

Level of trace metals Hg, Cd, Pb, Cu, Fe and Zn in yellowfin tuna (*Thunnus albacares*) and swordfish (*Xiphias gladius*) collected from Sri Lanka

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Abstract

Trace metals, mercury (Hg), cadmium (Cd) and lead (Pb) have no known essential role in living organisms, but are toxic even at low concentrations. Trace metal levels in sea water may be in lower quantity, but affects marine organisms as well as human, due to its tendency to accumulate in marine organisms. The concentrations of total Hg, Cd, Pb, copper (Cu), iron (Fe) and zinc (Zn) in samples of muscle tissues, located below the dorsal fin area were determined by Atomic Absorption Spectrometry in 20 yellowfin tuna (*Thunnus albacares*) and 27 swordfish (*Xiphias gladius*) collected from several areas of Sri Lanka, namely, Galle, Mutwal, Negombo and Trincomalee.

Iron showed the highest mean concentrations in both *X. gladius* (15.74±11.71 mg/kg) and *T. albacares* (12.82±7.94 mg/kg), followed by Zn (5.57±1.28 mg/kg, 3.97±1.08 mg/kg respectively). The mean Hg concentrations in edible parts of *T. albacares* and *X. gladius* were 0.30 and 0.73 mg/kg and the levels of Cd concentration were 0.01 and 0.08 mg/kg respectively. The mean Pb, Cu, Fe and Zn concentrations of *T. albacares* and *X. gladius* were 0.09 and 0.10 mg/kg, 1.44 and 1.69 mg/kg, 12.82 and 15.74 mg/kg and 3.97 and 5.57 mg/kg respectively.

The detected levels of Cd and Pb in flesh of *T. albacares* and *X. gladius* were much lower than the Provisional Tolerable Weekly Intake (PTWI) values, thus indicating that there was no risk of consuming these fish species. With respect to Hg, although there was no risk in consuming *T. albacares*, levels in *X. gladius* were moderately high.

Keywords: Trace metals, Yellowfin tuna, Swordfish, PTWI

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Introduction

Sri Lanka, being an island and having an exclusive economic zone of 517,000 km² was able to produce 384,670 tons of fish in the year 2010, which was an increase in fish production by 13.2% compared to the previous year. The growth rate of fish production in coastal, deep sea/offshore and inland and aquaculture sub-sectors were 52.62, 33.75 and 13.62% respectively (MOFAR, 2010). Fish is considered as one of the main protein sources of food for people in Sri Lanka with around 54% of their animal protein requirement being provided by fish through their daily diet (MOFAR, 2010). The per capita consumption of fish was 9.7 g/day, according to the food balance sheet of 2006 and total energy supplied by fish was about 55.6 calories per day. Although Medical Research Institute (MRI) of Sri Lanka recommends an increase of per capita consumption of fish to 21 kg/year, the per capita availability of fish has been about 10.8 kg/year in 2010 (MOFAR, 2010).

Annual production of yellowfin tuna (*Thunnus albacares*) and swordfish (*Xiphias gladius*) in Sri Lanka in 2008 were 33,027 and 779 tons respectively (FAO, 2009). Yellowfin tuna, commonly known in Sri Lanka as “*Kelawalla* or *Howalla*”, are epi-pelagic fish that inhabit the mixed surface layer of the ocean above the thermocline. The yellowfin tuna is one of the largest tuna species, reaching weights of over 200 kg (www.fishbase.org). Tuna is an excellent source of lean protein, vitamins and minerals and also contains a significant amount of Omega-3- fatty acids. Swordfish (*Xiphias gladius*) is known as “*Kadu Koppara* or *Sappara*”, are elongated, round-bodied fish that lose all teeth and scales by adulthood. They reach a maximum length of 455 cm and weight of 650 kg (www.fishbase.org).

Heavy metals are those generally regarded as having atomic numbers between 22 to 92 on the periodic table (Suhaimi *et al.*, 2006). At low levels, some heavy metals, such as Cu, Fe and Zn are essential nutrients, but at higher concentrations may act as enzyme inhibitors. Other metals, such as Hg, Cd and Pb have no known role in living organisms and are toxic even at low concentrations (Benjamin, 2003). Metal pollution of the sea shall be less, but directly effects on marine ecosystems and humans very extensively (Hoda *et al.*, 2007). Water pollution arising from accidental spillage of chemical wastes,

periodic precipitation contaminated with air-borne pollutants, discharge of industrial or sewage effluents, agricultural drainage, domestic waste water and gasoline from fishery boats can affect levels of metals in fish (Handy, 1994; Jent *et al.*, 1998). Furthermore, heavy metals have the tendency to accumulate in several marine organisms, especially in fish at the top of the food chain, and can later enter people consuming them causing serious health hazards (Itrat *et al.*, 2003).

This study was, therefore, conducted to assess the level of trace metal pollution in yellowfin tuna and swordfish found in Sri Lanka. The trace metals Hg, Cd, Pb, Cu, Fe and Zn were determined in an edible portion of the fish. The results were compared with international standards to determine whether the consumption of these fish posed a significant public health threat. The provisional values for tolerable weekly intakes (PTWI) were also used in this study to assess the relative safety of the yellowfin tuna and swordfish supply. PTWI depends on the amount, consumption period and contamination level of consumed food (Turkmen *et al.*, 2008). The PTWI values for Hg, Pb, Cd, As, Cu, Zn and Fe published by the Joint FAO/WHO Expert Committee on Food Additives are given in Table 1.

Table 1. PTWI values (mg per kg body weight per week) for selected metals

Metal	PTWI
Hg	0.005 (WHO, 1972)
Pb	0.025 (FAO/WHO, 2004)
Cd	0.007 (WHO, 1989)
Cu	3.5 (WHO, 1982)
Zn	7 (FAO/WHO, 2004)
Fe	5.6 (FAO/WHO, 2004)

Materials and Methods

Determination of Cd and Pb were performed using graphite furnace atomic absorption spectroscopy (GT-AAS) on a Varian GTA 120 atomic absorption spectrophotometer, equipped with a computer controlled auto sampler (Varian PSD-120). The determination

of total Hg was performed using a cold vapor Atomic Absorption Spectrometry system (CV-AAS: Varian VGA 77) and other metals were determined using fast sequence flame atomization techniques (FA-AAS) on a Varian 240 FS atomic absorption spectrophotometer.

All the chemicals used in the analyses were analytical reagent grade or purer. Standard solutions of the metals analysed containing 1000 mg/L were obtained from Fluka, Switzerland. All glassware used were first soaked overnight in a liquid detergent solution and thoroughly rinsed with tap water. The glassware was then soaked in 10% (v/v) HNO₃ overnight, rinsed with de-ionized water, dried in oven and the plastic-wares were air dried prior to use.

Performance of analytical procedure was maintained throughout the analysis period. Reference samples were not available in the laboratory; recovery limits of the spiked samples ranged between 80-120%.

The samples of yellowfin tuna (n=20) and swordfish (n=27), used in this study, were obtained from Galle, Mutwal, Negombo and Trincomalee areas in Sri Lanka during the year 2010. Fishes were randomly picked, packed in ice and transported to the Analytical Chemical Laboratory of the National Aquatic Resources Research and Development Agency (NARA). Upon arrival at the laboratory, the total length and total weight were first recorded. Individual fish were then filleted and a sample, devoid of skin and bone, was taken from the center of the block of muscle in the right fillet.

Approximately one gram of muscle was weighed into a microwave digester tube, 10 mL of concentrated nitric acid (65%, AR-Sigma) added and kept for 15 minutes in a fume hood for pre-digestion. After pre-digestion, samples were digested using a microwave accelerated system (CEM-Mass XP-1500+). Each sample was analyzed in duplicate. Two blanks and two spiked tubes prepared in a similar manner, but without the muscle sample were included in each batch of analysis. The microwave conditions were 800 psi, 200 °C temperature and 10 min holding time.

The digests were allowed to cool to room temperature and pressure was released carefully by opening the valve. Then digested fish samples were transferred into 50 mL volumetric flask. Digestion tube was rinsed 3 times with deionized water and filtered. Then it was diluted up to the mark with deionized water and transferred into polypropylene bottles and stored in a refrigerator until metal determination by using the Atomic Absorption Spectrophotometer. Using a series of standard metal solutions, a calibration curve for each metal was obtained. After the standard curve was obtained readings were obtained for blanks, samples and spikes.

The following formula was used to calculate the mean amount of fish that can be consumed without exceeding the recommended heavy metal concentration.

$$A = \left(\frac{1}{x} \times y \right) \times b \quad \text{Eq.1}$$

A – Amount of fish to be consumed for a corresponding body weight

x- Mean heavy metal concentration per kilogram of fish

y- Provisional Tolerance Weekly Intake (PTWI) for each heavy metal

b- Body weight

$$A = \frac{(z \times b)}{y} \quad \text{Eq.2}$$

X – Percentage of protein requirement fulfilled by fish consumption

y – Protein percentage in fish

z- Recommended weekly protein intake

b- Body Weight

According to the above formula the amount of fish to be consumed to meet the requirement was calculated.

Results and Discussion

The precision of analytical methods (GT-AAS, CV-AAS, FA-AAS) were evaluated in terms of their limits of detection and percentage recovered in the spiked samples. All recovery values were fell within the range 80-120% and were considered satisfactory.

Length and weight of fish

The swordfish was the largest of the sample species studied and had a mean body length of 131.2±36.8 cm and mean body weight of 51.0±19.6 kg. Maximum length and weight were 180.0 cm and 96 kg, respectively, while minimum length and weight were 64.0 cm

and 16 kg, respectively. The mean body weight and body length of yellowfin tuna were 118.8 ± 30.9 cm and 43.2 ± 11.0 kg respectively. The range of values for body length and weight of yellowfin tuna were 171.0-64.0 cm and 66.0-21.5 kg, respectively. The latter species is the second most coveted by industrial fisheries (Jessica *et al.*, 2006). Normally females have a higher growth rate than males and reach to greater lengths (Jessica *et al.*, 2006), but in the present study, sexual difference of samples was not considered.

In total, 20 yellowfin tuna and 27 swordfish samples were collected and analyzed for Hg, Cd, Pb, Cu, Zn and Fe in their muscle tissues. The mean concentration of the analyzed metals, Standard Deviation (SD), maximum (max) and minimum (min) values obtained are given in the Table 2.

Table 2. The levels of metals in each species (wet weight basis, w/w).

Species		Hg	Cd	Pb	Cu	Fe	Zn
		$\mu\text{g/kg}$			mg/kg		
Yellowfin tuna (n=20)	Mean	297.46	11.22	86.65	1.44	12.82	3.97
	$\pm\text{SD}$	135.36	8.13	118.88	1.10	7.94	1.08
	Max	534.96	26.00	404.29	3.56	26.29	8.09
	Min	87.25	<LOD	<LOD	0.09	2.21	3.06
Swordfish (n=27)	Mean	733.13	79.57	95.05	1.69	15.74	5.57
	$\pm\text{SD}$	329.37	63.33	104.63	1.11	11.71	1.28
	Max	1431.28	292.79	353.60	4.64	49.46	9.17
	Min	261.13	3.76	<LOD	0.60	1.71	3.93

LOD= limit of detection

Results indicate that the highest mean total Hg levels (733.13 ± 329.37 $\mu\text{g/kg}$) were present in swordfish. The maximum concentration of Hg (1431.28 $\mu\text{g/kg}$) was observed in a fish with 174 cm length and 78 kg weight. Overall, 22.2% of swordfish studied exceeded the maximum allowable limits (1 mg/kg), (EU regulation; 1881/2006) and by Sri Lankan government regulations (Fisheries and Aquatic Resources Act, No. 2 of 1996). The mean Hg concentration in the muscle of yellowfin tuna was 297.46 ± 135.36 $\mu\text{g/kg}$ and range was 87.25-534.96 $\mu\text{g/kg}$, but none of the samples exceeded the maximum allowable limits. Jessica *et al.*, (2006) have reported a possible correlation

between Hg levels and length of the fish. But in the present study, the number of samples was not sufficient to assess such a relationship.

The threshold level of Cd for yellowfin tuna and swordfish are 0.1 and 0.3 mg/kg and for Pb is 0.3 mg/kg. None of the samples studied exceeded the maximum allowable limits for these metals. In other studies, invertebrate species such as mollusks and crustaceans have shown the presence of high levels of Pb and Cd (Blanco *et al.*, 2008; Suhaimi *et al.*, 2005). Ray (1994) has reported that the trend for bioaccumulation of Cd among aquatic organisms was in the order of mollusks (10^3 - 10^4), crustaceans (10^3) and fish (10^2).

The maximum mean value for Cu was observed in swordfish (1.69 mg/kg) while the minimum mean value was observed in yellowfin tuna (1.44 mg/kg). The mean level of Fe (12.82 mg/kg) and Zn (3.97 mg/kg) were higher in yellowfin tuna.

There are very few similar measurements reported from Sri Lanka in earlier studies so that meaningful comparisons are not possible. Our results, however, compare well with values reported in certain other countries (Table 3).

Table 3. Reports of heavy metal levels in yellowfin tuna and swordfish in previous studies.

Fish species	Metal	Reported metal concentration (mg/kg)
Yellowfin tuna	Hg	0.249 (Suhaimi <i>et al.</i> , 2005), 0.51 (Jessica <i>et al.</i> , 2006), 0.18 (Cai <i>et al.</i> , 2007), 0.25 (Adams, 2004), 0.05 (Senadheera, 2005), 0.297 (This study)
	Cd	0.25 (Jessica <i>et al.</i> , 2006), 0.16 (Waqar <i>et al.</i> , 2006), 0.02 (Han <i>et al.</i> , 1998), 0.011 (This study)
	Pb	0.09 (Jessica <i>et al.</i> , 2006), 0.53 (Waqar <i>et al.</i> , 2006), 0.087 (This study)
	Cu	1.99 (Jessica <i>et al.</i> , 2006), 0.27 (Waqar <i>et al.</i> , 2006), 0.26 (Han <i>et al.</i> , 1998), 1.44 (This study)
	Fe	39.6 (Jessica <i>et al.</i> , 2006), 12.82 (This study)
	Zn	64.1 (Jessica <i>et al.</i> , 2006), 5.23 (Han <i>et al.</i> , 1998), 3.97 (This study)
Swordfish	Hg	0.62 (Mendez <i>et al.</i> , 2001), 1.30 (Meng <i>et al.</i> , 2006), 1.61 (Jessica <i>et al.</i> , 2006), 0.93 (Monteiro and Lopes, 1990), 0.22 (Senadheera, 2005), 0.733 (This study)
	Cd	1.04 (Jessica <i>et al.</i> , 2006), 0.080 (This study)
	Pb	0.12 (Jessica <i>et al.</i> , 2006), 0.05 (Storelli <i>et al.</i> , 2005), 0.095 (This study)
	Cu	0.64 (Jessica <i>et al.</i> , 2006), 1.69 (This study)
	Fe	22.4 (Jessica <i>et al.</i> , 2006) 15.74 (This study)
	Zn	41.7 (Jessica <i>et al.</i> , 2006), 5.57 (This study)

Amount of fish that can be consumed without exceeding PTWI for each heavy metal were calculated for the following body weights: 10, 20, 30, 40, 50 and 60 kg. The recommended protein intake (WHOFAO, 2007) is 0.8 g of protein/kg per day with higher values (2 to 3.1g of protein/kg body weight per day) for athletes building up their muscle mass (Tarnopolsky *et al.*, 1992). The amount of fish that should be consumed to fulfill recommended weekly protein intake was calculated using values of 23.6 and 18% protein for yellowfin tuna and swordfish, respectively (Mahaliyana, 2014). According to the formula used the quantities of yellowfin tuna or swordfish that need to be consumed to meet the requirements are given in Table 4.

Table 4. Quantity of fish needed to be consumed (in kg) to meet weekly protein requirement

Body weight of person (kg)	10	20	30	40	50	60	70
Yellowfin tuna	0.02	0.09	0.21	0.38	0.59	0.85	1.16
Swordfish	0.03	0.12	0.28	0.50	0.78	1.12	1.52

PTWI recommended by FAO/WHO (1998) for Hg is 0.005 mg/kg body weight/week and the calculated quantity of fish that can be consumed without exceeding the recommended limits for Hg are given in Table 5.

Table 5. Quantity of fish that can be consumed per week without exceeding recommended limits for Hg, Cd and Pb (assuming that no other protein is consumed)

	Body weight (kg)	10	20	30	40	50	60	70
Hg	Yellowfin tuna	0.17	0.33	0.50	0.67	0.83	1.00	1.17
	Swordfish	0.07	0.14	0.21*	0.27*	0.34*	0.41*	0.48*
Pb	Yellowfin tuna	2.87	5.75	8.62	11.49	14.37	17.24	20.11
	Swordfish	2.63	5.26	7.89	10.53	13.16	15.79	18.42
Cd	Yellowfin tuna	6.36	12.73	19.09	25.45	31.82	38.18	44.55
	Swordfish	0.88	1.75	2.63	3.50	4.38	5.25	6.13

* Unable to fulfill the body protein requirement without exceeding the PTWI limits

It can be seen from the calculations shown in Table 5 that if individuals with body weights of 30 kg or more consume only Swordfish to meet their protein requirement, the quantity of Hg ingested would exceed the recommended PTWI limit. The calculations in the Table 5 also show that there were no such problems with Hg in the case of yellowfin tuna or with any of the other two metals studies.

Conclusions

The concentration of the trace metals studied in swordfish and yellowfin tuna are below the maximum permissible limits specified by the European Union and Sri Lankan export regulations. Since swordfish contained relatively high levels of Hg, if this fish alone was eaten to fulfill the protein requirements, the Hg intake of those with body weights of 30 kg would exceed the recommended limits.

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