Heavy metal bioaccumulation in commercial Lethrinidae fish species in Mauritius

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Abstract

Concentrations of heavy metals arsenic, cadmium, lead and mercury and trace elements chromium, copper, nickel and zinc were tested in the muscle tissue of four commercial edible lethrinids fish species from different region of Mauritius. Sky emperor (*Lethrinus mahsena*) was collected from coastal regions as well as offshore regions (banks) for this study. Blackspot emperor (*Lethrinus harak*) and spangled emperor (*Lethrinus nebulosus*) were also studied for their popularity in the fish market. Condition factor was calculated for each fish and the highest value obtained was in *Lethrinus mahsena* collected from offshore regions (2.598 cm/g). Flame atomic absorption spectrometry was used to analyse copper, nickel and zinc while, graphite furnace technique was used for cadmium, chromium and lead. Mercury levels were evaluated with the cold vapour technique and arsenic with the thermal hydride cell. The level of lead, mercury, copper, nickel and zinc ranged from 0.0011-0.0024 mg/L, 0.0016-0.0036 mg/L, 0.080-0.389 mg/L, 0.566-1.192 mg/L and 0.219-0.422 respectively in wet weight. Inter-species variations in levels of heavy metals and trace elements were observed for nickel and no significant variations occurred for mercury, lead and zinc. Concentrations of heavy metals and trace elements were all within the permissible level except for nickel. As per this study, the Food Act 1998 of Mauritius must be reviewed in order to incorporate the Maximum Permissible Level for nickel.

Introduction

Increased heavy metal levels in the aquatic environment is attributed to human activities such as domestic effluents, agricultural runoff, offshore oil and gas exploration, industrials (paints, fertilisers, pesticides, textile, leather and pharmaceuticals) and mine drainage (Ansari *et al*., 2004). Erosion, volcanism and magmatic activity is said to cause atmospheric metal pollution responsible for dissolved metals such as arsenic (As), cadmium (Cd), copper (Cu), Iron (Fe), Nickel (Ni) and Zinc (Zn) to be present in the oceans (Chiarelli and Roccheri, 2014). Rise in the level of heavy metals in the marine environment has caused severe problem to the marine organism and humans (Bashir *et al*., 2013). Due to their capability to bio-accumulate heavy metals, edible fishes have gained serious concern and importance as the consumption of wild and aqua-cultured fish increased (Elnabris *et al*., 2012). Arsenic, cadmium , mercury (Hg) and lead (Pb) are categorised as harmful substances whereas chromium (Cr), copper, nickel and zinc are said to be beneficial to the human body system (Duffus, 2002; Duruibe *et al*., 2007). Lead is susceptible to children as it affects the developing nervous system due to rapid growth and metabolism. In addition, accumulation of cadmium in the human body has negative effects on several organs such as kidney, lungs, brain and kidney. Mercury is considered most toxic and ingestion in high concentrations affect developing fetus. However, if the concentration of trace elements such as copper is present in high concentration, this can cause adverse effects such as chronic anemia. Similar studies had been done in scombrids in Mauritius (Bhoyroo *et al*., 2015) and results showed levels of trace elements and heavy metals within permitted threshold levels for safe consumption, whereas threshold for Chromium level was not listed in Food Act 1998 of Mauritius.

Lethrinidae fish are widely consumed and are considered among the favourite dish of Mauritian. The Food and Agricultural Organisation (FAO) International Standard Statistical Classification for Aquatic Animals and Plants (ISSCAAP) has represented Lethrinidae fish family as demersal fishes and classified them as bottom-feeding (bottom-dweling) carnivorous fish. Due to their feeding habits and long life (30 years), Lethrinids can be used as an indicator of heavy metal bioaccumulation in the aquatic environment since they feed on mainly molluscs, crustaceans, sea urchins, hard-shell invertebrates and sometimes fishes (Carpenter and Allen, 1989). Increase in population together with increase in industrialization, have resulted in an increase in pollution to the marine environment and are causing potential risk for the marine organism and human health (Matoka *et al*., 2014). Mauritius, being a small island is experiencing rapid growth in industry, tourism and population with consequent increase in production of more waste and the release of untreated sewage to the marine environment. The suggested Maximum Permissible Level (MPL) for heavy metals in the human diet as a safety regulation by international and national agencies such as the World Health Organisation (WHO), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the Ministry of Agro-industry and Food Security (MAIFS) together with the Mauritian Food Act 1998 are set to identify the concentration of heavy metals in foods. The Fulton's condition factor is used to calculate the relationship between the length and the weight of the fish in order to estimate the condition and feeding intensity of the fish (Ighwela *et al*., 2011).

The length and weight relationship is important in fisheries biology to assess the relationship between the weight and length and to find potential variances between different stocks of the same species (Ighwela *et al*., 2011). Moreover, the condition factor provides information about the feeding behaviour and growth rate, and is affected by the physiology and stage of growth of a fish (Da Coasta and Araújo, 2003;

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Key words: Heavy metals, Lead, Mercury, Nickel, Lethrinids.

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Vasantharajan *et al*., 2013). The assumption made to assess the condition factor of a fish is that heavier the fish of a particular length, the better the condition factor (Froese, 2006). Barnham and Baxter (1998) have adopted a standard to classify the condition factor of salmon. The condition factor (k) was used to predict the health status and to estimate the feeding intensity of the fish (Abowei, 2010; Jin *et al*., 2015).

Materials and Methods

Selected fish species (Figure 1) were sampled from local markets and fishermen. The mass of the fishes (g) was recorded in an electronic balance and the lengths (cm) were determined using a fish board before dissection. The condition factor (k) was calculated using the following equation:

k=(weight in grams) * 100/(length in cm) 3

100 g muscle tissues from each fish, mainly the mid dorsal muscle was cut into pieces and placed in a polyethylene bag for further analysis in the laboratory. Stomach and part of the intestine were dissected to study the feeding content of each species. All equipment used was cleaned properly and acid washed before reuse to prevent contamination. One hundred g of each species were dried separately in an oven (CARBOLITE) at 70°C till constant weights were attained. Each dried sample was blended in an electric blender and for rapid and effective ashing. Then, 10 g of each blended sample was placed in a 50 mL crucible, labeled clearly with a heat resistant steel ball paint marker and were transferred in a muffle furnace (CARBOLITE S302RR) and ashed at 450°C for 8 hours (García-Montelongo *et al*., 1994). After 8 hours, each crucible was removed and 2 mL of HNO_3 was added to the sample, allowed to evaporate to dryness in a sand bath and transferred again into the muffle furnace to obtain carbon free solution (Perkin-Elmer, 1996). Once the crucible was cooled at room temperature, the white ash was treated with 5 ml HNO_3 in a sand bath at 60°C, filtered with ashless filter papers to eliminate any un-dissolved residues and volume made up to 25 mL with distilled water. Samples were then transferred in plastic bottles of 50 mL and placed into the refrigerator at 4°C for further analysis. The element selected was analysed using different techniques of AAS as suggested by Olowu *et al*. (2009).

Detection of metal through flame atomic absorption spectroscopy

Flame spectroscopy is an analytical technique used for the qualitative and quantitative determination on an element that uses thermal energy in the form of flame to excite free atoms to higher energy levels (Nick, 1995). The metals detected using the flame emission spectroscopy are cadmium, chromium, copper, lead, nickel and zinc. However, it must be noted that cadmium, chromium and lead was not detected when using flame spectroscopy and hence the graphite furnace technique was used. The working range was from 1 ppm to 5 ppm on heavy metals in fish in the marine environment. Standard solutions were prepared in parts per million. Metals such as cadmium, chromium, copper and lead were further analysed using the graphite atomic absorption spectrophotometer. The standard solution was prepared in parts per billion and the working range was from 1 ppb to 5 ppb.

Detection of arsenic and mercury through hydride atomic absorption spectroscopy

Hydride atomic absorption spectroscopy system is a continuous flow of

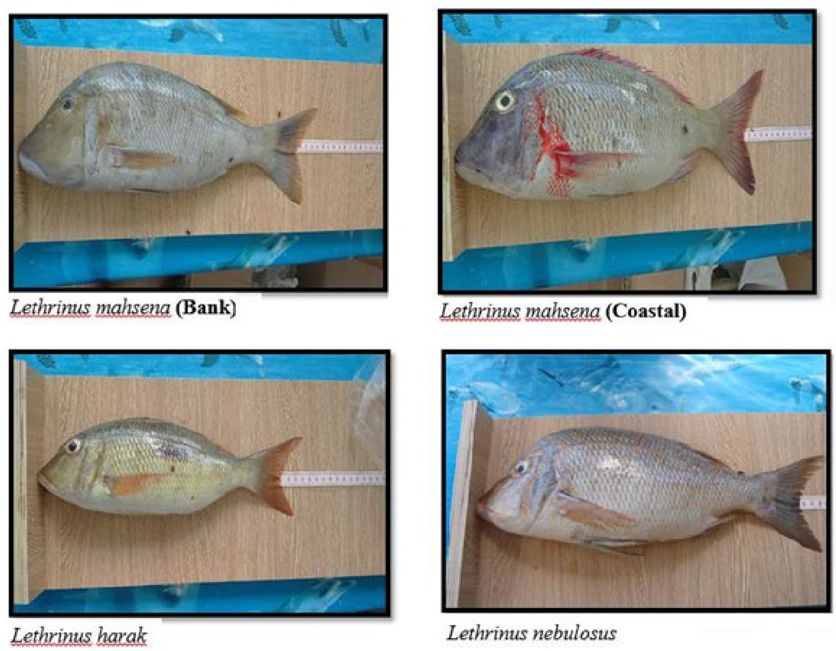

Figure 1. Plates showing the four fish species being studied for heavy metal and trace elements bioaccumulation.

Table 1. Condition factor of fish sampled.

Fish species	K (cm/g) Summer	Winter
Lethrinus mahsena Bank	0.999	2.598
Lethrinus mahsena Coastal	1.881	2.023
Lethrinus harak	1.488	1.121
Lethrinus nebulosus	1.321	0.812
Lethrinus mahsena Bank	1.627	1.06
Lethrinus mahsena Coastal	1.662	1.444
Lethrinus harak	1.424	2.285
Lethrinus nebulosus	1.226	1.46
Lethrinus mahsena Bank	1.561	1.761
Lethrinus mahsena Coastal	1.558	1.438
Lethrinus harak	1.453	1.564
Lethrinus nebulosus	1.19	1.674

K, condition factor. The condition factor of the fish ranged from 1.0 cm/g to 2.6 cm/g. Only for *Lethrinus mahsena* (*Bank*) in summer and *Lethrinus nebulosus* in winter, the condition factor was below 1 (0.999 cm/g and 0.812 cm/g respectively.

vapour generation system that increases the sensitivity of the atomic absorption technique for mercury and hydride forming metals such as arsenic. Sodium borohydride ($NaBH_4$) operation was used instead of the stannous chloride ($SnCl_2$) and the operation involved reaction of the analyte in an acidified solution with $NaBH_4$ to form gaseous hydride. Arsenic was detected using the thermal hydride cell system and mercury was detected using the cold vapour technique. The standard solution was prepared according to the working standard ranging from 1 ppb to 5 ppb.

Results and Discussion

Analysis of heavy metals such as As, Cd, Hg, Pb and trace elements such as Cr, Cu, Ni, and Zn through the atomic absorption spectrometer in muscle tissue of the four fish species namely the sky emperor (*Lethrinus mahsena, Bank*), sky emperor (*Lethrinus mahsena, coastal*), blackspot emperor (*Lethrinus harak*) and the spangled emperor (*Lethrinus nebulosus*) in different region of Mauritius, confirmed the presence of trace elements primarily of Cu, Ni and Zn and heavy metals Hg and Pb. The bioaccumulation of heavy metals and trace elements in muscle of fish is mainly related to 1) feeding habits, 2) the rate at which the organism metabolise the metals and 3) the surrounding water condition (Asante *et al*., 2014). Other factors to consider include environmental factors such as salinity, hardness, temperature, pH, water and basic factor such as age of the fish (Akan *et al*., 2012;). The condition factor, K, provides information on the growth rate and the feeding behaviour of the fish and a value greater than 1 cm/g point to well being and robustness of the fish (Vasantharajan *et al*., 2013). Fish sampled were in good condition (Table 1) with K-value greater than 1.4 cm/g and only two fish species had K value less than 1.0 cm/g and were classified as poor fish (Barnham and Baxter, 1998). Thus fish taken for this study were healthy and had a proper feeding behaviour and faced ideal environmental condition, which justify for the bioaccumulation of trace elements and heavy metals in the muscle tissue of the fish. Moreover, three fish species: *Lethrinus mahsena Bank, Lethrinus mahsena Coastal* and *Lethrinus harak* had condition factors greater than 2.0 cm/g (2.598 cm/g, 2.023 cm/g and 2.285 cm/g) which can account for their feeding behaviours and rapid growth rates. The feeding habit of the lethrinids is attributed to the accumulation of arsenic and cadmium. Lethrinidae fish consume crustacean, mollusc, fish and shellfish (King and Fraser, 2002; Addison and Tindall, 1990). CODEX STAN 193-1995 (2009) confirmed the presence of arsenic and cadmium in crustaceans, fish, mollusc and shellfish (Table 2). Positive correlations were obtained between fork length and heavy metal concentrations; and similarly fresh weight and heavy metal bioaccumulation showed positive co-relation for most heavy metals except zinc (Table 3).

The level of zinc (P=0.884), lead (P=0.254) and mercury (P=0.290) accumulated in the muscle tissues were not significantly different (P>0.005) among the four fish species. The highest concentration of Zinc (0.496±0.198 mg/kg), lead (2.804±1.030 µg/kg) and mercury (4.228±1.012 µg/kg) were all recorded in *Lethrinus nebulosus*, and lowest in *Lethrinus mahsena Bank* for zinc (0.259±0.024 mg/kg), *Lethrinus mahsena Coastal* for lead (1.313±0.402 µg/kg) and *Lethrinus harak* for mercury (1.942±1.776 µg/kg) (Figure 2).

Uptake of heavy metals and trace elements is related to fish age, mass and length and aquatic environment. This clearly supports the fact that higher concentration of zinc, lead and mercury were recorded in *Lethrinus nebulosus* and lowest concentration of lead in *Lethrinus mahsena Coastal.* Kumar *et al*. (2013) also supported the fact that there are significant differences between species and heavy metals accumulation in fish. Differences in the heavy metal concentrations in studies among same species in different regions can be attributed to associated environmental factors.

In Mauritius, the Maximum Permisible level (MPL) of heavy in fish and foodstuff is regulated by the Food Act 1998. International agencies such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Union (EU), the Ministry of Agriculture, Fisheries and Food (MAFF) in Britain and the CODEX Alimentarius Commission (2011) have set regulation on heavy metals and trace element in the human diet for security issues. Each international guideline can be compared with the Mauritian Food Act 1998 as a result of differences in threshold levels of heavy metals.

At the outset, copper is a widely used element worldwide and is responsible for the development of bones in the human

Table 2. Levels of heavy metals in the different Lethrinus species.

Species		N	Minimum	Maximum	Mean	SD
Lethrinus mahsena (bank)	Copper	6	.07	.50	.2408	.17375
	Zinc	6	.21	.65	.3403	.16475
	Nickel	6	.98	1.60	1.2367	.26126
	Mercury	6	.98	4.00	2.8730	1.12229
	Lead	6	2.05	3.15	2.3918	.40361
Lethrinus mahsena (coastal)	Copper	6	.10	.49	.2395	.16164
	Zinc	6	.05	.57	.3620	.19832
	Nickel	6	.71	1.40	1.0205	.27297
	Mercury	6	.79	4.37	3.3617	1.35339
	Lead	6	.78	2.42	1.5855	.68566
Lethrinus harak	Copper	6	.08	.39	.2030	.13130
	Zinc	6	.01	.55	.3682	.20401
	Nickel	6	.55	1.14	.8672	.24819
	Mercury	6	.12	4.35	2.2118	1.44368
	Lead	6	.72	3.48	2.0262	.92427
Lethrinus nebulosus	Copper	6	.08	.77	.3895	.25981
	Zinc	6	.22	.65	.4273	.17693
	Nickel	6	.87	1.96	1.3877	.39559
	Mercury	6	2.57	5.15	3.5968	1.07054
	Lead	6	1.66	4.26	2.4912	.96763

SD, standard deviation.

Table 3. Correlation between size and heavy metal concentrations.

		Mercury	Copper	Lead	Nickel	Zinc
Fork length	Pearson correlation	.298	.543**	.357	.441*	.106
	Significance (2-tailed)	.157	.006	.086	.031	.624
Fresh weight	Pearson correlation	.320	.424*	.284	.191	-.120
	Significance (2-tailed)	.128	.039	.178	.371	.575
	N	24	24	24	24	24

*Correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed).

body but can have negative effect if the MPL is exceeded. The Mauritian Food Act has stated a tolerable level of copper of 30 mg/k (ppm), MAFF (1995) 20 mg/kg wet weight and the Provisional Tolerable Daily Intake set by the CODEX 135- 1995 ranges from 0.05 mg/kg to 0.5 mg/kg. The concentration of copper obtained in wet weight for copper ranged from 0.265 mg/kg to 0.389 mg/kg in summer and 0.079 mg/kg to 0.273 mg/kg in winter. As a result, the concentration of copper generated during the two seasons did not exceed the threshold level for International and local agencies and is near the level set by the CODEX 135- 1995 for the daily intake of copper.

Furthermore, zinc is an important element needed in very minute amount in the human body but again if the level of the metal is exceeded, this can lead to severe health effect. Taking into consideration the Food Act settle in Mauritius, the tolerable level of zinc accepted in food stuff is at 100 mg/kg (ppm) and for MAFF (1995) is at 50 mg/kg. Other agencies such as the CODEX 135- 1995 has set a Provisional Tolerable Daily Intake for zinc at 0.3 mg/kg to 1.0 mg/kg. Hence, the result obtained during summer and winter ranged from 0.219 mg/kg to 0.422 mg/kg wet weight of zinc. Hence, it can be concluded that fish sampled were safe for consumption and according to the value set up by the CODEX 135-1995, it was safe to consume daily.

Moreover, the concentration of mercury detected was expressed in µg/kg(ppb) and then was converted to mg/kg (ppm). The level of mercury detected for the four fish species ranged between 0.0016 µg/kg to 0.0036 µg/kg wet weight. According to the Mauritian Food Act 1998, the acceptable level of mercury in fish is set at 1.0 mg/kg (ppm). According to the CODEX 135-1995, the provisional tolerable weekly intake for mercury is at 0.005 mg/kg. Therefore, since the four fish species have level of mercury within the range, it can be deduced that the fish is well safe in the human diet. Likewise, the tolerable concentration of lead is at 2.0 mg/kg (ppm). Besides, the threshold level of lead set up by the WHO (1989) is also at 2.0 mg/kg (ppm) and the CODEX 135- 1995, the provisional tolerable weekly intake for lead is at 0.025 mg/kg. The concentration of Lead detected was expressed in µg/kg (ppb) and then was converted to mg/kg (ppm). Lead concentrations in this study ranged from 0.0011 µg/kg to 0.0024 µg/kg wet weight, well below the limits. The leading area of concern for this study is nickel since the level obtained for this study is quite high. The Mauritian food act 1998 and the CODEX 135-1995 have no MPL set for nickel but conferring to the WHO (1989), the MPL set for the metal ranged from 0.5 mg/kg to 1.0 mg/kg wet weight in muscle tissue of fish. Henceforth, the result obtained for this study showed 0.676 mg/kg to 1.192 mg/kg which clearly indicate that the fish taken for the research is close to the threshold set by WHO (1989). The Food Act should be amended to consider the thresholds for Nickel.

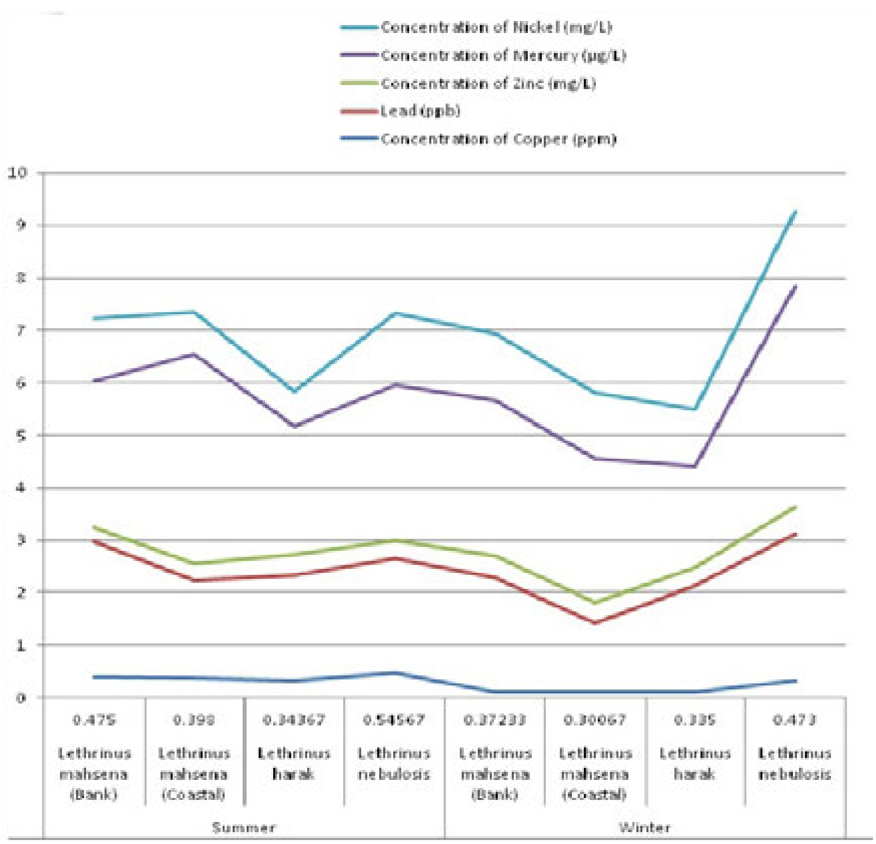

Figure 2. Chart showing the average levels of heavy metals and trace elements recorded in the four different species of Lethrinids. Peaks in heavy metal concentration are associated to increased size of the fish specimen. Lethrinus nebulosis is the largest among the four species.

Conclusions

This study highlights the importance of heavy metal bio-accumulation, shows a positive co-relation between size and heavy metal accumulation but also suggests that The Mauritian Food Act should be amended to consider thresholds for Nickel even though it is not considered a highly toxic metal and does not cross the threshold set by WHO.

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