}	2.7×10^{-6}	0.025	4×10^{-3}

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported
Note : PTWI : (JECFA, 2003; 2000; 1982)
: RfD : (USEPA, 2000; 1997

Estimation of metal intakes depends on the metal concentration, food consumption, and body weight (Yi *et al.*, 2017). According to Table 4.23, the estimated daily intake is lower than the corresponding RfD. Therefore, the results indicates that the studied fish's consumption might not have an adverse effect on human health.

In this study, the comparison of the calculated estimated intake values for inorganic As, Cd and Pb with the value provided by JECFA shows that these metals are still below the guidelines values. This would indicate that the consumption of *Pangasius* sp. and *Hemibagrus* sp. from both habitats at the present rate is safe for Malaysians. However, the EDI calculation in this study only evaluates for fish, which accounts for only a fraction of contamination intake through daily dietary consumption. Therefore, it is important to consider metal concentrations in other elements of aquatic system such as water or sediment column within he risk assessment. However, based only on the risk estimation for fish, the daily intake of fish from the study area might not cause any detrimental health hazards. Nevertheless, further research should be conducted to ensure the locals' health.

4.4.6.2 Target Hazard Quotient (THQ)

The risks of non-carcinogenic health risks associated with fish consumption are assessed based on target hazard quotients (THQs). It is unlikely that humans will experience any adverse health effects if THQs assume a level of exposure below one (THQ < 1) (Wei *et al.*, 2014). Higher THQ values mean a higher probability of experiencing long term non-carcinogenic effects. As macro-elements such as Na, Mg, K and C do not normally approach toxicological effects, more attention is given to other elements. The THQ values in this work are given in Table 4.24.

As can be seen, the THQ values are significantly lower than 1, therefore, it can be concluded that the metals evaluated in the edible parts of *Pangasius* sp. and *Hemibagrus* sp. from studied sites pose no health effects for consumers. However, more attention should be given to the consumption of *Hemibagrus* sp. from Selangor River. Based on Figure 4.14, it is observed that the HQ of As and Se for *Hemibagrus* sp. from Selangor River are near to one. This implies that the consumption of *Hemibagrus* sp. from Selangor River over a lifetime is likely to cause deleterious effects for Malaysians based on the THQ values. It is shown that sources of As and Se pollution are derived from anthropogenic sources (Leong *et al.*, 2007). The risk corresponding to their exposure may be related to their high tolerability (Low *et al.*, 2015). In addition, exposures to Cr, Fe, Ni, Cu, Zn, Cd, and Pb via fish ingestion could be deemed as non-significant since each of them has small THQ values. However, from the calculation of health risk due to consumption of studied fish, it is clear that the THQ values do not exceed 1 for all metals which indicates consuming both fish species do not pose health effects.

Table 4.24: The Target Hazard Quotient of metal ingestion *Pangasius* sp.and *Hemibagrus* sp.

	Target Hazard Quotient (THQ)					
	Selangor				Pahang	
	Pangasius sp.		*Hemibagrus*sp.		Pangasius sp.	
	Aquaculture pond	Wild-caught	Aquaculture pond	Wild-caught	Aquculture pond	Wild-caught
Cr	2.0×10^{-4}	2.0×10^{-4}	1.2×10^{-4}	4.3×10^{-4}	5.8×10^{-7}	5.8×10^{-7}
Fe	0.020	0.017	0.078	0.04	0.027	0.022
Ni	3.3×10^{-4}	1.2×10^{-4}	56.4×10^{-4}	15.3×10^{-4}	1.2×10^{-4}	1.2×10^{-4}
Cu	1.5×10^{-2}	0.1×10^{-2}	2.6×10^{-2}	1.8×10^{-2}	3.1×10^{-2}	2.0×10^{-2}
Zn	0.07	0.06	0.09	0.07	0.07	0.06
As[a]	0.014	0.043	0.07	0.70	0.01	0.01
Se	0.16	0.34	0.36	0.60	0.28	0.25
Cd	2.6×10^{-3}	2.4×10^{-3}	2.7×10^{-3}	2.8×10^{-3}	2.7×10^{-3}	0.1
Pb	6.4×10^{-4}	6.1×10^{-4}	5.1×10^{-2}	7.0×10^{-4}	6.7×10^{-4}	6.7×10^{-4}

[a] : Inorganic As (assuming 10% of the total As are inorganic As)
- : not reported

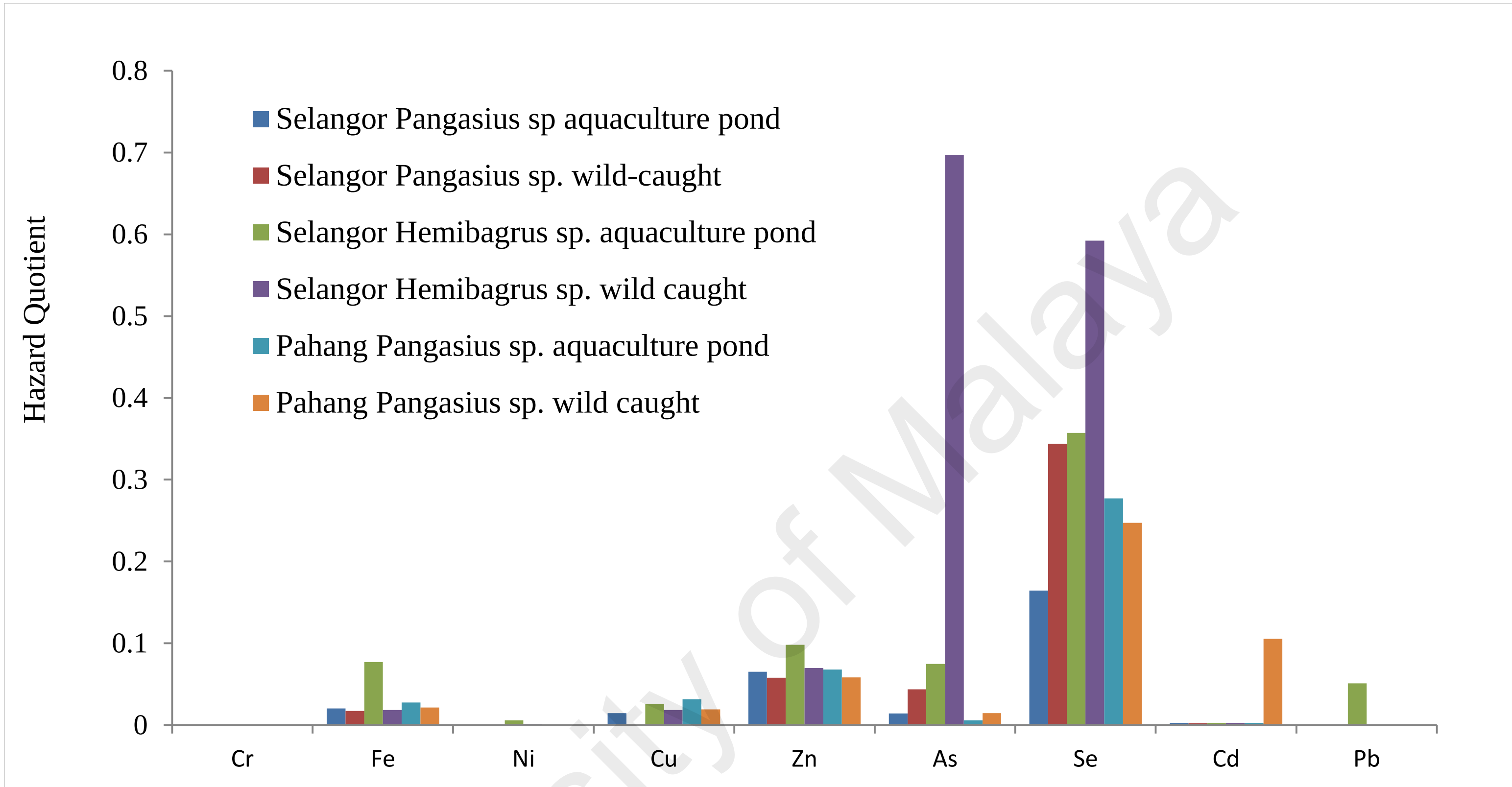


Figure 4.14 : Hazard Quotient of *Pangasius* sp. and *Hemibagrus* sp. in two studied sites. THQ safe limit (THQ <1)

CHAPTER 5 : CONCLUSION

Study on the variation of metals in water, sediment and fish in Sungai Selangor and Sungai Pahang as well as nearby aquaculture ponds had been undertaken in this work. As far as water quality parameters (temperature, conductivity, TDS, DO and pH) are concerned, these variables record worse condition in aquaculture ponds and may be attributed to aquaculture practices and management. One of the significant observations from the two-way dendogram analysis is that the water from aquaculture pond was the main depository for Na, Mg, K and Ca regardless of the geographical location of the study area. However, a contrasting trend was observed in the riverine sediments where levels of Na, Mg, K and Ca were high. This suggests that the metals concentrations retained in the sediment fractions were basically independent of the current inputs and normally reflects the long-term metal deposition within the riverine system.

Additionally, results from HCA identified three distinct clusters by elements in the sediments irrespective of their sampling sites. It is suggested that Na, Mg, K and Ca (Cluster 1) originated from additives applied in the aquaculture pond water management, while in Cluster 2, Cr, Cu, As, Ni, Se, Cd, Zn, Pb have similar behaviour indicating that they are derived from anthropogenic sources while existence of Fe in cluster 3 mostly originated from natural sources. In brief, there are two sources of pollution in the sediments due to metal loadings which are through natural processes and anthropogenic activities.

Moreover, combined information obtained from water, sediment and fish samples can describe the pattern of distribution and accumulation of metals. As demonstrated in this work, discrimination of samples by using principal component analysis provided a classification of the three groups based on their metal accumulation.

Apart from that, the results obtained illustrated the association between the concentrations measured in the tissues of *Pangasius* sp. and *Hemibagrus* sp. and the level of metal contamination in the water column and sediments. The distributions of metal concentration followed the order of; sediment > fish > water. The metals concentrations in sediment were much higher than metals in water and the fish samples. The study revealed that there was a considerable variation in the concentration of studied elements in water, sediments and fish. This variation might be due to the different sources of contaminants that are being transferred to the river at the different sampling site.

As demonstrated in this work, PCA provides a simple way to visualize the clustering tendencies among the different tissues of fish species, and helps to indicate which elements contribute most of the variation. It can be observed that there are some trends in metal distribution in the particular organs and the distribution pattern with respect to the study area. The evidence provided by this study demonstrated that the variation in the accumulation of metals followed the order of; liver > gills > muscle. It is observed that liver tissues were associated with high Fe, Cu, and Zn, gills with high Ca, Ni and Cr and muscles with high Mg, As and K. Since the BCF and BSAF values of *Hemibagrus* sp. shows more variation on metal accumulation than *Pangasius* sp., this suggests that *Hemibagrus* sp. Has the potential to be a useful bioindicator of metal pollution in environmental assessment and monitoring. The data also show association between metal levels in the fish and the concentrations of metal contaminants measured in the water and sediment at the sampling sites.

Results from preliminary risk assessments suggest that risks posed by metals via consumption of *Pangasius* sp. and *Hemibagrus* sp. from the studied sites were within tolerable regions. Elements of Zn, Cu and Se have been identified with higher factors than other metals. This suggested that trace elements are more extensively absorbed by fish for their biological function. Daily intake of all metals showed that in the case of regular consumption there should be no harmful effects on the health of consumers of the fishes studied. Although levels of calculated EDI and THQ are not high, serious care must be taken considering the fact that there are people who regularly consume large amounts of fish. However, more attention should be given to the consumption of *Hemibagrus* sp. from Selangor River as it was observed that the THQ of As and Se for *Hemibagrus* sp from Selangor River were near to one. Thus, it is advisable to regulate intake of *Hemibagrus* sp. so as to reduce the risk of deleterious health effects. It is suggested that As and Se are the best describers in characterizing the potential of *Hemibagrus* sp. as bioindicator of metal pollution in aquatic system.

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