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Trace Metal Contents in Muscle Tissues of Inland Fish Species in the North central Province of Sri Lanka

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ABSTRACT

Chronic Kidney Disease (CKD) has recently shown a remarkable increase in some areas in the North Central Province (Anuradhapura and Polonnaruwa districts), Sri Lanka. The consumption of fresh water fish contaminated with trace metals is considered as a possible causal factor for this. The concentration of mercury (Hg), lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), cobalt (Co), zinc (Zn), iron (Fe) and arsenic (As) in the muscle tissues of the five commonly consumed freshwater fish species, *Oreochromis* spp., *Heteropneustes fossilis*, *Glossogobius giuris*, *Channa striata* and *Macrognathus aral* were determined using Atomic Absorption Spectrophotometry (AAS). This study indicates that toxic metals' Hg, Cd and Pb were detected in very low concentration in muscle tissues and the recorded values were lower than the maximum permissible level (Hg< 0.5 mg/kg, Pb< 0.2 mg/kg and Cd< 0.05 mg/kg) established in Sri Lanka. The amount of fish that would fulfil the weekly protein requirement of an average child and adult was calculated according to the WHO guidelines. The calculated levels of contamination by Hg, Pb, Cd, As, Cu, Zn, Cr and Fe in the quantity of fish needed to fulfil the protein requirement was lower than the Provisional Tolerable Weekly Intake (PTWI) value established by WHO, EU and FAO and none of above guidelines not covered the level of Co.

Keywords: Chronic Kidney Disease (CKD), contamination, freshwater fish, protein requirement

INTRODUCTION

Reservoir fisheries in Sri Lanka includes both capture fishery and the culture fishery (Fernando and Indrasena, 1969) and *Oreochromis* species (family Cichlidae) are the most abundant species in all reservoirs of Sri Lanka (De Silva and Sirisena, 1987). Inland fisheries are mainly practiced in the North Central Province (NCP) of the country as there are many man-made reservoirs located in this province. As a result, the NCP contributed 37% of total inland fish production in 2008 (NARA, 2009). Moreover, in 2008, the highest inland fish production was recorded in the Anuradhapura district (9380 metric ton) while the Polonnaruwa district claimed for the second highest fish production (7320 metric ton) in the same year (NARA, 2009). Reservoir fish are the main source of animal protein of the rural people living in the dry zone of the country (Allinson *et al.*, 2009) where most of the people living in that area are farmers.

Consumption of fish contaminated with toxic heavy metals has found to be posing a risk to human health. Fish assimilate toxic metals by ingestion of food and particulate material suspended in water and by ion exchange of dissolved metals (Allinson *et al.*, 2009).Trace metals such as lead (Pb), cadmium (Cd) and mercury (Hg) are non-essential and considered as harmful elements (Andre *et al.*, 2005). Trace metals such as cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) are essential nutrients for organisms including humans, but are toxic if consumed in large quantities (Silva and Shimizu, 2004).

In the recent past, a high incidence of chronic kidney disease (CKD) has been reported from the North Central and Uva provinces of Sri Lanka and consumption of reservoir fish, especially *Oreochromis* spp., is believed to be a possible cause (Bandara *et al.*, 2008). Contamination of fish, therefore is of particular concern to both the fisheries industries and public health (Vinodhini and Narayan, 2008). Furthermore, fish are

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frequently used in bio-monitoring for the presence of contaminants in aquatic environments, particularly in relation to effluent discharges (Silva and Shimizu, 2004).

It appears that the trace metal contents in Tilapia have mostly been detected in Sri Lanka but a poor attention has been paid on other freshwater fish species. Moreover, to the best of our knowledge, there have been very few published studies on trace metals in freshwater fish from the NCP in Sri Lanka. Silva and Shimizu, (2004) have analysed trace metals in nine freshwater fish species (family: Anguillidae, Cichlidae, Cyprinidae, Gobiidae and Siluridae) from Victoria reservoir, Sri Lanka which is mainly used for generation of hydropower but not for aquaculture. Since Cd, Cr, Cu, Hg and Pb were not detected in flesh of fish, the authors concluded that Victoria reservoir is not contaminated with above mentioned metal ions or the concentration of these metals was not enough to cause bioaccumulation (Silva and Shimizu, 2004). Wijesinghe *et al.* (1999) have determined the concentration of several trace metals in four species (*A. nebulosa, O. niloticus, P. dorsalis and T. rendalli*) of freshwater fish with different feeding habits from Weras Ganga in the Colombo District, Sri Lanka. They found varying concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, Ti and Zn in different organs such as muscles, gills, gonads, kidney and liver. In addition, Allinson *et al.*, (2009) studied metal concentration of two species of Tilapia (*O. mossambicus* and *O. Niloticus*) in three river basins of Sri Lanka (Kala Oya, Mahaweli Ganga and Walawe Ganga) and (Jinadasa and Edirisinghe, 2012) studied Cd, Pb and Hg level of *Oreochromis* sp. from four districts including Anuradapura and Polonnaruwa from NCP, Sri Lanka. The results of those studies showed that metal concentration of *Oreochromis* species was well below the WHO permissible values. In contrast, Bandara *et al.* (2008) recorded a higher Cd level in muscle tissues of *O. niloticus* in Karapithkada and Thuruwila reservoirs in Anuradapura District, Sri Lanka.

This study focused on Hg, As, Cd, Pb, Cr, Co, Cu, Fe and Zn in muscle tissues of five selected fish species sampled from the NCP of Sri Lanka and to determine the quantity of fish of each species that would be safe for consumption.

MATERIALS AND METHODS

Freshwater fish species that are common in reservoirs of the NCP namely, *Oreochromis* spp. (Tilapia), *Heteropneustes fossilis* (Stinging catfish/Hunga), *Glossogobius giuris*, (Bar Eyed Goby/Weligouwa), *Channa striata*, (Snakehead Murrel / Loolla) and *Macrognathus aral* (One-stripe Spiny Eel/ Theliya) were selected for this study .These were collected from six reservoirs of the Anuradhapura district, and five reservoirs of the Polonnaruwa district in the NCP of Sri Lanka (Fig. 1).

Samples of *Oreochromis* spp. (n=145), *Heteropneustes fossilis* (n=39), *Glossogobius giuris* (n=26), *Macrognathus aral* (n=6) and *Channa striata* (n=13) were collected from commercial landings at Rajanganaya, Wilacchiya, Thuruwila, Padawiya, Kalawewa, and Kumbicchiyankulama from the Anuradhapura District and Parakrama Samudraya, Minneriya, Kaudulla, Girithale and Madhuru Oya from the Polonnaruwa district during March to November 2009. They were packed in polythene bags and transported to the Analytical Chemistry Laboratory, National Aquatic Resources Research Development Agency (NARA) in an insulated box with crushed ice.

In the laboratory, fish were cleaned and their total length, standard length and total weight were recorded. Then the muscles of each fish were separated using a plastic dissecting instruments and one portion was used to determine the protein content of the samples, according to the AOAC official methods of analysis 923.03 using UDK 132 (VELP Scientifica, Usmate, Italy) semi-automated Kjeltec system. Another portion was oven dried at 105 °C to a constant weight to determine moisture content (AOAC, 2000). The dried samples were powdered using a mortar and pestle and packed in airtight bags until further analysis. Fish samples were digested using CEM XP-1500 (CEM, Matthews, USA) microwave accelerated system. Around 0.5 g of an oven dried fish sample was weighed accurately to four decimal places in a teflon vessel. Then 10 mL of 65 % conc. HNO_3 (AR, Sigma) was added and allowed to stand for 15 minutes in a fume hood for pre digestion. Then the teflon vessel was connected to a microwave digester and digestion were carried out (200 °C, 25 min). The resulting digested fish samples were transferred to 50 mL volumetric flasks and made up to the mark with deionized water. Two reagent blank samples and two spiked samples were prepared with each batch in the same way. The spiked samples were routinely analysed as the method of validation procedure. The recovery limits were maintained between 75-125% during the analysis of all sets of samples. Limit of Detection (LOD; mean blank + 3S) was calculated for all metals and results of less than LOD were considered as not detected by the instrument.

Nine metals namely Pb, Cd, Hg, As, Cr, Co, Cu, Fe and Zn were analysed using an Atomic Absorption Spectrophotometer (Varian240 FS, Varian Inc., Walnut Creek, CA, USA). In this study, graphite tube atomizer (Varian GTA-120) was used for Pb, Cd, Co and Cr determination. Vapour generation accessory (Varian VGA 77) with closed end cell was used for Hg determination and vapour generation accessory (Varian VGA 77) with open-end cell was used for As determination. Spectra AA Varian atomic absorption spectrometer with a flame (AAS-240 FS) was used for Zn, Cu and Fe determination. The calibration curves for the absorption of all metals were performed with a standard solution of particular metal at optimum wavelength. Subsequent to the calibration, the reagent blank samples, unknown samples and spiked samples were aspirated into the AAS and the readings were recorded.

Statistical analysis was conducted using Microsoft Excel 2007 version and Minitab version 14.0.

RESULTS AND DISCUSSION

Average length, weight, moisture and protein content of fish samples are given in Table 1. According to the results, *C. striata* has the lowest average moisture content of 79.60%, while *G. giuris* has the highest average moisture content of 82.99%. However, there were no significant differences in the moisture content of muscle tissues among the fish species (P>0.05). The finding is in agree with previous studies which revealed the moisture content in the muscle tissues of fish species is around 80 % (FAO, 2001).

Figure 1. Sampling areas in the North Central Province of Sri Lanka.

Table 1. Average length, weight, moisture and protein content (wet weight) of fish species sampled (± standard deviations)

Species	Total Length (cm)	Standard Length (cm)	Weight (g)	Moisture (%)	Protein %
Oreochromis spp.	23.3 ± 4.2	20.5 ± 3.8	273.30 ± 147.90	81.32 ± 6.73	17.2 ± 1.8
H. fossilis	22.6 ± 3.2	20.6 ± 3.0	79.58 ± 35.52	82.45 ± 3.14	16.9 ± 0.9
G. giuris	22.5 ± 3.6	18.1 ± 2.8	101.62 ± 49.83	82.99 ± 2.00	14.6 ± 0.2
C. striata	38.8 ± 6.2	34.2 ± 5.5	612.70 ± 281.81	79.60 ± 3.14	17.7 ± 2.7
M. aral	40.5 ± 7.1	38.5 ± 7.3	163.09 ± 69.19	80.90 ± 1.16	17.0 ± 0.6

The mean metal concentrations detected in muscle tissues of fish species samples from the NCP are given in Figure 2. The highest mean Hg concentration was recorded from *C. striata* while the highest mean Co and Cr concentrations were recorded from *Oreochromis* spp. Arsenic was detected only in *G. giuris*. The highest mean lead concentration was recorded in *Oreochromis* spp. *H. fossilis* and *M. aral*. The highest mean Zn and Cu concentrations were recorded from *M. aral* and *H. fossilis* respectively.

Out of the metals considered Hg, Cd, As and Pb are considered to be toxic to humans(Andre *et al.*, 2005). However, according to this study, concentrations of all toxic metals are recorded below their maximum permissible level of muscle meat of studied fish species (Hg< 0.5, Pb< 0.2, Cd< 0.05 mg/kg in wet weight basis of fish muscles) established in Sri Lanka (MOFAR, 1996). Furthermore, among these toxic metals, Hg concentration was observed to be relatively higher than Cd and Pb levels. There is clear evidence from a reservoir in temperate regions that Hg concentrations in the water column and sediments, and thence fish, rise after impoundment (Allinson *et al.*, 2010). AlthoughBodaly *et al.* (1995) suggest that for temperate lakes remote from direct anthropogenic influences, Hg concentrations in fish are related to lake temperature, not catchment characteristics, but Allinson *et al.* (2002) hint that correlation between land use pattern and fish metal concentrations in these anthropogenically impacted, tropical lakes also. The Hg concentrations observed in this study are generally consistent with those reported in *O. mossambicus* from Badagiriya, Chandrikawewa, Kiribbanarawewa, Meegahajandura and Ridiyagama reservoiurs in Southern Sri Lanka in 1998 (Allinson*et al.* 2008). However the Cd concentration of *Oreochromis* sp. in this study is much lower than the reported value of Bandara *et al.* (2008). They reported Cd values of 0.0575mg/kg and 0.2022 mg/kg in *O. niloticus* from Karapithkada reservoir and Thuruwila reservoir respectively, from Anuradapura District.

Previous studies have concluded that there is no correlation between the age of the reservoir and metal concentration (Allinson *et al.*, 2009). Although some reservoirs sampled in this study were reconstructed long ago, Kaudulla and Minneriya were first constructed in the year AD 273 and 276, respectively (reconstructed 1958, 1903). Fish and other aquatic organisms are contaminated by toxic metals mainly due to human activities such as the use of metal containing pesticides, industrial waste, urban and agricultural runoff and dumping etc. Specially lead arsenate, mercury bichloride, ferrous sulphite and copper sulphate contained in pesticides that are used in paddy cultivation. In addition, reservoir water can be contaminated by acidification of soil by acid rain, runoff minerals due to soil erosion, exhaust of smoke from forest fires, fossil fuels and vehicles (Prasad, 2008).

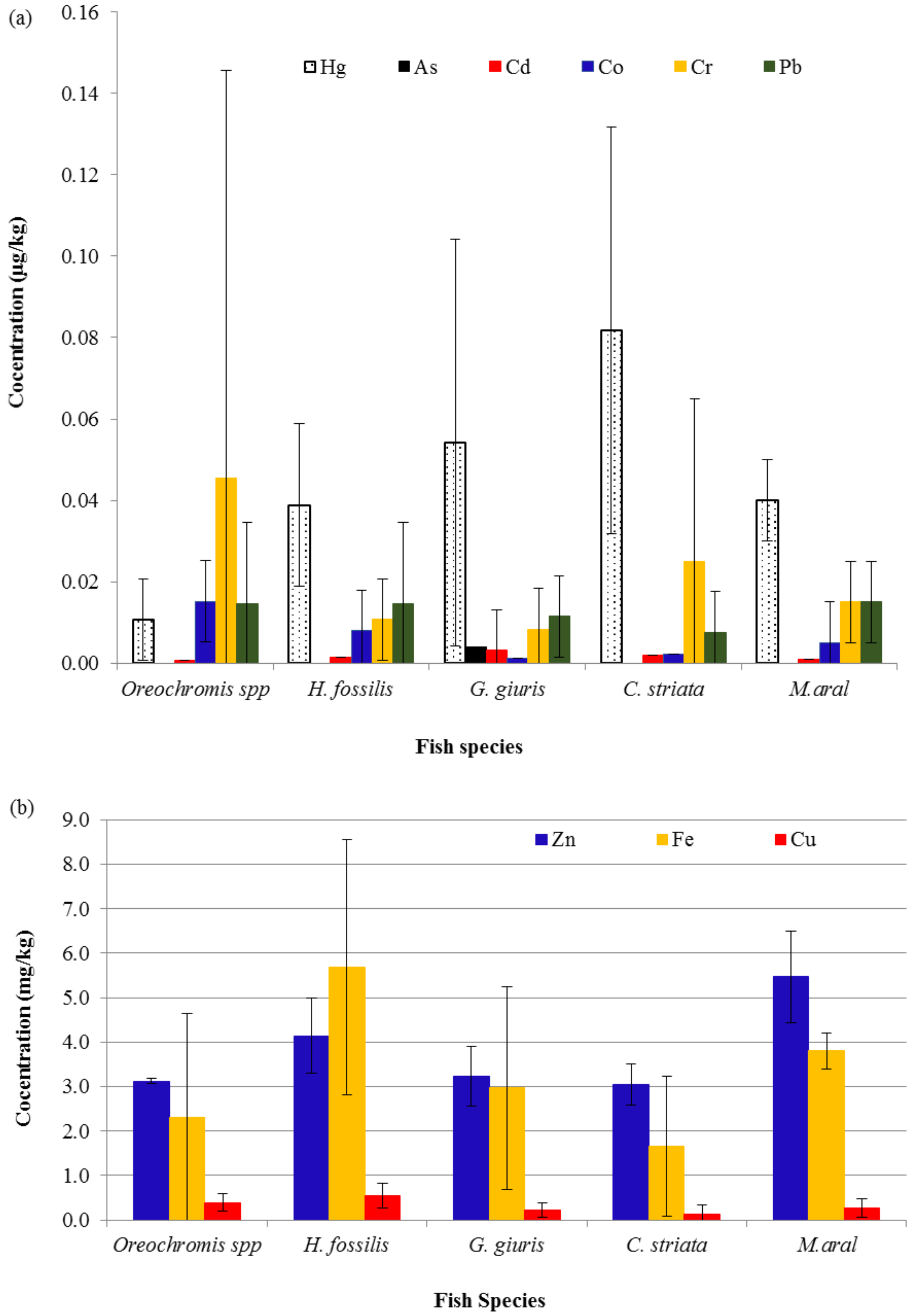

Figure 2. Variation of different trace metal contents in the muscles of examined fish species, (a) Hg, As, Cd, Co, Cr and Pb and (b) Zn, Fe and Cu

The trace metals levels detected in fish species during this study were compared the phylogenetically related species from the Sri Lanka and other countries elsewhere in world as shown in Table 2.

The recommended protein intake is 0.8g of protein per kilogram of healthy body weight per day (WHO, 2007). Therefore, the amount of fish that should be consumed to fulfil recommended weekly protein intake was also calculated (Table 3). The Joint FAO/WHO, 1998 Expert Committee on Food Additives have established provisional tolerable weekly intake (PTWI) values for Hg, Pb and Cd as 0.005, 0.025 and 0.002 mg/kg, bodyweight per week. Accordingly, the amount of fish that can be consumed without exceeding permissible levels for Hg, Cd and Pb are given in Table 4.

According to Tables 3 and 4, a child weighing 10 kg can consume 4.66 kg of *Oreochromis* spp. per week and a person weighing 60 kg is able to consume 27.93 kg of *Oreochromis* spp. per week without exceeding permissible levels of Hg according to MOFAR (1996)guidelines. However, a person of 10 kg and 60 kg need only 326 g and 1953 g of *Oreochromis* spp. to fulfil their protein requirements per week respectively.

According to the calculations based on permissible levels of these toxic metals and the weekly protein requirements of an average child and adult, the present levels of contamination with Hg, Pb and Cd in the four species of fish analyzed does not appear to exceed the permissible levels. Therefore, there is no apparent health risk due to these metals as a result of consumption of those fish species from the reservoir of NCP if consumed within the calculated quantities.

Table 2. Comparison of metal concentrations obtained in this study with other studies

Fish species	Metals level in muscle (mg/kg; wet weight)								Country and (the Reference)
	Hg	As	Cd	Cr	Pb	Zn	Fe	Cu	
O. niloticus	0.45	_	0.02	_	0.15	0.15	_	0.01	Egypt (Khallaf *et al.*, 2003)
O. niloticus	_	_	0.06	_	_	_	_	_	Sri Lanka (Bandara *et al.*, 2008)
O. niloticus	_	_	0.20	_	_	_	_	_	Sri Lanka (Bandara *et al.*, 2008)
O. mossambicus	_	0.59	0.35	0.11	0.6	47.25	3.8		Pakistan (Arain *et al.*, 2008)
O. mossambicus	0.02	0.26	0.05	_	_	4.17	4.13	0.7	Sri Lanka (Allinson *et al.*, 2002)
O. niloticus	0.01	<0.90	<0.15	_	_	4.59	4.9	1.1	Sri Lanka (Allinson *et al.*, 2009)
Oreochromis spp.	0.26	-	0.034	-	0.06	-	-	-	Sri Lanka (Jinadasa and Edirisinghe, 2012)
Oreochromis spp.	0.011	ND	0.001	0.046	0.015	3.126	2.308	0.39	Present study
H. fossilis	_	_	1.1	1.54	2.05	_	_	_	India (Begum *et al.*, 2009)
H. fossilis	0.039	ND	0.001	0.011	0.015	4.145	5.676	0.549	Present study
C. marulius	_	_	1.7	1.32	2.67	_	_	_	India (Begum *et al.*, 2009)

Table 3. Quantity of fish fillet of each species required to fulfil the recommended protein requirement of 10 kg child and 60 kg adult per week, calculated based on the protein content of muscle tissue of each species.

Fish	Protein content of the muscle of fish (%)	Quantity (g) of fish required for	
		a child (≈ 10 kg)	an adult (≈ 60 kg)
Oreochromis spp.	17.2	326	1,953
H. fossilis	16.9	331	1,988
G. giuris	14.6	384	2,301
C. striata	17.7	316	1,898
M. aral	17.0	329	1,976

Table 4. Amount of fish (kg) that could be consumed exceeding permissible levels of Hg, Cd and Pb

Species	Weekly consumption of fish without exceeding permissible levels					
	Child with a body weight of 10 kg			Adult with a body weight of 60 kg		
	Hg	Pb	Cd	Hg	Pb	Cd
Oreochromis spp.	4.66	17.11	26.53	27.93	102.66	159.18
G. giuris	4.61	21.81	6.29	5.53	130.85	37.76
M. aral	1.25	16.67	20.00	7.50	100.00	120.00
C. striata	0.61	32.66	10.50	3.67	195.95	63.02
H. fossilis	1.29	17.15	13.75	7.73	102.88	82.50

CONCLUSION

Nine toxic metals were detected in fish from a NCP in variable amounts. The concentration of non-essential metals such as Hg, Cd, As and Pb and essential metals such as Cu, Fe, Zn, Co and Cr were below the maximum permissible levels stipulated by JECFA (2006). The level of heavy metals found in the NCP was generally low and well within the limits for contaminants in food set by regulatory bodies. By consuming any of the fish species such as *Oreochromis* spp, *G. giuris, M. aral, C. striata* and *H. fossilis,* the recommended protein requirement can be fulfilled without exceeding the PTWI values of these essential and non-essential metals.

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