



Accumulation of Heavy Metals in Farmed *Lates calcarifer* of a Tropical Coastal Lagoon

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ABSTRACT

Heavy metals content in seafood, especially fish species has been of increasing concern to the human health. Nowadays, with increasing dependency towards farmed fish for sources of dietary protein and essential minerals, this heavy metals contamination in fishes are still questionable. This study aimed to investigate the accumulation of heavy metals in farmed fish, *Lates calcarifer* different organs from Setiu tropical coastal lagoon, Terengganu, Malaysia throughout its out-growing phase in the farm from the fingerling release up to their marketable size. Selected heavy metals namely Cu (Max:291.5±99.31), Zn (Max: 84.89±12.76), As (Max: 26.01±5.170), Cd (Max: 1.634±0.014), Hg (Max: 0.165±0.029) and Pb (Max: 0.634±0.550) in the fish was analyzed using ICP-MS after Teflon bomb closed digestion. The metal accumulation in each organ were generally found in the order of liver > gill > muscle. The concentrations of Cd and Zn in the liver were found to increase with fish size in each organ based on the association found in the principal component analysis (PCA). Meanwhile the overall findings observed negative correlation with *L. calcarifer* growth in fish size for all other heavy metals and organs. The amount of As in the fish muscle throughout its growth can be potentially harmful to humans with the highest averaged concentration at 3.29±0.65 mg/kg dw above the standard set by the Malaysian Food Regulation (1985) of more than 1 mg/kg. Meanwhile, all the other heavy metals were relatively safe and the concentrations well below the standard set by both national and international guidelines.

Keywords: Heavy metals, Farmed *Lates calcarifer*, Tropical coastal lagoon, Human health risk.

INTRODUCTION

Heavy metal pollution have been well-

discussed in the past decade to be a persistent issue that has come with global industrialization (Tarley *et al.*, 2001). Heavy metals though naturally available



has since been found at much elevated levels with many anthropogenic input in the environment whereby the aquatic environment has largely taken the impact of such pollution. As inorganic chemical substances that are non-biodegradable, heavy metals accumulate in the environment that eventually ends up in the body of organisms which would continue to magnify as it moves up each trophic level such as fishes and ultimately to us humans. Some metals are essential to living organisms but they can be toxic at elevated levels in the body and some in which have no known biological function and are toxic even at low concentration. Most water bodies of rivers, lakes, lagoons which eventually lead out to ocean become a natural sink for these metal pollutants to collect and accumulate. Setiu Lagoon is surrounded by mangrove-dominated swamps which has become predominant sites for aquaculture where it becomes potential collecting sites for industrial disposal and urban wastes causing the aquatic environment there to act as a pollution sink (Moiseenko and Kudryavtseva, 2001; Poh *et al.*, 2019).

In a study by Ahmad *et al.*, (2016), 78% of Malaysians consumes fish at least twice per week and with fish consumption of 54 kg/person/year (Teh, 2012). Fishes have been known to bioaccumulate metals therefore fish consumption might be the one of the main source of metal intake into the human body and as many of the fisheries resources have shown increased reliance towards aquaculture which have been slowly overtaking capture fisheries, there is little research which focuses on the metal pollution and bioaccumulation in fishes from that of cultured origin. However, there has been one study conducted in Malaysia by Taweel *et al.*, (2011) on the tilapia fish in selected sites of Bangi, Selangor where the study compares metal content in the fish from those of cultured pond, rivers and lakes. A more recent study by Sobihah *et al.*, (2018) have also observed the importance of heavy metals accumulation in estuarine maricultured fish from Pulau Ketam, Malaysia. Other notable studies focusing on marine fish aquaculture can be found from China (Liang *et al.*, 2017; Wong *et al.*, 2001) and Malaysia (Mok *et al.*, 2012). Though fishes may accumulate metals through its dietary

intake, as well as the bioavailability of the metals in its water environment and surrounding sediments (Bervoets and Blust, 2003) the size of organism have been found to affect their penchant towards metal accumulation (Kalay and Canli, 2000; Canli and Atli, 2003; Farkas *et al.*, 2003), and this leads to the need to understand the relationship between organism size and metal accumulation.

There is yet to have any definite relationships established between metal concentration and fish size though it has been found that the accumulation in fish reaches a steady state at a certain age (Bervoets and Blust, 2001). Therefore this study aims to investigate the effect of fish growth on the metal accumulation in fish in a farmed environment. This would help in identifying at which size the metal levels in the fish could be of concern to the human health.

MATERIALS AND METHODS

Sample Collection and Preparation

The fishes were taken from 3 cage farms where the fingerlings from the same batch of seedlings were grown out to their maturity. The first fish samples taken were for fishes in their first month after the seedling release (> 100mm) into the farm where the fishes have already acclimatized to the surrounding lagoon. The farming of the *L. calcarifer* follows a rotation system where they are sorted into different fish sizes as they grow at different rates.

The fish samples were always taken in the morning after feeding with disregard to the lagoon's tidal influence. A total of 20 samples *L. calcarifer* were collected every month for a period of 7 months throughout their out-growing phase in the farm from their fingerling release up to their marketable size of about 400g. Increasing fish bodyweight of the fish taken every consecutive month is taken as the proxy of age of the fish farmed in the area. Fig. 1 depicted the flow of the sample collection throughout each subsequent growth in fish size. Samples collected were then be transported back to the laboratory immediately in pre-cleaned polyethylene bags.

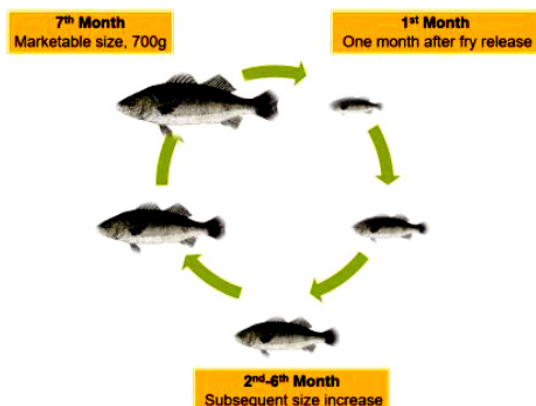


Fig. 1. Sample collection method of increasing fish size with each growth in fish size

The samples were measured in standard length, weighed (to the nearest 0.1 g) and then dissected for different organs of the gills, liver and muscle. All the samples were then dried in the oven at 60°C until a constant weight is achieved (dry weight) and then later homogenized by grounding into powder. Milli-Q water or de-ionized water was used to prepare all aqueous solutions. All the glassware and plastics were cleaned (acid-washed) in 10% nitric acid solution before being used.

Sample Analysis

0.05 g of the homogenized fish tissue sample was weighed into the acid-washed Teflon digestion vessels. 1.5 mL of suprapur nitric acid (HNO₃) acid, Merck KGaA, Germany were then added into each vessel via closed Teflon bomb digestion, custom made, Japan (Ong *et al.*, 2014; Ong *et al.*, 2015; Ong *et al.*, 2017). The prepared samples would then be placed in the oven for 8 h at 100°C to allow complete digestion of the fish tissues. The digested samples were then transferred into centrifuge tubes and diluted with Milli-Q water up to 10 mL. The samples would then be ready for the determination of metal concentrations measured using the Inductively Coupled Plasma Mass Spectrometry, ICP-MS, Perkin Elmer ELAN 9000, USA (Kamaruzzaman *et al.*, 2009; Ong *et al.*, 2013). for selected metal elements (Cu, Zn, As, Cd, Hg and Pb). The principal component analysis (PCA) is the statistical analysis applied to analyze the relationship

between the heavy metal accumulations in each organs of the fish with increasing fish size.

RESULT AND DISCUSSION

Average Fish Size

The measurements of fish growth are categorized by size in which the increase in fish size over time act as a proxy of age of the fish farmed in the area. The categorized fish growth can be seen in Table 1 where the first fish size is from the first month after fingerling release up to the average marketable size. After fish size 5 is the size at which the fishes would be typically harvested and sold in the market and thus, the size commonly accessible for consumers.

Table 1: Growth in fish size from fingerling up to their marketable size

Growth Phase(n=20)	Weight (g)
1	27.50±2.600
2	68.49±14.00
3	144.3±31.20
4	237.9±34.90
5	473.2±85.90
6	513.4±72.80
7	591.2±122.6

The average fish weight was recorded with an exponential growth where the fish samples peaked at an average of about 600g in the last stage as depicted in Fig. 2. This is the fish size that would be typically harvested and sold in the market and thus, the size commonly accessible for consumers. The average size of the fish taken from the fish farm is summarized in Table 2.

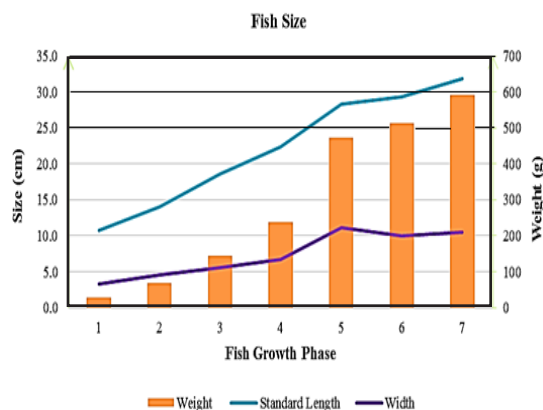


Fig. 2. Size of fish growth in their weight, standard length and width

Table 2: Summary of average fish size

Growth Phase (n=20)	Weight (g)	Standard Length (cm)	Width (cm)
1	27.50±2.600	10.79±0.300	3.300±0.100
2	68.49±14.00	14.04±1.800	4.495±0.600
3	144.3±31.20	18.60±1.600	5.530±0.600
4	237.0±34.90	22.38±1.000	6.730±0.500
5	473.2±85.90	29.35±2.300	10.00±0.800
6	513.4±72.80	28.30±1.400	11.08±0.700
7	591.2±122.6	31.80±1.800	10.50±1.100

Metal Accumulation with Fish Growth

The heavy metal accumulation in the fish organs is distinguished by each growth phase showed in Table 3. The heavy metal accumulation trend in the organs can be from highest in the order of liver > gills > muscle throughout the growth in fish sizes. Meanwhile the values of heavy metal in the

L. calcarifer were in the order of Cu > Zn > As > Pb > Hg > Cd.

Copper, Zn and Cd were found to accumulate highest in the liver compared to all other organs. The levels of As, Hg and Pb however were found highest in the fish muscle that can be worrying to the human health from their consumption. Most of the heavy metals were found to accumulate higher in smaller fish sizes and much lower in larger fish sizes to the point of the heavy metals detected were found below the detection limit (BDL). This can be seen for Cu, Zn, and Cd in the muscle in growth phase 5 and 6, almost all heavy metals in the gills in fish sizes 6 and 7 (except Zn) and for As, Hg and Pb in the liver during growth phase 6 and 7.

Table 3. Average heavy metal concentrations in each organ with increasing growth phase, (n=20)

Organ	Growth Phase	Heavy Metal Concentration (mg/kg *dw)					
		Cu	Zn	As	Cd	Hg	Pb
Muscle	1	0.320±0.610	2.210±4.920	0.180±0.480	0.004±0.021	0.019±0.007	0.525±2.302
	2	2.910±1.210	24.88±6.730	26.01±5.170	0.009±0.006	0.246±0.094	0.314±0.217
	3	0.570±0.940	21.89±7.570	17.17±2.700	0.006±0.006	0.026±0.018	0.406±0.518
	4	2.840±11.72	13.27± 2.810	16.93±3.680	0.001±16.93	0.136±0.016	0.030±0.102
	5	**BDL (<0.026)	12.29±7.120	20.02±3.660	0.003±0.009	0.165±0.029	0.062±0.049
	6	BDL	BDL (<0.120)	6.410±6.360	BDL (<1x10 ⁻⁵)	0.073±0.087	0.045±0.193
	7	BDL	3.470±3.110	12.88±9.530	BDL	0.082±0.138	0.130±0.118
Gills	1	5.040±3.570	49.32±29.66	1.270±1.590	0.020±0.017	0.030±0.015	0.438±0.738
	2	4.890±1.790	76.33±9.610	2.130±0.540	0.020±0.060	0.091±0.129	0.388±0.361
	3	4.920±9.040	57.85±6.150	1.510±0.290	0.010±0.060	0.021±0.008	0.218±0.256
	4	0.840±1.000	48.62±4.270	1.550±0.260	0.010±0.018	0.026±0.011	0.127±0.262
	5	1.390±0.720	53.24±7.610	3.290±0.650	0.009±0.050	0.049±0.011	BDL (<2x10 ⁻⁵)
	6	2.290±2.490	73.88±23.24	BDL (<0.007)	0.002±0.013	BDL (<9x10 ⁻⁵)	0.380±0.285
	7	BDL	58.17±13.80	BDL	BDL	BDL	BDL
Liver	1	90.59±46.30	40.04±14.93	2.240±1.740	0.059±0.029	0.022±0.010	0.634±0.550
	2	291.5±99.31	84.89±12.76	3.770±1.650	0.276±0.101	0.098±0.027	0.229±0.150
	3	111.4±54.56	37.24±6.450	1.630±0.290	1.634±0.014	0.023±0.007	0.126±0.136
	4	206.2±121.3	62.90±13.30	5.380±1.700	0.217±0.061	0.062±0.019	0.006±0.057
	5	35.88±25.87	47.31±8.300	6.540±2.370	0.145±0.056	0.072±0.013	0.001±0.069
	6	128.4±103.9	77.49±20.57	BDL	0.493±0.116	BDL	0.080±0.095
	7	147.9±215.3	75.66±21.76	BDL	0.906±0.356	BDL	BDL

*dw – dry weights; **BDL – below detection limit

The accumulated heavy metal concentration accumulated was compared through each growth in fish size (1-7) to better understand the accumulation trends towards each organ as the *L. calcarifer* grows bigger in size. Each growth phase represents a certain mark in fish growth, where possible changes in metabolic capabilities and function in the organs is possible as they further develop and grow. The Fig. 3 shows the plot of loadings of the heavy metals in each organ along with the changes in fish size in two

principal components (PCs), where PC1 (35.37% of variance) and PC2 (14.46% of variance) accounting for 49.84% of the total variance.

Looking into the changes and growth in fish size on the heavy metal accumulation in the fish the study finds that the heavy metals studied showed to have little association between them with the exception of Cd and Zn in the liver as seen from the loading plot in Fig. 3. Cd and Zn in the liver were

the only heavy metals found to be closely associated and is greatly influenced by the overall growth in fish size of their length and weight. This indicates that the changes in fish size have an effect on the accumulation of Cd and Zn in the fish liver for the *L. calcarifer* farmed in Setiu Lagoon. This was similarly observed of bream *Abramis brama* L. from a low contaminated site where the associations related to age and size were negative for Zn (Farkas *et al.*, 2003).

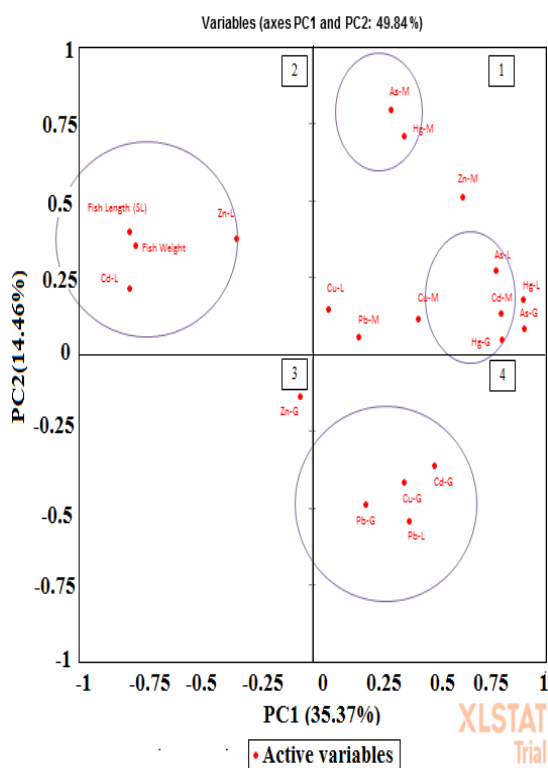


Fig. 3. Plot of loadings on the first two principal components

The level of As and Hg in the muscle were found to be closely associated where their close association can also be seen in their similar levels in the liver as seen in Fig. 3. The fish muscle has been noted to accumulate higher levels of As and is an efficient site of As storage which may lead to the higher As accumulation in the fish muscles than in the liver (Ciardullo *et al.*, 2008; Mok *et al.*, 2012). A study by Mok *et al.*, (2012) observed that higher As levels in farmed *L. calcarifer* in the East Peninsular Malaysia were found to be mainly influenced by the culture system in the area of net cages in the sea than anthropogenic sources. Other fishes studied in Terengganu waters have also observed relatively higher levels of As accumulated in the fish muscle

despite the studied comparison between seasons (Ong *et al.*, 2018). Arsenic levels in two fish species from Miankaleh Wetland were also found to be the highest in the muscle (Alipour *et al.*, 2016).

The level of As concentration in the liver and muscle were found to exceed the permissible level of more than 1.0 mg/kg wet weight (MFR, 1985) and as they accumulate with increased fish size this pose to be a worrying threat to fish health and inevitably human health from its consumption. Arsenic levels in each organ were accumulated in the order of liver > muscle > gills. Arsenic can be found naturally and especially more in the marine environment though there is no known biological function or metabolic roles and can be toxic in excess amount (Yudovich and Ketris, 2005). Though much less toxic to fishes compared to other heavy metals, As pose a threat to human health as it is a known carcinogen that can cause cancer to the skin, bladder, lung and liver (de Rosemond *et al.*, 2008, Farrell *et al.*, 2012). Despite being a non-essential element and a known toxic carcinogen to humans, As is often found high in seafood and is less acutely toxic in fishes as they are stored in organic form as Arsenobetaine (de Rosemond *et al.*, 2008, Wood *et al.*, 2012).

The higher levels of As and Hg in the fish muscle in this study are of worrying concern to the overall human health from the consumption of the *L. calcarifer* farmed in Setiu Lagoon. The increase in levels of As and Hg in the fish muscle with increase in fish size was however found to not be influenced by the overall increase in fish size as seen in the PCA loading plot.

Negative relation between heavy metal concentration and fish size were also observed where the metal levels decrease with increasing fish growth (Gracia-Montelongo *et al.*, 1994; Canli and Atli, 2003; Farkas *et al.*, 2003). One study had showed the negative relationship between heavy metal accumulations with fish size where the heavy metals (Cr, Mn, Ni, and Pb) levels were found to decrease with increase in fish length of *Labeo umbratus* (Nussey *et al.*, 2000). Coincides to that, the similarly lowered Pb concentration with increased in *Poecilia reticulata* fish size were also reported by Widianarko *et al.*, (2000). This is suggested by the growth dilution effect where fish growth as well as possible lowered metabolic activity could possibly

lead to dilution of heavy metal concentration in the fish tissues (Mok *et al.*, 2012). This is unless the heavy metal levels in the surrounding environment supersedes than the capacity of these factors in which may cause increase in heavy metal accumulation in the fish.

The overall outcome of this study has depicted no significant influence from the changes in fish size on the levels of heavy metal concentrations and have only showed to affect Cd and Zn in the liver.

Positive relationship between fish size and heavy metals accumulation have been observed where increasing fish size influences increased concentrations of heavy metals in the fish. The order of Hg levels in each organ were found as muscle > liver > gills. Typically, Hg are found to accumulate higher in concentration with increase in fish size and age (Björnberg *et al.*, 2003; Erdogru and Erbilir, 2006; Łuczynska and Brucka-Jastrzebska, 2006; Fokeena *et al.*, 2015; Ahmad *et al.*, 2016; Le *et al.*, 2018) though despite such this study showed otherwise for Hg accumulation in all 3 organs. Instead, the accumulation of Hg in the liver and gills were found to have a significant negative relationship with increasing fish size where lowered Hg levels were found in larger fish sizes. Catfishes from Yangtze River also observed similar negative relations for Hg levels with increasing fish size which were assumed to be influenced by its metabolic activities (Yi and Zhang, 2012). Hg have been found to be relatively higher in the muscles compared to other organs which makes it ideal as an indicator of Hg contamination (Łuczynska and Brucka-Jastrzebska, 2006). A study on farmed *O. niloticus* (tilapia) showed similar results where Hg was the most accumulated with the highest concentration in the muscle were the where it was also found to exceed the permissible limit in fish (Kaoud and El-Dahshan, 2010).

Metal accumulation in the fish have showed to vary in concentration changes with fish growth and have pointed how different organs have varying accumulation trend as the fish grows in size. Metabolic activity is a major factor in influencing the metals accumulation in the fish. Metabolic activity in younger and smaller individuals are usually higher than in older and larger individuals which may lead to higher metal accumulation in smaller and younger

fishes as most metabolic rate in organisms are more than often size-specific (Nussey *et al.*, 2000; Widianarko *et al.*, 2000; Mok *et al.*, 2012). Each organs have different and varying metabolic rates where the gills and liver with much higher metabolic rates tend to accumulate more metals than those less capable as in the muscle tissue. The liver is an active site for metal metabolism with specialized tissues and binding protein metallothionein allowing for higher metal accumulating capacity while the fish muscle is less active site for metabolic activities and are typically not the main site for metal accumulation with the exception of organic mercury (Ploetz *et al.*, 2007; Bashir and Alhemmal, 2015).

Based on the national and international standards as in Table 4, the permissible amount of metal elements throughout the fish growth were found to meet the standards available with the exception of Arsenic. Despite the relatively higher levels of Hg in the fish muscle, they were still found to be within the limits set.

Table 4: Comparison with national and international standard limits (mg/kg wet weight.)

	MFR (1985)	EU(2006)	FAO/WHO(1989)	FSANZ(1987)
Cu	-	-	-	-
Zn	-	-	-	-
As	1	-	-	2
Cd	1	0.05	2	2
Hg	1	0.5	0.5	1
Pb	2	0.3	0.3	0.5

The permissible limit of As levels found in food and fish product should be a at a minimum of 1.0 mg/kg wet weight. (MFR, 1985) base on Table 4. But this study found that the *L. calcarifer* in Setiu Lagoon were at three times higher than the permissible limit. A study on heavy metal accumulation in commercial fishes in south west Malaysian coast also showed similar results (Kamaruzzaman *et al.*, 2011). Then mud crabs from the same area in Setiu Wetland (Ong *et al.*, 2017) were also found to have high levels of Cu and Zn that were above the permissible limit by Malaysian Food Regulation though they were still found within permissible limit in this study. Organic As (arsenobentine) absorption into the body from consumption of fish and crustaceans however have showed to be efficient in its absorption (>70%) and appears in the urine after 2 days (European Food Safety Authority, 2009). Despite such, urinary As is limited to only its recent exposure and uptake

through consumption (Cascio *et al.*, 2011) or as the consequential production from the metabolism of arsenosugars and arsenolipids found in seafood (Lai *et al.*, 2004, Taylor *et al.*, 2017). Therefore, considerations should be taken for the consumption of this *L. calcarifer* fish farmed in the area without neglecting the importance of fish in our daily diet.

CONCLUSION

The study findings showed that fish growth and subsequently the fish size does affect the heavy metal accumulation in the fish where the relationship in each organ varies with each heavy metal. Positive relationship was observed for Cd and Zn in the liver as the main site for metabolic activity and in regulating contaminants in the body. Meanwhile the overall findings observed negative correlation with *L. calcarifer* growth in fish size for all other heavy metals and organs. Despite such, the all the heavy metals accumulated in the fish throughout its growth were all well within the permissible limit set by national and international standards with the exception of As that far exceeded the Malaysian Food Regulation (1985) of 1 mg/kg. The high levels of As in the fish muscle were prominent even at

earlier stages of its growth phase and found to only accumulate further as the fish grows in size. The heavy metal accumulation in each organ were found in the following order of liver > gills > muscle.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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