

## Evaluation Of Metal Concentrations In Two Aquaculture Pond Systems Containing Catfish Using Principl Component Analysis

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### Abstract

*In the present study, the concentration of Cd, Fe, Ni, Pb, Zn and Hg in various tissues of catfish from two different aquaculture pond systems were determined using the wet digestion method - atomic absorption spectrometry. The objective of this study is to determine the differences between the two aquaculture systems in terms of metal accumulation in the catfish's muscle, liver and gills tissues. The patterns of metal accumulation in all types of tissues as well as in the aquaculture pond systems were revealed through principal component analysis. The results suggested that variations in the patterns of metal accumulation are strongly dependent on the type of tissues. The results revealed that the concentration of metals in catfish from the earthen pond was relatively higher than those from the fibre pond. Thus, it is suggested that the differences in metal accumulation between two different aquaculture pond systems are probably due to differences in environmental conditions and fish diet as well*

**Index Terms:** Aquaculture, bioaccumulation, fish, metal,

### Introduction

In Malaysia, increased metal pollution has been discovered in freshwater fish, however, data and publications on the issue are still limited. Data from several researchers demonstrated that the distribution trend of metals in fishes has increased yearly (Tukimat, Rahayu, Zaidi, & Sahibin, 2002; Tukimat, Norazira, Muzneena, & Sahibin, 2006). Previous research have acknowledged that fish are able to accumulate higher concentrations of metals than that present in water columns and sediment (Olaifa & Onwude, 2004). Meanwhile, the freshwater aquaculture industry in Malaysia has grown due to the increasing demand for protein. In addition, the freshwater aquaculture industry in Malaysia can be described as being very diverse, for the example, catfish, carp and tilapia are usually found to be bred in different types of aquaculture systems (Iliyasa, Mohamed, & Terano, 2006). The total quantity of catfish produced in 2012 was approximately 73,816 tonnes, thereby making them the largest contributor to aquaculture product in Malaysia (Iliyasa et al, 2006). Due to the rising popularity and intake of this species, it is important to evaluate its metal concentration in order to obtain a risk assessment of the fish to better inform its consumer.

### Material and methods

#### Sample Collection and Processing

Field study and sampling were conducted in June 2018. The catfish samples were collected from two different aquaculture ponds located in Jeli (earthen pond) and Tanah Merah, Kelantan (fibre pond). The slaughtered catfish donated by local fish farmers were individually wrapped in low-density polyethylene sampling bags, kept in an ice box and transported to the laboratory on the same day. The fishes' total length and weight were recorded before stored under -2°C until dissection was performed. The removed muscle, gills and liver tissues were cleaned several times using deionised water. Consequently, each dried dissected sample was grounded and homogenised using an octagonal agate mortar and pestle and stored under a dessicator in amber jars

### Laboratory Procedure

To prepare analytical samples, 0.5 g of dried samples were weighted accurately. Digestion was performed on a hotplate using a mixture (1:1 v/v) of 65 % nitric acid and 98 % sulphuric acid. The samples' temperature was increased to 60 °C in 30 the space of minutes. After cooling to room temperature, 10 ml of nitric acid was added to the samples which were then reheated to 120 °C. The temperature was increased further to 150 °C until the samples turned black. After that, hydrogen peroxide was added to the samples until the samples were in clear solutions. After digestion, the samples were cooled down and filtered into 50 ml volumetric flask. The volume was made up to the mark with deionised water.

### Statistical Analysis

Principal component analysis (PCA) was conducted to transform the original data matrix (composed of 30 samples and 6 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 JMP software package version 9.0.

### Results and Discussion

Biometric data of catfish samples taken from both the earthen and fibre ponds are summarised in Table 1. Although the mean weight of fish originating from the earthen pond was found to be slightly greater than those taken from fibre pond, there were no substantial differences noted in the effect of metal concentration in the studied tissues (Ann0, Young, Bloom, & Mercier, 2006). Moreover, size variations rarely show correlation with metal concentrations in fishes, since their internal metal concentrations are regulated biologically (Yi & Zhang, 2012).

Table 1 Biometric parameters for catfish from different pond

Biometric parameter	Habitat	
	Earthen Pond	Fiber Pond
Weight (g)	89.40 ± 0.2	60.70 ± 0.1
Length (cm)	25.6 ± 0.0	20.5 ± 0.1

Values are mean ± SD ,  $n = 30$

All reported values are referred to dry base, mean ± s.d,  $n = 30$ , n.d – not detected

The mean concentrations of metals in the muscle, liver and gills of catfish from the earthen and fibre ponds are summarised in Table 2. Cadmium, iron, nickel, lead, zinc and mercury were selected as the analyte elements. Subjected to regulation, excretion, and storage mechanism, different tissues showed significant differences ( $p < 0.001$ ) in metal accumulation (Low, Zain, & Abas, 2011). Generally, metal concentrations were found to be higher in active metabolite organs such as gills and liver compared to muscle tissue (Dural & Goksu, 2007; Ahmed et al, 2016). The results demonstrate that generally, active tissues (liver and gills) contain higher concentrations of Cd, Fe, Ni, Pb for both habitats.

Table 2 The concentration of metals in catfish from earthen and fibre pond

	Tissue (mg/L)	Cd	Fe	Ni	Pb	Zn	Hg
Earthen	Muscle	0.04 ± 0.0	0.25 ± 0.2	0.12 ± 0.1	0.08 ± 0.0	0.12 ± 0.1	(8.7 ± 0.0) x 10 <sup>-6</sup>
	Liver	0.10 ± 0.1	0.60 ± 0.2	0.38 ± 0.2	0.10 ± 0.0	0.10 ± 0.0	(4.7 ± 0.0) x 10 <sup>-6</sup>
	Gill	0.55 ± 0.1	0.41 ± 0.1	0.59 ± 0.2	0.37 ± 0.1	0.11 ± 0.0	(8.3 ± 0.1) x 10 <sup>-6</sup>
Fibre	Muscle	n.d	n.d	n.d	0.26 ± 0.1	0.16 ± 0.1	(4.8 ± 0.0) x 10 <sup>-6</sup>
	Liver	n.d	0.18 ± 0.1	n.d	0.30 ± 0.0	0.20 ± 0.0	(1.0 ± 0.1) x 10 <sup>-6</sup>
	Gills	n.d	0.45 ± 0.1	n.d	0.28 ± 0.0	0.33 ± 0.1	(3.1 ± 0.1) x 10 <sup>-6</sup>

Essential metals such as Fe and Zn are needed for fish metabolism, while non-essential elements (Pb, Cd) have no function in the biological system (Romeo, Siau, Sidourmou, Gnassia, & Barelli, 1999). Information on the metal elements existing in cultivated catfish is essential for environmental management programmes and human consumption.

The pattern of metal accumulation in fishes varies greatly depending on the species as well their type of tissues. A previous report by Idris (2016), showed that liver tissues were the main storage area for metal, while the muscle tissues recorded the lowest level of metal accumulation. Mormede and Davies, (2001), suggested that the reason why liver tissues appear to be the target organ is its functions of detoxifying and accumulating food, while muscles are at most concern to humans due to human consumption.

For better understanding, a PCA was conducted to assess the distribution of metals in the studied tissues and fishes origins. PCA is usually discussed in terms of loadings and scores since they complements each other. The Scree test proposed by Catell, (1966), suggests that only the first two PCs account for 76 % of the total variability of associated metal concentrations in the fishes' origins (Fig. 1).

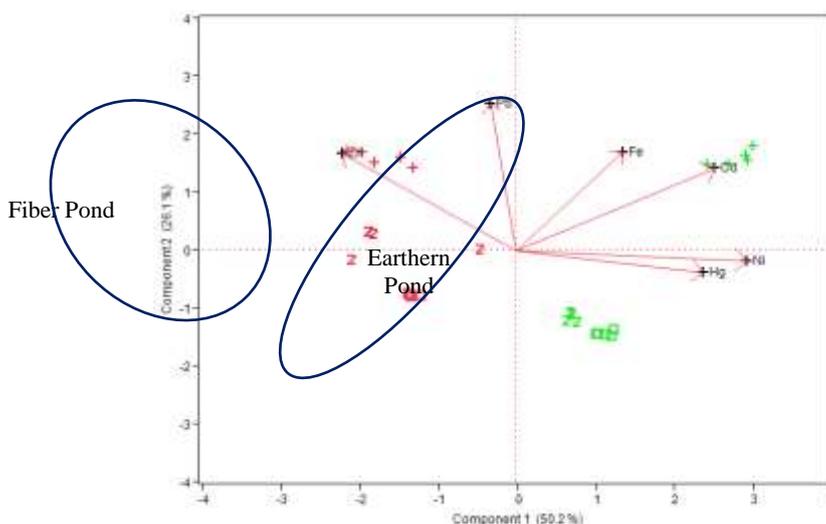


Figure 1: PCA bi-plot for all samples ( $n = 30$ ). The symbol + denotes gills samples, symbols □ denotes muscle gills and symbol z denotes to liver tissues.

The model demonstrated that the variation in metal accumulation depend on the fishes' origins. As seen in Figure 1, scores of fish samples from earthen pond in PC1 axis were spread in the region characterized by positive loadings of Fe, Cd, Hg and Ni. A similar trend was observed for the gills, muscle and liver samples, where the bi-plots (Fig. 2, 3, and 4) also indicate that fish samples from the earthen pond showed positive score on PC1.

The results also demonstrated that fishes from the earthen pond accumulated higher metal concentrations than those from the fibre pond. These observations are consistent with the findings reported by Idris, (2016), who posited that, most metal elements accumulate higher in fishes from cultivated ponds. Several researchers have also reported varying metal concentrations in tissues between different fish habitats. Diet is claimed to be the main reason for these observed distinctions (Dincer, Cakli, & Cadun, 2010; Martinez et al, 2010). Metal concentrations found in fish normally reflect the human inputs/activities conducted at the sites (Idris, 2017).

Gills act as the first organ to be in contact with the water and sediment particles. Therefore, gills can be relevant sites of interaction with metal ions as it is a sensitive respiratory organ with an ion regulatory membrane (Pereira, Pablo, Valie, & Pacheco, 2010). As shown in Fig. 2, the gill samples taken from fish from the earthen pond have more positive scores on PC1 than the fish from fibre pond, thus explaining 84 % of the total variance. The gill samples were also shown to have positive loadings of Hg, Pb, Ni, and Cd.

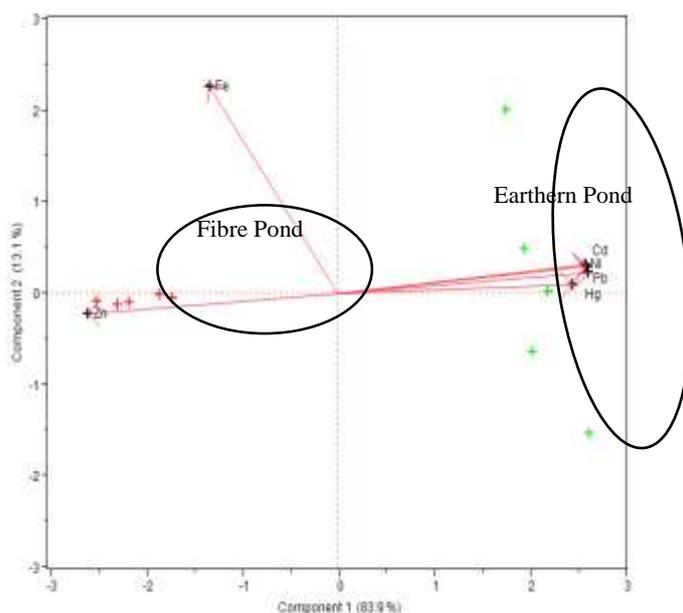


Figure 2: PCA bi-plot for gills samples.

The PCA bi-plot conducted for liver samples obtained two clusters (Fig. 3). The liver samples from the fibre pond have more negative scores on PC1 than fishes from the earthen pond, which explains 83 % of total variance in the loadings of Pb and Zn. These results also demonstrate that the fish samples from the earthen pond accumulated higher metal concentrations than fish samples from the fibre pond.

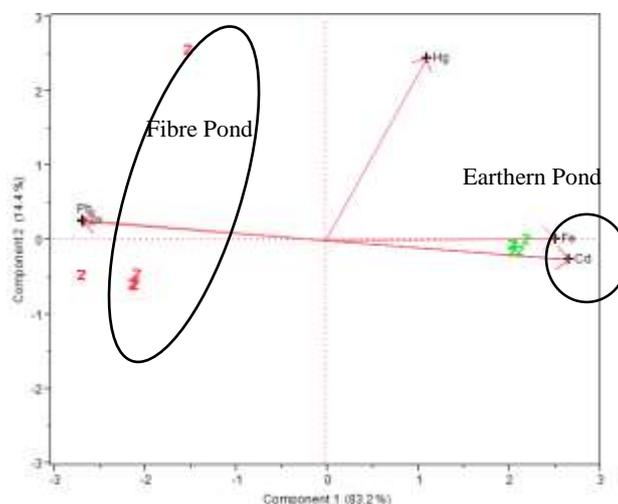


Figure 3: PCA bi-plot for liver samples.

A previous study by Begum (2013), reported that the liver appears to be the main storage of metal elements, while the lowest level of metals is normally associated with the muscle tissue. Based on the permissible level limits recommended by the Malaysian Food Regulation and the USEPA (2000) risk based concentrations, all metals included in this study were below the permissible levels. The bi-plot in Figure IV indicates that the muscle samples taken from fish from the earthen pond were starkly different from other tissues, with high and positive scores on PC1 due to loadings of Hg, Cd and Ni. Although previous studies have agreed that metals released from the surficial sediment may influence the accumulation of metals in catfishes from earthen ponds, it is likely that the uptake of metal from enriched feed contributes more significantly to the bioaccumulation of metals in muscle tissues (Low, Zain, Abas, Salleh, & Teo, 2015).

For instance, the catfish samples from the earthen pond had higher concentration levels of the studied metals (except Pb and Zn) compared to samples collected from fibre pond. The variation in bioaccumulation profiles could be attributed to the bioavailability and chemistry characteristics of metals in each pond (Low et al, 2015). Thus, it is emphasised that there might be a relatively higher risk associated with the consumption of catfish from earthen ponds compared to catfish from fibre ponds.

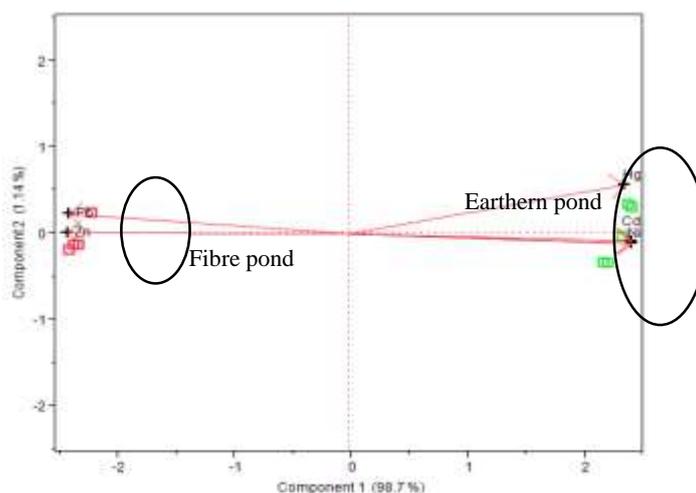


Figure 4: PCA bi-plot for muscle samples.

### Conclusion

The concentration levels of Cd, Fe, Ni, Pb, Zn, and Hg in the edible muscles of catfish were recorded to be below the prescribed limits and are thus safe for consumption. The results of the study showed that the PCA can indicate patterns in data, thus providing information on the clustering of patterns among different organs of catfishes from two different cultivated ponds. The PCA results suggested that the concentration of metals in each studied tissues is completely associated with the type of tissue regardless of its origins. The results revealed that the metal concentrations in catfish from the earthen pond were generally higher than of the fibre pond. This suggests that the differences in metal concentrations between samples from the two cultivated ponds are due to differences in environmental conditions as well as habitat.

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