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ASSESSMENT OF METAL DISTRIBUTION IN VARIOUS TISSUES OF WILD-CAUGHT AND CULTURED *Pangasius* sp.

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Abstract: This study aims to evaluate the spatial distribution of metals (Na, Mg, K, Ca, Cr, Fe, Ni, Cu, Zn, As, Se, Cd and Pb) in various tissues (muscle, liver, gills) of *Pangasius* sp. living in Sungai Selangor and a nearby aquaculture farm using microwave-assisted digestion/inductively-coupled plasma-mass spectrometry. The results demonstrated that the pattern of metal accumulation is dependent on the type of tissue, although such variations are subjected to their metabolism. In general, the liver contained higher concentrations of metals, followed by gills and muscle tissue. The concentration of heavy metals (As, Pb) indicated the presence of anthropogenic input. In terms of risk assessment, the toxic metal concentrations in the muscle of *Pangasius* sp. were significantly lower than hazardous limits. In general, *Hemibagrus* sp. from Sungai Selangor was found to contain elevated concentrations of hazardous metals such as As, Se and Pb ($p < 0.05$) than those from the aquaculture farm.

Keywords: Accumulation, metal, fish, bioindicator, sustainability, microwave-assisted digestion/inductively coupled plasma-mass spectrometry.

Introduction

In Malaysia, high levels of toxic metals have been found in freshwater fish, indicating a rise in pollution of water sources. The fish absorbed the contaminants from their food and surrounding waters, and cumulatively deposited them in their organs and skeletal tissues (Alasalvar *et al.,* 2002; Rosli *et al.*, 2018). Studies of metal concentration in fish in this country may be traced back to the late 1970s, when Babji *et al.* (1979) conducted their study in Peninsular Malaysia. Previous studies have demonstrated an annual increase of metal distribution in local fish (Tukimat *et al.*, 2002 & 2006). Even so, despite the wide range of fish species available, not many research had compared the metal concentrations of fish from two habitats.

In Malaysia, the aquaculture sector is a fast-expanding industry due to its importance as a source of food and protein. Among the freshwater species produced is *Pangasius* sp. which has a huge market demand (Roberts & Vidthayanon, 1991; FAO, 2014). Variations in metal content between cultured and wild-type fish is always a subject of argument. Some studies have demonstrated the differences in terms of chemical composition and nutritional value (Dincer *et al.*, 2010). Nevertheless, there are also other differentiating factors, such as biological and seasonal variations (species, size, age, sex), food sources, environmental conditions (chemical and physical quality of water), production technologies and catching areas (Zeynali *et al.*, 2009).

One of the concerns of the present study is the difference in metal accumulation between wild-type and aquacultured fish. Basically, cumulative metal deposition patterns in both types of fish differed considerably due to many factors. It has been reported that the deposition of metal in wild-type fish is due to its ambient habitat, while aquacultured fish mainly derived it through diet, such as from commercial-feed pellets (Low *et al.*, 2016). To fill the scarcity of data in this subject, a study on metal accumulation in farmed and wild-type species is, therefore, needed. Due to the rising popularity and intake of *Pangasius* sp., the spatial distribution of metal elements in fish from Sungai Selangor and a nearby aquaculture farm is investigated to understand the health risks derived from the consumption of the said species.

Materials and Methods

Sampling Sites

Sungai Selangor mostly flowed through naturally-vegetated areas and originates from an altitude of 1500 m. It starts from the highlands of Kuala Kubu Baru in the state of Selangor, Peninsula Malaysia, passing farms, industrial areas and towns before flowing into the Straits of Malacca via Kuala Selangor. The river's catchment area is about 1700 km^2, which provided raw water for agriculture and an aquaculture farm as shown in Figure 1. Nevertheless, this river had been exposed to various contaminants, particularly agricultural and industrial effluents, besides domestic waste (Leong *et al.*, 2007; Ashraf *et al.*, 2012).

Sample Collection and Preparation

Field study and sampling were conducted for four months (June to September), which comprised five sampling points for each location, with 20 samples taken in total. The basic water quality parameters (temperature, conductivity, total dissolved solid (TDS), salinity, dissolved oxygen (DO), and pH were measured in-situ using a Hydrolab multiparameter unit (OTT Hydromet, Kempten, Germany). Water samples were collected using polyethylene bottles, which were rinsed twice with the water samples before collection. The closed bottles were submersed, then opened to collect the water samples and recapped sub-surface (USEPA, 1999). Then, the water samples were stored at 8 ºC for elemental analysis. The *Pangasius* sp.

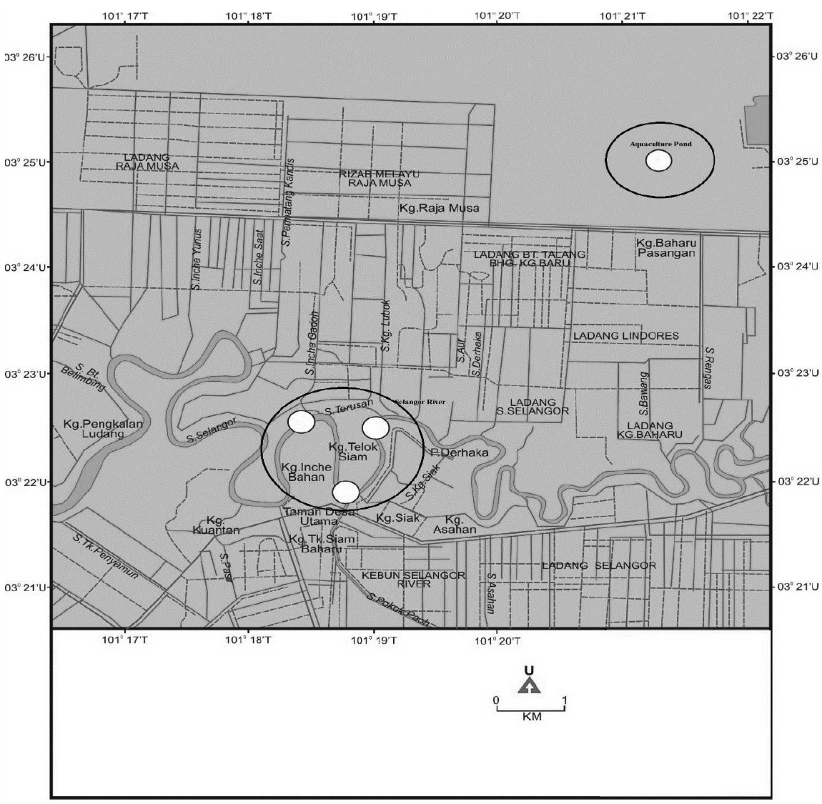

Figure 1: Map of the study area showing the location of the aquaculture farm and river sites where samples were taken

samples were obtained from local fishermen and fish farmers. The fish samples were wrapped in a polyethylene sampling bag, packed in an ice box and transported to the laboratory on the same day. Before the specimens were stored at -20 ºC, their total length and weight were measured. Liver, gills and muscle tissue were removed and cleaned several times with ultrapure water (UPW). These tissue samples were freeze-dried (CHRiST) before being homogenised with a mortar and pestle, and stored in a dessicator.

Reagents and Standard Materials

All chemicals used were of analytical grade. The UPW with a resistivity of more than 18 MΩ cm was obtained from the PURELAB® UHQII System (Elga LabWater, High Wycombe, United Kingdom). Suprapur® quality solutions of HNO_3 (65 %), H_2O_2 (30 %) and HCI (30 %) were obtained from MERCK (Merck KGaA, Darmstadt, Germany). Calibration standards were prepared from appropriate dilutions of environmental calibration standard solutions that consisted of 1000 mg L^{-1} Fe, Ca, K, Mg, and Na, and 10 mg L^{-1} of As, Cd, Cr, Cu, Ni, Pb, Se and Zn. Argon gas and helium collision gas purities were higher than 99.999 %. For the trace metal validation method, DORM-3 and DOLT-4 certified reference materials were used. All plastics were soaked overnight in 10 % nitric acid (v/v), thoroughly rinsed with the UPW and dried prior to use.

Microwave-Assisted Digestion

An analysis of the water samples was conducted according to USEPA Method 3015 (1994) using a Mar Xpress Microwave Accelerated Reaction System (CEM Corporation, Matthews, NC, USA). About 9.00 mL of water sample and 1.00 mL of 65 % HNO_3 were pipetted into a pre-cleaned digestion vessel for microwave-assisted digestion (MAD). The temperature was first set to reach 160 ºC in 10 minutes. It was then gradually increased to 170 ºC for the next 10 minutes. After digestion, the sample was cooled and filtered through a 0.45 µm PTFE membrane into a 25 ml volumetric flask. The sample's volume was added up to the mark using deionized water.

The fish samples were analysed according to the method described by Low *et al.*, (2012). MAD was performed using the same microwave system as done on the water samples. About 0.25 g of dried samples were weighed directly into 55 mL self-regulating control PFA® vessels and treated with 2.50 mL HNO_3, 0.50 mL HCI and 7.00 mL UPW. The samples' temperature was increased to 185 ºC for 10.5 minutes and held for 14.5 minutes under a microwave power of 800 W. After the samples were cooled down to room temperature, the solutions were filtered (using 0.45 µM PTFE membrane) and diluted with UPW to a volume of 50.0 mL. All digestate solutions were stored below 8 ºC and analysed using ICP-MS within five days (Idris *et al.*, 2017).

Calculations and Data Analyses

All calculation and statistical analyses were performed using Microsoft® Excel® 2010 and SAS® JMP® version 9 software package (SAS Inc, Cary, NC, USA). An analysis of variance (ANOVA) was carried out to assess the significant differences between the studied samples of *Pangasius* sp. taken from the two identified habitats. For unsupervised study, HCA was applied with training set to overview the clustering and distribution pattern of the studied elements. HCA converted the original data into one-dimensional dendograms based on Ward algorithm and Euclidean distance.

Results and Discussion

MAD-ICP-MS Performance

The analytical performance of the microwave assisted digestion/extraction of fish was checked using DORM-3 dogfish muscle and DOLT-4 dogfish liver. The results obtained for all analysed metals were in the certified range, with recovery values ranging from 92 % to 106 %. This meant the results of the study were considered to be accurate with respect to the true concentrations in the samples (Mziray & Kimirei, 2016).

Water Quality

The results of water quality for Sungai Selangor and nearby aquaculture farm are displayed in Table 1. There were noticeable discrepancies observed in the physical and chemical characteristics of samples from both sampling sites, even though the aquaculture farm relied on the same river as its water source.

The differences could plausibly be due to spatial variability between sampling sites and the water management programme for fish farming. One of the noteworthy observations was that the mean for physico-chemical parameters of water at the aquaculture farm was always greater than that of the river, and all measured parameters were found to be significantly different ($p < 0.05$) except pH. This might be caused by specific aquaculture practices and management. This trend might be associated with the application of water quality enhancers, such as lime, gypsum and salt, to control the alkalinity, hardness, turbidity, nitrite and bacteria levels, besides the phytoplankton loads of the farm water (Boyd, 1995).

Introduction of highly soluble salts into aquaculture farm ponds not only changed the physico-chemical parameters of the water, but also the concentrations of Na, Mg, K, and Ca. Apart from aquaculture practices, it was believed that surrounding activities emanating from industrial, agricultural and residential settlements could have also impacted the concentration of metals in the water (Idris *et al.*, 2017; Mishra *et al.*, 2008).

Fish Morphometric and Metal Distributions in the Tissues

To overcome the differences in variability associated with size variation in this study, an effort was taken to choose *Pangasius* sp. samples of comparable size. Their morphometric data are as summarised in Table 2. Although the mean length of wild-type *Pangasius* sp. was slightly longer than the cultured fish from the aquaculture ponds, no significant differences were found in their Condition Factor (CF) and Hepatosomatic Index (HSI) values.

The mean metal concentrations in different tissues of *Pangasius* sp. are presented in Figure 2. In particular, the mean concentrations of bioactive elements (As, Cd, Pb, Fe, Cu and Zn) were significantly higher ($p < 0.001$) than the concentrations recorded in muscle and gill tissues. Although the concentrations were found to vary over a wide range, however, there were general trends, where the level of metals in liver > gills > muscle according to their physiological and metabolism roles (Jayaprakash *et al.*, 2015).

Such trends had been extensively documented in other fishes as well (Idris *et al.*, 2017, Mziray & Kimirei, 2016; Pannetier *et al.*, 2016;), where studies had identified that the liver and gills, which acted as active metabolic

Table 1: The result of water quality.

	Sungai Selangor **N 03° 21.88 E 101° 18.73**	**Aquaculture farm** **N 03° 25.05 E 101° 21.20**
Temperature (°C)	29.93 ± 0.05	32.14 ± 0.01
Conductivity (µS cm^{-1}) *	202.67 ± 39	523.50 ± 0.7
TDS (g L^{-1}) *	0.12 ± 0.02	0.31 ± 0.01
Salinity (ppt) *	0.09 ± 0.02	0.22 ± 0.01
DO (mg L^{-1}) *	3.0 ± 0.20	7.7 ± 0.50
pH **	5.5 ± 0.32	6.8 ± 0.03

Mean ± standard deviation
* significant at $p < 0.05$

Table 2: Biometric information of cultured and wild-type *Pangasius* sp.

	Wild-type	**Cultured**
Length (cm)	76.5 ± 6.4	68 ± 1.40
Weight (kg)	3.75 ± 0.5	3.34 ± 0.20
Condition Factor (CF) (g/mm^3)	0.80 ± 0.05	1.07 ± 0.14
Hepatosomatic Index (HSI)	0.69 ± 0.08	1.07 ± 0.14

Mean ± standard deviation, *n* = 20

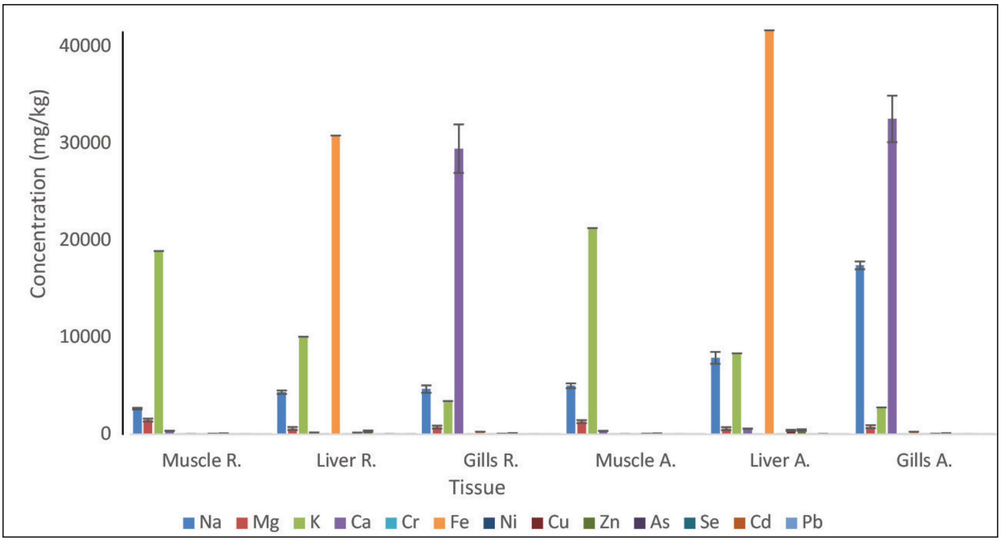

Figure 2: Metal concentrations in freeze dried *Pangasius* sp tissues. 'R' denotes wild-type fish while 'A' refers to aquacultured fish

tissues, could accumulate a high level of metals (Dural *et al.*, 2007), whereas muscle had low affinity to absorb and retain metals (Uluturhan & Kucuksezgin, 2007).

The two-way dendogram in Figure 3 shows that the liver of *Pangasius* sp. was the main depository of Cd, Cu, Se, Zn, Fe and Pb. Meanwhile, due to metabolic functions, elements of Na, Ni, Ca and Cr were accumulated in the gills, while Mg and K were found to be higher in muscle tissues. The differences in tissue metal concentration could be explained by the dissolved metal ion exchange capacity of the lipophilic membrane and the reaction of combined metals with the metallothionein protein in different tissues (Malik *et al.*, 2014).

Likewise, the liver samples of fish from the aquaculture farm were associated with higher contents of Na, Mg and K (Figure 3). The variations of these elements in the liver of cultured fish samples were probably due to aquaculture practices and management, such as the use of metal-enriched fish feed and metal-based antifoulants (Low *et al.*, 2015 ; 2016). This trend was consistent with the results in Figure 2. Higher accumulation rates in the liver of fish were associated with chemical interactions between the elements and oxygen carboxylates, nitrogen, amino groups and sulfur of the mercapto group in metallothionien proteins (Al-Yousuf *et al.*, 2000; Silva *et al.*, 2016).

Another noteworthy observation that was consistent between Figure 2 and 3 was on the fish muscle samples, which were primarily loaded with Mg and K. Mg and K were macro-elements needed for survival as they played an essential role as enzyme co-factors and structural components of cell membranes and extracellular fluids (National Research Council, 1977). Referring to Figure 3, the gill samples were found to have higher Na, Ni, Ca and Cr mineral content. The metal concentration in gills were directly dependent on ambient conditions. Thus, the habitat of fishes could be indicated based on metal accumulation in the gills (Low *et al.*, 2016).

In addition, the liver of wild-type fish had higher metal concentrations than those from the aquaculture farm. A similar pattern was also noticed in the gills. Again, these results highlighted the fact that wild-type *Pangasius* sp. caught from Sungai Selangor had a higher tendency to accumulate metals than those bred in the aquaculture farm. Metal exposure was a main reason for these variations. Based on previous research on Sungai Selangor, the metal input could be associated with industrial and agricultural effluents (Leong *et al.*, 2007). Therefore, the metal concentrations in water sources played an important role in influencing its accumulation in fish tissues (Vicente-Martorell *et al.*, 2009; Rajeshkumar & Li, 2018).

Therefore, these trends suggested that food quality, feeding habits and metal concentrations in the water played an important role in affecting metal accumulation in fish tissues (Vicenta–Martorell *et al.*, 2009). Several studies had reported differences in metal accumulation between tissues of cultured and wild-type fish, and the diversity in background conditions and fish diet were identified as the main reason for the differences (Dincer *et al.*, 2010; Martinez *et al.*, 2010). On the other hand, cultured fish were found to have higher contents of Na, Cu, Zn and Fe (Figure 3) where basically, these elements were related to the fish diet and metal levels in the water column (Yildiz, 2008). Thus, the significant variations of the mentioned elements could be due to metal concentrations in the water and fish feed (Idris *et al.*, 2017).

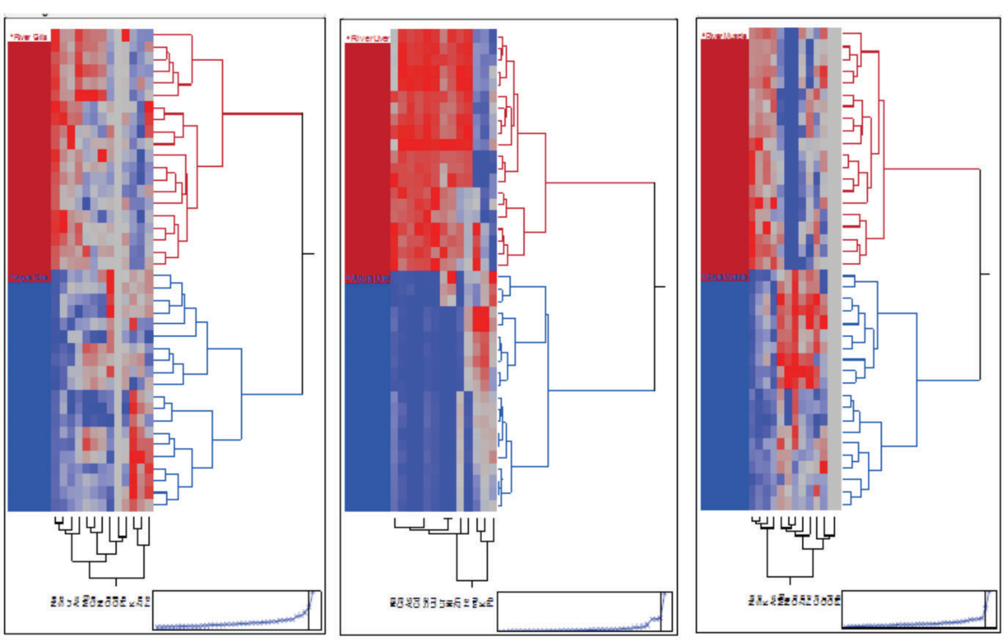

Figure 3: Dendogram of *Pangasius* sp. (a) gills (b) liver (c) muscle sample from Sungai Selangor and aquaculture farm

Conclusion

This study showed that metal distribution in *Pangasius* sp. was strongly associated with the type of tissue and fish habitat. The variability in site background and diet was regarded as the main reason for the observed differences. The distribution of metal concentrations followed the order of liver > gills > muscle. The evidence provided by this study demonstrated that the accumulation of metals was associated with metal input in the water, as the tissues of wild-type *Pangasius* sp. had greater metal concentrations (Na, Cu, Zn, Fe) than those from the aquaculture farm. Therefore, it was noteworthy to conclude that the fish habitat did strongly influence the metal concentration in fish tissues.

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