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METALS ACCUMULATION IN CULTURED TIGER GROUPER, *Epinephelus fuscoguttatus* WITH ESTIMATED WEEKLY IN TAKE LEVELS FROM EAST COAST OF PENINSULAR MALAYSIA

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Abstract: This study focuses on quantifying metals concentration in muscles, gills and livers of cultured tiger grouper, *Epinephelus fuscoguttatus*. *E. fuscoguttatus* were sampled from the Semerak River, Kelantan (n=5) and the Setiu Wetland, Terengganu (n=20) in April and May 2016 respectively. The samples were digested according to microwave digestion method and analysed for iron (Fe), copper (Cu), zinc (Zn), lead (Pb) and nickel (Ni) using inductively coupled plasma-mass spectrometry Perkin Elmer Elan 9000 (ICP-MS). The bioaccumulation of metals (mg/kg, dry weight) follows the order of Fe> Zn> Cu> Ni> Pb and the concentration trend significantly increased from muscle to gill to liver, respectively ($p<0.05$). Liver was found as a good bioindicator of Fe, Cu and Zn accumulation in both study areas. In contrast, Pb and Ni accumulation in *E. fuscogutatus* from Semerak River were found higher in muscle than liver with 2.48±1.99 mg/kg dry weight and 16.3±4.10 mg/kg dry weight, respectively compared to 0.99±0.78 mg/kg dry weight and 2.47±1.42 mg/kg dry weight, respectively. It suggests potential sources of Pb and Ni into the study area that deserved further investigation. Level of Fe, Cu, Zn, Pb in *E. fuscoguttatus* were found below the standard permissible limit of Malaysian Food Regulation (MFR 1985) and World Health Organization (WHO 1989). The permissible limit for Ni is unavailable in MFR 1985, thus we have referred to WHO 1989. Level of Ni was found exceeding the WHO 1989, suggesting potential risk health effect on the consumer. However, the Estimated Weekly Intake (EWI) values are below the established provisional tolerable weekly intake (PTWI) indicates that *E. fuscoguttatus* from aquaculture cage in selected areas are safe for human consumptions. Further monitoring should be conducted in Semerak River as Pb and Ni were found highly concentrated in non-target organ, muscle.

KEYWORDS: Metal accumulation, tiger grouper, *Epinephelus fuscoguttatus*, estimated weekly intake, South China Sea

Introduction

Metals contamination has attentively gained interest among researchers due to their ability to bioaccumulate in aquatic organism (e.g. fish, bivalve, seaweed) which may pose threats to human health through daily diet consumption (Nurulnadia *et al.,* 2016; Griboff *et al.,* 2017). Recently, fish are widely used in biomarker-based monitoring due to their importance as the main source of protein for human and their sensitivity towards environmental changes (Valon *et al.,* 2013). Realizing this, Ptashynski *et al.* (2002) emphasised the importance of assessment of metal accumulation in fish for the purpose of food safety and security. Although metals such as iron (Fe), copper (Cu) and zinc (Zn) are essential parts of biological systems, lead (Pb) and nickel (Ni) are toxic even in trace amounts (Effendy *et al.,* 2010; Yilmaz *et al.,* 2010). Essential metals are taken up by the fish via sediment and water but non-essential metals also may accumulate in the tissues through the same route (Canli & Atli, 2003). When the concentration exceeds the threshold level, even the essential metals will eventually cause intoxication.

Kamaruzzaman *et al.* (2011) suggested that studies of metals concentration in fish are crucial for nature management and human consumption. Studies on metals accumulation on fish is not something new, but recently the risk factors' calculation for the population has gain attention among researchers (Agusa *et al.,* 2005; Cheung *et al.,* 2008; Amirah *et al.,* 2013; Damodharan & Reddy, 2013; Omar *et al.,* 2013; Ahmad & Sarah, 2015). In general, several established permissible limit (e.g. MFR 1985; WHO 1989) values were referred to predict the safe level for human consumption. However, the value does not always represent the risk for human health (Copat *et al.,* 2012). In this respect, the Estimated Weekly Intake (EWI) was calculated and compared to the provisional tolerable weekly intake (PTWI) recommended by WHO (2017). The similar assessment had been applied in Mediterranean region (Copat *et al.,* 2012; Renieri *et al.,* 2014) and Turkey (Ateş *et al.,* 2015). For that reason, in the present study we aimed to determine the metals (i.e. Fe, Cu, Zn, Pb &Ni) concentration in muscle, gill, and liver tissues of cultured tiger grouper, *Epinephelus fuscoguttatus* from Kelantan and Terengganu aquaculture cages and to calculate its EWI.

Materials and Methods

Study Area

Sampling was conducted in Semerak River, Kelantan and Setiu Wetland, Terengganu (Figure 1) which flow ultimately into South China Sea. Semerak River is located in the Pasir Puteh district, Kelantan while Setiu Wetland located in Setiu district, Terengganu. Semerak River is located approximately 50 km from Kota Bharu and 15 km from Kuala Besut, making the river a natural border between Kelantan and Terengganu. The estimated aquaculture area is 900x200 meters along Semerak River with two bridges connecting the river banks. The aquaculture site is located at one tributary of main Semerak River. It is a semi-enclosed lagoon with total area of 1.2 km^2 with an average depth of 3.12 meters (Samsudin *et al.,* 2017). Setiu Wetland is a part of the Setiu River basin. Measuring 23,000 ha and the water column within the wetland is shallow and well mixed (Suratman *et al.,* 2016). Setiu Wetlands is the largest natural wetlands in the East Coast region of Peninsular Malaysia, combining various ecosystems freshwater, seawater, brackish water, and a 14 km lagoon. The cultured fish are usually marketed to locals and nearby markets.

Early 2015, an oil and gas supply has commenced operation near to Semerak River, Kelantan. The existence of the supply base was followed by the construction of semi enclosed breakwater and sand dredging activity near the river mouth. This activity is predicted to disrupt the ecology as well as contributing pollutants into the environment. Whereas, aquaculture cage owners from Setiu Wetland, Terengganu claimed to suffer a total loss of cultured products and cages during massive flood happened in 2014. Recompensing to the event, sand dredging activity is continuously operated. The dredging activities are known to cause new embedded pollutants to emerge as particulate in water and potentially accumulate in marine organisms such as fish. To this respect, the analysis was done in aquaculture areas from Semerak River, Kelantan and Setiu Wetland, Terengganu.

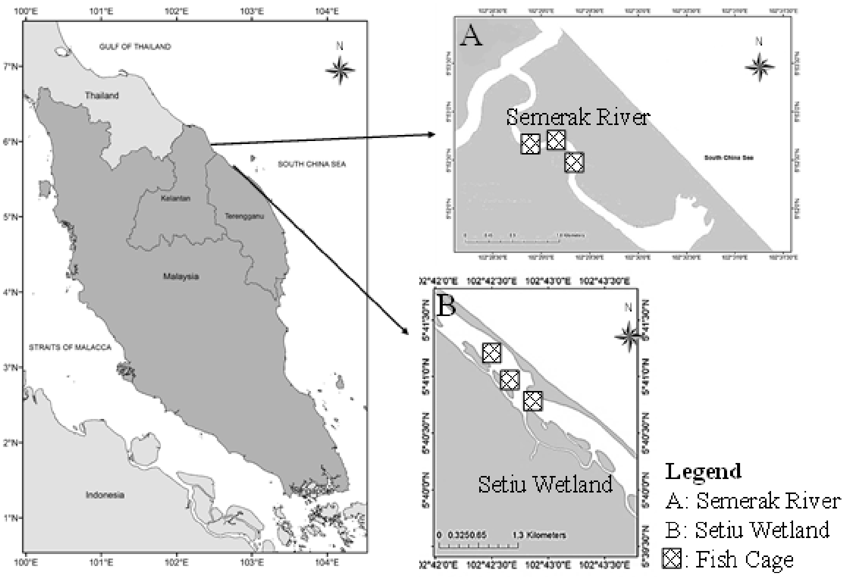

Figure 1: The map shows the sampling sites and sampling locations at the Semerak River and Setiu Wetland in Kelantan and Terengganu, respectively (A: Semerak River, Pasir Puteh, Kelantan and B: Setiu Wetland, Setiu, Terengganu).

Samples Collection and Preparation

Cultured *E. fuscoguttatus* were purchased from the Semerak River cage (n=5) and Setiu Wetland cage (n=20). Small sample size from Semerak River was due to the sampling was done after harvesting period, thus only five individuals were found suitable for the analysis. The range of body weight and total length of *E. fuscoguttatus* were 178 to 380 g and 22 to 29 cm in the Semerak River; and 160 to 250 g and 21.5 to 27.3 cm in the Setiu Wetland, respectively. To represent health index of the fish, condition factors (K) were calculated by multiplying 100 to body weight (g) of individual *E. fuscoguttatus* over their [total length]^3 in centimetre (cm). Prior to analyses, glassware was soaked in 10% nitric acid (HNO_3) for 24 hours to remove non target compound that could possibly interfere with the analysis. *E. fuscoguttatus* were dissected using ceramic knife in order to collect muscle, gill and liver samples. The tissues samples were then dried in the oven at 60°C until constant weight (dry weight) was obtained. The dried samples were then grounded using porcelain mortar and pestle into powder form.

Metals Digestion and Analysis

Metals concentration in the muscle, gill and livers were extracted by applying the microwave digestion method as described by Türkmen *et al.* (2005) with some modifications. Approximately 0.05 g of each tissue samples were placed in Teflon beaker and introduced to high throughput carousel (HTC) safety shield. The samples were digested using 7 mL of nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) in a maximum pressure of 100 bar and 300°C closed digestion vessels (SK10, MILESTONE, Monroe, CT, USA).

After the digestion process completed, the supernatant was thoroughly transferred into 10 mL polypropylene tube and mashed to 10 mL with mili-Q water. Samples were measured using inductively coupled plasma-mass spectrometer, ICP-MS (Perkin Elmer Elan 9000). The quality assurance test was performed using standard reference materials dogfish liver (DOLT-4) with percentage of recovery ranges between 94.8% to 116%. Analyses were performed in four replicates of standard samples with the standard deviation of all analysis within ± 25%. The results were presented in dry weight (dw) and wet weight (ww) in Table 1 and dry weight values were converted to wet weight by multiplying dry weight to moisture percentage over 100. The moisture percentage ranging between 58.3% to 78.2%. Student's T-test was used to examine the significant difference of metal concentration in tissues and study areas. All statistical analyses were performed using IBM SPSS Statistics 24.

To determine the health risk consumption to human, the EWI values for each metal was calculated using the mean concentration of each metal in muscles multiplied by the amount of aquatic-food-product consumption in Malaysia (0.96 kg/week) and divided by average weight of an individual (62.65 kg) referring to Mok *et al.* (2012). The calculated values were compared with the standard PTWI as set by WHO (2017). The calculation was made using wet weight concentration of metals to comply with the values provided by WHO (2017).

Results and Discussion

Condition factor was calculated to be 1.53 and 1.48 in Semerak River and Setiu Wetland, respectively. Hence, *E. fuscoguttatus* collected from both sites were categorised under well-proportioned fish (Barnham & Baxter, 1998). Table 1 shows the compilation data of metals concentration in grouper from several studies and comparisons with the standard permissible limits. The standard permissible limit act as a baseline value to safeguard human health. Based on our results, most of the metals accumulate highest in the liver, followed by gill and muscle. *E. fuscoguttatus* was found to accumulate the highest concentration of Fe in the liver from both study areas, Semerak River (569±512 mg/kg dw) and Setiu Wetland (322±118 mg/kg dw). Fe had an inclination to accumulate in the liver due to the natural function of liver in blood cells and haemoglobin synthesis (Eneji *et al.,* 2011; El-Moselhy *et al.,* 2014), while accumulation of Zn and Cu were found to be concentrated in gills as it is the main site for interaction with metal ions (Hamza-Chaffai *et al.,* 1995; Fernandes *et al.,* 2008). The results of our study showed that liver and gill accumulate significantly higher concentration of metals compared to muscle ($p<0.05$) suggesting that metals accumulate at different levels in different tissues (Yilmaz *et al.,* 2010).

Chavan and Muley (2014) also observed histopathological alteration of liver and gills when exposed to toxicants as these organs are crucial for specific function. Liver is an important site of accumulation, biotransformation, and excretion of toxicant in fish, while gill aids in respiration, osmoregulation, and also excretion (Figueiredo-Fernandes *et al.,* 2007; Saleh & Marie, 2015; Bano *et al.,* 2017).

Table 1: Comparison of metals concentration in muscle, gill and liver of tiger grouper, *Epinephelus fuscoguttatus,* mg/kg dw (unless noted as ** = ww) to other studies with standard permissible limits.

Fish	Organ	Fe	Cu	Zn	Pb	Ni	Location	Reference
Tiger grouper (*E. fuscoguttatus)*	Muscle	192±19.8	1.97±0.33	31.8±24.6	2.48±1.99	16.3±4.10	Semerak River	Present study
		(ww = 41.9)	(ww = 0.429)	(ww = 6.93)	(ww = 0.541)	(ww = 3.55)		
	Gill	176±34.3	31.9±21.7	86.1±15.5	0.97±0.53	5.48±3.98		
		(ww = 62.1)	(ww = 11.3)	(ww = 30.4)	(ww = 0.342)	(ww = 1.93)		
	Liver	569±512	16.0±13.9	71.7±40.5	0.99±0.78	2.47±1.42		
		(ww = 166)	(ww = 4.67)	(ww = 20.9)	(ww = 0.289)	(ww = 0.721)		
	Muscle	103±95.1	2.51±2.16	30.7±24.6	0.76±0.32	3.45±2.29	Setiu Wetland	
		(ww = 30.7)	(ww = 0.748)	(ww = 9.15)	(ww = 0.226)	(ww = 1.03)		
	Gill	217±48.3	3.02±0.82	68.0±21.3	0.83±0.44	9.93±5.91		
		(ww = 73.3)	(ww = 1.02)	(ww = 23.0)	(ww = 0.281)	(ww = 3.36)		
	Liver	322±118	38.3±25.8	93.7±43.4	2.65±2.32	6.73±4.84		
		(ww = 134)	(ww = 16.0)	(ww = 39.1)	(ww = 1.11)	(ww = 2.81)		
Orange-spotted grouper *(E. coioides)*	Muscle	-	0.34±0.01	-	0.20±0.02	-	Oman	Abdul-Wahab *et al.,* 2013
Greasy grouper *(E. tauvina)***	Muscle	4.00-5.00	0.10-0.20	4.00-5.00	2.00-9.00	9.00-39.0	The Persian Gulf	Agah *et al.,* 2009
	Liver	25.0-440	1.60-33.5	16.1-93.0	0-30.0	0.034-33.5		
Blacktip grouper *(E. alexandrines)* **	Muscle	16.8±3.66	0.12±0.07	4.71±0.97	0.53±0.11	0.42±0.15	Turkey	Tepe, 2009
	Liver	61.5±16.5	3.36±0.93	19.4±7.63	1.91±0.34	1.31±0.43		
E. sp. **	Muscle	3.35±0.79	0.29±0.05	2.42±0.22	0.88±0.12		Shalateen, Red Sea, Egypt	El-Moselhy *et al.,* 2014
	Gill	44.5±8.05	1.88±0.32	29.0±1.24	4.86±1.45	-		
	Liver	291±47.6	9.60±2.33	59.9±10.0	3.08±0.78			
	Muscle	2.54±1.29	0.23±0.01	3.98±0.61	0.43±0.05		Suez, Red	
	Gill	27.0±3.93	2.32±0.35	29.3±4.20	4.86±1.45	-		
	Liver	418±37.4	8.51±1.13	64.6±6.46	1.45±0.20			
Tiger grouper *(E. fuscoguttatus)* **	Muscle	-	0.26±0.02	3.57±0.23	0.11±0.04	-	East Malaysia	Mok *et al.,* 2012
	Liver	-	20.4±4.72	76.7±18.0	0.02±0.01			
Standard permissible limits								
Malaysian Food Regulation (MFR 1985)		-	30.0	100	2.00	-		Taweel *et al.,* 2013
World Health Organization (WHO 1989)		100	30.0	100	2.00	0.50-1.00		Mokhtar *et al.,* 2009

**ww = wet weight

Differ from Fe, Cu and Zn, *E. fuscoguttatus* from Semerak River accumulated the highest concentration of Pb and Ni in the muscle with 2.48±1.99 mg/kg dw and 16.3±4.10 mg/kg dw, respectively (Table 1). This suggests that the exposure of Pb and Ni probably have passed through the body defence systems, as muscle is not the main target organ for accumulation (Kalay *et al.,* 1999; Monferrán *et al.,* 2016). High concentration of Pb in the muscle was also in agreement with Semerak River sediment (28.7±4.06 mg/kg dw) compared to Setiu Wetland sediment (5.20±2.52 mg/kg dw) (Afiqah, 2017; Amiera, 2017). In this respect, there might be some pollutant-releasing sources of Pb at Semerak River that influence accumulation of Pb via sediment. Excessive Pb concentration in the body might cause major health problem in haematological, nervous and renal systems (Duruibe *et al.,* 2007). Exposure to high level of Ni also might lead to reproductive toxicity, hepatoxicity, gene toxicity, neurotoxicity, nephrotoxicity and risk of cancer (Guo *et al.,* 2015). Availability of Pb and Ni might come from agricultural, industrial, domestic sewage discharge, atmospheric deposition, feeding habits, species, age and size of fish, feed and physio-chemical parameters of the aquatic environment (Zhang *et al.,* 2013; Varol *et al.,* 2017).

Compared to previous published reports (Table 1), the concentration of Fe, Cu and Zn in muscle and gill in the present study are higher than studies from Oman (Abdul-Wahab *et al.,* 2013), The Persian Gulf (Agah *et al.,* 2009), Turkey (Tepe, 2009), Egypt (El-Moselhy *et al.,* 2014) and East Malaysia (Mok *et al.,* 2012). Most of the compared literatures have sampled wild grouper from open sea and gulf, hence possibly accumulated lower metals concentration compared to cage cultured grouper in the present study (Qiao *et al.,* 2007). Additionally, Mok *et al.* (2012) stated that most of the water pollution sources were from West Malaysia which cause variation of metal concentration in *E. fuscoguttatus* from East Malaysia.

The wet weight concentrations of Fe, Cu, Zn and Pb in the present study were found below the standard permissible limit established by MFR 1985 (Taweel *et al.,* 2013) and WHO 1989 (Mokhtar *et al.,* 2009) (Table 1). However, Ni concentration in all three organs were found exceeding the standard permissible limit by WHO 1989 with very low values (0.5 to 1 mg/kg) than this study. Thus, we can suggest that Ni potentially cause detrimental health effect on consumer and require further investigation. After all, the sources of metals effluent were not investigated in this study.

Provisional tolerable weekly intake (PTWI) was introduced by Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Joint FAO/WHO Meeting on Pesticides Residues (JMPR), European Union and national regulatory agencies as safety approach to establish acceptable intakes of substances that exhibit thresholds of toxicity (Herrman & Younes, 1999). The standard PTWI values of respective metals are shown in Table 2. Estimated weekly intake (EWI) generally calculated in order to estimate the dietary exposure level that can be ingested in a week without appreciable health risk (Ateş *et al.,* 2015). The EWI was calculated based on the metal concentration in muscle tissues. The weekly per capita consumption used in calculation was based on FAO (2007) with the value of 0.96 kg/week and mean body weight of 62.65 kg for Malaysian (Azmi *et al.,* 2009).

Table 2: Comparison of EWI of tiger grouper, *E. fuscoguttatus* with PTWI (mg/62.65 kg body weight/week).

Metals	**Fe**	**Cu**	**Zn**	**Pb**	**Ni**
PTWI standard (WHO, 2017)	5.6	3.5	7.0	-	-
Semerak River (EWI)	0.642	0.006	0.106	0.008	0.054
Setiu Wetland (EWI)	0.470	0.011	0.140	0.003	0.016

The calculated EWI was found to be below the PTWI level, yet we are not able to compare Pb and Ni EWI as there is no standard provided by WHO (Table 2). This finding demonstrates that the level of weekly intake of metals (i.e. Fe, Cu, and Zn) is within the safe limit. While implementing this calculation, PTWI might shows variation of values with human age and type of metals residing in different fish (Castro-González & Méndez-Armenta, 2008). This result could provide margin of safety to the public, as they may avoid or eat only small portion of fishes that tend to accumulate more metals. In this case, although cultured *E. fuscoguttatus* from selected aquaculture cage are safe for consumption, a potential risk may arise in future if domestic waste and construction activities in the region are not well managed.

Conclusion

In conclusion, this study discovered that the level of metals accumulation follows the order of Fe > Zn > Cu > Ni > Pb which are based on liver > gill > muscle. This finding suggest fish liver as the best organ to monitor metal pollution in aquaculture ecosystem. In addition, the level of Pb and Ni need to be monitored as the muscles sample from Semerak River were found exceeding concentration in gills and livers. Ni level in all tissues from both study areas also exceeded permissible limit set by WHO (1989). The EWI values for Fe, Cu, and Zn were found below the PTWI values suggest that consumption of tiger grouper from this region pose no immediate threat to human. Although the results of EWI found in this study are reassuring, the estimated intakes do not take into account of food other than tiger grouper. As a regular customer, it is important to note that a higher consumption of fish would be risking of detrimental health effects. Besides, due to small number of samples collected from Semerak River, this finding must be clearly justified by increasing the number of individual *E. fuscoguttatus.* Further monitoring programmes should also be conducted especially on the potential sources of Pb and Ni in Semerak River.

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