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LEVEL OF HEAVY METALS IN BAMBOO SHARKS (*Chiloscyllium* sp.) IN STRAITS OF MALACCA, MALAYSIA

(Kepekatan Logam Berat di dalam Yu Cicak (*Chiloscyllium* sp.) di Selat Melaka, Malaysia)

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Abstract

Sharks which are the top predators in the ocean food chain are still high in demand as delicacies (shark meat) in restaurants despite the efforts to protect them. Anyhow, sharks accumulate a significant amount of metals in their bodies. Hence, a study was carried out to examine the presence and concentration of heavy metals (Cd, Cu, Pb and Zn) in bamboo sharks (*Chiloscyllium*), the most commonly landed species. A total of 43 *Chiloscyllium* samples were collected from the local fishermen in Lumut, Perak. In this study, the two edible tissue samples, namely the muscle and fins were dissected and analysed using inductively coupled plasma – mass spectrometry (ICP-MS) following a Teflon bomb closed digestion with HNO_3 acid. Based on the results, the concentration of heavy metals in muscle tissues arranged in a diminution order is Zn > Cu > Pb > Cd. Meanwhile, the concentration pattern of metals in fin tissues followed the Zn > Pb > Cu > Cd sequence. The average concentration of metals in the fin tissues was higher than that of muscle tissues. All metals examined were associated with the sharks' length and weight, whereby the findings also indicated that all metals exhibited positive correlation except for Pb concentration in muscle and Zn concentration in fin tissues. The Pollution Load Index (PLI) was used as the predictor to assess the contamination status in the tissue samples. The average PLI value calculated for the muscle and fin tissues ranged between 3.2 and 12.4, respectively. These values also indicated that the level of contamination is very much under control, hence, no monitoring is needed. Therefore, all findings from this study may be used as a reference for future studies, especially on the west coast of Peninsular Malaysia waters.

Keywords: Straits of Malacca, sharks, heavy metals, pollution load index

Abstrak

Ikan yu adalah pemangsa teratas dalam rantaian makanan dan boleh mengakumulasi sejumlah besar logam berat dalam badan mereka. Terdapat juga permintaan tinggi daging ikan yu sebagai makanan istimewa di restoran. Oleh itu, kajian ini bertujuan untuk menentukan kepekatan logam berat (Cd, Cu, Pb, dan Zn) dalam tisu yu cicak (*Chiloscyllium*) yang paling banyak didaratkan. Sebanyak 43 sampel *Chiloscyllium* telah dikumpulkan daripada nelayan tempatan di Lumut, Perak. Dalam kajian ini, dua tisu iaitu daging dan sirip ikan yu telah dipilih untuk dianalisa dengan menggunakan spektrometri jisim-gadingan aruhan plasma (ICP-MS) selepas proses pencernaan tertutup bom Teflon dengan asid HNO_3. Corak kepekatan logam dalam tisu daging dalam urutan Zn > Cu > Pb > Cd. Manakala kepekatan logam dalam tisu sirip ikan menurun dalam urutan Zn > Pb > Cu > Cd. Pada umumnya, kepekatan logam dalam tisu sirip adalah lebih tinggi berbanding tisu daging. Kandungan logam dalam setiap organ dikaitkan dengan panjang dan berat ikan yu dan semua logam dalam tisu daging menunjukkan korelasi positif, manakala Pb dalam tisu daging dan Zn dalam tisu sirip menunjukkan korelasi negatif kepada panjang dan berat ikan yu. Status pencemaran ikan yu cicak juga dinilai menggunakan Indeks Beban Pencemaran (PLI) sebagai petunjuk pencemaran. Purata nilai PLI untuk tisu daging adalah 3.2, dan untuk tisu sirip adalah 12.4 dan ini menunjukkan tahap pencemaran berada di bawah kawalan dan pemantauan tidak diperlukan. Hasil dapatan kajian ini boleh digunakan sebagai rujukan pada kajian akan datang terutamanya di perairan barat Semenanjung Malaysia.

Kata kunci: Selat Melaka, ikan yu, logam berat, indeks bebanan pencemaran

Introduction

Heavy metal pollution in the marine ecosystem is deemed as a significant environmental problem. The concern, however, is regarding the toxic accumulation of heavy metals in the organism's body and within the ecosystem [1]. These toxic heavy metals are introduced into the estuarine and aquatic environments through anthropogenic activities such as the development of coastal areas. Moreover, the urban, municipal and industrial wastes discharged into the aquatic environment contain a substantial amount of heavy metals contaminating the marine environment [2].

Having said that, the heavy metals input into water easily accumulate in aquatic organisms like sharks. Lopez et al. [3] stated that sharks readily accumulate these elements in their tissues due to their diet as they prey on various trophic organisms. The level of heavy metal accumulation in each organism may differ depending on its diet and habit. Hence, sharks being the top predator consuming most of the organisms would be taking in a higher level of heavy metal concentration into their system [4], especially muscle and fin tissues [5]. Therefore, sharks are suitable bio-indicators of heavy metal contamination in the aquatic environment [6].

In this study, the bamboo shark (*Chiloscyllium*) was chosen as the study sample to determine the level of heavy metals. Based on our survey together with an officer from Department of Fisheries Malaysia located in Lumut, Perak and their statistical data [7], *Chiloscyllium* is the most landed sharks which can be found at the study area. The statistics [7] also documented that the Perak state landed the most number of sharks species, 530 metric tonnes or 58% of the total catch compared to other states along the Straits of Malacca. According to our survey, *Chiloscyllium* is one of the species most fishmongers seek as it is one of the most popular fish among the public at several landing points.

Materials and Methods

Sample collection and preparation

A total of 43 *Chiloscyllium* samples were collected from the local fishermen at Lumut, Perak, Malaysia (Figure 1) in October 2018 which are usually collected as trawl bycatch. Once collected, the samples were labelled and stored in an icebox at low temperature before dissection and analysis in the laboratory [7]. Prior to the dissection, the samples were defrosted at room temperature. The total body length and weight of samples were measured. The sex of the shark was also determined by checking for the presence of clasper. Only the muscle tissues and fin samples were dissected using a ceramic knife. For each shark, a total of 50g muscle and fin tissues were transferred to a clean sample container. The samples were then rinsed with distilled water to avoid contamination. Next, the muscle and fin samples were dried in an oven at 60 °C until a desired constant dry weight was obtained. Once the samples were dried, pestle and mortar were used to homogenise the sample into powder form. All the apparatus used in sample preparation were cleaned using ethanol after every sample.

Analytical procedures

The Teflon bomb digestion method for biological samples was suggested by Okamoto and Fuwa [8]. This method determines the metal concentration of biota samples in liquid form. Hence, this study employed the modified procedures as described by Kamaruzzaman et al. [9] and Ong et al. [10]. Firstly, 0.05g of the homogenised sample was weighed and transferred into 25 mL Teflon beaker. Then, 1.5 mL of Suprapur 65% nitric acid (HNO_3) was added into the Teflon beaker. Since HNO_3 is a strong acid, this step was performed in a perchloric fume hood. Then, the Teflon beaker was sealed tight to prevent acid leakage during the acid digestion process.

Blank and certified reference material (DOLT-4 Dogfish liver) samples were processed simultaneously to test the accuracy of the procedure. All the Teflon bombs were heated in an oven at 100 °C for 8 hours to aid the digestion process. After 8 hours of heating, the Teflon bombs were let to cool down to room temperature, where the acid-digested solution in the Teflon bombs was transferred into separate centrifuge tubes. Deionized water was added to a 10mL mark. Finally, the concentrations of heavy metals namely Cadmium (Cd), Copper (Cu), Lead (Pb) and Zinc (Zn) were obtained using inductively coupled plasma mass spectrometry (ICP-MS) model Elan 6000. Table 1 lists the detection and quantification limits for the ICP-MS instrument used in the sample detection.

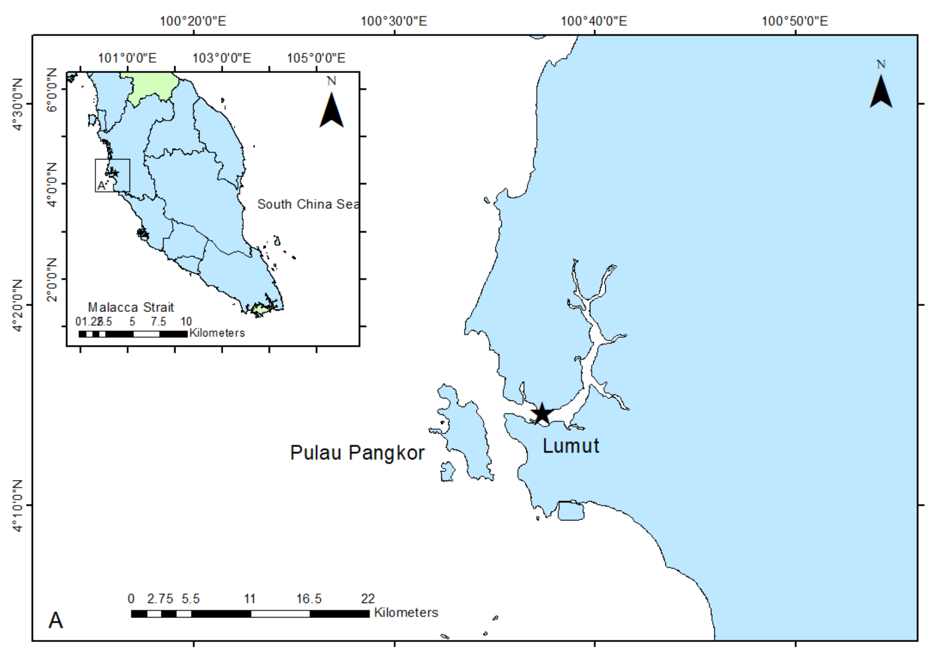


Figure 1. Map showing the location of the sampling area at Lumut, Perak

Table 1. Detection and quantification limits for the ICP-MS Elan 9000 instrument

Element	**Mass**	**Detection Limit (μg/L)**	**Quantification Limit (μg/L)**
Cd	114	0.003	0.01
Cu	63	0.21	0.69
Pb	208	0.02	0.05
Zn	64	0.26	0.85

Results and Discussion

Table 2 illustrates the biological data of the 43 *Chiloscyllium* samples collected in this study, including the length and weight. The average length and weight were estimated at 54.5cm and 758g, respectively. The length of the largest *Chiloscyllium* in this study was 82.0 cm, while the smallest recorded was 38.3 cm. Meanwhile, the weight ranged from 213 to 2483g. The length and weight of each *Chiloscyllium* were recorded to determine the correlation with the studied metals.

Table 3 indicates the result of the recovery test for the selected heavy metals using 1946 DOLT-4 Dogfish liver as the Standard Reference Material (SRM). The recovery test is used to control the accuracy of the methodology. The recovery percentage of the selected metals namely Cd, Cu, Pb and Zn ranged between 87.2% and 112.5%. According to Yohannes et al. [11], recovery percentage ranging between 80% - 115% indicate good accuracy and that the data obtained is acceptable.

The concentration of heavy metals in the edible tissues of *Chiloscyllium* are tabulated in Table 4 and Table 5, respectively. Based on the results, muscle samples indicated a decreasing trend of Zn > Cu > Pb > Cd, whereby this metal concentration trend in muscle tissue was in agreement with previous studies [12, 13, 14]. The fin samples, on the other hand, demonstrated a trend of Zn > Pb > Cu > Cd. Of the four heavy metals, Zn and Cu appeared to be present in high concentrations because they are essential metals [15]. These heavy metals are fundamentally necessary for fish growth development [14] and as micronutrients to fishes that is obtained through water and diet [16].

However, the non-essential metals such as Pb and Cd were present in low concentrations as they do not have any biological importance and are not needed by an organism [17]. The concentration of Pb was higher than that of Cu in the fin samples possibly due to the ambient Pb concentration in the sampling location, Lumut. Lumut is a coastal town with notable ship activities for fisheries and tourism. Hence, the accumulation of such metals in the waters at a reasonably high concentration in a developed coastal area indicates the impacts of human activities. Additionally, several researchers have also mentioned the accumulation of heavy metals in marine ecosystems were associated with urban runoff, water treatment systems, industrial effluents and waste, shipping and agricultural runoff [18-22].

Compared to the concentrations of metals (Zn, Cu, Pb and Cd) between the samples, fin tissues contained higher concentrations of metal than that of the muscle. This observation could probably be because fin is the final destination where all metals accumulate [7]. However, the concentration of metals can vary in shark tissues due to several factors, including the size of the shark, sex, species, feeding habit and habitat [23, 24].

Table 2. Size of *Chiloscyllium* sp. collected in this study

	Length (cm)	**Weight (g)**
Average	54.5±9.3	758±469
Min	38.3	213
Max	82.0	2483

Table 3. Recovery test using Standard Reference Material (SRM) 1946 DOLT-4 Dogfish liver

Metals	**Measured Value (µg/g dry wt.)**	**Certified Value (µg/g dry wt.)**	**Recovery (%)**
Copper (Cu)	28.8±1.5	31.2±1.1	92.3
Zinc (Zn)	110±8.0	116±6.0	94.8
Cadmium (Cd)	21.2±1.2	24.3±0.8	87.2
Lead (Pb)	0.18±0.07	0.16±0.04	112.5

Table 4. Concentration of metals in bamboo shark muscle

Samples	**Concentration of Metals in Muscle (µg/g dry wt.)**			
	Cu	**Zn**	**Cd**	**Pb**
Average	0.75 ± 0.33	28.30 ± 16.20	0.07 ± 0.04	0.72 ± 0.35
Min	0.31	9.50	0.02	0.31
Max	1.65	110.40	0.16	2.33

Table 5. Concentration of metals in bamboo shark fins

Samples	**Concentration of Metals in Fins (µg/g dry wt.)**			
	Cu	**Zn**	**Cd**	**Pb**
Average	4.16 ± 3.74	62.20 ± 25.00	0.31 ± 0.33	9.53 ± 12.90
Min	0.75	24.50	0.02	0.26
Max	20.61	138.80	1.45	63.54

Figure 2 to Figure 5 represent the correlation graphs between the concentrations of selected metals (Cu, Zn, Cd and Pb) in edible shark tissues against shark size. The correlation of Zn and Pb against shark size varied between muscle and fin sample. Kamaruzzaman et al. [13] also published similar findings that there was no or weak relationship between Zn and Pb against the size of the shark.

According to Adel et al. [15] and Endo et al. [25], the concentration of accumulated Cu and Cd in shark muscle were related to shark body length. However, Cd concentration pattern in *Chiloscyllium* from the Straits of Malacca indicated a weak positive correlation between shark size and edible tissue of shark, which might be associated with the shark's feeding preference and habits. Generally, *Chiloscyllium* species feed extensively on molluscs and cephalopods, which contains a high concentration of Cd in their body [15].

On the other hand, the positive correlation between Cu with shark size and a comparatively higher Cu level in their body might be due to the diet of bamboo sharks [26]. Ong & Gan [7] mentioned that *Chiloscyllium* is a demersal shark species that consume a higher proportion of crustaceans and molluscs in their diet [26]. According to Vas [27], crustacean and molluscs usually contain high Cu content due to the presence of haemocyanin in their tissues. Hence, the crustaceans and molluscs dependent diet may be accounted for the high level of Cu in *Chiloscyllium* sp.

As previously reported by Mendoza-Carranza et al. [28], the primary transfer route of heavy metals in the aquatic environment is through the diet of an organism, which is mainly through vertical (between different trophic levels) and horizontal (within the same tropical level) transfer [29]. Similar observations can also be concluded for the bamboo sharks as the aforementioned literature [12, 15, 26, 27]. Moreover, molluscs are also deemed as good bioindicators for metal pollution in seawater as they easily absorb heavy metals in the aquatic environment [30]. Since molluscs are identified as ecologically important organisms and are preyed upon by bamboo sharks [12], bamboo sharks end up as ecological basin accumulating much of the heavy metals in the aquatic environment.

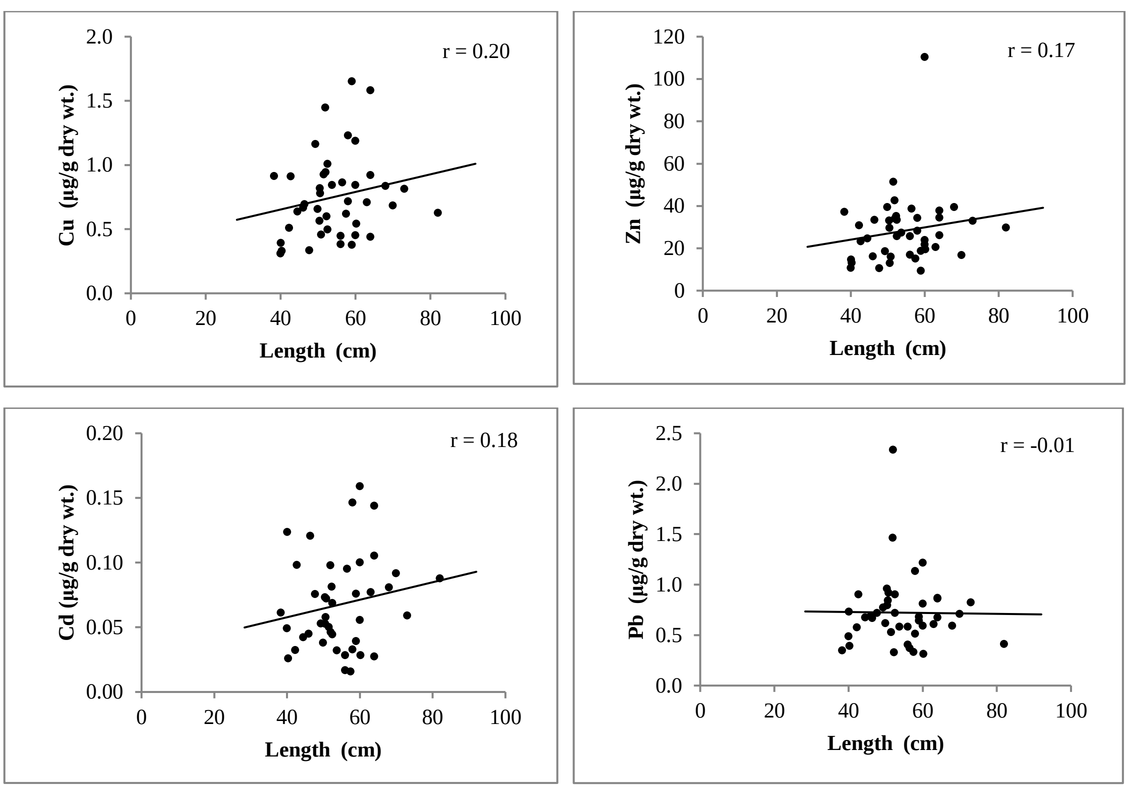


Figure 2. Correlation graph between concentration of selected metals (Cu, Zn, Cd, and Pb) in shark muscle and length of shark

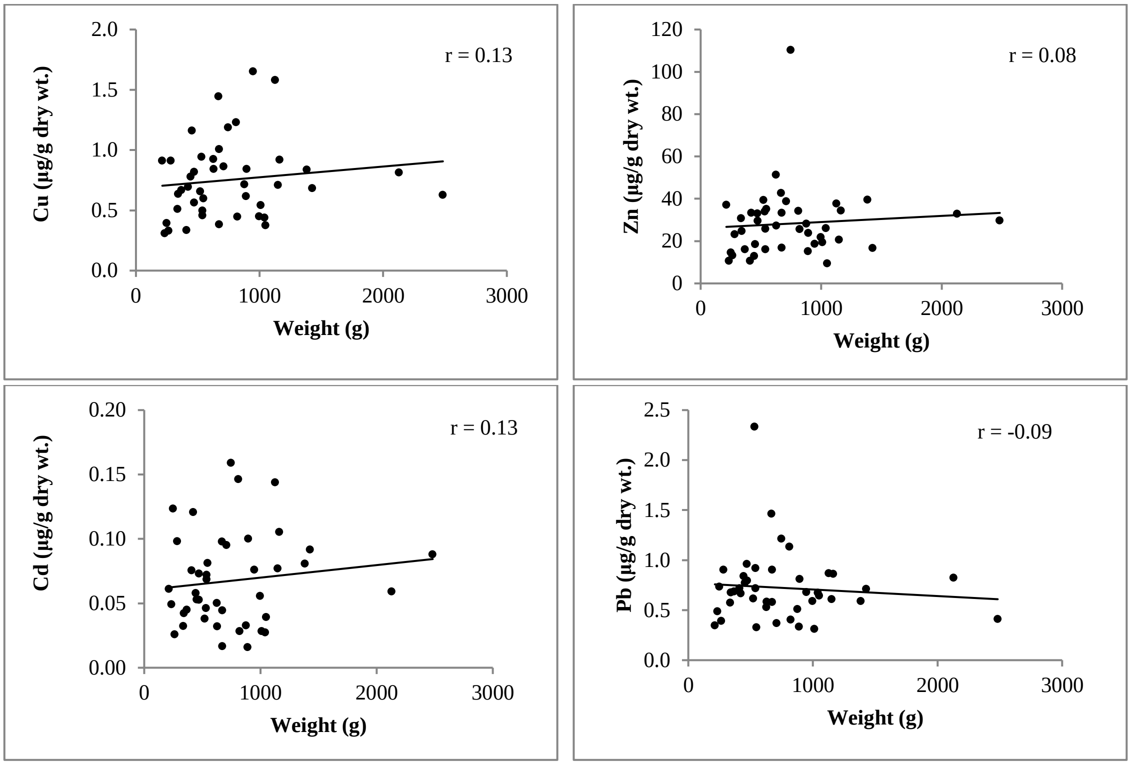
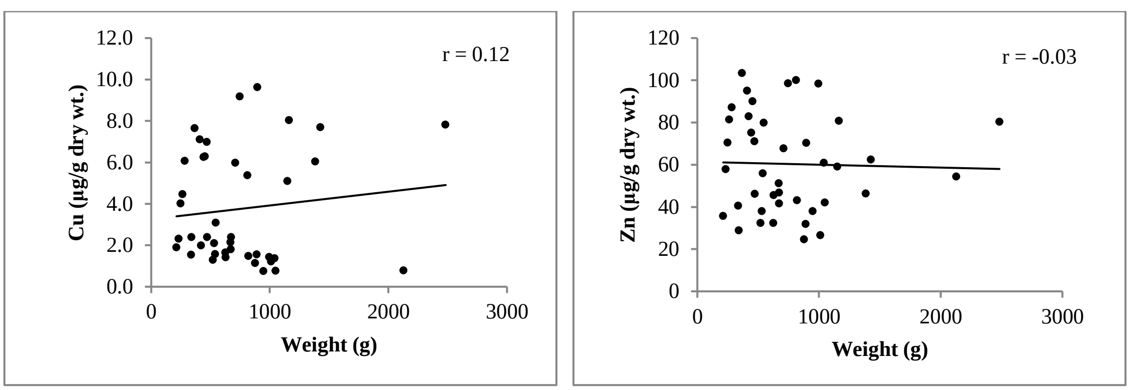


Figure 3. Correlation graph between concentration of selected metals (Cu, Zn, Cd, and Pb) in shark muscle and weight of shark



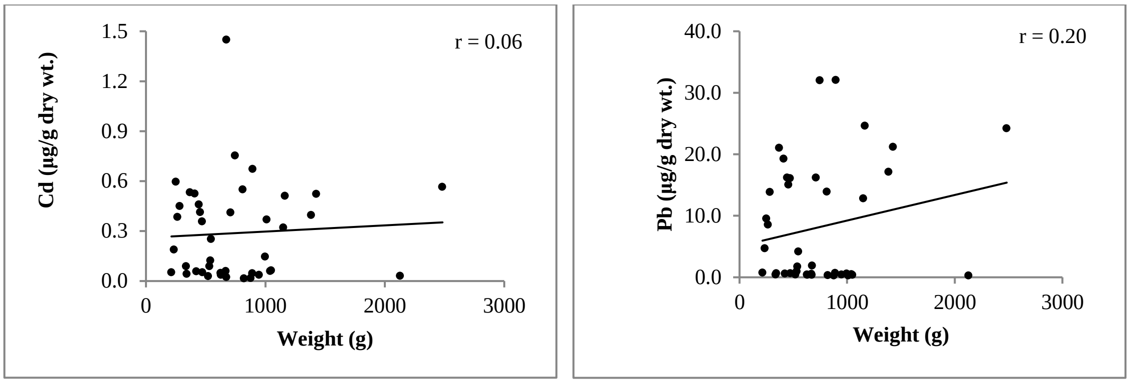


Figure 4. Correlation graph between concentration of selected metals (Cu, Zn, Cd, and Pb) in shark fin and length of shark

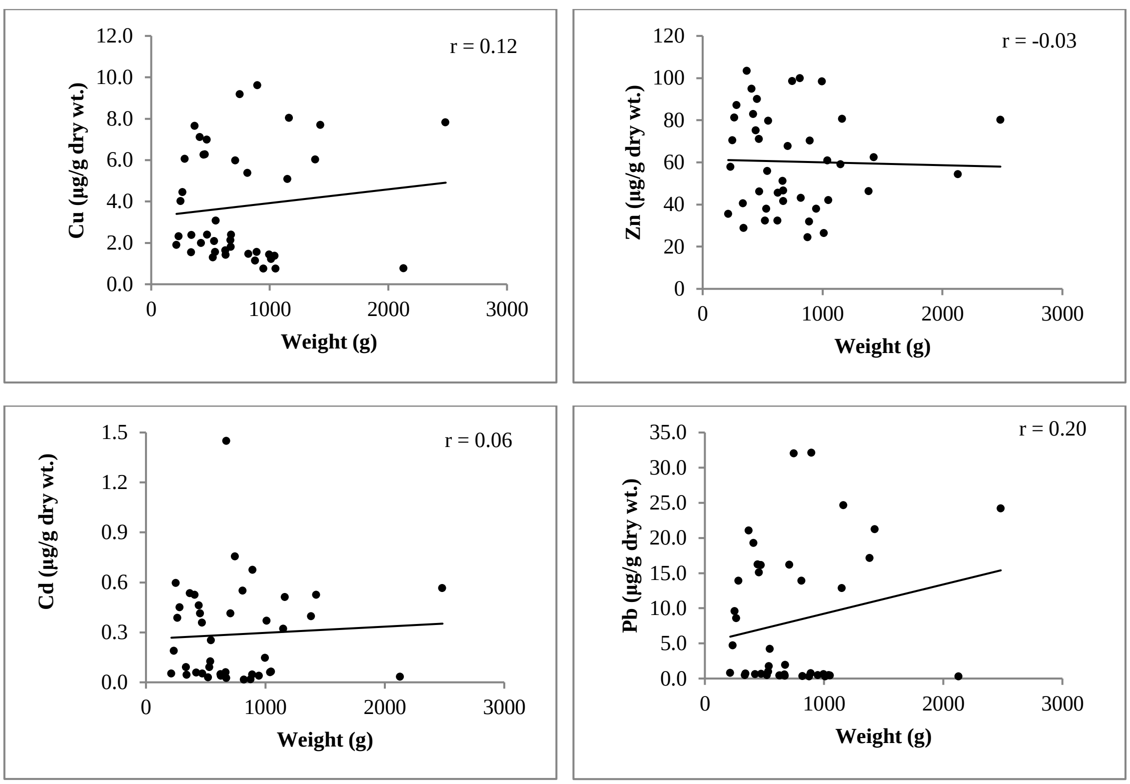


Figure 5. Correlation graph between concentration of selected metals (Cu, Zn, Cd, and Pb) in shark fin and weight of shark

Table 6 presents the correlation between heavy metals in muscle and fin samples, respectively. According to the table, all four metals exhibited a positive correlation among them, indicating a common source of the metals [31]. Among the metals, Cu-Pb demonstrated a strong correlation value of 0.70, further suggesting that these two metals might originate from the same source. According to Yap et al. [32], Cu and Pb might have originated from anthropogenic sources, such as industrial activities and sewage systems.

Table 6. Correlation values between heavy metals in *Chiloscyllium* sample

	Cu	**Zn**	**Cd**
Zn	0.56		
Cd	0.57	0.47	
Pb	0.70	0.48	0.47

The Pollution Load Index (PLI) compares the pollution levels among different sites or at different times [33]. The index provides a general and clear view of the levels of total heavy metal pollution. PLI values below 50 indicate that no action is required, values of 50 and above suggest the requirement of monitoring, while values of 100 and above indicate extreme pollution [34]. PLI was determined using the method described by Angulo [35] based on the Equation 1 and 2.

The calculated PLI values from the *Chiloscyllium* samples are provided in Table 7. Generally, the PLI values of the fins were higher than that of the muscle samples. However, according to the overall PLI values, no action is required in this study area as the average values were far below 50.

$$Pollution\ Load\ Index\ (PLI) = \sqrt[n]{CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n} \quad (1)$$

where, n = number of metals

$$Contamination\ factor\ (CF) = \frac{Concentration\ of\ metal}{Concentration\ of\ background} \quad (2)$$

Table 7. The Pollution Load Index (PLI) of edible tissue of *Chiloscyllium* sp.

	PLI	
	Muscle	**Fin**
Average	3.2 ± 0.9	12.4 ± 10.4
Min	2.6	1.7
Max	4.7	35.0

Conclusion

Based on the findings, *Chiloscyllium* samples obtained from Lumut, Perak, Malaysia, demonstrated a concentration of heavy metals in the muscle and fin samples with a descending order of Zn > Cu > Pb > Cd and Zn > Pb > Cu > Cd, respectively. The concentrations of heavy metals in the fins were higher than that was accumulated in the muscle. Moreover, the uptake of heavy metals is size-dependent and accumulates in the fins at a higher rate. Nevertheless, some previous studies indicated that shark size is not the only factor influencing the heavy metal content in sharks. Other variables such as the shark biological, environmental factors and dietary preferences need to be taken into account to assess the accumulation of heavy metals in sharks. However, based on the PLI values recorded in this study, no monitoring is required currently in the study area. Nevertheless, frequent research could ensure that the degree of pollution in well under control in marine environments. The findings from this study can also be used to estimate the intake of heavy metals by a human *via* the consumption of bamboo shark meat since it is a delicacy for the public.

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References

1. Kotze, P., Du Preez, H. H. and Van Vuren, J. H. J. (1999). Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. *Water SA*, 25(1): 99-110.
2. Guthrie, R. K., Davis, E. M., Cherry, D. S. and Murray, H. E. (1979). Biomagnification of heavy metals by organisms in a marine microcosm. *Bulletin of Environmental Contamination and Toxicology*, 21(1): 53-61.
3. Lopez, S. A., Abarca, N. L. and Meléndez, R. C. (2013). Heavy metal concentrations of two highly migratory sharks (*Prionace glauca* and *Isurus oxyrinchus*) in the Southeastern Pacific Waters: Comments on public health and conservation. *Tropical Conservation Science*, 6(1): 126-137.
4. Stevens, J. D., Bonfil, R., Dulvy, N. K. and Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (*chondrichthyans*), and the implications for marine ecosystems. *ICES Journal of Marine Science*, 57(3): 476-494.
5. Bengil, F. and Mavruk, S. (2019). Warming in Turkish Seas : Comparative multidecadal assessment. *Turkish Journal of Fisheries and Aquatic Sciences*: 1-10.
6. Storelli, M. M., Giacominelli-Stuffler, R. and Marcotrigiano, G. (2002). Mercury accumulation and speciation in muscle tissue of different species of sharks from Mediterranean Sea, Italy. *Bulletin of Environmental Contamination and Toxicology*, 68(2): 201-210.
7. Ong, M. C. and Gan, S. L. (2017). Assessment of metallic trace elements in the muscles and fins of four landed elasmobranchs from Kuala Terengganu waters, Malaysia. *Marine Pollution Bulletin*, 124(2): 1001-1005.
8. Okamoto, K. and Fuwa, K. (1984). Low-contamination digestion bomb method using a teflon double vessel for biological materials. *Analytical Chemistry*, 56(9): 1758-1760.
9. Kamaruzzaman, B. Y., Rina, Z., Akbar John, B. and Jalal, K. C. A. (2011). Heavy metal accumulation in commercially important fishes of south west Malaysian coast. *Research Journal of Environmental Sciences*: 1-8.
10. Ong, M. C., Yong, J. C., Khoo, X. Y., Tan, Y. F. and Joseph, B. (2014). Selected heavy metals and polycyclic aromatic hydrocarbon in commercial fishes caught from UMT enclosed lagoon, Terengganu, Malaysia. *Advances in Environmental Biology*, 8(14): 91-98.
11. Yohannes, Y. B., Ikenaka, Y., Nakayama, S. M. M., Saengtienchai, A., Watanabe, K. and Ishizuka, M. (2013). Organochlorine pesticides and heavy metals in fish from Lake Awassa, Ethiopia: Insights from stable isotope analysis. *Chemosphere*, 91(6): 857-863.
12. Adel, M., Copat, C., Saeidi, M. R., Conti, G. O., Babazadeh, M. and Ferrante, M. (2018). Bioaccumulation of trace metals in banded persian bamboo shark (*Chiloscyllium arabicum*) from the Persian Gulf: A food safety issue. *Food and Chemical Toxicology*, 113: 198-203.

13. Kamaruzzaman, B. Y., Ong, M. C. and Rina, S. Z. (2010). Concentration of Zn, Cu and Pb in some selected marine fishes of the Pahang coastal waters, Malaysia. *American Journal of Applied Sciences*, 7(3): 309-314.
14. Ong, M. C. and Gan, S. L. (2016). Heavy metals concentration in four landed elasmobranchs from Kuala Terengganu waters, Malaysia. *International Journal of Applied Chemistry*, 12(4): 761-772.
15. Adel, M., Oliveri Conti, G., Dadar, M., Mahjoub, M., Copat, C. and Ferrante, M. (2016). Heavy metal concentrations in edible muscle of whitecheek shark, *Carcharhinus dussumieri* from the Persian Gulf: A food safety issue. *Food and Chemical Toxicology*, 97: 135-140.
16. Clearwater, S. J., Farag, A. M. and Meyer, J. S. (2002). Bioavailability and toxicity of dietborne copper and zinc to fish. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 132(3): 269-313.
17. Mohammed, A. and Mohammed, T. (2017). Mercury, arsenic, cadmium and lead in two commercial shark species (*Sphyrna lewini* and *Caraharinus porosus*) in Trinidad and Tobago. *Marine Pollution Bulletin*, 119(2): 214-218.
18. Chow, T. J., Earl, J. L. and Bennett, C. F. (1969). Lead aerosols in marine atmosphere. *Environmental Science and Technology*, 3(8): 737-740.
19. Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, 68: 167-182.
20. Tong, S., von Schirnding, Y. E. and Prapamontol, T. (2000). Environmental lead exposure: A public health problem of global dimensions. *Bulletin of the World Health Organization*, 78(9): 1068-1077.
21. Alemdaroglu, T., Onur, E. and Erkakan, F. (2003). Trace metal levels in surface sediments of Lake Manyas, Turkey and tributary river. *International Journal of Environmental Studies*, 60: 287-298.
22. Alina, M., Azrina, A., Mohd Yunus, A. S., Mohd Zakiuddin, S., Mohd Izuan Effendi, H. and Muhammad Rizal, R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the Straits of Malacca. *International Food Research Journal*, 19(1): 135-140.
23. Al-Yousuf, M. H., El-Shahawi, M. S. and Al-Ghais, S. M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *Science of the Total Environment*, 256(2–3): 87-94.
24. Canli, M. and Atli, G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six mediterranean fish species. *Environmental Pollution*, 121(1): 129-136.
25. Endo, T., Kimura, O., Ohta, C., Koga, N., Kato, Y., Fujii, Y. and Haraguchi, K. (2016). Metal concentrations in the liver and stable isotope ratios of carbon and nitrogen in the muscle of silvertip shark (*Carcharhinus albimarginatus*) culled off Ishigaki Island, Japan: Changes with growth. *PLoS ONE*, 11(2): 1–18.
26. Cornish, A. S., Ng, W. C., Ho, V. C. M., Wong, H. L., Lam, J. C. W., Lam, P. K. S. and Leung, K. M. Y. (2007). Trace metals and organochlorines in the bamboo shark *Chiloscyllium plagiosum* from the southern waters of Hong Kong, China. *Science of the Total Environment*, 376(1–3): 335-345.
27. Vas, P. (1991). Trace metal levels in sharks from British and Atlantic waters. *Marine Pollution Bulletin*, 22(2): 67-72.
28. Mendoza-Carranza, M., Sepúlveda-Lozada, A., Dias-Ferreira, C. and Geissen, V. (2016). Distribution and bioconcentration of heavy metals in a tropical aquatic food web: A case study of a tropical estuarine lagoon in SE Mexico. *Environmental Pollution*, 210: 155-165.
29. Jara-Marini, M. E., Soto-Jiménez, M. F. and Páez-Osuna, F. (2009). Trophic relationships and transference of cadmium, copper, lead and zinc in a subtropical coastal lagoon food web from SE Gulf of California. *Chemosphere*, 77(10): 1366-1373.
30. Conti, M. E., Stripeikis, J., Finoia, M. G. and Tudino, M. B. (2012). Baseline trace metals in gastropod mollusks from the Beagle Channel, Tierra del Fuego (Patagonia, Argentina). *Ecotoxicology*, 21(4): 1112-1125.

31. Nyangababo, J. T., Henry, L. and Omutange, E. (2005). Lead, cadmium, copper, manganese, and zinc in wetland waters of Victoria Lake Basin, East Africa. *Bulletin of Environmental Contamination and Toxicology*, 74(5): 1003-1010.
32. Yap, C. K., Ismail, A., Tan, S. G. and Omar, H. (2002). Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environment International*, 28: 467-479.
33. Wuana, R. A. and Okieimen, F. E. (2011). Heavy metals in contaminated soils : A review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology,* 2011: 402647.
34. Goher, M. E., Farhat, H. I., Abdo, M. H. and Salem, S. G. (2014). Metal pollution assessment in the surface sediment of Lake Nasser, Egypt. *Egyptian Journal of Aquatic Research*, 40(3): 213-224.
35. Angulo, E. (1996). The Tomlinson Pollution Load Index applied to heavy metal, “Mussel- Watch” data: A useful index to assess coastal pollution. *Science of the Total Environment*, 187(1): 19-56.