The elemental distribution study of heavy metals (Fe, Cu, Cd, As, Cr, Ni and Hg) in various fish species at local market in Muar, Johor using SEM-EDX

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Abstract. The polluted marine environment causes bioaccumulation of heavy metals particularly by fish, which is consumed by humans as sources of nutrients for humans, particularly as a source of protein. Thus, the investigation of the toxic heavy metals concentration levels in fish is essential. This study aimed to evaluate the heavy metals (Cd, Cr, As, Ni, Fe, Hg and Cu) contents in five different fish species, namely Rastelligar kanagurta, Selaroides leptolepis, Megalaspis cordyla, Decapterus russell and Euthynnus affinis. Each sample has been dried and grinds to 100 microns of powder form. The elemental composition of the heavy metals was determined using the rapid and versatile analytical technique, which is Scanning Electron Microscopy with Energy Dispersive X-ray analysis (SEM-EDX) and the results were expressed as an atomic percentage. The percentage concentrations of Cd, As, Cr and Ni were found to be higher in R. kanagurta which are 0.01%, 0.02%, 0.03% and 0.02%, while Cu was found to be higher in S. leptolepis which is 0.29%. For Hg element analysis, it was found that this element only exists in D. russelli species with atomic percentage of 0.02%. The atomic percentage concentration of As in R. kanagurta and S. leptolepis are 0.02% and 0.01%. The element of Cr, Fe and Ni were detected in M. cordyla with atomic percentage of 0.01%, 0.02% and 0.02%. In E. affinis, Fe was the highest concentration of metals detected with 0.02%, while other elements such as Cr, Cu and Ni recorded similar the atomic percentages concentration at 0.01%. This study revealed that R. kanagurta species contributed most of the elements compared to other species. The accumulation of these toxic heavy metals from this fish species could contribute to the toxicity of heavy metals in the human body. However, since all heavy metal concentration measurements in this study are in atomic percentages level, hence it can be considered that the measurements are below the acceptable limit that suggested by WHO, FAO and UNSCEAR.
1. Introduction
Heavy metals pollution is a major concern worldwide due to toxicity, persistent nature, non-biodegradable and accumulative behaviors [1]. It is caused by industrialization, urbanization, agriculture, and other human activities especially in developing countries [2]. Heavy metals from these activities will be discharged into the sea or rivers, accumulated in water, sediments and aquatic food chain resulting in sublethal effects or death in the fish population [3]. Heavy metals enter the aquatic food chain through the direct consumption of water and food or through the muscle or gills [4]. Fish are at the top of the aquatic food chain and considered as one of the bioindicators of pollutants [5][6]. Besides that, fish is the major source of protein for humans. Thus, the human body is easily susceptible to heavy metals concentration in fish [7]. Therefore, analysis of the heavy metal in fish will reflect the level of sediment and water of the aquatic environment from which they are sourced [8].

Accumulation of heavy metals in fish are influenced by their nutritional habits, ecological requirements, concentrations of heavy metals in water and sediment, fish’s life, season and physicochemical properties of water [9][10][11][12]. Among the heavy metals, cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As) and copper (Cu) have a higher potential to accumulate in fish above the standard limit [13][14][15]. Some trace elements such as zinc, iron, chromium, manganese, cobalt, nickel and copper are required by marine organisms. For the aquatic organism, these elements are required in a trace amount for normal growth and development. However, elements such as arsenic, mercury and cadmium do not give any biological importance. All the heavy metals are potentially toxic to most organisms at some level of exposure and absorption [16][17]. An excessive intake of heavy metals will cause serious threats such as renal failure, liver damage, cardiovascular disease and even death to human [18].

Fishes can hold toxic materials in a small concentration to high concentrations in their body without harming them, but the toxicity will affect the humans who feed on them. A small amount of heavy metals can be held either temporarily or permanently in their body. Thus, fish in the polluted water will accumulate high concentration of heavy metals along the time [19]. For example, a high concentration of Hg, As and Cd could be harmful to the living cells, and prolonged exposure to the body can cause illness or even death [20]. The high Cd content could damage the kidney, liver, causing diarrhea and nausea [21]. Besides that, metals such as Cd, Hg, As, Ni, As and Cr that are commonly found in human diets have been reported to be carcinogenic [22][23]. The distribution of heavy metals in fish differs due to habitat and feeding habits [19]. Information on the distribution of heavy metals in fish resources from local market is still lacked in Malaysia. Hence, in this work the investigation of heavy metals composition quantification in several fish species from local market in Muar, Johor has been conducted using rapid and versatile analysis technique, SEM-EDX.

2. Materials & methods

2.1. Sample collection
The sample of fish species were obtained from wet market at Bukit Gambir, Muar. Five kind fish samples have been collected consist of Selaroides leptolepis, Rastelligar kanagurta, Megalaspis cordyla, Decapterus ruselli and Euthynnus affinis as shown in Figure 1. The collected samples were preserved in ice container and kept frozen in -20°C before analysis. The total length (cm) and weights (gm) for each fish samples were measured before dissection as tabulated in Table 1.
Table 1. The description for all fish sample species.

| Scientific name           | Local name | Common name       | Length (cm) (min-max) | Weight (g) (min-max) |
|---------------------------|------------|-------------------|-----------------------|----------------------|
| Selaroides leptolepis     | Selar      | Yellowstripe scad | 20-23                 | 106-145              |
| Rastellagar kanagurta     | Kembung    | Indian mackerel   | 20-25                 | 110-160              |
| Megalaspis cordyla        | Cencaru    | Torpedo Scad      | 23-30                 | 110-300              |
| Decapterus ruselli       | Selayang   | Slander scad      | 17-22                 | 61-127               |
| Euthynnus affinis        | Tongkol    | Mackerel tuna     | 30-41                 | 350-925              |

2.2. Sample preparation
All fish samples were washed using distilled water to remove the skin surface dirt. Head, viscera, gills and flesh segments were cut into identical segments. The samples are freeze dried for 48 hours to remove the moisture content. Then, the dried samples were ground into 100 microns of powder form. The fish samples need to be in the homogenous powder form for elemental composition analysis purpose. Before the grinding process, it is important to make sure that the fish samples were completely dried.

2.3. Elemental composition analysis
In this study, Scanning Electron Microscopy with Energy Dispersive X-ray analysis (SEM-EDX) (EM-30AX, Coxem, Korea) was used to quantify the metal concentrations. The powder form samples were mounted in the stubs with conductive carbon tape. Then, the samples were sputter-coated with gold to avoid charging during the analysis. EDX system are attached to SEM instruments where the imaging capability of the microscope is used for morphology analysis. In this research, the elemental composition of the sample was analyzed by three measurements in order to achieve a good statistical analysis. Three fine spot areas were selected, and the average is determined. Data were collected at 20 kV accelerating voltage and 12 cm working distance. Figure 2 shows a typical EDX spectrum where each energy represents different element and therefore the analysis of elemental compositions was calculated by the percentage of the atomic elements, at %.
3. Results and discussion

3.1. Morphology analysis for every fish species
The morphology structure obtained for all fish species were shown in Figure 3(a)-(e). From this morphology analysis, it is seen that the particle size for all types of fish species was different and the distribution of each particle is uneven. The morphology structure for S. leptolepis exhibited a smoother surface compared to other, while D. ruselli and E. affinis have a similar particle size.

![Figure 2. EDX spectrum for fish samples from SEM-EDX](image)

![Figure 3. Morphology image of all fish species (a) R. kanagurta, (b) S. leptolepis, (c) M. cordyla, (d) D. ruselli, (e) E. affinis.](image)
3.2. Elemental percentage concentration for all fish species

The quantitative analysis of heavy metals was given in atomic percentage (%). The result in Table 2 shows that the level of Cd, Hg, As, Cu, Fe, Ni and Cr vary according to the samples. The concentrations of Cd, Hg, As, Cu, Fe, Ni and Cr were classified according to different fish species. The same applies to the study done by the previous researchers where the heavy metals distributions were varied among different fish species due to metabolism and feeding patterns of the fishes [24].

| Elemental compositions (%) | Fish species | Mean Concentrations (%) |
|----------------------------|--------------|-------------------------|
|                            | Rastelligar kanagurta | Selaroides leptolepis | Megalaspis cordyla | Decapterus Ruselli | Euthynus affinis |                        |
| Cadmium                    | 0.010±0.001   | 0.010±0.001   | ND                 | ND                 | ND              | 0.010                  |
| Mercury                    | ND            | ND            | ND                 | 0.020±0.007        | ND              | 0.020                  |
| Arsenic                    | 0.020±0.007   | 0.010±0.001   | ND                 | ND                 | ND              | 0.015                  |
| Chromium                   | 0.030±0.020   | 0.010±0.004   | 0.010±0.001        | 0.010±0.001        | 0.010±0.001    | 0.014                  |
| Copper                     | 0.220±0.014   | 0.290±0.077   | ND                 | ND                 | 0.010±0.001    | 0.173                  |
| Iron                       | 0.040±0.035   | 0.010±0.001   | 0.020±0.007        | 0.020±0.007        | 0.020±0.007    | 0.022                  |
| Nickel                     | 0.020±0.007   | 0.020±0.007   | 0.020±0.007        | 0.010±0.001        | 0.010±0.001    | 0.016                  |

ND=Not detected

From the observation, the trends for the concentrations of heavy metals for all fish samples were in descending order which are Cu > Fe > Hg > Ni > As > Cr > Cd. The mean concentrations of the heavy metals found in fish species were 0.173 (Cu), 0.022 (Fe), 0.020 (Hg), 0.016 (Ni), 0.015 (As), 0.014 (Cr) and 0.010 (Cd).

*R. kanagurta* was found to have a greater atomic concentration of heavy metals as compared to the other fish species. Most of the heavy metals detected in *R. kanagurta* is mainly consist of Cd, As, Cr, Cu, Fe and Ni with atomic percentage concentration of 0.010±0.001, 0.020±0.007, 0.030±0.020, 0.220±0.014, 0.040±0.035 and 0.020±0.007 respectively. Additionally, Figure 4 shows that Cu was found in higher concentrations than the other heavy metals. The distribution of heavy metals in descending orders for *R. kanagurta* was Cu > Fe > Cr > As > Ni > Cd. This trend was similar to the outcome obtained from [25], where Cu was detected to be the highest among the other metals. The finding obtained from *R. kanagurta* suggests that the cause of high Cu reading is due to the high Cu concentration in the fish’s catching area environment. This opinion is supported by [26] where Cu is highest detected in fish due to the fish species taken from waters enriched in this element. It was proposed in [24] that Cu is essential in all organisms in trace amounts and it serves as a constituent of respiratory enzyme complex in the human body and due to its role in facilitating iron uptake, deficiency of Cu can cause impaired growth, anaemia-like symptoms, bone abnormalities and vulnerable to infections.

Meanwhile, Cd, As, Cr, Cu, Fe and Ni were detected in *S. leptolepis* with percentage concentration of 0.010±0.001, 0.010±0.001, 0.010±0.004, 0.290±0.077, 0.010±0.001 and 0.020±0.007 respectively. Figure 5 showed that metals Cu and Ni were found at higher percentage concentrations than the other heavy metals. The level of accumulation of these heavy metals for *S. leptolepis* species in descending order was Cu > Ni > Cd > As > Cr > Fe. In the present study, arsenic (As) and Cd were only detected in *R. Kanagurta* and *S. leptolepis*. The existence of arsenic (As) in this particular fish may be resulting in poisoning to consumers as reported by national environmental protection agencies [28]. The observed percentage concentration of heavy metals detected in *M. cordyla* is shown in Figure 6. The heavy metals compound in this particular fish species is mainly consist of Cr, Fe and Ni with a percentage concentration of 0.010±0.001, 0.020±0.007 and 0.020±0.007 respectively. The general observed trends
for the metals for *M. cordyla* was Fe > Ni > Cr. The lowest percentage concentration of Cr (0.010±0.001) was recorded while Fe and Ni showed the same percentage concentration which was 0.020±0.007.

As for *D. Ruselli* species, the heavy metals concentration observed were Hg, Cr, Fe and Ni with percentage concentrations of 0.020±0.007, 0.010±0.001, 0.020±0.007 and 0.010±0.001 respectively. The trends for the metals for *D. ruselli* in descending order were Hg > Fe > Cr > Ni. The result shows Hg and Fe recorded the same concentration as shown in Figure 7. Cd, As and Cu were not detected in *D. ruselli*. Mercury is carcinogenic and produces adverse effects during developmental stages as a result of acute and chronic exposure [29]. In the present study, the mercury had only been detected in *D. ruselli*.

While for the percentage concentration of heavy metals identified in *E. affinis* is shown in Figure 8. The detected heavy metals were Cr, Cu, Fe and Ni with percentage concentration of 0.010±0.001, 0.010±0.001, 0.020±0.007 and 0.010±0.001 respectively. The heavy metals in *E. affinis* followed an order of Fe > Cr > Cu > Ni. In *E. affinis*, Fe was the highest concentration of metals detected with 0.020±0.007 while Cr, Cu and Ni record the same concentration which is 0.010±0.001. Ahmed & Bat (2015) found a similar finding, with Fe being the highest concentration found in fish. In the current analysis, chromium was found at low levels in all fish samples, close to those examined in Turkey [27] and Malacca [25].

In general, all fish species had Fe, Ni and Cr in them. The maximum concentration of Cu was observed in *R. Kanagurta* and *S. leptolepis* and was not detected in *M. cordyla*, *D. Ruselli* and *E. affinis*. From the observation, it indicated that different levels of metals were found in different fish species. The differences in the heavy metals content are due to the accumulation of each metals in different fish species that related to its ecology surrounding such as habitat and nutrient in the sea water [19, 30]. All heavy metals concentration is considered below the acceptable limits suggested by WHO, FAO and UNSCEAR [31][32][33].

![Figure 4. The elemental distribution of heavy metals in *R. Kanagurta*](image1)

![Figure 5. The elemental distribution of heavy metals in *S. leptolepis*](image2)

![Figure 6. The elemental distribution of heavy metals in *M. cordyla*](image3)

![Figure 7. The elemental distribution of heavy metals in *D. Ruselli*](image4)
As Fe, Ni, Hg. As Cr, Cu consumed. Accumulated in this particular fish can be considered very low concentration and still safe to be content, hence it could give a risk of disease to human beings. However, heavy metal contribute amount of Hg was found in compared to the other fish species.

In this research, the overall, it was found that most of the heavy metals elements is found in percentage concentration of Cd, As, Cr and Fe in R. kanagurta was found to be the highest compared to the other fish species. Whilst, Cu was the highest in S. leptolepis. On the other hand, some amount of Hg was found in D. ruselli, while, Cr, Fe and Ni are mainly observed in all fish samples. Overall, it was found that most of the heavy metals elements is found in R. kanagurta, while M. cordyla contributes the lowest heavy metals concentration. As R. kanagurta has the highest heavy metals content, hence it could give a risk of disease to human beings. However, heavy metals content that accumulated in this particular fish can be considered very low concentration and still safe to be consumed.

4. Conclusion
In this research, the existence of heavy metals in fish samples was successfully investigated. The distribution of heavy metals accumulation in all fish species were established and the descending order of heavy metals concentration are as the following order of Cu > Fe > Hg > Ni > As > Cr > Cd. The atomic percentage concentration of Cd, As, Cr and Fe in R. kanagurta was found to be the highest compared to the other fish species. Whilst, Cu was the highest in S. leptolepis. On the other hand, some amount of Hg was found in D. ruselli, while, Cr, Fe and Ni are mainly observed in all fish samples. Overall, it was found that most of the heavy metals elements is found in R. kanagurta, while M. cordyla contributes the lowest heavy metals concentration. As R. kanagurta has the highest heavy metals content, hence it could give a risk of disease to human beings. However, heavy metals content that accumulated in this particular fish can be considered very low concentration and still safe to be consumed.

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**Acknowledgements**

The authors would like to thank the financial support by GPPS (Vot: 497) provided by University Tun Hussein Onn Malaysia.