Distribution of Cu, Zn and Ni in Asian Swamp Eels, *Monopterus albus* from Bachok and Pasir Mas, Kelantan, Malaysia

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Abstract. This work investigated the metal concentration in Asian swamp eel (*Monopterus albus*) tissues, from paddy cultivation areas in Kelantan. Five selected tissues, such as liver, gills, kidney, skin and muscle were examined for Zn, Cu and Ni concentrations. Zn concentration was found to be the highest in the liver of eels from Bachok and Pasir Mas, while Cu concentration was highest in the liver and kidney, respectively. In addition, non-essential metal like Ni was identified in high amount in the eel gills and kidney. However, low level of Zn, Cu and Ni concentrations were identified in the muscle tissues. Overall, Asian swamp eels from Bachok and Pasir Mas did not show much variation in Zn, Cu and Ni accumulation in the five selected tissues. Nevertheless, the deposition of agrochemical fertilisers and pesticides should be regularly controlled by responsible agencies to avoid any metal poisoning.

1. Introduction
The Asian swamp eel is a common fish that can be found in paddy fields and is widely consumed by the majority of population in Kelantan. Besides, fish is an important part of human diet because of its high nutritional quality [1]. In addition, fish is also enriched with some essential minerals, vitamins and unsaturated fatty acids [2].

Pollution is a great environmental issue that leads to unsolved solutions and concerns to humans. Daily anthropogenic activities are one of the top listed factors for heavy metal accumulations in hydro systems that cause a potential threat to aquatic organisms and human health by encouraging a broad range of adverse impacts [3]. Heavy metals are easily diffused or enter the human body through food consumption that is contaminated by heavy metals, especially in fish. There are two heavy metal pathways that enter the aquatic food chains, which are direct consumption of water and food through the digestive tract and non-dietary routes across permeable membranes, such as the muscle and gills [4].

Regular fertiliser and pesticide applications during paddy season may cause the accumulation of pollutants (heavy metals) in paddy fields, resulting in high bioaccumulation in Asian swamp eels. According to Sow et al. [5], the repetitive use of agrochemicals during paddy cultivation had escalated the amount of pollutants in the soils. For example, ploughing soils can cause pollutants from previous
paddy cycles to resurface, while dependency on chemical fertilisers for seedlings and growing seasons adds to the amount of pollutants. Since fish are relatively situated at the top of the aquatic food chain, they normally can accumulate heavy metals from food, water and sediments [6,7].

Fish ingests important micronutrients, such as Zn, Cu, Pb, Cd and Hg for metabolic functions from its diet or surroundings which will eventually accumulate in the tissues [8,9]. There are several factors that can influence metal uptake, such as sex, age, size, reproductive cycle, swimming patterns, feeding behaviour and living environment (geographical location) [10-11,7]. Therefore, fish are considered as one of the best heavy metal contamination indicators in coastal environments [12,13]. This study was conducted to examine the contents of metals (Cu, Zn and Ni) in five selected organs (gills, liver, kidney, muscle and skin) of *M. albus* obtained from a paddy field located at Bachok and Pasir Mas, in Kelantan.

2. Material and Method

2.1 Sampling location
This study was conducted in paddy fields located at Kg. Bekelam, Bachok (N 06° 02’ 30.9” E 102° 21’ 28.9”) and Pasir Mas (N 06° 6’ 36.2484” E 102° 7’ 5.4876”), in Kelantan. The selection of paddy field area was based on the area consumption of agrochemical fertilisers, pesticides, insecticides and herbicides by in paddy cultivations and the paddy field was located at a nearby residential area and far from heavy industrial activities. Moreover, a river runs along the paddy field and it has become the water resource for the farmers’ water supply to the paddy field. However, paddy field in Bekelam was not far from the road. Meanwhile, the paddy field in Pasir Mas was more rural than at Bekelam and located quiet far from the road. The Asian swamp eel samples were collected monthly for four months beginning from March to June 2018 and at 10 stations (S1-S10) for each sampling.

2.2 Water sample
The water samples were collected by using 500ml washed polypropylene bottles which were securely labelled with a unique sample number. Then the water samples were directly filtered and acidified with hydrochloric acid (HCl) to preserve most trace metals and reduce microbial activities. The water samples were preserved in a chiller at a temperature of 4°C until metal determination was carried out.

2.3 Asian swamp eel
The eels, *Monopterus albus* were collected by using a tool known as tukil which is a cylindrical long tube made from PVC with several holes at the bottom and a cone bamboo on top. Small amounts of bait, usually of smoked fish, were inserted into the tool to attract the eels. The tukil was placed at certain points in the paddy field and were left overnight and collected on the next day. The eels were placed in polyethylene plastic bags and brought back to the laboratory for measurement. The length and weight of the eels were recorded before dissection. The Asian swamp eels were rinsed several times with distilled water before dissection. The eels were dissected by using a dissecting set and each organ was placed on small-sized aluminium folds and labelled. The samples were oven dried at 60°C for at least 72 h. Subsequent to drying, the samples were ground into fine powder by using a pestle and mortar.

2.3.1 Acid Digestion of *M. albus*
Each of the eel organs was weighed. Organ of 1 g each was put into a beaker. Then 10ml of concentrated nitric acid (HNO₃) (69%) was added to the samples and they were slowly heated at 40°C for 1 h. After 1 h, the temperature was increased up to 80°C in the second hour. The temperature was later increased to 100°C in the third hour and lastly to 140°C in the fourth hour, which made a total of 4 h of heating on the hot plates. Furthermore, after getting a clear solution, the digested samples were diluted with distilled water up to 40ml and cooled for approximately 15 min. Next, the samples were
filtered by using Whatman No. 42 filter paper and a syringe filter. The filtrate was stored in a chiller at 4°C until metal determination was carried out by using AAS.

2.4 Statistical analysis
The data was shown as mean ±SD (standard deviation) and analysis of variance (ANOVA, p< 0.05) was interpreted for the association between pairs of variable identification.

3. Result
Table 1 and Table 2 show the concentrations of Cu, Zn and Ni in water samples collected from Bachok and Pasir Mas. Table 3 lists the concentrations of Cu, Zn and Ni in Asian swamp eels collected from Bachok and Pasir Mas. The concentration of Cu was estimated in the range of 7.54 µg/g – 15.10 µg/g, Zn 57.24 µg/g – 309.44 µg/g and Ni 1.27 µg/g – 8.60 µg/g for Bachok. For eels collected from Pasir Mas, the levels of Cu were estimated in the range of 1.22 µg/g – 25.34 µg/g, Zn 55.15 µg/g – 362.73 µg/g and Ni 0.83 µg/g – 41.05 µg/g.

Table 1. Heavy metals concentration (mg/L) with standard deviation (SD) of Cu, Zn and Ni in Kg Bekelam, Bachok, Kelantan

| Sampling | Cu    | Zn    | Ni   |
|----------|-------|-------|------|
| March    | 0.02  | 0.02  | 0.02 |
| March    | 0.02  | 0.08  | 0.01 |
| April    | 0.1   | 0.05  | 0    |
| May      | 0.14  | 0.07  | 0    |
| June     | 0.09  | 0.06  | 0.04 |
| Mean concentration | 0.07±0.06 | 0.06±0.02 | 0.01±0.02 |

Table 2. Heavy metals concentration (mg/L) with standard deviation (SD) of Cu, Zn and Ni in Pasir Mas, Kelantan.

| Sampling | Cu    | Zn    | Ni   |
|----------|-------|-------|------|
| March    | 0.01  | 0.02  | 0.04 |
| March    | 0.01  | 0.02  | 0.04 |
| April    | 0     | 0.02  | 0.06 |
| May      | 0.04  | 0.01  | 0.03 |
| June     | 0.05  | 0.02  | 0.02 |
| Mean concentration | 0.02±0.02 | 0.02±0.01 | 0.04±0.02 |

Table 3. Cu, Zn, and Ni concentration (µg/g d.w ±S.D) in selected organs of *M. albus* from Bachok and Pasir Mas, Kelantan

| Organ   | BACHOK          | PASIR MAS        |
|---------|-----------------|------------------|
|         | Cu   | Zn   | Ni   | Cu   | Zn   | Ni   |
| Kidney  | 9.01±5.89<sup>b</sup> | 168.42±187.42<sup>b</sup> | 6.19±7.24<sup>c</sup> | 25.34±45.48<sup>d</sup> | 163.42±183.07<sup>b</sup> | 41.05±88.87<sup>c</sup> |
| Gills   | 7.54±7.02<sup>d</sup> | 131.88±60.32<sup>c</sup> | 8.60±5.29<sup>b</sup> | 4.84±3.49<sup>c</sup> | 127.80±23.11<sup>c</sup> | 10.59±9.85<sup>b</sup> |
| Liver   | 15.10±3.63<sup>a</sup> | 309.44±200.12<sup>d</sup> | 2.60±1.56<sup>c</sup> | 14.73±15.51<sup>b</sup> | 362.73±350.12<sup>a</sup> | 13.95±29.34<sup>b</sup> |
| Skin    | 11.27±9.82<sup>b</sup> | 80.52±20.65<sup>d</sup> | 3.28±2.08<sup>c</sup> | 5.70±4.0<sup>c</sup> | 69.5±32.94<sup>ab</sup> | 4.95±8.47<sup>c</sup> |
| Muscle  | 10.19±15.55<sup>b</sup> | 57.24±36.97<sup>c</sup> | 1.27±1.66<sup>d</sup> | 1.22±1.25<sup>d</sup> | 55.15±25.24<sup>d</sup> | 0.83±1.33<sup>d</sup> |
4. Discussion
The present study showed that the accumulation of heavy metals (Cu, Zn and Ni) in the selected M. albus tissues from the liver, gills, kidney, muscle and skin. The mean values for each metal concentration in the examined tissues are presented in Table 3. The aggressive and regular application of chemical fertilisers and pesticides during the paddy seasons had significantly contributed to the contamination of the paddy ecosystem, including seasonal accumulation of heavy metals, such as Cu, Zn and Ni [14,15]. Wang et al. [16] and Uysal et al. [17] reported that essential and non-essential metals were demonstrated to accumulate along the trophic chain in freshwater ecosystem.

Among the metals studied, Zn was found to be highly bio-accumulated in the tissues as compared to Cu and Ni for Bachok and Pasir Mas, respectively. However, Zn displayed a significant difference (p<0.05) among the studied tissues, except in skin and muscle (Table 3). Zn is an essential element that is required to survive and is regulated by physiological mechanisms in most living organisms [18].

In this study, the Zn concentration in livers of the collected Asian swamp eels from Bachok was 309.44 µg/g and that from Pasir Mas was 362.73 µg/g. Results showed that the Zn concentration was the highest among studied tissues (kidney and gills), and showed higher levels than studies conducted by Salam et al. [19] on the liver of Euthynnus affinis species or mackerel tuna from Tok Bali Port. Zhao et al. [7] stated that the liver played a major role in metabolic processes and showed a tendency to accumulate metals at higher levels. Meanwhile, the liver also acts as an environmental indicator of water pollution as it can accumulate various pollutants at higher levels than the surrounding environment and plays an important role in storage contamination, detoxification, redistribution, transformation and act as an active pathological site [20,21]. In comparison with the study conducted by Yin et al. [14] on M. albus collected from Tumpat, Kelantan, the Zn concentration level in gills (98.33 µg/g) and kidney (93.62 µg/g) were found to be lower than the present study. Furthermore, the skin tissue in the present study indicated higher levels than the finding by Yin et al. [14] which was 59.30 µg/g . However, lower Zn concentration was observed in muscle tissues collected from Bachok and Pasir Mas than the M. albus muscle tissues from Tumpat [14].

Copper (Cu) is recognised as an essential element that is required by a wide variety of enzymes and other cell components for a vital function in all living things. But, when Cu is taken in excessive amount, it will damage human health and pose serious health problems, including poisoning, nausea, acute stomach pains, diarrhoea and fever [22]. In Bachok, the Cu concentration in liver of M. albus was found to be the highest, followed by tissues from the skin, muscle, kidney and gills, while insignificant difference (p>0.05) was observed for the skin and muscle. Next, for Pasir Mas, the highest Cu concentration was observed in the M. albus kidney, followed by liver, skin, gills and muscle. Surprisingly, in the present study the Cu concentration in the five selected tissues were found higher than the study conducted by Salam et al. [19] in Euthynnus affinis (mackerel tuna) species, Pampus argenteus (silver pomfret), and Leiognathus daura, (goldstripe ponyfish) collected from Tak Bali Port and in a study conducted by Yin et al. [14] in M. albus from Tumpat, Kelantan. The high bioaccumulation of Zn and Cu were found in liver of M. albus from Bachok might due to the MT, a binding protein [23] that stores metals to cater to the needs of enzymes and metabolic requirements [24]. The finding of high Zn and Cu concentrations in the liver of the Asian swamp eel was in line with that discovered by Sow et al. [5] for M. albus. Although, heavy metal uptakes are dependent on various factors, such as age, geographical distribution, feeding nature, trophic levels and species specific factors that vary from organ to organ [25].

Nickel (Ni) is a non-essential element needed by living organisms and possess various adverse effects when it is consumed in high concentration. In this study, high Ni concentration was found in kidney tissues of Asian swamp eels from Pasir Mas than Bachok. Sow et al. [26] reported that high Ni concentration was observed in M. albus gill tissues. As both of gills and kidney are known as target organs of accumulating of heavy metals, it is not surprising these tissues are capable to highly bio-accumulate Ni from the aquatic ecosystem. Besides, the high Ni concentration levels might be extracted from agricultural activities that demand the utilisation of excessive agrochemical fertilisers and pesticides to produce superior crops [26]. Furthermore, the low Ni concentrations were found in
muscle and skin of *M. albus* from Bachok and Pasir Mas than the study finding conducted by Yin et al. [14], which were 20.84 µg/g and 22.98 µg/g.

Velusamy et al. [27] asserted that the variance in uptake of elements among species depended on several fish biological traits, for instance, mobility, habitat and trophic aspects. Therefore, as the Asian swamp eels live in paddy fields with varied seasons, their exposure to heavy metals is indeed high [5]. Therefore, these eels showed high level of metals in their tissue samples. Furthermore, as these eels have increased longevity, the metals accumulate in their bodies as pollutants [5].

5. Conclusion
As a conclusion, the outcomes of this study highlight the following: (1) the high presence of Zn was noted in the liver, followed by kidney and gills for both locations, while Cu in the liver and kidney was noted for Bachok and Pasir Mas; (2) accumulation of non-essential metal, such as Ni, was high in gills for Bachok and kidney for Pasir Mas; (3) a minimal level of Zn, Cu and Ni was found low in muscle tissues than in liver, gills, kidney and skin. Nevertheless, the intake of the muscle tissues of Asian swamp eels should be consistently regulated to avoid further critical health issues. The deposition of heavy metals through the excessive use of agrochemical fertilisers should be constantly observed and controlled by responsible government agencies.

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