# A Comparative Study of Cd and Pb Concentration in Selected Commercial Marine Fishes from Wet Markets and Supermarkets in Klang Valley, Malaysia

Victor JN[a*], Nor Hasyimah AK[b], Pearline Ng HC[a], Lee CY[a], Teh YY[a]
[a]*Greencity International College, No. 9, Jalan Gereja, 50100 Kuala Lumpur*
[b]*Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, Cheras,56000 Kuala Lumpur*

*Corresponding author: victor@greencity.edu.my
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**ABSTRACT**: Assessment of heavy metals content in different commercial fish species are of particular interest since fish is important to the human diet and nutrition, apart from being a bioindicator for marine pollution and contamination and food safety. Most contemporary studies focus either on single markets, ports, seaside markets or direct sampling from natural habitat. Very few studies were conducted on fish samples obtained from both wet markets and supermarkets; hence this present study aims to compare the heavy metal concentration of marine fish from these places. Selected organs isolated from *Rastrellinger kanagurta*, *Epinephelus sexfasciatus*, *Lates calcarifer*, and *Decapterus maruadsi* were analysed for Cd and Pb using Flame AAS via dry ashing-acid digestion method. Results from wet markets and supermarkets were statistically compared for any significant difference ($p<0.05$). Cd and Pb contents ranged from 0.489 – 2.366 mg/kg and 0.616– 2.638 mg/kg respectively for wet market samples while supermarket samples ranged from 0.121– 2.667 mg/kg and 0.813 – 3.124 mg/kg. Mean comparisons between markets were also found to be significantly different though Cd and Pb contents in the edible organs fell within the safety limit for human consumption when compared to the standard permissible limits from the Fourteenth Schedule of Malaysian Food Regulations 1985.

**Keywords:** Acid digestion, dry ashing, Flame AAS, heavy metals, supermarkets, wet markets.

## Introduction

It is well known that fish provide essential source of cheap and readily available proteins alongside with minerals, omega-3 fatty acids, amino acids, and vitamins which are important in maintaining human health and well-being (Hajeb *et al.*, 2009; FAO, 2010). The reputation of fish as health food has well established itself as a key nutritional requirement in most parts of the world and thus its consumption is highly recommended by health practitioners, nutritionists and dieticians to combat non-communicable diseases, pushing aside meat, poultry and eggs. Malaysians, being blessed with abundance fish supplies of various species and other seafood products, consume about 60-70% of proteins from such sources (Tukiman *et al.*, 2006; Zuraini *et al.*, 2006).

Fishes are predatory by nature. It is positioned at the top level of the aquatic food chain. For this reason, fishes have the ability to bioaccumulate heavy metals that biomagnifies along the food chain and store the absorbed metals in various parts of their body - mainly in organs and muscles. This bioaccumulation ability and the stored concentration however, are species and organ functional dependent.

The predicament of heavy metal poisoning and its effects on human health from long term fish consumption should nevertheless, be the priority of monitoring research to quantify those non-essential heavy metals in fish organs. Furthermore, such a study when carried out periodically may be useful to assess the trend and the state of current marine environment pollution, as well as on the product handling and management this minimizing unnecessary heavy metal exposure to human health through frequent fish consumption.

The exponential growth of global human population since the last century has unprecedentedly exerted multiple destructive pressures by various economic activities on the natural environment. The marine ecosystem itself is not being spared from anthropogenic pollutions and these are becoming even more intensified (Raja *et al.*, 2009). Industrial wastewater discharge, sewage effluent and emissions that contain heavy metals from various source points have greatly affected the quality and equilibrium of marine food web that further affects the natural structure and functions of marine biotic communities at a larger scale. Marine fishes therefore are susceptible and vulnerable to the chemical changes made by these heavy metals contaminants which bioaccumulate and biomagnify along the aquatic food chain (Kennish, 1998; Agusa *et al.,* 2007). These toxic elemental chemicals are subsequently reaching human body through consumption of contaminated fish. The effect may lead to serious deterioration of human health status (Virtanen, 2007; Raja *et al.*, 2009; Alinnor and Obiji, 2010).

Wet market, as described by Shackleton *et al.* (2009) is the typical local market setting whereby the market produce sold by vendors are displayed either on the countertop, stalls, or on the floor. Such settings vary upon locations. Supermarket on the other hand, denotes the large-format of modern retail by having a larger self-service store in selling groceries, seafood, poultry and dairy products and household goods with customers typically assumed to travel up and down the aisles of the store premise (Burger *et al.*, 2004; Reardon *et al.*, 2004; Larson *et al.*, 2005; Arda, 2006). Such facilities are commonly found in urban settings, where they provide a 'one-stop' convenience center for shoppers to obtain their grocery needs. With the majority of Malaysians obtaining and consuming fish from either commercial sources, it is of worth to carry out an assessment of heavy metals content in fishes from these sources.

Klang Valley, a region in the state of Selangor is located in the southwestern part of the Malaysian Peninsula. It is a basin that covers an area of 2842 $km^2$ (Abas and Simoneit, 1996). Kuala Lumpur, the metropolitan capital city of Malaysia lies in the heart of this region together with adjacent major economic towns such as Petaling Jaya, Damansara, Subang Jaya, Shah Alam and Klang (Nasr Yousef *et al.,* 2002; Sharifi *et al.,* 2006). Being the center for commerce, education, administrations, industries, recreation and tourism, Klang Valley boasts the highest human population in Malaysia with a total figure of 6.825 million as of July 2010 (Department of Statistics Malaysia, 2011). This is unquestionably the results of rapid development that attracts both locals and internationals for employment, investment, recreational and residency opportunities.

Residents of this region especially those of Kuala Lumpur and its adjacent townships enjoys modern lifestyles, public facilities, seamless transport connectivity and shopping centers. Those working class urbanites of medium and high income earners as well as the younger generation, are more inclined to obtain their food needs from supermarkets and other similar premises (i.e. malls and hypermarkets) due to the clean and tidy settings, attractive price offerings, as well as better facilities (e.g. secured indoor vehicle parking, and washrooms) provided by the management of the premises. This is opposed to the conditions exhibited in wet markets which are typically wet, mushy, having unpleasant odours, lack and/or unsecure vehicle parking facilities.

Reported occurrences of contaminated commercial fish around the world had successfully raised the safety concern of many quarters, especially health advisors and food authorities (Burger *et al.*, 2004). This present preliminary study, therefore, was conducted with the objective to draw comparison between the levels of harmful heavy metals in edible fish obtained from wet markets and supermarkets in Klang valley.

## Materials and Methods

*Fish samples and sampling*

Four commercially important fish species were used in this study, namely *Rastrellinger kanagurta*, *Decapterus maruadsi*, *Epinephelus sexfasciatus* and *Lates calcarifer*. The fish samples were randomly selected with reference to their respective standard lengths in mind: *Rastrelliger kanagurta* (24 cm), *Decapterus maruadsi* (25 cm), *Epinephelus sexfasciatus* (21 cm), and *Lates calcarifer* (25 cm). All samples were purchased from nine different wet markets and supermarkets in Klang Valley. The fish samples were packed in clean zipped polythene bags and transported to UCSI University Chemistry Laboratory in an ice-filled polystyrene insulation box. Fish samples were immediately transferred into a designated clean laboratory freezer upon arrival and stored at temperature of -2 °C prior to sample preparations.

*Test samples preparation*

Stored frozen fish were allowed to thaw at ambient room temperature in its respective zipped polythene bags, after which careful scale removal were done. De-scaled fish were subsequently rinsed with ultrapure water prior to dissection for the isolation of the following internal organs as test samples: brain, gills, intestines, kidneys, liver and flesh muscles. Dissections were carried out with utmost care and in a clean manner to avoid any unnecessary injuries and puncture on the organs of interest as well as to prevent any possible metal contaminations on the isolated test samples. Clean and sterilized stainless steel surgical blades, scissors and tweezers were used and changed for every new fish samples.

The isolated organs were carefully sliced and minced manually and weighed accurately to 3.00 ± 0.05 g (wet weight) in individual *aqua regia* sanitized porcelain crucibles and subsequently subjected to oven drying at 180 °C for four hours. The dried samples were later ashed at 500 °C for 12 hours in a muffle furnace (ThermConcept, Germany). Cooled ashes were then digested with 1.5 mL of concentrated 65% $HNO_3$ analytical grade (Merck Chemicals, Germany) and subsequently mixed and diluted with ultrapure water to 30 mL. These diluted test samples solution were then filtered through Whatman® No. 595 filter paper prior to AAS analysis.

*Glasswares sanitization, sample blanks and chemical preparation*

All glassware were soaked overnight in *aqua regia* solution of 1:1 analytical grade 37% HCl and 65% $HNO_3$ (Merck Chemicals, Germany) for sanitization, while porcelain crucibles were soaked in the same solution for three hours. Soaked glassware and crucibles were then rinsed with ultrapure water, and were left to air-dry in a drying oven for 12 hours prior to usage. The preparation procedures of sample blanks were similar to that of the test samples, and these were used for spectrometry background correction. Standard solutions for Cd and Pb were prepared from stock solutions of 100 ppm, respectively. The test samples solution were then analysed thrice for Cd and Pb content using air-acetylene flame AAS (Perkin Elmer AAnalyst 100). Detected metals were expressed as $mg \cdot kg^{-1}$ wet weight (Suhaimi *et al.*, 2005).

*Statistical analysis*

All data obtained were analysed using two-way ANOVA using SPSS Statistical software ver. 14 to determine the significant differences ($p<0.05$) of the means value of each metals present within the organs of the four selected fish species. A post hoc analysis using Scheffe's test was used to further verify the analysed mean differences between the organs. All data were presented in wet weight basis as it was more useful and presentable for health risk considerations since animals as well as human consume organisms in their natural state. Wet weight moreover, was chosen as it was a more convenient fisheries products safety assessment in accordance with the objective of this study; that of assessing and evaluating the safety of commercial marine fisheries with respect to the level of Cd and Pb detected in the sampled four species for this study (CRESP, 2006).

## Results

The mean concentration levels of Cd and Pb detected in the samples tested from the four fish species were analyzed according to the type of markets is shown in **TABLE 1**. The overall mean concentration of Cd and Pb detected in four different paired organs samples were also tabulated and compared based on their respective type of markets. Comparisons of Cd mean concentrations (mg/kg) in test organs of four fish species between wet markets and supermarkets is shown in **FIGURE 1**.

**TABLE 1:** Mean concentrations levels of Cd and Pb in four fish species sampled from wet markets and supermarkets

| Types of markets | Organs | [1]Cadmium mean concentrations (mg/kg) | | | | [2]Lead mean concentrations (mg/kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Rastrellinger kanagurta* | *Epinephelus sexfasciatus* | *Lates calcarifer* | *Decapterus maruadsi* | *Rastrellinger kanagurta* | *Epinephelus sexfasciatus* | *Lates calcarifer* | *Decapterus maruadsi* |
| Wet markets | Brain | 0.489±0.099 | 1.246±0.044 | 0.654±0.163 | 0.657±0.067 | 1.007±0.049 | 1.344±0.159 | 1.386±0.146 | 1.278±0.161 |
| | Gills | 1.482±0.119 | 1.074±0.062 | 0.874±0.073 | 1.162±0.108 | 1.461±0.078 | 1.419±0.121 | 1.097±0.095 | 1.151±0.063 |
| | Intestine | 1.160±0.111 | 0.820±0.043 | 0.699±0.044 | 0.964±0.086 | 1.256±0.064 | 1.060±0.017 | 0.865±0.086 | 1.484±0.033 |
| | Kidney | 1.289±0.060 | 2.366±0.151 | 0.766±0.044 | 0.790±0.073 | 1.366±0.084 | 2.638±0.073 | 0.912±0.051 | 1.599±0.103 |
| | Liver | 1.062±0.139 | 1.959±0.212 | 0.767±0.120 | 1.225±0.089 | 1.212±0.069 | 2.145±0.065 | 0.995±0.078 | 1.635±0.073 |
| | Flesh Muscle | 0.863±0.042 | 0.536±0.024 | 0.497±0.031 | 0.119±0.007 | 1.506±0.095 | 0.616±0.046 | 1.045±0.088 | 1.419±0.070 |
| Super-markets | Brain | 0.940±0.040 | 1.408±0.031 | 1.146±0.101 | 1.631±0.111 | 1.010±0.043 | 1.577±0.087 | 1.398±0.059 | 1.428±0.115 |
| | Gills | 2.080±0.052 | 1.201±0.031 | 1.138±0.056 | 2.460±0.116 | 1.795±0.085 | 1.434±0.144 | 1.183±0.075 | 1.554±0.130 |
| | Intestine | 1.849±0.054 | 1.153±0.039 | 1.001±0.036 | 1.865±0.150 | 1.657±0.107 | 1.368±0.052 | 1.191±0.045 | 1.742±0.088 |
| | Kidney | 1.767±0.087 | 2.667±0.097 | 1.044±0.040 | 1.796±0.142 | 1.475±0.084 | 3.124±0.217 | 1.092±0.074 | 1.747±0.078 |
| | Liver | 1.245±0.144 | 2.153±0.163 | 1.057±0.032 | 2.016±0.175 | 1.368±0.072 | 2.306±0.098 | 1.156±0.035 | 1.775±0.073 |
| | Flesh Muscle | 0.982±0.055 | 0.774±0.052 | 0.574±0.033 | 0.121±0.016 | 1.594±0.053 | 0.813±0.070 | 1.144±0.085 | 1.399±0.166 |

[1,2]Mean values ± SD were obtained from three replications.

Comparisons of Pb mean concentrations (mg/kg) in test organs of four fish species between wet markets and supermarkets is shown in **FIGURE 2**. Kidney samples from both wet markets and supermarkets had the highest mean concentration levels of Cd followed by liver tissue samples, as shown in **TABLE 2**. The level of Cd in kidney samples from wet markets and supermarkets were 1.303±0.155 mg/kg and 1.819±0.139 mg/kg respectively. The Cd levels detected in liver samples were 1.253±0.121 mg/kg and 1.618±0.126 mg/kg. Thus, Cd has higher bioaccumulation in the kidney tissues as compared with the liver tissues.

The highest mean concentration levels of Pb were detected in kidney samples from wet markets and supermarkets with the tabulated amount of 1.628±0.150 mg/kg and 1.860±0.185 mg/kg, respectively. This study also found that Pb had tendencies to bioaccumulate in liver tissues as well with respective concentrations of 1.497±0.106 mg/kg and 1.651±0.106 mg/kg detected from the wet markets and supermarkets samples.

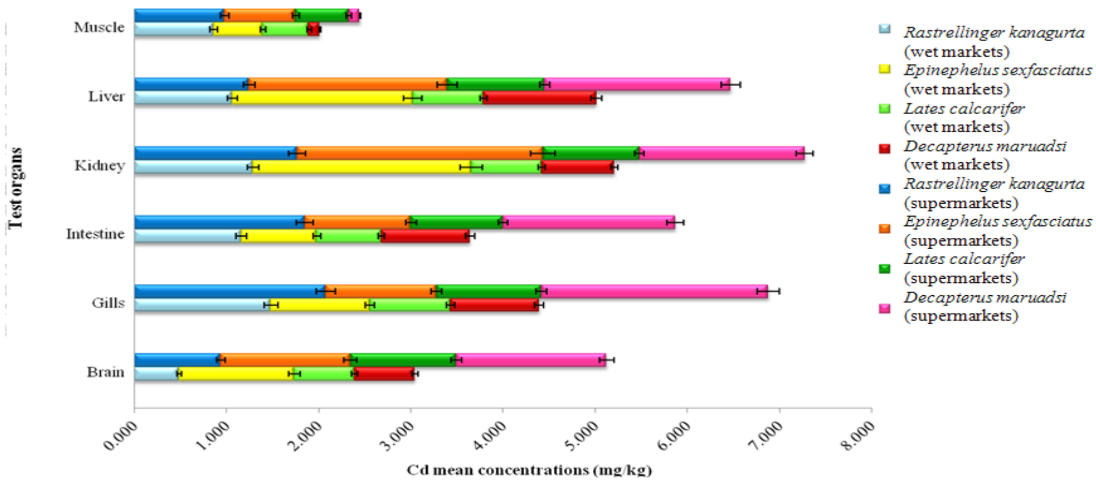


**FIGURE 1:** Comparisons of Cd mean concentrations (mg/kg) in test organs of four fish species between wet markets and supermarkets

**TABLE 2:** Overall comparison between paired test organs from wet markets and supermarkets samples around Klang Valley

| Pairs | Organs | N | Mean (mg/kg) | |
|---|---|---|---|---|
| | | | Cadmium | Lead |
| Pair 1 | [1]Brain | 20 | 0.761±0.081[a] | 1.254±0.071[c] |
| | [2]Brain | 20 | 1.281±0.070[a] | 1.353±0.061 [c] |
| Pair 2 | [1]Gills | 20 | 1.148±0.066[a] | 1.282±0.056[b] |
| | [2]Gills | 20 | 1.719±0.134[a] | 1.492±0.072[b] |
| Pair 3 | [1]Intestine | 20 | 0.910±0.053[a] | 1.166±0.059[b] |
| | [2]Intestine | 20 | 1.467±0.982[a] | 1.489±0.062[b] |
| Pair 4 | [1]Kidney | 20 | 1.303±0.155[a] | 1.628±0.150[b] |
| | [2]Kidney | 20 | 1.819±0.139[a] | 1.860±0.185[b] |
| Pair 5 | [1]Liver | 20 | 1.253±0.121[a] | 1.497±0.106[b] |
| | [2]Liver | 20 | 1.618±0.126[a] | 1.651±0.106[b] |
| Pair 6 | [1] Flesh Muscle | 20 | 0.504±0.062[a] | 1.147±0.088[c] |

| | | | |
|---|---|---|---|
| [2] Flesh Muscle | 20 | 0.613±0.076[a] | 1.238±0.082[c] |

[1]Organs collected in all four fish species from wet markets
[2]Organs collected in all four fish species from supermarkets
[a,b]Mean values within same pair are significantly different ($p<0.05$)
[c]Mean values within same pair are not significantly different ($p>0.05$)

The sequence of Cd and Pb concentrations detected in all test organs used in this study was kidney > liver > gills > intestine > brain > muscles. Overall, higher Cd and Pb concentrations were detected in all test organs obtained from supermarkets fish compared to wet markets fish. Considerable variations of fish exposure towards heavy metals contaminations between different type of markets were suspected in this study as the detected Cd level were significantly different ($p<0.05$) in all six test organs. The Pb level detected in brain and flesh muscles samples examined in fish collected from both wet markets and supermarkets on the other hand, had no significant differences ($p>0.05$). There were significant differences in the overall comparison of Cd and Pb mean concentrations between fish sampled from wet markets and supermarkets. The mean concentrations of Cd and Pb obtained in this research were further compared with several available guidelines on heavy metals permissible limits (mg/kg) for food safety (wet weight basis) as shown in **TABLE 3**. The levels of Cd and Pb detected from this study were still appreciably within the permissible limits stipulated by the Malaysian Food Regulation 1985 apart from USFDA 1990 and HKEPD 1997.

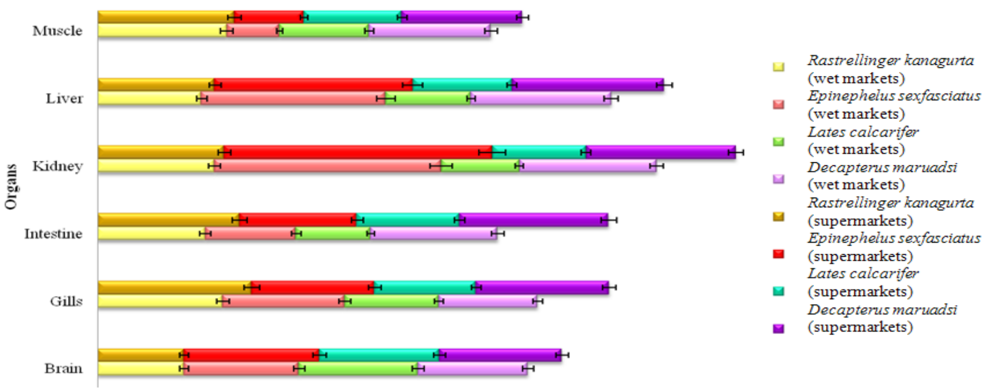


**FIGURE 2:** Comparisons of Pb mean concentrations (mg/kg) in test organs of four fish species between wet markets and supermarkets

**TABLE 3:** Guidelines on heavy metals permissible limits (mg/kg) for food safety (wet weight basis)

| **Organisations** | **Permissible limits (mg/kg)** | |
|---|---|---|
| | **Cadmium** | **Lead** |
| Fourteenth Schedule (Regulation 38), Malaysian Food Regulation 1985 | 1.00 | 2.00 |
| United States Food and Drug Administration 1990 | 3.70 | 1.70 |
| Hong Kong Environmental Protection Department 1997 | 2.00 | 6.00 |
| Food and Agriculture Organization 1983 | 0.50 | 0.50 |

**Discussion**

Rapid developments in industrial and agricultural sectors have undoubtedly contributed to the increased pollution in the natural environment. This includes heavy metals pollution level in marine environment despite the nature of heavy metals which leach slowly into thw aquatic ecosystem. Furthermore, the presence of microplastics in the marine environment amplifies the chances and possibilities of ish to uptake heavy metals through water ingestion owing to its small size and large surface area that may act as a carrier for heavy metals (Gregory, 1996; Browne *et al.*, 2009; Fendall and Sewell, 2009; Cole *et al.*, 2011). Hence, heavy metals contamination and its toxicity assay have received much attention among researchers by using fish as the dominant bioindicator species. Cd and Pb are the two metallic species commonly studied by researchers. These metallic species could present at significant level in the aquatic environment and this poses high risk of toxicities towards the aquatic organisms (Zhou *et al.*, 2008).

Fish kidney, which mainly contains excretory tissues that are involved in filtering liquid waste materials from the blood, is located along the dorsal wall of the fish body and the liver which lies below the fish stomach acts as the vital homeostasis site (Dallinger *et al.,* 1987; Reynders *et al.,* 2006). Higher tendencies of bioaccumulation for Cd and Pb found in the fish kidney and liver tissues are in accordance with the common functions of these two organs to carry detoxification process. Cd and Pb are able to form strong fixation with the free protein-thiol group present inside the kidney and liver tissues which contains metallothioneins binding proteins (Iwegbue, 2008).Metallothioneins binding proteins are also found in fish gills (Dallinger *et al.*, 1987) that are able to trap heavy metals particles through surface adsorption. Marine fishes are susceptible to heavy metal cationic exposure from the surrounding sea water. These waterborne cationic heavy metal fractions are able to adsorb on the gills surfaces, particularly when marine fish drink considerable amounts of sea water. Therefore, fish gills also serve as one of the important routes of waterborne Cd and Pb bioaccumulations during respiration and osmoregulation processes (Dallinger *et al.,* 1987; Reynders *et al.,* 2006; Di Giulio and Hinton, 2008).

Fish intestine, which is part of the digestive tract acts as a transient site for heavy metal bioaccumulations in fish body compared to other organs tested in this study. Cd and Pb are able to form complexation with the intestinal small peptides and amino acids due to their high affinity for protein thiol-group that present inside the fish intestine. Therefore, fish intestine is associated with bioaccumulation of heavy metal fractions through alimentary tract. The rate of these heavy metal uptakes are controlled by the specific transport system across the intestinal epithelium via simple diffusion mechanism (Filipovic and Raspor, 2003; Marijic and Raspor, 2007).

Fish brain, which is enclosed in the skull cavity functions as the control center that process automatic functions and higher behaviors information. The blood brain barriers which are present in the fish brain are involved in protecting the vulnerable brain tissues from perturbations of heavy metal particulates, preventing fish against neurotoxicity effects. Furthermore, the metallothioneins within the brain tissues are found not as inducible as those metal binding proteins located in the kidney and liver. This further supported the results collected from this study in which lesser amount of Cd and Pb were detected in the fish brain when compared with the kidney and liver tissues (Filipovic and Raspor, 2003; Marijic and Raspor, 2007).

Fish flesh is composed of striated muscle tissues and is also the edible part of fish by humans. The flesh is frequently employed by researchers as one of the test organs in human health risks assessment related to marine fish consumptions (Altindag and Yigit, 2005). The surface of fish skin is coated with a mucous layer that acst as the first line of defense to protect the integrity of fish flesh muscle tissues from its surrounding contaminants. This mucous layer naturally serves as a barrier that hinders the entrance of heavy metals particulates into the flesh muscle tissues by forming complexes with heavy metals.

The presence of this mucous layer will lead to lesser bioaccumulation of heavy metals in fish flesh compared to the other organs tested. This further justifies the lowest concentration of Cd and Pb detected in fish muscle tissues although higher Cd and Pb level were detected from the supermarkets fish samples. However, the amount of Cd and Pb that were bioconcentrated in the sampled fish flesh were still within the permissible limits as stated in the Fourteenth Schedule (Regulation 38) of Malaysian Food Regulation 1985 (Schlenk and Benson, 2001; Altindag and Yigit, 2005; Uysal *et al.,* 2008).

Based on these findings, higher bioconcentrations of Cd and Pb were detected in fish samples purchased from respective supermarkets around Klang Valley as compared to wet markets samples. However, clear justifications to support such outcome still remains unknown as similar studies have yet to be done by other researchers. Moreover, this study mainly assessed and compared the distributions of Cd and Pb levels in commercial fish sampled between wet markets and supermarkets due to budget, instrumental and time constraints. Further detailed studies are strongly needed to justify the following closest postulations and explanation made in order to support the results of this study.

The influent of perimortem and post-mortem conditions which include rigor mortis, ambient and storage temperatures as well as the effects of freezing on cellular metabolism are among the speculations made which are suspected to be related with higher amount of Cd and Pb detected in supermarkets fish. Erikson and Misimi (2008), Cappeln and Jessen (2002), Abe and Okuma (1991) and Harada (1988), have studied the relationship of perimortem and post-mortem conditions with rigor mortis in fish flesh muscles. Their findings reported that ATP metabolism still occurred when the fish flesh are subjected to temperature ranges of 0 to 30°C. Thus, the variations and fluctuations in ambient temperatures between supermarkets and wet markets are suspected to facilitate the movements of heavy metals in dead fish.

The majority of the wet markets settings in Klang Valley have warmer ambient temperatures in which this condition is associated with shorter ATPase activities in dead fish because the optimal conditions for enzymatic reactions and also the substrates are completely used up in much shorter time. Contrastingly, supermarkets have cooler temperature settings which prolongs the ATPase activities and enable more heavy metals to be transported across cell membranes until the remaining ATPs are totally depleted. Supermarket facilities have the advantage in regulating and maintaining their in-house ambient temperature through the centralized air-conditioning systems that slows down the enzymatic and bacterial spoilage.

The presence of cell-surface functional groups on both, gram-positive and negative-positive bacteria are also thought to cause intracellular accumulation of trace metal fractions under anaerobic conditions in which these trace metals are used as the electron acceptors in enzymatic reactions. The mobilization and transportation of metal particulates in aquatic systems are also strongly associated with the production of extracellular polysaccharides (EPS) by most microorganisms that form strong fixation through the EPS-metal interactions (Ford and Ryan, 1995).

The enzymatic activities of ATPase ionic pumps are also speculated to be affected by storage temperatures since the movements of Cd and Pb across cells occur through active transport. This process required the utilization of ATP supply which is also facilitated by P-type ATPase ionic pump, majority are from the IB ATPase subfamily since Cd and Pb are both divalent soft Lewis acids (Gatti *et al.*, 2000). Overall, the availability of ATP, temperatures and pH influences the phosphorylation and dephosphorylation processes occur during perimotem and post-mortem in dead fish.

Freezing is also another closest postulation which is associated with higher Cd and Pb contents in supermarkets fish. The increase in concentrations of dissolved organic and inorganic salts is observed when liquid water changes into ice (Tull, 1996; Potter and Hotchkiss, 1995; Love and Haraldsson, 1958). The size of ice crystals basically is determined by the freezing method whereby these ice crystals can easily cause cell rupture that further permits movements of cytoplasmic fluid that possibly contain heavy metals, especially when the fish is being thawed. Slow freezing method that results in the formation of large ice crystals introduce more damage to the delicate tissue cells meanwhile quick freezing method reduces the size of these ice crystals and results in better product's quality in terms of textures, freshness, coloration and the tenderness of meat.

Some supermarkets retailers have been suspected to practice refreezing including intermittent freezing of the unsold fish stocks overnight. This is with regards to the availability of cold storage facilities in their premises. Such refreezing practices however will lead to the increment of Cd and Pb contaminants from the adjacent fishes that were mixed together with the surrounding environment.

Furthermore, supermarkets retailers were also found to mix the new batch of fish stocks with the leftover unsold fishes as per on-site observations made during field sampling. This can be observed from the physical conditions of those re-sold fishes which were often dull in appearance, shrunken abdomen, torn flesh, loss of meat firmness and elasticity, white pale eyes, and darken (at times pale) gills with liquid dripping from body orifices when fish was held upright.

Contrastingly, the scenario observed from wet markets was different from those of supermarkets. The selling activities commonly started as early as pre-dawn and usually will finish by noon when the surrounding environment during this period was still considerably cool. It has been speculated as well that the ambient temperature of wet market hasten the microbiological and enzymatic spoilage in fishes. Moreover, the practice of restoring the unsold fishes are not commonly favored among wet market retailers instead they preferred to either spread ice chunks or sprinkle pipe water on the fishes during their operation hours.

Microbiological and enzymatic spoilage in fish are the two factors which are influenced by temperature. Thus, final freezing temperature is a critical point in maintaining the freshness of fish. Hall (1997) further elaborated that fish contains about 75 percent of water by body weight whereby 90 – 95 % of this water freezes at $-25^{o}C$. The chemically bond water which binds with hydrogen bonds, carbonyl and amino groups of proteins are not available for freezing. Thus, temperature of -18$^o$C have been employed as part of safety measure in most storage and transportation of fish stocks. Nevertheless, -18$^o$C is not considered as low enough to inhibit the non-enzymatic reactions even at a very low rate provided that the bound water inside the cells is still available to serve as substrates for the reaction to occur (Potter and Hotchkiss, 1995). Hence, this could be a reason for higher Cd and Pb contain found in supermarkets.

## Conclusion

This study highlighted the comparison of Cd and Pb levels in fish sold between wet markets and supermarkets within Klang Valley that will serve as a baseline reference for future continuous studies. The levels of Cd and Pb detected in different test organs of the four selected fish species were significantly higher in supermarket samples compared to those samples of wet markets. The edible fish muscles in all of the fish samples examined in this study were found not heavily contaminated with Cd and Pb as the levels were within the permissible safety limits.

The significant difference of Cd and Pb contents in fish sampled between wet markets and supermarkets might be influenced by several factors during perimortem and postmortem fish handling stages. The actual scenario of freezing and storage practices by trawlers remained unknown that could also further influence heavy metals concentrations in the fish captured. Nevertheless, dietary hazards due to Cd and Pb exposures upon consumption of the selected fish species sold in wet markets and supermarkets around Klang Valley are still possible to occur. Hence future similar studies are highly recommended to further justify the amount of heavy metals that bioconcentrated in marine organisms particularly fishes that could reflect the state of marine pollution.

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