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SIZE AND ORGAN-SPECIFIC HEAVY METAL ACCUMULATION PATTERNS IN LONGTAIL TUNA (*Thunnus tonggol*) FROM TERENGGANU

(Saiz dan Organ-Khusus Corak Pengumpulan Logam Berat dalam Ikan Aya Hitam (*Thunnus tonggol*) dari Terengganu)

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Abstract

Longtail tuna, *Thunnus tonggol*, belongs to the *Scrombidae* family and is commonly served in special Terengganu delicacies, such as in *Nasi Dagang* gravy. Primarily, the predator fish is found in shallow waters. The Terengganu state is located on the east coast of Peninsular Malaysia, where the oil and gas industry is a primary economic bolster. The rapid urbanisation, large-scale developments, and tourist attraction in the area might contribute to the heavy metal pollution in Terengganu waters. The present study was conducted to determine the concentration of six heavy metals, such as copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) in longtail tuna muscle, gills, liver, and stomach, by employing inductively coupled plasma-mass spectrometry (ICP-MS). The highest concentration of Cu, Cd, and Pb was recorded in the liver. The muscle samples revealed the highest As and Hg concentrations, while the gills documented the maximum Zn concentration. The results also revealed that the heavy metals possessed a significant positive relationship with the tuna size (p <0.05). For instance, Hg demonstrated a strong positive association with fish size, recording a 0.577 r-value. Conversely, the longtail tuna samples indicated apparent growth dilution effects for Cu and Pb, proved by the negative correlations between their concentrations and the fish length. This study successfully outlined comprehensive approaches to better understand the controlling factors, including fish size and targeted organs in determining the concentration of heavy metals in longtail tuna.

Keywords: heavy metal, inductively coupled plasma-mass spectrometry, pollution, South China Sea, *Thunnus tonggol*

Abstrak

Ikan Aya hitam, *Thunnus tonggol*, dikategorikan dalam keluarga *Scrombidae* dan lazimnya dihidangkan sebagai hidangan istimewa di Terengganu seperti dalam kuah Nasi Dagang. Lazimnya, spesis pemangsa ini dijumpai di kawasan air cetek. Terengganu terletak di Pantai Timur Semenanjung Malaysia yang mana industri minyak dan gas merupakan penyumbang ekonomi utama. Pembandaran yang pesat, pembangunan berskala besar, dan tarikan pelancongan di negeri tersebut mungkin menjadi antara punca pencemaran logam berat dalam perairan Terengganu. Kajian ini dijalankan untuk menentukan kepekatan enam logam berat; kuprum (Cu), zink (Zn), kadmium (Cd), plumbum (Pb), arsenik (As) dan merkuri (Hg) di dalam isi, insang, hati, dan perut ikan

Aya hitam menggunakan ICP-MS. Keputusan menujukkan bahawa kepekatan Cu, Cd dan Pb paling tinggi dicatatkan di dalam hati. Isi ikan merekodkan kepekatan As dan Hg paling tinggi, manakala insang mencatatkan kepekatan Zn yang paling tinggi. Keputusan kajian ini juga menunjukkan bahawa semua logam berat mempunyai hubungan positif yang ketara dengan saiz ikan Aya hitam (p <0.05). Contohnya, Hg mempunyai hubungan positif yang kukuh dengan saiz ikan dengan nilai r ialah 0.577. Sebaliknya, ikan Aya hitam menunjukkan kesan pencairan pertumbuhan untuk Cu dan Pb dengan hubungan negatif antara kepekatan logam berat dengan saiz ikan. Kajian ini telah Berjaya menunjukkan pendekatan menyeluruh dan kefahaman yang lebih mendalam terhadap faktor-faktor yang mempengaruhi kepekatan logam berat di dalam ikan Aya hitam, termasukalah saiz ikan dan organ sasaran.

Kata kunci: logam berat, spektroskopi jisim- plasma gadingan aruhan, pencemaran, laut China Selatan, *Thunnus tonggol*

Introduction

Recently, heavy metal pollution has attracted global attention. Uncontrolled heavy metal contamination could pose critical threats to ecosystems and detrimental health impacts on humans. Increasing levels of heavy metal effluents in marine environments from several anthropogenic sources, such as industrial activities, domestic sewage, and agriculture decreased the quality of fish and other aquatic organisms. Moreover, the growing number of vehicles and metal leachings from various sources, including landfills, waste dumps, and runoffs, could contribute to the increasing heavy metal concentrations in marine environments, which might harm marine life organisms and the ecosystem as a whole [1].

Metals could bioaccumulate and biomagnify in food chains and food webs, posing risks to aquatic organisms and human health [2, 3]. Heavy metals also damage multiple organs, even at low exposure concentrations. Fish in metal-polluted environments could be exposed to metals through food chains or via uptake from contaminated water. The introduction of heavy metals into fish organs is predominantly through adsorption and absorption, hence the accumulation rate is defined by the uptake and depuration rates [4].

In Terengganu, longtail tuna, locally known as *ikan Aya hitam* (*Thunnus tonggol*), is one of the most popular fish. The fish is commonly served in special delicacies, including the gravies for *Nasi Dagang* and *Nasi Lemak* and as *ikan singgang*. A survey documented that the Terengganu locals consumed approximately 437.4 g/person of longtail tuna weekly [5], which constitutes 62.49 g of their daily intake. The consumption rate was slightly higher than the average amount of fish consumed by other Malaysians in 2009 at 60.0 kg/person/day [6].

Longtail tuna is a species belonging to the *Scomberoidae* family and the *Thunnus* genus. The fish could reach a maximum total length of 142 cm and is the second smallest of eight *Thunnus* species [7]. The longtail tuna has a relatively slower growth rate than other tuna species. The species is also considered a suitable indicator of environmental pollution by wastewater, microplastic, or heavy metals as they reportedly accumulate higher concentrations of heavy metals [8, 9].

Heavy metal concentrations in longtail tuna could indicate exposure time and the metal contents in the sediments and water columns of the areas from which the fish are sourced. The size of the tuna emulates the exposure period to the contaminated water. At the same time, the concentrations of heavy metals in their organs allow health condition assessments of the area from which they were collected. This study emphasised the relationship between the accumulation of heavy metals and the size of the longtail tuna and targeted organs.

Materials and Methods

Sampling area

The longtail tuna, *Thunnus tonggol*, samples employed in this study were bought from local fishermen of vessel Class B operating in Kuala Terengganu waters (see Figure 1). The specimens were purchased from the fishermen on the same day of capture and promptly brought to the laboratory on ice before being dissected (see Figure 2).

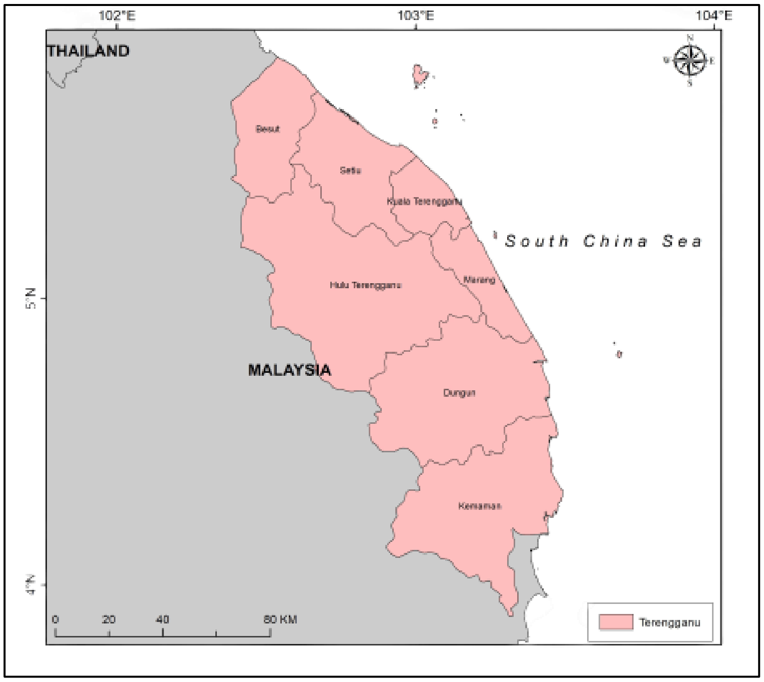

Figure 1. The longtail tuna samples were caught in Kuala Terengganu waters and landed at Lembaga Kemajuan Ikan Malaysia (LKIM) Complex, Pulau Kambing, Kuala Terengganu

Figure 2. The longtail tuna (*Thunnus tonggol*), or locally known as *ikan Aya*, *ikan kayu*, or *ikan tongkol hitam* in Malaysia

Sample collection and preparation

The present study obtained 75 longtail tuna specimens from fishermen before measuring and recording the total length and weight of the samples (see Table 1). Subsequently, the gills, liver, stomach, and muscles were dissected with clean ceramic equipment (see Figure 3). The tissue samples were oven-dried at 60℃ for three days or until the samples recorded a constant weight. The dried specimens were then homogenised with mortar and pestle before digestion.

Table 1. The total length and weight ranges of the longtail tuna samples

Location	***n***	**Total length (cm)**	**Total weight (g)**
Terengganu waters	75	21.1–49.5	339–1537

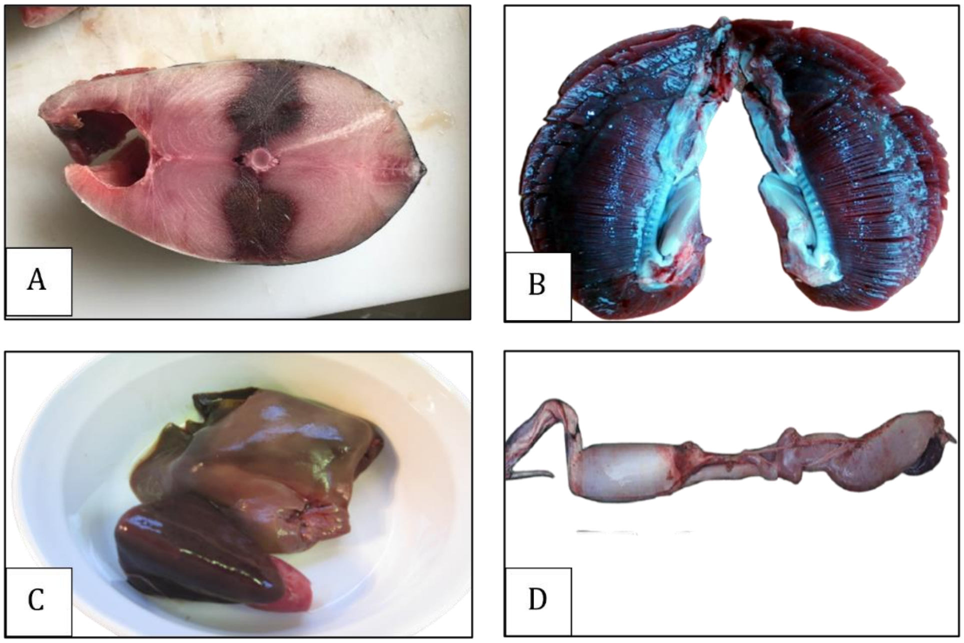

Figure 3. The A: muscle, B: gills, C: liver, and D: stomach samples of the longtail tuna

Samples digestion and heavy metal detection

During the digestion process conducted in this study, 0.05 g of the homogenised dried sample was weighed and mixed with 1.5 mL of Suprapur nitric acid (HNO_3; Merck KGaA, Germany) in an acid-washed Teflon beaker. The Teflon beaker was closed with a cap in a Teflon bomb before being placed into an oven for 8 h at 100 °C to allow complete digestion of the samples [10-12]. The digested samples were cooled to room temperature, transferred into centrifuge tubes, and diluted with deionised water up to 10 mL. Finally, the samples were stored at under 4 ℃ before selected metal element (Cu, Zn, Cd, Pb, As, and Hg) measurements with inductively coupled plasma-mass spectrometry (ICP-MS) (ELAN 9000) [13].

The standard reference material employed in this study, Dolt-4 Fish Liver SRM 1946 (National Research Council Canada), and blank specimens were digested following a procedure similar to the longtail specimens. The blank samples were prepared with the Suprapur HNO_3 without adding the fish samples. The standard solutions utilised in the present study were yielded via mixing the Suprapur HNO_3 with 0.05 g of DOLT-4 fish liver [14]. The current study constructed the standard to enhance confidence in the levels of heavy metals determined in the longtail tuna samples evaluated.

Statistical analysis

A Shapiro-Wilk (p-value <0.05) normality assessment is conducted to assess the distribution of data before constructing reference intervals and variables. Normality evaluations are required for accurate and reliable conclusions. Generally, non-normally distributed data are subjected to non-parametric assessments. This study applied Friedman and Spearmank's rank, which are non-parametric evaluations, based on the variables involved.

Heavy metal concentrations in the muscle, gills, liver, and stomach of the longtail tuna samples

The present study obtained non-normally distributed data. Accordingly, a Friedman evaluation, which is a non-parametric assessment, was conducted to determine the differences between the independent groups (muscle, gills, liver and stomach) when the dependent criterion (heavy metal concentration) was measured as a continuous parameter (ratio). The employment of the Friedman evaluation was appropriate for the present study, as the concentration of the heavy metals was measured in triplicates or more. The variables also recorded a significant difference when the p-value was under 0.05.

The selected heavy metal concentrations in longtail tuna of different sizes

The data in the present study did not assume normality. Consequently, a non-parametric linear correlation analysis, Spearman's rank, was applied to determine the strength and direction of the associations between two ranked variables: the size of the tuna samples (independent variable) and the concentration of the heavy metals (dependent variable) detected. The non-parametric evaluation was employed as two parameters in this study were continuous (ratio). The Spearman's rank assessment was also utilised to determine the strength of following monotonic relationships.

(i) Increasing longtail tuna size improves the concentration of heavy metals.
(ii) Improving longtail tuna size decreases the concentration of heavy metals.

The Spearman's correlation coefficient, r_s, which might be between +1 and −1, were defined as follows.

(i) A +1 r_s indicates a perfect association of ranks
(ii) A zero r_s zero denotes no association between ranks
(iii) A −1 n r_s demonstrates a perfect negative association of ranks

Results and Discussion

Heavy metal contents in the muscle and target organs of the longtail tuna from Terengganu waters

Heavy metal concentrations (Cu, Zn, Cd, Pb, As, and Hg) in the muscle, gills, stomach, and liver of the longtail tuna (*Thunnus tonggol*) specimens collected caught in Terengganu waters are listed in Table 2. The heavy metals were statistically significant (*p*-value <0.05) to the tissues of the longtail tuna samples, including the muscle, gills, liver, and stomach.

Based on the results, the muscle and organ samples recorded variations in the levels of heavy metal accumulated. For example, the muscle tissues recorded the highest accumulation of As and Hg than the other metals, while Zn demonstrated a higher tendency to accumulate in the gills. On the other hand, Cu, Cd, and Pb were highly concentrated in the liver.

Table 2. The average concentration of heavy metals detected in the muscle, gills, stomach, and liver of the longtail tuna (*Thunnus tonggol*) samples

	The Average Concentration of Heavy Metals (mg/kg) Dry Weight			
Metal	**Muscle**	**Gills**	**Stomach**	**Liver**
Copper	4.46 ± 6.67	1.98 ± 1.81	4.32 ± 5.32	11.2 ± 7.34
Zinc	13.3 ± 18.6	56.1 ± 71.4	31.4 ± 41.5	32.3 ± 22.2
Cadmium	0.060 ± 0.183	0.086 ± 0.129	0.310 ± 0.384	4.73 ± 5.30
Lead	0.075 ± 0.222	0.076 ± 0.118	0.026 ± 0.022	0.087 ± 0.117
Arsenic	10.5 ± 10.2	2.44 ± 1.41	7.98 ± 5.59	7.54 ± 4.90
Mercury	0.114 ± 0.16	0.020 ± 0.023	0.052 ± 0.048	0.080 ± 0.217

Heavy metals could accumulate in most organs in a fish, including the skin, gills, intestine, and kidneys, which might harm the fish physically and internally [15]. Moreover, even trace amounts of heavy metals could be toxic to fish. Heavy metal toxicity in marine organisms depends on several factors, such as exposition routes, nutritional status of the exposed organism, chemical species, genetics, age, and gender [16, 17]. The direct absorption of heavy metals in fish occurs through water columns, while an indirect route is via food chains. Nevertheless, heavy metal concentration variabilities in fish organs could also be due to the types of tissues and organs, which significantly depend on the concentration of heavy metals in the surrounding waters [18, 19].

Among the six elements evaluated in the present study, Hg and As recorded higher concentrations in fish muscle than the other targeted organs. Previous reports hypothesised that the higher tendency of Hg to accumulate in fish muscle is related to its high affinity to the thiol groups in amino acids, a protein component in muscle tissues [20-22] also suggested that the metals bioaccumulated in muscle tissues could be linked to the feeding habits of the species, contributing to metal level variations [23]. Large predatory fish, such as longtail tuna, consume numerous smaller fish, accumulating Hg in their muscles. Post-Hg assimilation in fish, the contaminant is distributed throughout the organism via blood and stored in various tissues, specifically the muscles [24]. Similarly, this study recorded the highest As levels in the muscle samples. The observations might be due to its diet since longtail tuna have a high feeding rate and are carnivorous and active predators [25].

Besides food, heavy metals could also be absorbed into fish organs via mechanical captures of suspended hydroxide particles in the gills and chemical ion absorptions via membranes [26, 27]. In this study, Zn recorded the highest concentration in fish gills at 56.1 ± 71.4 mg/kg dry weight. Fish gills consist of epithelial membranes primarily containing mucus-covered phospholipids. The gill surfaces are negatively charged, thus providing potential sites for interactions with positively charged metals [27]. Fish also assimilate metals through ionic exchanges across lipophilic membranes in their gills during respiration [28].

Dural et al. [29] found that the accumulation of heavy metals predominantly occurred in metabolically active tissues, such as gills and the liver. A higher metal accumulation in fish liver correlates with the presence of natural protein binding, known as metallothionein (MTs), which stores the metals to fulfil enzymatic and other metabolic demands in the fish [30]. The MTs are small cysteine-rich proteins that are critical in metal homeostasis and protection against heavy metal toxicity, DNA damage, and oxidative stress [30].

Metalloregulatory processes involve MTs by binding the natural proteins to heavy metals through the thiol groups in their cysteine residues. The MTs have a high affinity for heavy metals, hence possessing the ability to bind to xenobiotic heavy metals to protect against metal toxicity, especially Cd [31]. Furthermore, MTs attaching to physiological heavy metals, such as Cu, enable their participation in regulating cell growth and proliferation and protecting the fish against oxidative stress [32]. The MTs also act as an essential metal store to meet enzymatic and other metabolic demands [33]. Nonetheless, higher toxic element concentrations, including Cd and Pb, could lead to adverse health effects as they compete with calcium (Ca^{2+}) at enzymatic locations [34].

The relationship between the selected heavy metal concentrations and the size of the longtail tuna samples caught in Terengganu waters

Generally, the size of a longtail tuna could affect the heavy metal concentration in its muscles. The length of the longtail tuna is commonly considered the fundamental measure as its probability to change considerably is low [35]. The standard length of a fish is the measurement from its closed mouth to the end of the fleshy part of its body [36].

This study assessed the strength of associations between the variables, which were the size of the specimen and the concentration of heavy metals detected. Resultantly, three relationship strength ranks, low, medium, and strong, were obtained (see Table 3). A linear correlation analysis, the Spearman's rank correlation assessment, was employed in the current study to determine the relationship between heavy metal concentration and the longtail tuna size (standard length).

Table 4 demonstrates the relationship value (r) that indicates the strength of the correlation between the parameters evaluated. The p-values obtained represent the relationship significance between the variables. r-values approaching 1 indicated the crucial role of size and strong correlation with heavy metal accumulation.

Table 3. The *r*- and *r* squared (R^2)-values of the linear correlation between the longtail tuna size and the heavy metal concentration

***r*-value**	**R^2-value**	**Indication**
0.1–0.29	0.01–0.084	Low relationship association
0.3–0.49	0.09–0.24	Medium relationship association
0.5–1.0	0.25–1.0	Strong relationship association

Table 4. The Spearman's rank correlation coefficient (*r*) and significance levels (*p*) of the association between heavy metal concentrations in the muscle of the longtail tuna and their size

Element	**Muscle**	
	***r*-value**	***p*-value**
Cu	−0.435	0.000*
Zn	0.353	0.000*
Cd	0.237	0.001*
Pb	−0.192	0.006*
As	0.469	0.000*
Hg	0.577	0.000*

In this study, positive relationships were observed between the fish size and metal levels. Nevertheless, negative associations were demonstrated by Cu and Pb with the longtail tuna muscle samples. The results paralleled a report that documented positive correlations between the size of *Branchiostoma belcheri* with the concentration of Zn, Hg, and Cd [37]. Farkas et al. [32] also found a positive correlation in Hg, Cu, and Zn with the size of *Abramis brama* L. In another report, fish length was positively associated with the concentrations of Cr, Cu, Fe, and Pb but recorded a negative relationship with Cd, Cu, Cr and Pb levels [38].

Typically, increasing fish size reflects the age of the fish [30]. Heavy metal concentration assessments of marine fish from the Oman Gulf found that Hg levels increased with the age and size of the fish [39]. Douben et al. [40] suggested that metal accumulation in fish reaches a steady state after a certain age. Nonetheless, if metal concentrations in the surrounding water are higher than the capacities of growth associated-tissues and lowered metabolic activities in older (larger) organisms, it might not affect the dilution of the metal levels in the fish. Thus, continued metal accumulation might occur, and a positive relationship could be observed. Conversely, the significant negative relationship between Cu and longtail tuna size documented in this study could be due to the slow uptake rates of Cu in muscles compared to other tissue [41]. Furthermore, Cu elimination is reportedly efficient in muscle tissues [41].

Heavy metal concentration could also be affected by environmental contamination and exposure duration [42, 43]. Therefore, larger or older longtail tunas are expected to have accumulated higher metal concentrations than their smaller or younger counterparts. Since longtail tuna is a predator fish, rising heavy metal levels with increasing fish size could also be related to the greater consumption of contaminated prey from higher trophic levels [44].

Conclusion

The concentration of heavy metals in the longtail tuna fish specimens assessed in this study varied significantly. The differences were not only attributable to the size of the specimen but also to the targeted organs. The functions and properties of the organs, such as the presence of natural protein binding, metallothioneins, in fish liver, also influenced the remarkable ability of the organs to absorb more heavy metals. Heavy metal concentrations in the environment, such as sediment and the surrounding water, also affect heavy metal contamination degrees in fish. Nevertheless, the heavy metal levels in the areas were not measured in the current study.

The findings reported in this study provided the current status of heavy metal contents in longtail tuna from Terengganu waters. The data could assist the Malaysian Environmental Department in evaluating, monitoring, and controlling metal pollutant discharges into marine environments. The information also documents the quality of longtail tuna, which could be beneficial to the Department of Fishery Malaysia (DOF) in enhancing the quality of seafood sources.

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