



## EVALUATION OF HEAVY METAL CONCENTRATIONS IN WILD AND CULTIVATED *Hemibagrus* sp. USING PRINCIPAL COMPONENT ANALYSIS

(Penilaian Kepekatan Logam Berat dalam *Hemibagrus* sp. dari Habitat Semulajadi dan Kolam Ternakan Menggunakan Kaedah Analisis Komponen Utama)

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### Abstract

In the present study, the concentration of Cr, Cd, Cu, Zn, Pb, and As in various tissues of *Hemibagrus* sp. from two different habitat were determined with microwave assisted digestion-inductively coupled plasma-mass spectrometry. The objective of this research was to determine the differences between wild and cultivated in terms of heavy metal accumulation in muscle, liver and gills tissues. The metals accumulation pattern relating types of tissue and elements as well as fish habitats were revealed by principal component analysis. The results revealed that variation in metal accumulation pattern is strongly dependent on the type of tissues. The results suggested that Cu, Zn, and Pb are highly associated with liver tissue while Cr and As with muscle and gills. The differences in heavy metal accumulation observed between wild and cultivated *Hemibagrus* sp. were probably due to the differences in their environmental conditions.

**Keywords:** principal component analysis, pattern recognition, heavy metal, fish, farmed

### Abstrak

Kepekatan Cr, Cd, Cu, Zn, Pb, dan As dalam pelbagai tisu *Hemibagrus* sp. dari dua habitat yang berlainan adalah ditentukan dengan pencernaan bantuan gelombang mikro-plasma gadingan teraruh-jirim spektrometri. Objektif kajian ini adalah untuk menentukan perbezaan antara ikan dari habitat semulajadi dan kolam ternakan dari segi pengumpulan logam berat dalam tisu, hati, dan insang. Corak pengumpulan logam berkaitan jenis tisu dan elemen kajian serta habitat ikan ditunjukkan oleh analisis komponen utama. Keputusan menunjukkan bahawa perubahan dalam corak pengumpulan logam adalah bergantung kepada jenis tisu. Keputusan kajian mencadangkan bahawa elemen Cu, Zn, dan Pb adalah berkaitan dengan organ hati manakala elemen Cr dan As adalah berkaitan dengan tisu dan insang. Perbezaan dalam pengumpulan logam berat di antara *Hemibagrus* sp. dari habitat liar dan kolam ternakan adalah mungkin disebabkan oleh perbezaan dalam keadaan persekitaran mereka.

**Kata kunci:** Analisis komponen utama, corak taburan, logam berat, ikan, kolam ternakan

### Introduction

The rapid development of agriculture and industrialization has resulted in increasing of metal pollution in the aquatic ecosystems, which are significantly hazardous for fish and human [1]. Significant quantities of contaminant elements are discharged into river, which can be strongly accumulated and biomagnified along water, sediment and aquatic food chain, thus resulting in sublethal effects or death in fish populations [2-4]. Fish are commonly situated at top of the aquatic food chain and normal metabolism of fish may accumulate large amounts of certain metals from food, water and sediment [5]. Commonly, fish is known to be nutritious with essential proteins, polyunsaturated fatty acids and liposoluble vitamins [6]. Nevertheless, levels of metals in fish are of particular interest due to their high potential to enter and accumulate in food chains [7, 8]. Even low concentrations of these elements have damaging effects to humans and animals because there is no good mechanism for their elimination from the body [9]. On the other hand, fish can be regarded as a good bioindicator because it is easily sampled and found in abundant population, with the potential to accumulate metals and has a long life span with an optimum size for analysis [10]. In this study, heavy metal (Cr, Cd, Cu, Zn, Pb) of *Hemibagrus* sp. in certain tissues (gill, liver, muscle) from wild and cultivated habitat are compared. Accumulation pattern relating tissue and elements and the underlying structure related to their origins and tissues were evaluated with principal component analysis (PCA). The aim of PCA is finding the similarities and differences between *Hemibagrus* sp. of different habitats based on ICP-MS measurement so as to create classification and identification of key discriminants that distinguish between samples.

### Materials and Methods

The slaughtered *Hemibagrus* sp. was donated in June 2012 by a local fisherman and local fish farmer. The samples originated from the Selangor River and the cultivated pond located at Taman Agroteknologi Pertanian Bistari Jaya, Kuala Selangor, Selangor (Figure 1). The samples were stored in 20°C freezer and the length and weight were recorded before dissection. The tissues of muscle, gills, and liver samples were freezing dried (CHRIST, Germany) and the homogenized with a mortar and pestle. Dried samples were kept in amber jars in a desiccator before microwave digestion.

### Laboratory procedure

Ultra-Pure Water (UPW) with resistivity more than 18 MΩ was obtained from the PURELAB® UHQII system (ELGA®, UK). 65 % nitric acid and 30 % hydrochloric acid solution were of Suprapur® quality (Merck, Germany). The dogfish liver (DOLT-4) (NRCC, Canada) certified reference material (CRM) for trace metals were used to evaluate the analytical performance of the methods. The tissue samples were analysed according to the method described by Low et al. [11] in which microwave-assisted digestion (MAD) were carried out in a CEM Mar Xpress Microwave Accelerated Reaction System (CEM, Corporation, Matthews, NC, USA). Dried samples (0.25g) was weighted directly in each 55mL self-regulating pressure control PFA® vessel and digested with a reagent consists of 2.50 mL of 65 % HNO<sub>3</sub>, 0.50 mL of 30 % HCl and 7 mL UPW. Under microwave power of 800W, the microwave temperature was ramped to 185°C in 10.5 min and held for 14.5 min. After digestion, the sample was cooled before being transferred to a 50 mL polypropylene volumetric flask and the volume was made up to the mark with UPW. Diluted sample solutions were stored in polyethylene vials below 8°C and analysed by ICP-MS [12].

### Statistical analysis

Principal component analysis (PCA) was conducted to transform the original data matrix (composed of 120 samples and 5 variables) into a product of two matrices, one which contains information about metal concentrations (loading matrix) and the other about the samples (score matrix). All statistical calculations were performed using the software package JMP version 9.0.

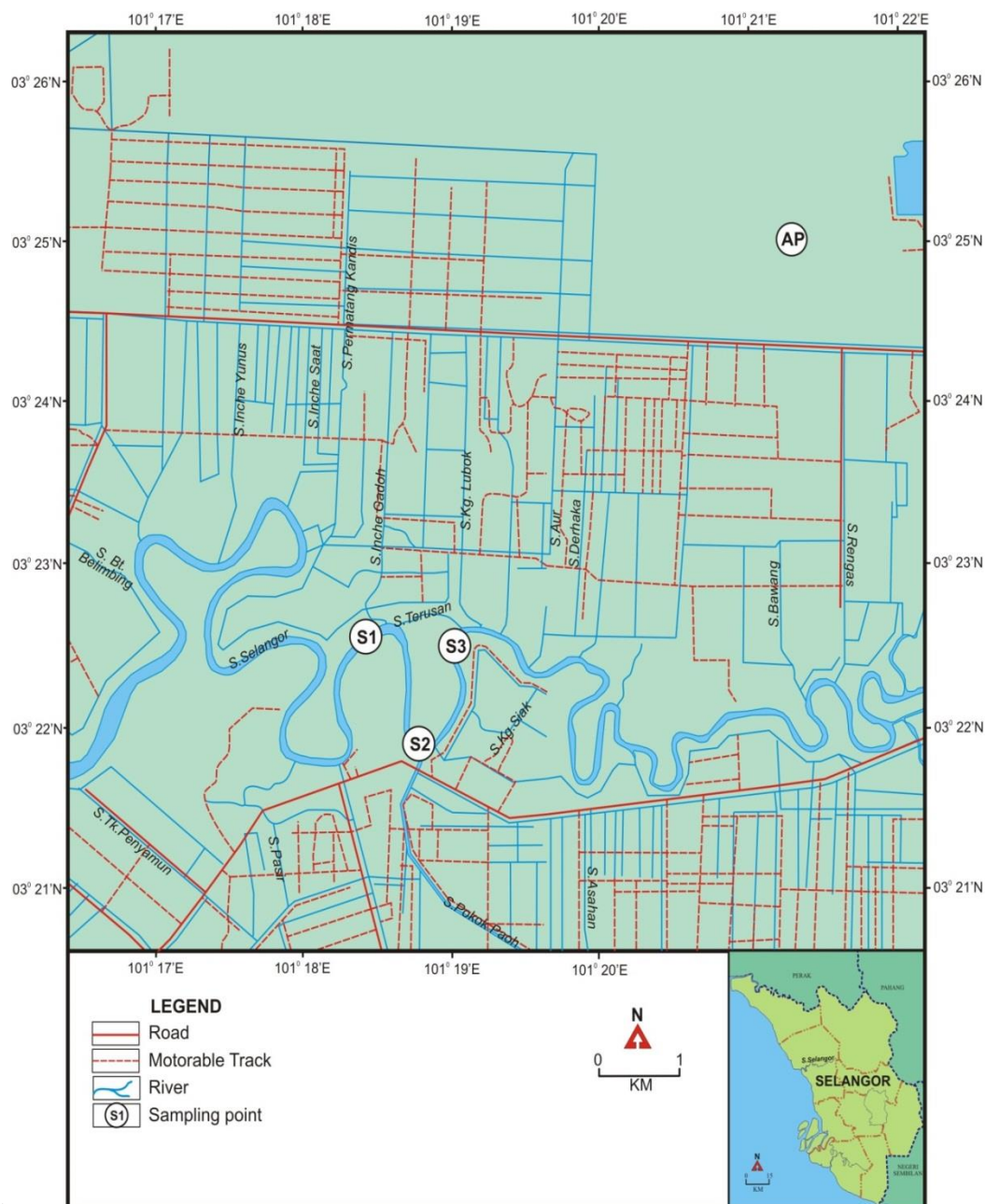


Figure 1. Location of sampling sites, letter S denotes to samples from wild, letter AP denotes cultivated fish.

### Results and Discussion

Certified reference materials of dogfish liver (DOLT-4) were used to check the accuracy of MAD-ICP-MS method. The elemental concentrations determined in the standard reference materials were in a good agreement with the certified values (Table 1).

Table 1. Analysis of certified reference materials

		Cr	Cu	Zn	Pb	Cd	As
DOLT -4	Certified values	-	31 ± 1	116 ± 6	0.16 ± 0.04	24 ± 0.8	9.7 ± 0.6
(mg kg <sup>-1</sup> )	Amount detected	-	32 ± 2	118 ± 7	0.16 ± 0.04	28 ± 1.6	9.6 ± 0.3
	Recovery / %	-	102 ± 6	102 ± 5	100 ± 6	116 ± 4	99 ± 3

95 % confidence intervals , n = 7, Note : - not certified

Morphometric data of *Hemibagrus* sp. from wild and cultivated samples are summarized in Table 2. Condition factor (CF) was calculated by the ratio of weight/length which is commonly used as an index of fish health, while hepatosomatic index (HSI) is defined as the ratio of liver weight to body weight that reflects the energy reserve in fishes [13]. Samples obtained from the cultivated pond were generally smaller than those caught from Selangor River. Although there are differences in size variation, there were no substantial differences noted in the effect of metal concentration in studied tissues [14].

Table 2. Biometric parameters for *Hemibagrus* sp. from different habitat

Biometric Parameter	Habitat	
	Aquaculture	Wild
Location	N 03°25.046 E 101° 21.203	N 03°21.883 E 101° 18.728
Condition factor (CF)	0.8 ± 0.2	0.7 ± 0.0
Hepatosomatic index (HSI)	0.7 ± 0.2	0.7 ± 0.1

95% confidence intervals, n = 20

Mean concentrations of heavy metals in the muscle, liver, and gills of wild and cultivated *Hemibagrus* sp. are presented in Table 3. Chromium, cadmium, copper, zinc, and lead were selected as the analyte elements. There are significant differences ( $p < 0.001$ ) concentrations obtained in different organs. The results have demonstrated that liver tissues generally contained higher concentrations of Cd, Cu, Zn, and Pb for both habitats except for higher Cr concentrations in the gill tissues. This result is also in a good agreement with Fernandes et al [15], where metal concentrations of Cu, Zn, and Pb were higher in liver than other studied tissues. Essential metals such Cu and Zn are needed in fish metabolism, while others such as Pb and Cr have no functional in biological systems. Our study showed that tissue of wild *Hemibagrus* sp. contained significantly higher content ( $p < 0.001$ ) of Pb and As, while tissue of farmed *Hemibagrus* sp. had significantly higher contents ( $p < 0.05$ ) of Cu. However, all metals may cause harmful effects on fish depending on their concentrations [16]. Overall metal concentrations are generally found to be higher in liver compared to other studied tissues regardless of the sample origins.

The accumulation pattern of metals varies greatly depends on the species as well type of tissues. Previous report by Begum et al. [17], showed that the liver appeared to be the main storage of heavy metal, while the muscle tissue with the lowest level. Yet, Mormede and Davies [18], suggested that the liver is the target organ, which functions to detoxify and accumulate [19], while muscles are at most concern to humans because it is the main tissues of human diet [20]. Based on permissible level limits for the muscle meat of fish, all metal studied were below the permissible level recommended by Malaysian Food Regulation and USEPA risk based concentrations. For a better understanding, PCA was conducted to evaluate the distribution of metals in the studied tissues. PCA is usually discussed in terms of loadings and scores since they complement each other. The scree test proposed by Cattell [21] suggest that with only the first two PCs accounted for 74% of the total variability of associated metal concentrations in tissues studies and 3 distinct clusters can be revealed (Figure 2). The model demonstrated that Cluster I is made up of liver tissues, Cluster II composed of scores of gill tissues, while the scores of muscle tissues are grouped in

Cluster III. The variation of these clusters, are totally associated with the type of tissues regardless their habitats. The variation of metal accumulation trends depends on the studied tissues. Combination of the results from Figure 2 and Table 3 suggest that the total metals accumulation is liver > gill > muscle.

Table 3. The concentration of heavy metals in freeze dried *Hemibagrus* sp.

	Tissue (mg kg <sup>-1</sup> )	Cr	Cu	Zn	Pb	Cd	As
Aquaculture	Muscle	0.3±0.0	1.2±0.2	33.0±3.8	0.2±0.1	< LoD	0.3±0.0
	Liver	0.3±0.1	(1.4±0.2) x 10 <sup>2</sup>	(1.8±0.2) x 10 <sup>2</sup>	1.2±0.3	< LoD	0.1±0.0
	Gill	0.4±0.1	2.9±0.4	56.1±4.1	0.2±0.0	< LoD	0.6±0.1
Natural	Muscle	0.3±0.0	0.8±0.1	22.8±1.0	< LoD	< LoD	2.3±0.5
	Liver	0.3±0.0	79.3±6.8	(1.5±0.1) x 10 <sup>2</sup>	5.0±0.6	< LoD	0.4±0.1
	Gill	0.4±0.0	1.6±0.1	49.8±3.0	0.9±0.1	< LoD	0.9±0.1

All reported values are referred to dry base, 95% confidence intervals,  $n = 20$ , LoD – Limit of Detection (Pb < 0.03 mg kg<sup>-1</sup>)

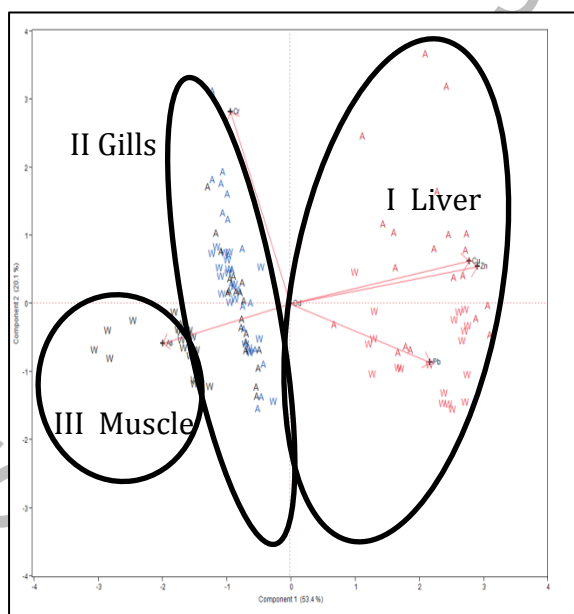


Figure 2. PCA bi-plot for all samples ( $n = 120$ ). The letter A denotes aquacultured fish, letter W denotes to wild fish.

Based on Figure 2, it is noticed that the scores of liver sample (Cluster I) in PC1 axis was spread in the region with positive loadings of Pb, Cu and Zn. High variation of these metals makes liver samples stand out more from others on the right-hand side of the scores plot. These observations are consistent with the findings reported by [22], that higher metal accumulation in liver tissues are due to the greater binding tendency of the metals with oxygen carboxylate, amino groups, nitrogen, and/or sulphur of the mercapto group in the metallothionein protein.

PCA bi-plot for liver samples demonstrates two clusters (Figure 3) were obtained. The liver samples from cultivated *Hemibagrus* sp. have positive scores on PC1 than wild *Hemibagrus* sp. which explained 52 % of the total variance

and have positive loadings of Cr, Cu, and Zn. The gills (Cluster II) and muscle samples (Cluster III) are discriminated from liver samples with negative score on PC1. Nevertheless, Cluster II and Cluster III overlap with high score of Cr and As. In fact, Cluster III mainly consists of two sub-clusters which can be distinguished by the magnitude of score on PC1 (53% of total variance). Similar trend was observed in gills samples, where the bi-plot also indicates 2 distinct cluster (Figure 4) (which explained 73% of the total variances. These results also demonstrated that cultivated *Hemibagrus* sp. from Selangor River accumulated high metal concentrations than wild *Hemibagrus* sp.

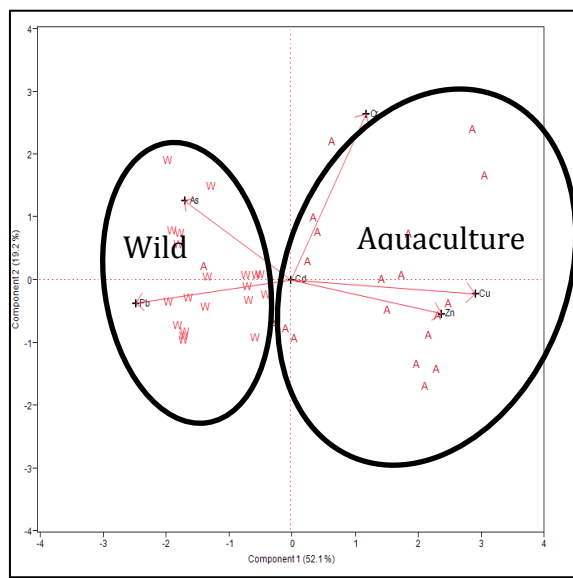


Figure 3. PCA bi-plot for liver samples ( $n = 40$ ). The letter A denotes aquacultured fish, letter W denotes to wild fish.

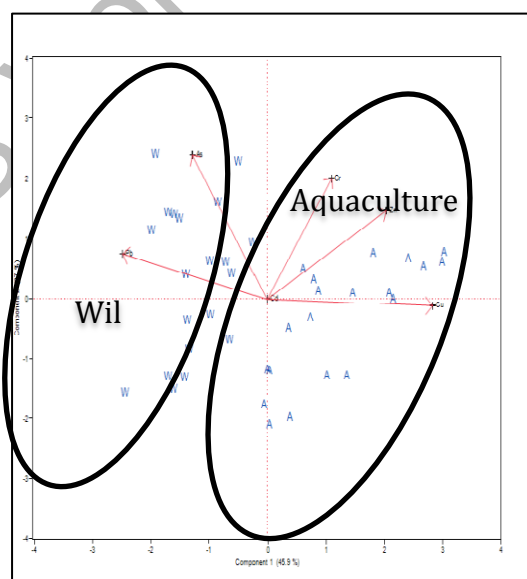


Figure 4. PCA bi-plot for gills samples ( $n = 40$ ). The letter A denotes aquacultured fish, letter W denotes to wild fish.

Refer to PCA bi-plot for muscle sample (Figure 5), it was demonstrated that two discrete cluster are separated by PC1 (75% of total variance), which high positive loadings of Cu, Zn, and Pb for cultivated fish while negative loading of Cr and As for wild fish. According to the Malaysian Food Act 1983 and Food Regulations 1985 [23], accumulation of heavy metal in muscle samples are below the permissible level.

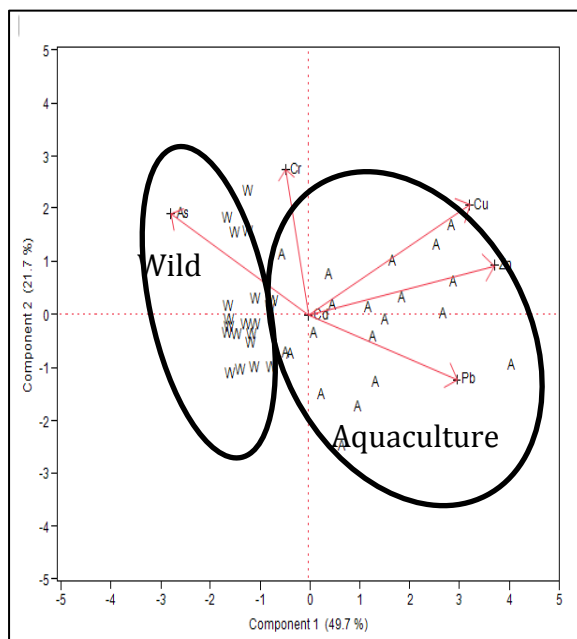


Figure 5. PCA bi-plot for muscle samples ( $n = 40$ ). The letter A denotes aquacultured fish, letter W denotes to wild fish.

### Conclusion

The results of the study show that PCA can identify patterns in our data, where PCA provides a way to visualize the clustering pattern among different organs of *Hemibagrus* sp from two different habitats, and easily identifies which metals contribute most to these variation. It also demonstrates the similar clustering tendencies related to the origin of the samples. The results revealed Cu, Zn, and Pb highly associated with liver tissues while Cr and As with muscle and gills. This suggests that the differences in accumulation of heavy metals between two habitats were probably due to the differences in their environmental conditions.

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