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Heavy Metals Uptake by Asian Swamp Eel, *Monopterus albus* from Paddy Fields of Kelantan, Peninsular Malaysia: Preliminary Study

Sow Ai Yin, Ahmad Ismail* and Syaizwan Zahmir Zulkifli

Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstrak: Belut sawah, *Monopterus albus*, adalah salah satu ikan yang biasa dijumpai di sawah padi, dan ikan ini sesuai digunakan sebagai bio-penunjuk untuk penyelidikan pencemaran logam berat di kawasan sawah padi. Penyelidikan ini dilakukan bagi menilai kandungan logam berat dalam belut sawah dari sawah padi di Kelantan, Malaysia. Keputusan telah menunjukkan zink [Zn (86.40 μg/g berat kering)] adalah logam paling tinggi terakumulasi dalam ginjal, hati, tulang, insang, otot dan kulit. Antara organ-organ yang telah dipilih, insang didapati mengandungi kepekatan tertinggi bagi plumbum (Pb), kadmium (Cd) dan nikel (Ni), manakala otot menunjukkan jumlah kepekatan logam terakumilasi terendah bagi Zn, Pb, kuprum (Cu), Cd dan Ni. Berdasarkan kepada Peraturan Makanan Malaysia, paras Zn dan Cu di bahagian-bahagian yang dimakan (otot dan kulit) adalah masih dalam julat yang selamat. Walaupun demikian, Cd, Pb dan Ni telah melebihi julat yang dibenarkan. Apabila dibandingkan dengan paras maksimum pengambilan (MLI), Pb, Ni, dan Cd dalam bahagian-bahagian yang dimakan masih selamat untuk dimakan. Penyelidikan ini menunjukkan *M. albus* dari sawah padi di Kelantan adalah selamat untuk dimakan oleh manusia dengan sedikit pengawasan.

Kata kunci: Logam Berat, Belut Sawah, Sawah Padi

Abstract: Swamp eel, *Monopterus albus* is one of the common fish in paddy fields, thus it is suitable to be a bio-monitor for heavy metals pollution studies in paddy fields. This study was conducted to assess heavy metals levels in swamp eels collected from paddy fields in Kelantan, Malaysia. The results showed zinc [Zn (86.40 μg/g dry weight)] was the highest accumulated metal in the kidney, liver, bone, gill, muscle and skin. Among the selected organs, gill had the highest concentrations of lead (Pb), cadmium (Cd) and nickel (Ni) whereas muscle showed the lowest total metal accumulation of Zn, Pb, copper (Cu), Cd and Ni. Based on the Malaysian Food Regulation, the levels of Zn and Cu in edible parts (muscle and skin) were within the safety limits. However, Cd, Pb and Ni exceeded the permissible limits. By comparing with the maximum level intake (MLI), Pb, Ni and Cd in edible parts can still be consumed. This investigation indicated that *M. albus* from paddy fields of Kelantan are safe for human consumption with little precaution.

Keywords: Heavy Metals, Swamp Eel, Paddy Fields

INTRODUCTION

Heavy metal pollutions in the environment have induced a great attention from many researchers from Malaysia and also other countries due to its toxicity to aquatic organisms specifically fishes that inhabit aquatic ecosystems. It is widely accepted that heavy metal uptake occurs mainly from water, food and sediment.

*Corresponding author: aismail@science.upm.edu.my

However, the efficiency of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism and the contamination gradients of water, food and sediment, as well as other factors such as salinity, temperature and interacting agents (Pagenkopf 1983).

Fish has been widely used as a bio-monitor in many studies to assess the levels of heavy metals pollution potential. Also, it is considered as one of the most indicative factors for pollution studies in freshwater system (Barak & Mason 1990; Evans *et al*. 1993; Rashed 2001). Most of the human population in the world consumes fish as part of their diet as they are the top consumers in the food chain. Presence of pollutants in aquatic ecosystems can cause aquatic organisms such as fish to be contaminated. Since humans are one of the last receivers in the food chain, they tend to accumulate more pollutants (example: heavy metals) in their tissues. Once heavy metals accumulate in human body, they can pose chronic toxicity when their presence exceeds the concentration levels required by body metabolisms. Nabawi *et al*. (1987) stated that assimilation of heavy metals in the human body is one of the causes of cancer.

In order to ensure the quality of rice produced, farmers tend to use huge amounts of pesticides and fertilizers. A variety of pesticides and fertilizers are applied every paddy season to get rid of pest organisms and diseases as rice is a staple and essential food consumed by majority of the world's population. In Malaysia, the apple snail (*Pomacea* sp.) is one of the pest organism that pose major threats to paddy crops. They are usually present in high population numbers and upon reaching a certain level, might cause serious problem to farmers. Applying excessive amount of pesticides to control this snail could potentially introduce significant amount of pollutants into the paddy field areas. This event will affect other non-target organisms. The Asian swamp eel (*Monopterus albus)* is one of the common aquatic species existing in paddy fields. Since swamp eels live in and move around paddy fields, it is easily exposed to those fertilizers and pesticides. In other words, they might be exposed to heavy metals contamination. Some of these metals could originate from pesticides used by farmers which include Decis 250, Lebaycid, Bayluscide and Tikumin.

In Korea, a report has showed that there was a heavy metal pollution in paddy planting areas because the planting areas were located nearby a (lead-zinc) Pb-Zn mine (Jung & Thornton 1997). In China, paddy areas located around Taihu Lake also faced similar problem. Pb pollution occured due to the discharge of Pb/Zn waste water from a mine in the Guangdong province (Shu 1997). Abbas *et al*. (2007) reported that, the farmers who live in the district of Sheikhupura, Pakistan, used polluted water from industrial effluents and human waste to irrigate rice fields. Such activities have been identified to have potential to increase heavy metals pollution in the paddy fields' soil and then to the paddy plants (Khairiah *et al*. 2009).

As the swamp eel is a popular fish consumed by the local people of Kelantan, therefore, it is necessary to assess the level of heavy metal accumulation in this fish collected from paddy fields. The main objective of this study was to determine the concentration of heavy metals in selected organs of *M. albus* which includes liver, kidney, gill, skin, bone, gonads and muscle. The information obtained from this study could give a clearer picture on the behaviour of heavy metals.

MATERIALS AND METHODS

Site Description

M. albus were collected from paddy fields located in Kelantan, Peninsular Malaysia (N06°08.454´ E102°8.430´). The paddy fields are situated nearby residential areas and far away from heavy industrial activities. There is a river nearby the paddy fields known as Sungai Jal. Water is sourced from Sungai Jal by farmers when there is a shortage of water supply to the paddy fields. The major sources of contamination might be from anthropogenic inputs which are pesticides and fertilizers used in rice fields and from transportation activities near the sampling sites. Ten stations (S1–S10) were identified for collecting eel samples. The stations are showed in Figure 1.

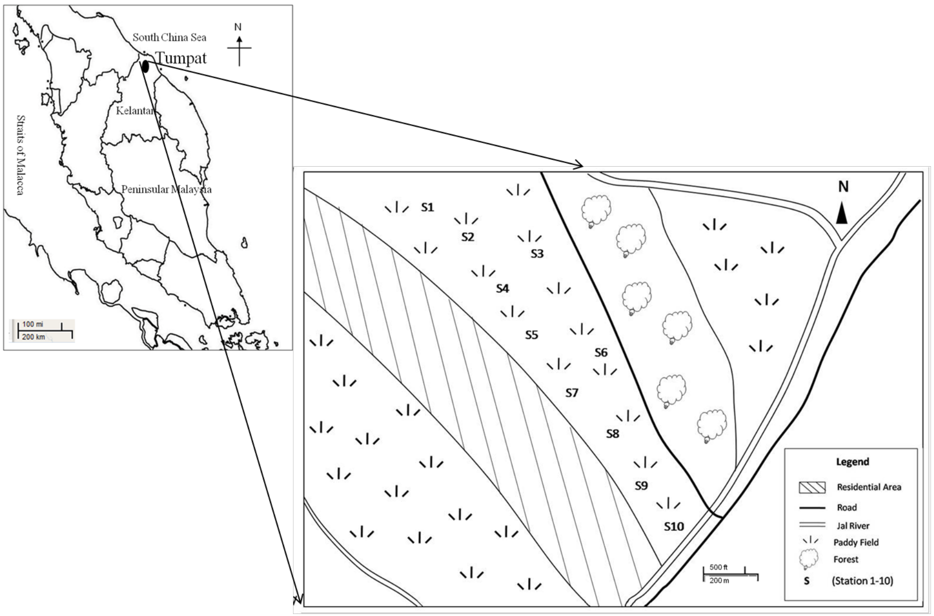

Figure 1: Sampling location of *M. albus* in paddy fields around Tumpat, Kelantan (Malaysia).

Sampling of *M. albus*

M. albus were collected by using a tool known as *tukil*. *Tukil* is a cylindrical tube made from PVC. The length of *tukil* is 36 inches long and the breadth is 2 inches. Small amounts of baits were inserted into *tukil* to attract the eel. Afterwards, these *tukil* were placed at selected positions in the paddy field areas. *Tukil* were collected on the next day and checked for presence of eel. A total of 21 eel individuals were collected, placed in plastic bags and transported back to the laboratory under cold temperature. In the laboratory, each sample was measured for its body weight and standard body length. The average body weight of *M. albus* was 117.98 g (82.90–142.80 g) and standard body length was 48.89 cm (44.90–53.40 cm).

Heavy Metal Determination
Each individual of *M. albus* was dissected with a clean stainless steel dissecting set. Selected organs (liver, gill, muscle, skin, kidney, gonad and bone) were removed from the body and placed on small-sized folded aluminium folds. The samples were dried in an air-circulating oven for at least 72 hours at 60°C until a constant dry weight is obtained. Afterwards, the samples were digested in concentrated nitric acid (69%, AnalaR grade, BDH Chemicals, UK). Next, the digestion tubes filled with samples were placed on a digestion block. The samples were heated at 40°C for 1 hour and later increased to 140°C for 3 hours (Yap *et al*. 2002). After digestion was completed, all digested samples were allow to cool to room temperature and were diluted with distilled water (DW) to a fixed volume (40 ml). The samples were then filtered using filter papers (Whatman No.1) and the filtrates were stored until metal determination was carried out. Concentrations of Zn, Pb, copper (Cu), nickel (Ni) and cadmium (Cd) in all digested samples were analysed by using an air-acetylene flame atomic absorption spectrophotometer (AAnalyst 880, Perkin-Elmer, USA). The data obtained from the analysis were converted into µg/g (dry or wet weight) basis. Prior to carrying out any analysis, all apparatus were acid-washed with 5% nitric acid by dipping for 16 hours and then rinsed with DW. Procedural blanks were analysed once every 10 samples for maintaining accuracy. The DORM-3 Certified Reference Material [National Research Council Canada (NRC 2007)] was used to determine the accuracy of the applied analytical procedures. The percentages of recoveries were 103.33% for Cu, 93.27% for Zn, 86.72% for Cd, 99.10% for Pb and 77.87% for Ni.

RESULTS AND DISCUSSIONS

Heavy Metals Content in Different Parts of Organs of *M. albus*
Mean concentrations of Zn, Pb, Cu, Cd and Ni in kidney, liver, bone, gill, muscle, gonads and skin of *M. albus* from Kelantan are shown in Table 1 and Figure 2. Gill was recorded to have the highest accumulations of metals such as Pb, Cd and Ni. Meanwhile, the highest accumulations of Zn and Cu concentrations were found in gonads and liver respectively. Concentrations of Pb, Cd and Ni were detected highest in gill, with the mean values of 68.69, 4.45 and 48.95 µg/g respectively. Lowest concentration of Zn, Cu and Ni were found in muscle. As for Pb and Cd, they were found lowest in the skin of the eel. In this study, gill of *M. albus* was known as the target organ of accumulation for many metals since a fish's gill indicates the concentrations of metals in the water where the fish lives in (Yap *et al*. 2004). Target organs such as liver, gonads, kidney and gills are known as metabolically active tissues and accumulate high levels of heavy

metals, as was observed in many experimental and field studies (Yilmaz 2009). The other reason for high metal concentrations (Pb, Cd and Ni) in the gills is due to the metal complexing with the mucus making it impossible to be removed completely from the lamellae before analysis (Heath 1987). Also, gills are defined as an uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of an organism (Heath 1987). Karadede *et al.* (2004) reported higher levels of metals in gills than muscle of mullet and catfish. The total lowest metal accumulation of heavy metals was in the muscle compared to others tissues (kidney, liver, bone, gill, gonad and skin) studied of *M. albus*. Therefore, muscle is identified as a non-active tissue in accumulating heavy metals (Roméo *et al*. 1999; Ünlü *et al*. 1996).

The Zn elements were enriched in most organs of *M. albus* with the mean values of 86.40 µg/g. Zn is an essential element that is required in order to survive and are carefully regulated by physiological mechanisms in most living organisms (Eisler 1988). However, when Zn is consumed in huge quantities, it can pose health effects and endanger both humans and organisms (Papagiannis *et al*. 2004). Qiao-qiao *et al*. (2007) found that the organs studied such as skin, gonad, encephalon and muscle of four species (*Cyprinus carpio*, *Carassius auratus*, *Hypophtalmichthys molitrix* and *Aristichthys nobilis*) of fish had highest Zn accumulation.

Based on Table 1, Zn contents were the highest in gonads, with the mean values of 103.80 µg/g. The highest Cu levels were recorded in liver with the mean values of 7.13 µg/g. Studies conducted by Papagiannis *et al*. (2004) showed that Zn and Cu levels were highest in liver and gonads. In this study, both Zn and Cu concentrations were revealed to be highest in gonads and liver respectively. Both Cu and Zn are micro-nutrients and toxicant, released from industrial and domestic sources but Zn is about five-fold less toxic than Cu (Yilmaz 2009). Since Zn is enriched in gonads, to overcome high intake of Zn through food, people can remove the gonads during food preparation. Besides, majority of people do remove the internal organs of eels and consume the muscle. Dietary habits may also play a role in metal concentrations in eels (Papagiannis *et al*. 2004). The liver showed the highest concentration of Cu and many studies have demonstrated that diet is the most important route of Cu accumulation in aquatic animals, and food choice influences body burden of Cu (Sindayigaya *et al*. 1994; Fisher & Reinfelder 1995). The large amounts of fertilizers used by farmers can also explain the higher mean concentrations of Cu and Zn in liver and gonads tissues compared to other tissues (gill, kidney, skin, bone and muscle) in this study, indicating both tissues have potential to be used to bio-monitor Zn and Cu. In Wong *et al*. (2001) study, gonad showed a high enrichment coefficient for Zn. Compound fertilizers, which constitute Zn and Cu as one of the elements, may elevate the levels of Zn and Cu. Romeo *et al*. (1994) opined that liver is the major organ involved in xenobiotic metabolism in fish and organisms can retain both metals, Cu and Zn, through specific binding proteins known as metallothioneins in their liver (Allen-Gil & Martynov 1995). Olsson *et al.* (1989) stated metallothioneins play a vital role in metal homeostasis and in protection against heavy-metal toxicity. On the other hand, heavy metals in edible parts (muscle and skin) of *M. albus* indicated that all metals had the lowest concentrations. The same pattern was observed in Tilapia (*Oreochromis mossambicus*) caught from Kelana Jaya Pond in a study conducted by Yap *et al*. (2004). Based on Table 2, the edible part (muscle) of *A. anguilla* collected from La Capelière (Henry *et al.* 2004) showed lower accumulation of heavy metals (Cu, Pb, Cd, Ni and Zn) compared to this study. Furthermore, the Cd and Pb in muscle of three species of fish (Dab, Flounder and Plaice) caught from Dunkirk (Henry *et al.* 2004) also showed similar trend in accumulation of heavy metals (Cd and Pb) except Cu, which was much higher than Cu in muscle of *M. albus* of Tumpat. Cd has a high potential for bio-concentration in fish and is accumulated in multiple organs (Yilmaz 2009). Gill tissues have higher tendency and capacity to accumulate Cd compared to liver and muscle tissues. Furthermore, Cd and Pb belong to the group of non-essential elements and are known as toxic metals, implying no known function in biochemical processes (Heath 1987; Schlenk & Benson 2001). Sunderman (2004) stated that shellfish and crustaceans also contain higher concentration of Ni in their edible flesh, with the potential causes being biological cycles, atmospheric fallout and industrial processes and waste disposal.

Table 1: Mean concentrations [metal/dry weight (μg/g)] with standard deviations (SD) in kidney, liver, bone, gill, muscle, gonad and skin of *M. albus* from Kelantan.

Organ	Zn	Pb	Cu	Cd	Ni
Kidney	93.62±22.43^{a}	40.09±19.89^{ab}	4.20±1.93^{ab}	2.83±1.978^{a}	34.14±25.17^{a}
Liver	94.92±13.39^{a}	29.55±20.13^{b}	<u>7.13±1.91^{a}</u>	1.78±1.18^{a}	23.04±18.60^{a}
Bone	85.49±28.02^{ab}	47.20±14.31^{ab}	2.53±0.95^{bc}	2.47±1.55^{a}	24.34±14.30^{a}
Gill	98.33±14.10^{a}	<u>68.69±25.98^{a}</u>	3.33±1.46^{b}	<u>4.45±2.54^{a}</u>	<u>48.95±34.92^{a}</u>
Muscle	59.31±10.36^{b}	22.73±10.90^{b}	0.84±0.53^{d}	1.61±1.07^{a}	19.71±14.37^{a}
Gonad	<u>103.80±18.27^{a}</u>	41.32±31.22^{ab}	4.84±2.34^{ab}	3.31±3.08^{a}	38.85±45.38^{a}
Skin	69.34±21.35^{b}	22.15±10.68^{b}	1.13±0.63^{cd}	1.55±0.85^{a}	20.73±16.05^{a}

Notes: *Post-hoc: Mean metal concentrations of different parts of tissue sharing a common letter for a particular metal are not significantly different, $p>0.05$. Underlined values represent the highest concentrations of heavy metals among the organs tested.

Guidelines and Human Consumption

The levels of Cd, Pb and Ni in muscle and skin were higher than recommended levels set by Malaysian Food Regulation (1985) (Table 2). Based on studies conducted by Zulkifli *et al*. (2010) on intertidal sediment collected from estuary of Kelantan River (DKB), Ni and Pb were found occurring at a very high concentration (above the interim sediment quality guidelines). Ismail (1994) stated that apart from ecology, biology and behaviour of the animals, the metal concentrations in sediment could be one of the factors contributing to metal bioaccumulation in animals. Since our sampling location is nearby the area, we assumed that the concentration of Ni and Pb in the paddy areas could be high and bioaccumulated into the existing organisms in the area. In this study, Zn and Cu levels in muscle and skin were below the safety limits.

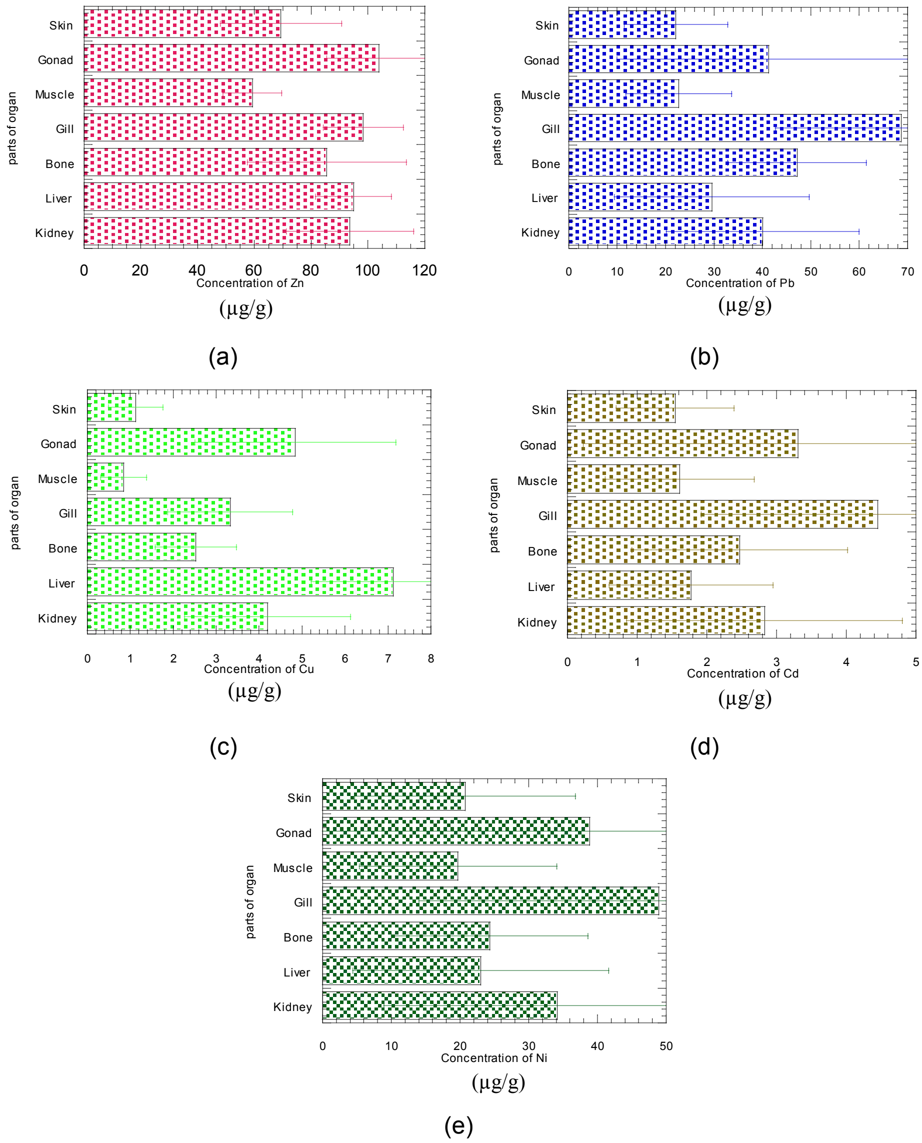

Figure 2: Distribution of the concentrations (mean µg/g dry weight ± SD) of (a) Zn, (b) Pb, (c) Cu, (d) Cd and (e) Ni in the different parts of organ of *M. albus* collected from paddy fields, Kelantan.

Table 2: Comparison of heavy metal concentrations (μg/g dw) in the edible parts (muscle and skin) of *M. albus* with other guidelines and studies.

Description	Element (μg/g)							Reference
	Tissue	W/D	Cu	Cd	Zn	Pb	Ni	
Present study	Muscle	Dry	0.84	1.61	59.31	22.73	20.84	This study
	Skin	Dry	1.13	1.55	69.34	22.15	22.98	This study
A. anguilla (La Capeliere)	Muscle	Dry	0.43	NA	55.4	0.79	0.83	Oliveira Ribeiro *et al.* (2005)
Dab (Dunkirk)	Muscle	Dry	0.94	0.003	NA	0.07	NA	Henry *et al.* (2004)
Flounder (Dunkirk)	Muscle	Dry	0.78	0.003	NA	0.02	NA	Henry *et al.* (2004)
Plaice (Dunkirk)	Muscle	Dry	1.2	0.007	NA	0.07	NA	Henry *et al.* (2004)
Permissible limits set by Malaysian Food Regulation		Wet	30	1	100	2		Malaysian Food Regulation (1985)
Permissible levels set by FAO/ WHO		Wet	30	2	1000	2	0.05	Wood (1974)
TUI			10000	–	40000	–	1000	Food and Nutrition Board (2001)
Permissible levels established by Brazilian Ministry of Health		Dry	150	5	250	10	–	ABIA (1991)

Notes: TUI: Tolerable upper intake level; NA: not available; W = wet; D = dry

When comparing with the data set by Brazilian Ministry of Health (ABIA 1991), the data in this study suggest the metals Cu, Cd, and Zn in muscle and skin were within the safety limits but the levels of Pb and Ni levels in muscle and skin were above the safety limits. Therefore, this information reflects that the edible parts have the potential to cause low toxicity. This situation could be due to the pesticides and fertilizers applied by farmers to paddy fields in order to get rid some pest organisms and yield high quality crops. According to the studies conducted by Ismail (1994) on snails of Kuala Klawang's, fertilizers and pesticides used could be the cause of higher concentration of heavy metals in animals.

Based on the estimated daily intake (Table 3), the accumulation of metals in the parts (muscle and skin) consumed by human for each metal was 11.86 μg/day (Cu), 18.94 μg/day (Cd), 771.91 μg/day (Zn), 269.31 μg/day (Pb) and 262.93 μg/day (Ni). The estimated daily intake of metals in the edible parts consumed by human was lower compared to the recommended value of maximum level intake (MLI). This indicates that, the edible parts of *M. albus* are safe to be eaten by people. The levels of Cu, Cd, Zn, Pb and Ni were below the recommended limit set by MLI. Therefore, *M. albus* are safe to be consumed by humans. Based on the results obtained from this study, the intakes of Cu, Cd, Zn, Pb and Ni are within the recommended safety limits of MLI but people should control their intake of Pb and Ni to avoid Pb and Ni toxicity. If Pb is consumed in excessive amounts, it can causes behavioural deficits in vertebrates, decrease survival and growth rates and, cause learning and metabolism disabilities (Qiao-qiao *et al*. 2007). Acu-Cell Nutrition (Acu-Cell Nutrition 2000) reported that, Ni have the possibility to cause cancer of the sinuses, throat and lungs. In addition, other health effects such as asthma, angina and/or other cardiac symptoms are a result of nickel interfering with Vitamin E activity.

Table 3: The estimated daily intake of edible tissues (muscle and skin) of *M. albus* in comparison with the recommended metal intake values (µg/day).

Element	EDI (muscle)	EDI (skin)	EDI (total)	MLI[a]
Cu	5.06	6.79	11.86	3200
Cd	9.67	9.28	18.94	18–200
Zn	355.88	416.03	771.91	17000
Pb	136.39	132.92	269.31	300
Ni	125.05	137.88	262.93	450

Notes: EDI: estimated daily intake; MLI: maximum level of intake without detriment to health.
[a]Senczuk (1999) based consumption of 6 g dry weight per day.

CONCLUSION

Overall, Zn was the highest accumulated heavy metal in the selected organs of *M. albus*. Gill was found accumulating highest concentrations of Pb, Cd and Ni, whereas Cu was highest in gonad and Zn was highest in liver. Based on the comparison with permissible levels set by Malaysian Food Regulation and MLI, all metals are within the safety limit with little precaution in term of intake volume per day needed. Therefore, regular monitoring of *M. albus* should be practiced in order to avoid health problems to consumers.

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