

## **Heavy Metals Concentration in Four Landed Elasmobranchs from Kuala Terengganu Waters, Malaysia**

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### **Abstract**

Concentration of essential metals (Cu and Zn) as well as non-essential metals (Cd, Hg and Pb) in four different elasmobranchs species, purchased from Pulau Kambing LKIM Complex, Kuala Terengganu, had been investigated in this study. Species of elasmobranchs used in this study included spot-tail sharks (*Carcharhinus sorrah*), milk sharks (*Rhizoprionodon acutus*), whitespotted bamboo sharks (*Chiloscyllium plagiosum*) and also whitespotted guitarfish (*Rhynchobatus australiae*). Five organs (muscle, fins, gills, liver and stomach) from each fish were dissected and its metals level was analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Zn contained the highest concentration in most of the organs of elasmobranchs, followed by Cu. Besides, relationship of heavy metals in five organs of the fish and their length was also studied. Present study revealed that Hg in muscle and fins as well as Cu in gills and stomach showed positive relationship with the length of fish. Pb in liver was negatively correlated with the length of the fish. It can be concluded that distribution of heavy metals is different in organs, species and size of the fish.

**Keywords**— elasmobranchs, heavy metals, Kuala Terengganu, relationship

### **INTRODUCTION**

Heavy metals contain density of more than 5 g / cm<sup>3</sup> [13]. Generally, heavy metals can be divided into essential and non-essential metal [15]. Metals such as mercury (Hg), lead (Pb), cadmium (Cd) which are high in density and very toxic in low

concentration are known as heavy metals or non-essential metals. On the other hand, some of the trace elements such as copper (Cu) and zinc (Zn) also known as heavy metals or essential metals due to their importance in regulating the metabolism in our body but toxic when in high concentration [11].

All heavy metals can be present naturally or enter into food chain by human activities such as mining, fossil fuel combustion, industrial processes, storm runoff, atmospheric deposits, landfills leaching, aquaculture, agriculture and municipal waste [9,27,29]. They cannot be destroyed, they will either saturate inside the sediment or enter into food chain and accumulate in human bodies [19]. Storelli et al. [30] also stated that heavy metals are very persistent in aquatic environment, they will accumulate in the tissue of living organisms. Gorur et al. [10] also added that heavy metals are non-biodegradable. Besides that, heavy metals are very dangerous and toxic because they will bioaccumulate in which they will deposit in the organisms' body [19].

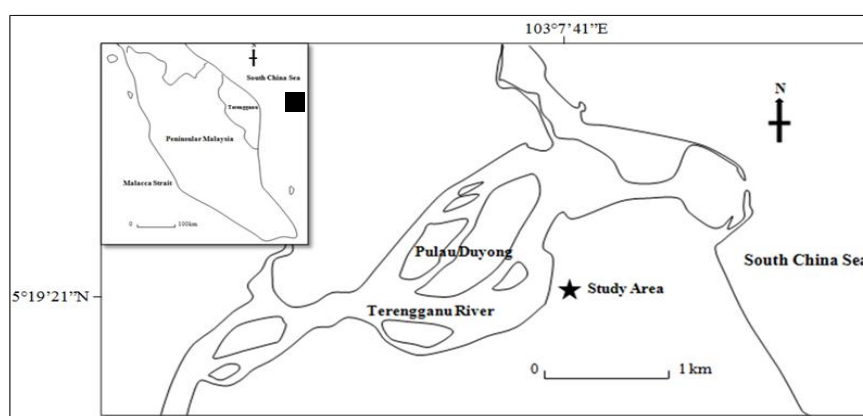
Due to the characteristics of heavy metals which tend to accumulate and magnify through the trophic level, fishes will be a good bio indicator to assess the contaminant level of heavy metals in aquatic environment. This is because they are available in different ages and sizes as well as occupy different trophic levels [3]. Fishes like elasmobranchs have always been chosen in biomonitoring heavy metals in marine environment due to its top position in the food chain [31]. They live longer than others and have a slower growth rate, their bodies most probably have accumulated a significantly high level of heavy metals [21]. Some studies have showed that elasmobranchs tend to accumulate heavy metals and toxins higher than other marine organisms [7,8,33]. Concentration of heavy metals in elasmobranchs may vary according to their sex, species, body length and living habitat [12,18,22]. Sharks and rays that live in different places will have different level of heavy metals because every marine environment will be affected by different amount of contaminants [31].

In the present study, concentration of heavy metals in five organs (muscle, fins, gills, liver and stomach) of each elasmobranch was studied. Muscle and fins are chosen because they are the main part consumed by public [20]. Liver is selected because it is important in detoxification and metals storage [17]. Gill is the first organ that interacts with heavy metals from water and also the uptake site of the waterborne ions, so it will tend to accumulate heavy metals compared with others [15]. According to Rashed [28], stomach is the major organ that involved in xenobiotics metabolism in organisms. Besides that, relationship of heavy metals concentrations in five organs of fish between their sizes (length) was also investigated in the current study.

## MATERIALS AND METHODS

**Sampling and sample treatment:** Elasmobranchs were purchased from the fish market at Pulau Kambing LKIM Complex, Kuala Terengganu (Figure 1). It is the biggest fish market at Kuala Terengganu where all the fishes caught along Kuala Terengganu Waters will be centered here. Elasmobranchs were collected in the period

from May to July 2014. Frequent visit had been done throughout three months to ensure that each species consists of at least five fishes. After sampling, samples were transported back to the laboratory and frozen at  $-4^{\circ}\text{C}$  for further analysis [24]. Before dissection, fish samples were thawed at room temperature. Then, length and weight of the samples were measured. Besides that, sex of the elasmobranchs was also determined by looking at the presence of clasper. Elasmobranchs were dissected by using the stainless steel scalpels, scissors and forceps. Five organs of elasmobranchs were taken out, which included muscle, fins, gills, liver and stomach. To reduce the chances of samples being contaminated, samples were dissected carefully to avoid losing blood and also rinsed with distilled water after dissecting. All the dissected organs were dried in the oven at  $60^{\circ}\text{C}$  until a constant weight was achieved. After that, they were grinded into powder form for acid digestion [24].



**Figure 1:** Study area at Pulau Kambing LKIM Complex, Kuala Terengganu

**Analytical procedure:** 0.05 g of the finely grinded samples was weighed and digested with 1.5 ml of Suprapur Nitric Acid ( $\text{HNO}_3$ ) in Teflon beaker. Blank and standard reagents were processed simultaneously to control the accuracy of the procedure used [16]. Teflon beakers were sealed well by screw closure to avoid the leakage of the acid during digestion. All Teflon bombs were then heated in the oven at  $100^{\circ}\text{C}$  for 8 hours. After the digestion, Teflon bombs were cooled down to room temperature. The acid-digested samples were transferred into centrifuge tubes and diluted up to 10ml with deionized water. Level of heavy metals (Cu, Zn, Cd, Hg and Pb) in the five organs of the elasmobranchs were detected by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with the model of Perkin Elmer Elan 9000 in order to get a quick and precise data [24].

## RESULTS AND DISCUSSION

In this study, a total number of 23 elasmobranchs, which collected from Pulau Kambing LKIM Complex, Kuala Terengganu, had been analyzed with weight and

length, ranging from 0.28 to 2.63 kg and 42.8 to 79.8 cm. The biological information of the samples was presented in Table 1. They comprised four different species, which included spot-tail sharks (*Carcharhinus sorrah*), milk sharks (*Rhizoprionodon acutus*), whitespotted bamboo sharks (*Chiloscyllium plagiosum*) and also whitespotted guitarfish (*Rhynchobatus australiae*).

**Table 1:** Biological information of the collected samples with range of weight and total length

Common Name	Scientific Name	n	Weight (kg) (min-max)	Total Length (cm) (min-max)
Spot-tail shark	<i>Carcharhinus sorrah</i>	5	0.690-0.935	52.0-56.5
Milk shark	<i>Rhizoprionodon acutus</i>	8	1.90-2.63	71.1-79.8
Whitespotted bamboo shark	<i>Chiloscyllium plagiosum</i>	5	0.303-1.740	45.0-79.5
Whitespotted guitarfish	<i>Rhynchobatus australiae</i>	5	0.280-1.586	42.8-64.6

All the sex of the elasmobranchs was identified prior to dissection by checking the presence of its clasper. The maturity of elasmobranchs was also determined by measuring its total length. All the information was tabulated in Table 2.

**Table 2:** Information of the collected samples with its sex and maturity

Common Name	Scientific Name	n	Sex		Maturity	
			Female	Male	Juvenile	Adult
Spot-tail shark	<i>Carcharhinus sorrah</i>	5	2	3	5	0
Milk shark	<i>Rhizoprionodon acutus</i>	8	4	4	0	8
Whitespotted bamboo shark	<i>Chiloscyllium plagiosum</i>	5	2	3	3	2
Whitespotted guitarfish	<i>Rhynchobatus australiae</i>	5	2	3	5	0

According to Table 2, two females and three males of spot-tail sharks were collected in this study. All of them were still juvenile and haven't reached maturity yet. Next, sex of the milk sharks were evenly collected, which was four for both sexes. All of

them have reached maturity length. On the other hand, there were two females and three males of whitespotted bamboo sharks obtained. Three of them were still juvenile and two were adult. Two females and three males of whitespotted guitarfish were collected in this study. However, they were all still juvenile and haven't reached maturity yet.

In this study, level of the selected heavy metals (Cu, Zn, Cd, Hg and Pb) in muscle, fins, gills, liver and stomach of elasmobranchs was determined by using a fast and sensitive Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as tabulated in Table 3, 4, 5, 6 and 7 respectively. In the present study, not all the organs and fish species are consistently high in heavy metals concentration. Among all the elements, Zn is the most abundant in five organs of all species, ranging from 7.74±2.27 to 43.4±13.9 µg/g dry weight except fins and gills of milk sharks as well as liver of whitespotted bamboo sharks, which contained the highest amount of Cu. Cu and Zn, both are essential elements, are normally found to be more concentrated than others because they are needed by organisms to undergo metabolism [1]. Zn is needed in the production and degradation of the macronutrients like carbohydrates, lipids, protein and nucleic acid. Not only that, Zn is also important in metabolizing other micronutrients [6]. El-Sherif et al. [5] stated that Zn is needed in developing the embryo of organisms. On the other hand, Cu helps in transporting electrons in organisms' body [25]. Velusamy et al. [32] described that Cu is vital in the synthesis of haemoglobin. Moreover, Cu acts as the precursor of enzymes and regulated by the physiological mechanisms in living organisms [16]. Nonetheless, they are hazardous to animal and human if they are highly concentrated in their bodies [14].

**Tables 3:** Average elements concentration (µg/g dry weight) in elasmobranchs' muscle

Species	Concentration of Heavy Metals (µg/g dry weight)				
	Cu	Zn	Cd	Pb	Hg
Spot-tail shark	4.6±2.72	14.2±2.0	0.010±0.010	0.279±0.105	0.571±0.151
Milk shark	2.44±1.16	11.0±1.54	0.006±0.011	0.433±0.323	0.736±0.434
Whitespotted bamboo shark	2.14±1.72	19.2±10.1	0.174±0.158	0.256±0.298	3.26±1.981
Whitespotted guitarfish	1.15±0.431	7.99±2.50	0.010±0.019	0.115±0.023	0.202±0.079

**Tables 4:** Average elements concentration ( $\mu\text{g/g}$  dry weight) in elasmobranchs' fins

Species	Concentration of Heavy Metals ( $\mu\text{g/g}$ dry weight)				
	Cu	Zn	Cd	Pb	Hg
Spot-tail shark	31.3 $\pm$ 24.6	34.7 $\pm$ 10.1	0.040 $\pm$ 0.035	1.02 $\pm$ 0.675	0.362 $\pm$ 0.189
Milk shark	23.3 $\pm$ 8.88	21.0 $\pm$ 3.65	0.030 $\pm$ 0.017	1.31 $\pm$ 0.685	0.383 $\pm$ 0.144
Whitespotted bamboo shark	1.35 $\pm$ 0.788	19.5 $\pm$ 3.99	0.026 $\pm$ 0.022	0.576 $\pm$ 0.247	0.487 $\pm$ 0.324
Whitespotted guitarfish	7.62 $\pm$ 9.48	15.1 $\pm$ 5.63	0.065 $\pm$ 0.070	0.564 $\pm$ 0.458	<i>n.d.</i>

*n.d.*: not detected

**Tables 5:** Average elements concentration ( $\mu\text{g/g}$  dry weight) in elasmobranchs' gills

Species	Concentration of Heavy Metals ( $\mu\text{g/g}$ dry weight)				
	Cu	Zn	Cd	Pb	Hg
Spot-tail shark	28.7 $\pm$ 19.1	43.4 $\pm$ 13.9	0.035 $\pm$ 0.024	1.28 $\pm$ 1.07	2.22 $\pm$ 1.74
Milk shark	50.3 $\pm$ 60.8	36.3 $\pm$ 4.44	0.052 $\pm$ 0.04	0.647 $\pm$ 0.259	0.444 $\pm$ 0.18
Whitespotted bamboo shark	3.91 $\pm$ 2.17	21.5 $\pm$ 4.99	0.182 $\pm$ 0.067	0.219 $\pm$ 0.039	1.02 $\pm$ 0.488
Whitespotted guitarfish	24.2 $\pm$ 35.7	32.6 $\pm$ 5.08	0.123 $\pm$ 0.048	4.22 $\pm$ 8.38	0.271 $\pm$ 0.149

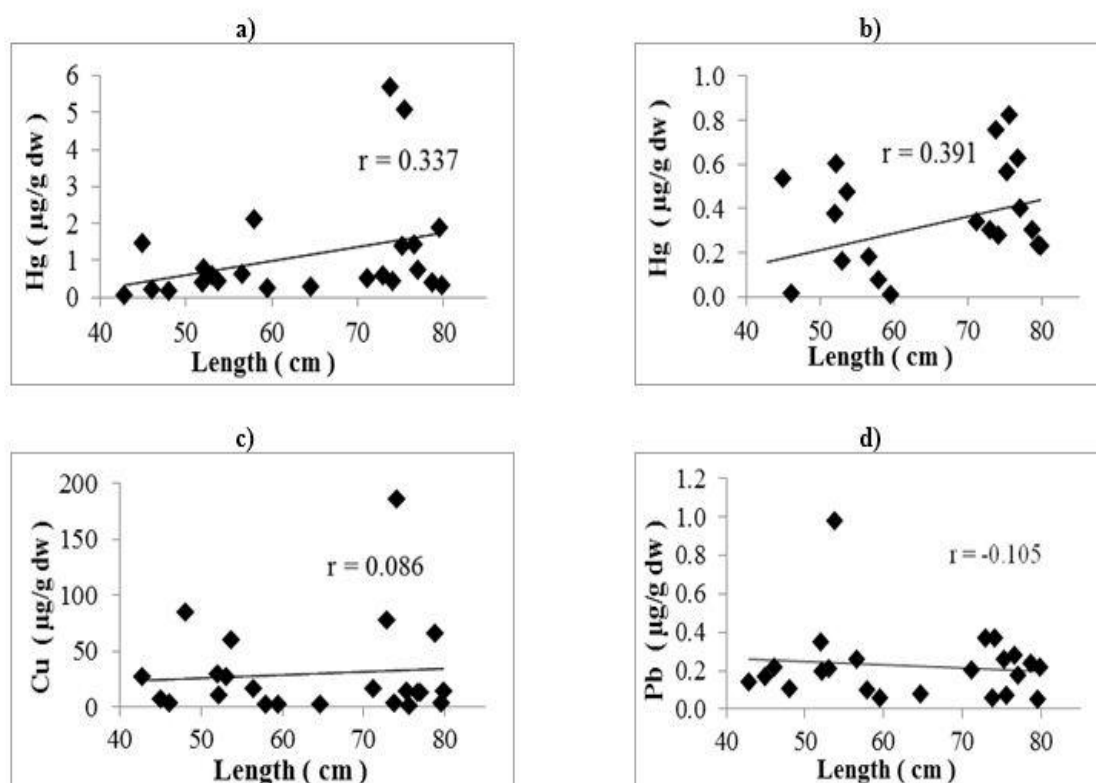
**Tables 6:** Average elements concentration ( $\mu\text{g/g}$  dry weight) in elasmobranchs' liver

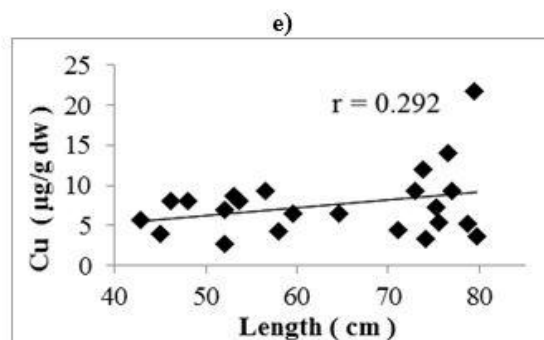
Species	Concentration of Heavy Metals ( $\mu\text{g/g}$ dry weight)				
	Cu	Zn	Cd	Pb	Hg
Spot-tail shark	6.1 $\pm$ 1.65	7.74 $\pm$ 2.27	0.009 $\pm$ 0.010	0.400 $\pm$ 0.328	0.260 $\pm$ 0.102
Milk shark	3.02 $\pm$ 0.867	9.28 $\pm$ 2.50	0.066 $\pm$ 0.080	0.265 $\pm$ 0.073	0.069 $\pm$ 0.088
Whitespotted bamboo shark	70.1 $\pm$ 110.2	38.3 $\pm$ 50.0	8.25 $\pm$ 15.0	0.091 $\pm$ 0.047	0.876 $\pm$ 0.566
Whitespotted guitarfish	8.80 $\pm$ 3.25	37.2 $\pm$ 12.2	1.3 $\pm$ 1.47	0.120 $\pm$ 0.063	0.289 $\pm$ 0.212

**Tables 7:** Average elements concentration ( $\mu\text{g/g}$  dry weight) in elasmobranchs' stomach

Species	Concentration of Heavy Metals ( $\mu\text{g/g}$ dry weight)				
	Cu	Zn	Cd	Pb	Hg
Spot-tail shark	7.14 $\pm$ 2.67	41.0 $\pm$ 21.6	0.026 $\pm$ 0.012	0.400 $\pm$ 0.328	0.333 $\pm$ 0.119
Milk shark	7.08 $\pm$ 3.65	39.2 $\pm$ 11.1	0.214 $\pm$ 0.366	0.286 $\pm$ 0.174	0.163 $\pm$ 0.134
Whitespotted bamboo shark	9.46 $\pm$ 7.63	22.8 $\pm$ 14.1	1.20 $\pm$ 0.494	0.066 $\pm$ 0.023	1.39 $\pm$ 0.658
Whitespotted guitarfish	6.96 $\pm$ 1.07	38.3 $\pm$ 11.2	1.59 $\pm$ 2.12	0.184 $\pm$ 0.087	0.352 $\pm$ 0.230

Figure 2 showed the relationship of heavy metals concentration in five organs of the samples with its length. Positive relationship can be found between Hg in muscle and fins as well as Cu in gills and stomach, whereas only a negative relationship was found in between Pb and liver of elasmobranchs.





**Figure 2 (a-e):** Relationship between heavy metals concentration in five organs (a) muscle, (b) fins, (c) gills, (d) liver, (e) stomach and elasmobranchs size (in the term of length)

Relationship of Hg in fins was the strongest compared to other organs and metals with the correlation value of 0.391. Correlation value of Hg in the muscle of elasmobranchs was found to be the second highest after fins. Higher concentration of metals found in older elasmobranchs might due to the stability of the metal and high accumulation ability of the metal in the food chain which cannot be removed from body [13]. On the other hand, negative relationship that found in metals and elasmobranchs suggested that metals are more abundant in younger samples compared to the elder one. This might due to the 'growth dilution effects' in which the older fish with bigger size may dilute the concentration of the bioaccumulated metals in their body compared to smaller ones [26]. It might also be related to the variations in the metabolism of the organisms with different age and hence affect the uptake and elimination of metals as suggested by Williamson [35]. This is due to smaller fish tend to be more active and will take in more oxygen in order to supply more energy for themselves to grow [4].

In this study, it showed that heavy metals distributed differently in all the organs, species and also size. As proposed by Wang and Rainbow [34], level of metals in organisms is controlled by the balance between uptake and elimination. Not only that, it also depends on the feeding habits like herbivores, carnivores, and omnivores as well as their living habitat [36]. Besides that, gender, growing rates of the fish and types of organs analyzed will also attribute to the different concentration of heavy metals [23]. Other than all the biological factors, level of water pollution, chemical form of the metals in the water, pH value, temperature of water, water transparency and even the catching season of the same fish species will also cause difference in the heavy metal level [2].

## CONCLUSION

Findings of current study represents valuable data of the concentration of heavy metals (Cu, Zn, Cd, Hg and Pb) in five organs (muscle, fins, gills, liver and stomach)



of the elasmobranchs that landed at Pulau Kambing LKIM Complex, Kuala Terengganu. Result of this study revealed that Zn is the most abundant in all the organs of elasmobranchs, followed by Cu as they are the essential metals needed by organisms to regulate metabolic activities. Besides that, relationship of heavy metals between five organs of elasmobranchs was determined. Length of the fish was used as a proxy of size. Hg in muscle and fins as well as Cu in gills and stomach were found to have positive relationship with the length of fish. Pb in liver showed negative relationship with the length of the fish. It can be concluded that distribution of heavy metals is different in organs, species and size of the fish.

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