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ASSESSMENT OF HEAVY METAL CONTAMINATION IN SQUID (*LOLIGO SPP.*) TISSUES OF KEDAH-PERLIS WATERS, MALAYSIA

(Penilaian Pencemaran Logam Berat dalam Tisu Sotong *(Loligo Spp.)* bagi Perairan Air Kedah-Perlis, Malaysia)

Jamil, T.*, Lias, K., Norsila, D., Syafinaz, N. S.

Center of Ocean Research, Conservation & Advances (ORCA)
Division of Research, Industrial Linkage, Community Network & Alumni
Universiti Teknologi MARA (Perlis), 02600 Arau, Perlis, Malaysia

**Corresponding author: jamiltajam@perlis.uitm.edu.my*

Abstract

This study was carried out to determine the concentration of selected heavy metals (Cd, Cu, Pb and Zn) in three different tissues collected from the ink-sac, head, and muscle of the squid. Squid samples were caught off the coastal waters of Kedah-Perlis, where only mature squids within the maturity size-range of 13 to 15 cm were used. From this study, the concentration of Zn (35.06±4.06 mg/kg in dry weight) was found significantly higher in all of the tissues, followed by Cu (15.10±13.28 mg/kg in dry weight), Cd (4.76±3.77 mg/kg in dry weight), and Pb with an average value of 4.01±0.08 mg/kg in dry weight. From this study, it was discovered that Zn and Cu concentrations in the tissues were lower than the maximum limit recommended by the Food and Agriculture Organization, Malaysian Food Regulation (1985), as well as other countries. Meanwhile, both non-essential elements namely Cd and Pb recorded higher values than that of the recommended concentrations. According to the Metal Pollution Index (MPI), the ink-sac indicated medium-range contamination, while the head and muscle tissues showed low contamination levels.

Keywords : Heavy Metal, Squid (Loligo spp) and Metal Pollution index

Abstrak

Kajian ini telah dijalankan untuk menentukan kepekatan logam berat terpilih (Cd, Cu, Pb dan Zn) dalam tiga tisu sotong yang berbeza iaitu kantung dakwat, kepala, dan otot. Sampel sotong telah dikumpulkan dari sekitar Perairan Kedah-Perlis. Hanya sotong yang bersaiz matang sahaja dipilih, iaitu sotong yang bersaiz di antara 13 - 15cm bagi tujuan analisis ini. Daripada kajian ini, kepekatan Zn (35.06 ± 4.06 mg/kg berat kering) telah didapati jauh lebih tinggi di dalam kesemua tisu, diikuti dengan Cu, Cd dan Pb dengan nilai purata masing-masing adalah 15.10 ± 13.28 mg/kg berat kering, 4.76 ± 3.77 mg/kg berat kering dan 4.01 ± 0.08 mg/kg berat kering. Kajian ini telah menunjukkan bahawa kepekatan Zn dan Cu dalam tisu sotong adalah lebih rendah daripada had maksimum yang disyorkan oleh Pertubuhan Makanan dan Pertanian (FAO), Peraturan Makanan Malaysia (1985), dan lain-lain negara. Sementara itu, kedua-dua elemen Cd dan Pb telah menunjukkan nilai kepekatan yang lebih tinggi daripada nilai yang telah disyorkan. Menurut Indeks Pencemaran Logam (MPI), kantung dakwat sotong telah menunjukkan tahap pencemaran pada julat sederhana, manakala tisu dari bahagian kepala dan otot menunjukkan terdapat pencemaran pada peringkat yang rendah.

Kata kunci: Logam Berat, Sotong (Loligo Spp), Indek Pencemaran Logam

Introduction

Nowadays, seafood especially squid *(Loligo Spp.)* from the Cephalopoda class (Figure 1) has a high demand due to their nutritional values. According to Guerra et al. [1], cephalopod resources increase continuously because of their importance and short life cycle. Squids have good-tasting flesh and that makes them commercially favourable among people since the early 80's [2]. Apart from that, they play an important role in the marine ecosystem as they become prey to some marine mammals [3].

Lourenço et al. and Hannalore et al. have stated that squids may provide some of the heavy metals required by the human body [3, 4]. However, humans are also indirectly more susceptible to heavy metal poisoning by consuming this organism [1, 6]. According to Paolo et al. [7], this species is said to be vulnerable to heavy metal contaminants because of their feeding habits. Meng et al.[8] has stated that contamination from heavy metals may occur due to the marine environment being continuously polluted by human activities. Heavy metals may then transfer to and accumulate in marine organisms through the food chain [9].

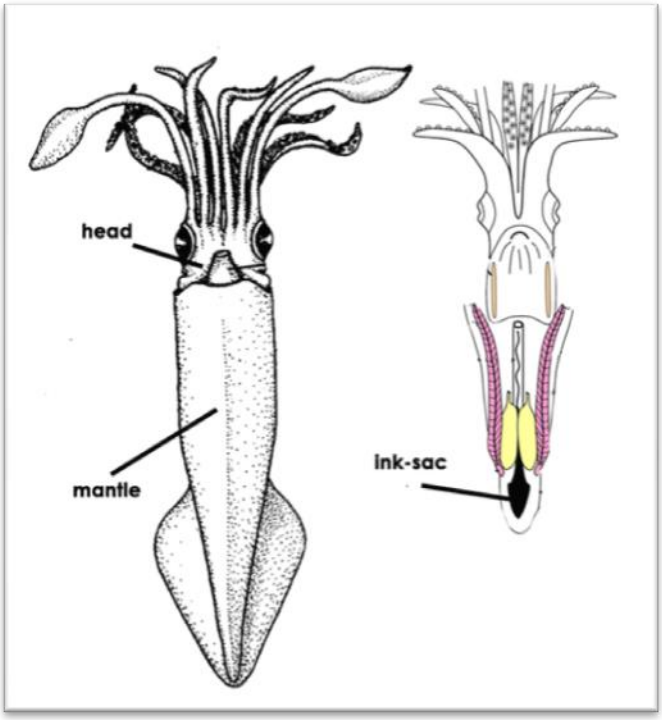

Figure 1: Squid *(Loligo Spp.)* from the Cephalopoda class

In this study, copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn) have become the main focus due to the fact that all of these elements are found accumulated excessively in squids [4, 10, 11, 12]. This phenomenon occurs because squids need Cu and Zn for their metabolic reaction [13, 14], while the concentration of Cd could be present due to the by-product of the reaction between Zn, Cu and Pb. According to Paolo et al. [7], Cd is also released from biogenic detritus in order to regenerate phosphate and nitrate. Pb, on the other hand, is present in the marine environment through atmospheric deposition and soil erosion, as well as vehicle exhaust, and industrial discharge [15].

According to Aaron et al. [16], these four elements have their own role in the human body, but they may become harmful if the amount taken is high. The imbalance of Cu consumption will lead to chronic diseases, such as Wilson disease, Alzheimer disease and Menkes Syndrome [17], while Cd, Pb and Zn may cause chronic kidney damage and even cancer [18]. Therefore, the amount taken should be minimized in order to avoid being adversely affected. In this regard, the assessment of heavy metals in seafood is needed in order to ensure the safety of the foods for the sake of human health.

Materials and Methods

Sample collection and preparation

Fresh squid samples were purchased directly from fishermen with their commercial catches landed at the fisheries harbours of Kuala Kedah. The samples were randomly selected according to their maturity size, where the average length of the samples ranged from 13 to 15 cm. This was in the range of commercial grades. Samples were then immediately iced and brought to the laboratory in insulated boxes. These were either analyzed fresh or kept frozen (-20^{o}C) until further analysis. At the laboratory, squid samples were carefully dissected in order to separate the ink-sac, head and mantle tissues. The samples were then dried in the oven at 80°C until a constant weight was obtained. This step should be carefully monitored as to avoid losses of volatile elements such as As, Cd, and Pb during analysis. When the samples have reached a constant weight, they were allowed to cool in the desiccators before they were crushed into a fine powder by using a porcelain mortar and pestle afterwards.

Sample analysis

In this study, the digestion and analytical procedures were adopted and applied from the Milestone Microwave Digestion Method (DG-FO-17). For this analysis, 0.5 g of the homogenized squid sample was weighed and put into the Teflon vessel. After that, 7 ml of HNO_3 (65%) and 1 ml of H_2O_2 (30%) were added into the Teflon vessel using a single channel pipette, 100-1000 microlitre (µl) of the brand CappAero which was ISO 9001; 2000 certified. Afterwards, the Teflon bombs were placed into the oven at 200˚C for 30 minutes. The samples were then poured into centrifuge tubes and filled-up to 10 ml with Mili-Q water. These procedures were later repeated for the DORM-3 (Fish Protein Certified Reference Material for Trace Metals). The analysis of Heavy metals concentration was then carried out by using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Perkin Elmer model Nexion 300X).

For method validation purpose, a certified reference material for trace metals, DORM-3 (Fish Protein Certified Reference Material for Trace Metals) was digested and heavy metal levels were determined in the same manner as our samples. The limit of detection for each heavy metal was calculated from three standard deviations of eight method blanks.

Heavy Metal Assessment Index

Metal Pollution Index (MPI) equation is used in order to identify the degree of pollution of both squid and cuttlefish samples [19]. The equation (1) is as shown below:

$$MPI = (Cf_1 \text{ X } Cf_2 \ldots\ldots\ldots\ldots Cf_K)^{1/K} \quad (1)$$

where Cf_1 is concentration value of the first metal, Cf_2= concentration value of the second metal and Cf_k= concentration value of the kth metal. The MPI index values are then tabularised according to what is shown in Table 1.

Table 1: MPI index value with the corresponding pollution criteria

Index Value	Degree of Pollution
MPI > 2	Not impacted
2 < MPI < 5	Very low contamination
5 < MPI < 10	Low contamination
10 < MPI< 20	Medium contamination
20 < MPI < 50	High contamination
50 < MPI < 100	Very high contamination
MPI > 100	Extreme contamination

Results and Discussion

Validity of Methods

For method validation, a certified reference material (DORM-3; National Research Council, Canada) was determined as a precision check. The percentage of recoveries (n = 4 for each metal) for certified and measured concentration of those metals was satisfactory, with the recoveries being 93.10 – 105.07%. Table 2 shows the recovery test results for SRM (1646a) analysis.

Table 2: Determined value, certified value and percent (%) recovery of heavy metal contents of the DORM-3 as validation for analytical technique

Heavy Metal	Measured Value (mg/kg)	Certified value (mg/kg)	% Recovery
Cd	0.27±0.04	0.290±0.02	93.10
Cu	14.6±0.21	15.5±0.63	94.42
Pb	0.401±0.32	0.395±0.05	101.52
Zn	53.9±2.10	51.3±3.10	105.07

Accumulation of heavy metals in squids

According to the results shown in Table 3 and Figure 2, there were significant differences ($p<0.05$) among the concentrations of heavy metals in the three parts of squid tissues (mantle, head, and ink-sac). The average concentrations of heavy metals in squids were 35.06 ± 4.06 mg/kg for Zn, 15.10 ± 13.38 mg/kg for Cu, 4.76 ± 3.77 mg/kg for Cd and 4.01 ± 0.08 mg/kg for Pb. Generally, the trend of heavy metals' mean concentration in squids can be seen as Zn>Cu>Cd>Pb. In regard of the accumulation of heavy metals in specific parts of the squid, the most potential part seemed to be the ink-sac, followed by the head and the mantle, with the following trend: ink-sac>head>mantle. From this study, Zn and Cu were found to be abundant in major tissues of the squid. In a previous study done by Craig and Overnell [20], it was also found that Zn and Cu concentrations were significantly high in squids.

Table 3: Heavy metal concentrations (mg/kg) in squid tissues

Squid Tissue	Metal			
	Zn	Cu	Cd	Pb
Mantle	30.6	4.29	1.39	3.93
Head	36.01	10.96	4.07	4.09
Ink-sac	38.56	30.06	8.83	4.02
Average	35.06 ± (4.06)	15.10 ± (13.38)	4.76 ± (3.77)	4.01 ± (0.08)

Figure 2 indicates that the ink-sac was the most contaminated tissue compared to head and mantle. From the observation, Zn concentration was found to be extremely high in the ink-sac with the value of 38.56 mg/kg. This might be due to the composition of melanin contents in the ink. According to Luçero et al. [21], melanin pigment is the main composition of the ink-sac of squids and other cephalopods. Furthermore, Slominski et al., [22] has stated that melanin in squid ink-sac is the derivation of oxidized L-DOPA (L-3,4-dihydroxyphenylalanine). The oxidation process of L-DOPA involves metal cations such as Cu, Zn, Mn and Fe [22, 23]. Therefore, the characteristic of melanin which enables it to bind with this element was one of the reasons why the ink-sac accumulates higher concentrations of heavy metals compared to muscle and head tissues [4, 22, 24].

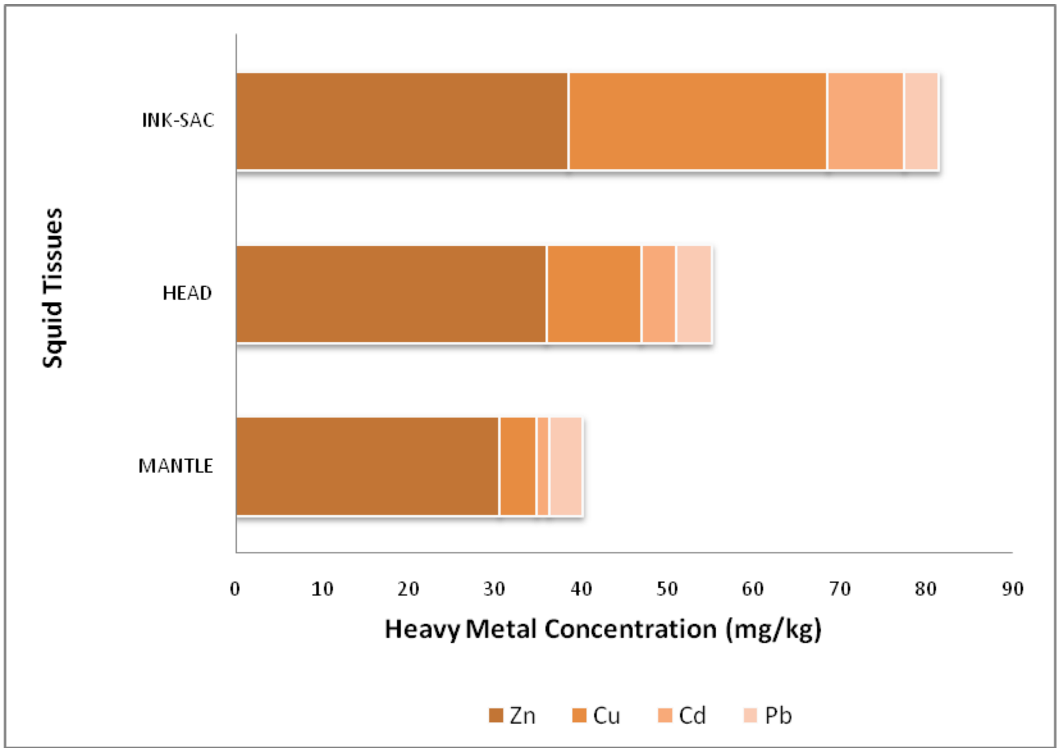

Figure 2: Accumulation of heavy metals in squid tissues

Meanwhile, Zn concentrations in the head tissues were slightly lower compared to the ink-sac with the value of 35.06 ± 4.06 mg/kg. This might be due to the eyes of squids which also contain melanin components. According to Daw and Pearlman [25], and Fox and Crane [26], the retina of the squid eye also contains melanin, but the amount is still little compared to that of the ink-sac. In general, among other heavy metal elements, Zn was found in higher values in squid tissues. According to Tukimat et al. [27], Zn element is always found abundant in marine organisms. Furthermore, Zn is one of the essential elements required by aquatic organisms for their metabolic processes [28].

Cu displayed the second highest in heavy metal content in squid tissues with a mean concentration of 15.10 ± 13.38 mg/kg. Just like Zn, metal cation of Cu is also one of the essential elements in the oxidation process of L-DOPA in producing melanin in the ink-sac and eye tissues of squids [22]. However, the mantle tissue revealed the lowest concentration of Cu, with the value of 4.29 mg/kg. This is due to the movement of oxygen through the mantle via haemocyanin. Squids and other cephalopods use haemocyanin protein in transporting oxygen in their bodies [29]. The accumulation of Cu in muscle tissues of squids still occurs even at low concentrations. This is due to the transportation of oxygen that takes place in the muscle. According to Mommsen et. al. and Ballantyne, muscle tissues of squids consist of both white and red muscle but majorly are white [30, 31]. Both of these muscles have different characteristics. White muscles lack mitochondria, low in blood supply but high in levels of glycolytic enzymes and do anaerobic respiration [30, 32]. Meanwhile, red muscles are high in myoglobin, mitochondria, and oxidative enzymes and do aerobic respiration [30, 32]. The lack of mitochondria can be the answer to why muscle tissues of squids accumulate the lowest levels of Cu.

Meanwhile, Cd recorded lower concentrations with a mean value of 4.76 ± 3.77 mg/kg. This is because generally all of the three squid tissues have a low ability to carry the accumulation of this element compared to the other tissues of the squid body. In a previous study done by Koyama et al. [33], it was stated that muscle, head and ink-sac tissues of squids were accumulated with Cd at the lowest concentration compared to the digestive tract, liver and gills. According to Kojadinovic et al. [34] and Bustamante et al. [35], Cd is found abundant in marine animals including cephalopods because Cd is required for their physiological and detoxification processes that occur in the squid body. Squids and other cephalopods need Cd for their homeostasis and detoxification processes.

Last but not least, the concentration of Pb was the lowest among the other heavy metals in squid tissues, with the value of 4.01±0.08 mg/kg. From the observation, the distribution of this metal was constant in all squid tissues. Koyama et al. [33], and Bustamante et al. [35] have stated that Pb will accumulate in squid tissues by means of seawater absorption through their skin. The low value of Pb concentration in the present study may indicate that the mantle of the squid is not the target organ for Pb accumulation [36].

Safety Issues for Human Consumption

Contamination levels of heavy metals in marine organisms including squids are usually compared with the permissible limits set by Food and Agriculture Organization (FAO), World Health Organization (WHO) and other countries. Malaysian Food Regulation [37] has also set a limit for contamination levels of heavy metals. Table 4 presents the comparison between a set of four selected heavy metal concentrations obtained from this study (Zn, Cu, Cd, Pb) and the recommended permissible limit set by several countries.

Table 4: Comparison of heavy metal concentrations with recommended permissible limit[1-6]

Heavy Metal	Present Study (mg/kg)	Permissible Limits (mg/kg)	References
Zn	35.06 ± 4.06	<100	1,2
Cu	15.10 ± 13.38	<30, <20	1,2
Cd	4.76 ± 3.77	1, 2, 3, 2	1,3,4,6
Pb	4.01 ± 0.08	2, 0.5, 1.5, 0.5	1,2,4,5

[1]Malaysian Food Regulation [37], [2]Minsitry of Public Health, Thailand [38], [3]Australia and New Zealand Food Authority Amendment No. 53 [39], [4]US Food and Drug Administration [40], [5]FAO. Report of the Codex Committee on Food Additives and Contaminants [41], [6]FAO. Report of the Codex Committee on Food Additives and Contaminants [42]

From Table 4, the average concentrations of Zn and Cu were within the permissible limits. However, for the non-essential metals, both Cd and Pb concentrations were found higher compared to the values of the recommended permissible limits. According to Castro González and Méndez-Armenta [43], Cd toxicity in humans may affect some organs such as kidney, lung, bones, brain, as well as the central nervous system. Meanwhile, Pb poisoning in humans may lead to diseases like gout, kidney problems and autistic offspring [44]. Furthermore, huge amounts of Pb accumulation in marine organisms can cause genetic alteration and reduce metabolic reactions, therefore reducing the quality of the flesh of commercial organisms, such as squids [45]. Therefore, due to Cd and Pb contamination, squids inhabiting the Kedah-Perlis coastal waters may not be a proper food source for humans.

Metal Pollution Index (MPI)

In order to evaluate the degree of heavy metal contamination in squid tissues, Metal Pollution Index (MPI) was applied. The contamination levels in squids taken from the Kedah-Perlis waters are displayed in Table 5. From the observation, the contamination in each tissue ranged from low contamination to medium contamination.

Table 5: The MPI values for squids in the Kedah-Perlis waters

Sample	MPI	Index Value	Degree of Pollution
Squid mantle	5.15	5 < MPI < 10	Low contamination
Squid head	9.01	5 < MPI < 10	Low contamination
Squid ink-sac	14.24	10 < MPI < 20	Medium contamination

The concentrations of heavy metals in mantle and head tissues were within the range of low contamination (5<MPI<10), where the MPI values are 5.15 and 9.01, respectively. However, the MPI value for ink-sac tissues was within the range of medium contamination (10<MPI<20).

Conclusion

The accumulations of heavy metals are significantly high in the ink-sac tissues compared to head and muscle tissues, except for Pb. The order of heavy metal content in squids is as follows: Zn > Cu > Cd > Pb. Compared to the recommended permissible limits, the concentration of Zn and Cu in squid tissues are still lower than that of safety standards. Meanwhile, the concentration of Cd and Pb are higher than that of safety standards. However, the average value of MPI has shown that the heavy metal concentration in squid tissues is at a low contamination level (5 < MPI < 10). Further research and close monitoring are therefore necessary to follow changes in contamination levels. Further investigation may also provide a better view of contaminant sources, particularly for cadmium and lead.

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