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*Corresponding author: B.K.K.K. Jinadasa, Analytical Chemistry Laboratory (ACL), Institute of Post-Harvest Technology (IPHT), National Aquatic Resources Research and Development Agency (NARA), Colombo 15, Sri Lanka
E-mail: jinadasa76@gmail.com

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Trace metals in the muscle tissues of skipjack tuna (*Katsuwonus pelamis*) in Sri Lanka

B.K.K.K. Jinadasa^{1*}, A.S. Mahaliyana², N.P.P. Liyanage² and G.D.T.M. Jayasinghe¹

Abstract: Non-essential trace metals, namely mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As), and essential trace elements copper (Cu), iron (Fe) and zinc (Zn) found in muscle tissues were analysed and compared between female, male skipjack tuna (SJT) in the Indian Ocean, Sri Lanka. Forty-four (20 female and 24 male) individual specimens of SJT were investigated using an atomic absorption spectrometer. The mean trace elements of the male fish were determined to include Hg, 0.12; Cd, 0.02; As, 0.85; Pb, <0.52; Cu, 5.45; Fe, 20.54 and Zn, 5.15 (mg/kg ww). The values for the female fish were determined to be Hg, 0.14; Cd, 0.03; As, 0.85; Pb, <0.52; Cu, 3.75; Fe, 21.82 and Zn, 8.11 (mg/kg ww). In terms of gender, the mean trace elements in the muscle tissue of male and female did not significantly vary ($p < 0.05$) except Cd and Zn. The results show that, according to European legislation, the muscle tissues of SJT are generally “safe” for human consumption.

Subjects: Food Analysis; Food Laws & Regulations; Seafood

Keywords: food security; seafood; fisheries; aquatic environment

1. Introduction

One of the seven species of tunas, the species *Katsuwonus pelamis* is of great commercial value. Capture fisheries produced 417,220 tonnes, and total fisheries production including offshore was 159,680 tonnes in 2012, Sri Lanka (MOFAR, 2013). This was an increase in seafood production by 8% compared to the previous year. The Sri Lankan fisheries sector is an important contributor to export sector and also the main source of animal protein for the Sri Lankan population. Yellowfin tuna

ABOUT THE AUTHOR

B.K.K.K. Jinadasa received his Bachelor of Science (BSc-sp), Master of Science (MSc) degrees and postgraduate diploma from the University of Ruhuna, University of Sri Jayewardenapura, Sri Lanka and United Nation University, Iceland, respectively. Since 2005, he stayed in National Aquatic Resources Research and Development Agency (NARA), Sri Lanka, as a senior scientist and technical manager of Analytical Chemistry Laboratory (ACL).

PUBLIC INTEREST STATEMENT

Although fish is considered as a major source of protein and other essential nutrients to human body, recent research has found that most of the major and more popular marine food fish have been contaminated with toxic trace metals such as Hg, Cd, Pb and As. With the present days increased health concerns of people, though fish have more health benefits, contamination of them with toxic trace metals by exceeding the recommended maximum allowable limits which have health risks on human can lead to their rejection from the local as well as export market. Among major tuna fisheries, skipjack tuna (*Katsuwonus pelamis*) is the largest fishery in all the oceans around the world and the largest marine fishery in Sri Lanka as well. The present study has shown that skipjack tuna does not pose any health risk due to contamination with Hg, Cd and As toxic trace metals.



B.K.K.K. Jinadasa

(*Thunnus albacares*), swordfish (*Xiphias gladius*), skipjack tuna (SJT) (*K. pelamis*) and marlin (*Makira* sp.) are the most important export fishes in Sri Lanka and make a significant contribution to foreign earnings (Jinadasa, Edirisinghe, & Wickramasinghe, 2014). Annual production of SJT in Sri Lanka in 2012 was 53,410 tonnes and it was 13% of the total marine capture fish production (MOFAR, 2013).

Heavy metal pollution in aquatic ecosystem has been recognized as a serious environmental problem. In many cases, heavy metals occur in natural water bodies at the levels below their toxic thresholds. The heavy metals are non-degradable, and hence they persist in environment for a long period of time and such low concentration may still pose risk of damage via uptake and subsequent bioaccumulation by organisms. Metal bioaccumulation can be important in two points of view: human consume accumulators and environmental quality (Jinadasa, Ariyaratne, & Ahmad, 2014). Potential benefits and risks analysis of fish consumption is required in order to establish appropriate dietary recommendations and public health as well as scientific information.

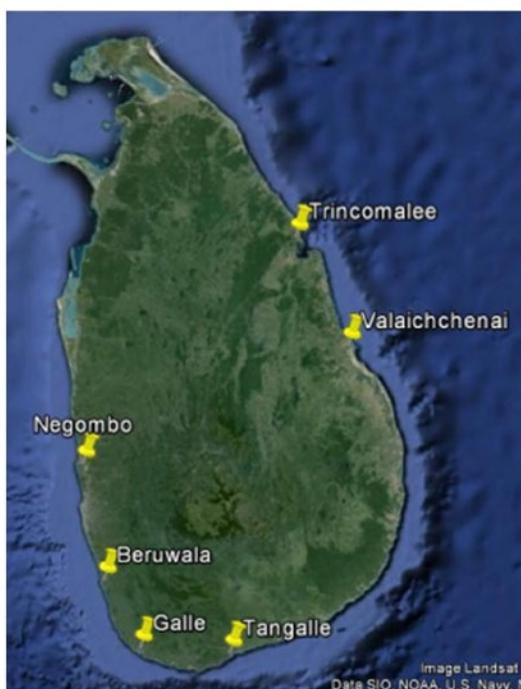
Fish that contain good nutrients such as proteins, vitamins, minerals and omega-3 fats help reduce the risk of certain cancers and cardiovascular diseases, whereas (Uzairu, Harrison, Balarabe, & Nnaji, 2009) certain toxic compounds in fish, non-essential trace metals, can generate negative consequences. Potential benefits and risks analysis of fish consumption is required in order to establish appropriate dietary recommendations and public health as well as scientific information (Wim, Isabelle, Zuzanna, John, & Stefaan, 2004). Being a frequently available and popular marine food fish in Sri Lankan fish market, SJT is required to be assessed for its levels of essential and non-essential trace metals. Hence, the present study was planned to analyse the trace metals concentrations, with the aim of assessing risks of consumption of SJT (*K. pelamis*) in Sri Lanka.

2. Materials and methods

2.1. Study area and sample collection

SJT fish samples were collected to represent fish landing sites in Valaichchenai, Trincomalee, Galle, Tangalle, Beruwala and Negombo areas around Sri Lanka in April–July 2014 (Figure 1). The total sample size (n) was 44 SJT individuals.

Figure 1. Map of sampling area.



Selected samples were separately covered using a clean polythene bag, placed with ice in an insulated container and were transported to the Analytical Chemistry Laboratory, at the National Aquatic Resources Research and Development Agency (NARA). Before dissecting each individual, the standard length, SL (cm), up to one decimal place was measured using measuring board and the total weight, TW (kg), was measured up to two decimal places by top loading balance (Citizen, CZ 1002, Japan). Then, the fish were dissected using a stainless steel knife and the sex of the individuals was identified by observing their gonads. All these three types of data (SL, TW and sex) were recorded for each individual that was sampled. Each sample was taken from individual SJT fish as an edible portion of one side of the body; flesh samples were taken from the area below the left dorsal fin of each individual SJT fish. Then each sampled portion was homogenized using a mixer grinder (Sonica domestic, India). From the homogenized sample nearly 250 g of muscle portion was obtained for the study. All these sampled homogenized portions were packed separately in polythene bags with proper labelling and were stored at -20°C until further analysis.

All glassware used in this research work were first soaked overnight in liquid detergent and then thoroughly rinsed with flowing tap water followed by the rinsing with distilled water. Then, the glassware were soaked in 10% (v/v) HNO_3 (Sigma) overnight and subsequently rinsed with de-ionized water. Then all the cleaned glassware and plasticware were oven dried at low temperature prior to use.

All standards and reagents were prepared using ultrapure water (Barnsted, Esay pure LF system, Dubuque, USA). All chemicals used in this study were of analytical reagent grade or better. The standard solutions of Hg, Cd, Pb, As, Cr, Fe, Cu and Zn at 1,000 mg/L (Fluka, Switzerland) were used for the construction of calibration curves.

2.2. Sample preparation

Approximately 1 g portion from each homogenized and pre-prepared muscle samples was taken into a microwave digestion tube and weighed separately using an analytical balance (AAA 300 L, Bradford, UK). Predigestion of the sample was performed by adding 10 mL of 65% (v/v) HNO_3 acid (AR, Sigma-Aldrich, USA) and allowing it for 15 min in fume hood. Each sample was analysed in duplicate. A microwave accelerated system, CEM XP-1500 (CEM, Matthews, USA) was used to digest the sample further after the predigestion. Predigested samples were digested under pressure in a closed vessel heated by microwaves. The digest was left to cool to room temperature and pressure was released carefully by opening the valve. Then the digested fish samples were transferred into 50 mL volumetric flask and the interior of the digestion tube was washed three times with de-ionized water and it was added to the same volumetric flask. Finally, the flask volume was made up to 50 mL with de-ionized water as diluents.

2.3. Trace metal analysis

Atomic absorption spectrophotometer (AAS) (Varian240 FS, Varian Inc., Mulgrave, Victoria, Australia) was used to determine the Hg, Cd, Pb, As, Fe, Cu and Zn in the prepared samples. A graphite tube atomizer (Varian GTA-120) was used for Pb, Cd and As determination. Vapour generation accessory (Varian VGA 77) with closed end cell was used for Hg 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 the metals were performed with a series of standard solutions of particular metal at optimum wavelength. The reagent blank samples and spiked samples were aspirated into the AAS subsequent to the calibration and the readings were recorded. Each analytical batch was consisted of spiked samples and reagent blanks. The quality control samples were digested by following the same procedure used in the preparation of fish samples for the trace metal analysis.

2.4. Quality control procedure

The accuracy of the analytical procedure was maintained using the following ways throughout the analysis period. Certified quality control material (canned fish muscle, T/07194 and canned crab meat, T/07192QC) from Food Analysis Performance Assessment Scheme (FAPAS, Sand Hutton, York, UK) for total Hg, Cd, As and spiked samples (for Pb, Fe, Zn and Cu) was routinely treated and analysed in the

same manner as the samples. The analytical chemical laboratory at NARA has participated in the proficiency testing programme of the FAPAS for total Hg, Cd and As within the same period with satisfactory results (the Z value for Hg: 0.0, Cd: -0.3 and As: -0.2), Proficiency Testing Report 07215, July–August 2014. The average field blank, derived from sample field blanks, and three times its standard deviation were used to evaluate the limit of detection (LOD). The limit of quantification (LOQ) was $3 \times \text{LOD}$.

2.5. Statistical analysis

Statistical analysis was performed using a program Statistical Package for Social Sciences, version 20 for Windows. The statistical significance of the correlation was reported at the $p < 0.01$.

3. Results and discussion

The suitability of the method was evaluated in terms of their respective LOQ, recovery value using certified quality control materials (only for Hg, Cd, As and Cu) and recovery value of spiked samples (Fe, Zn and Pb). The recoveries were maintained between 80 and 120% and the relative standard deviation values were less than 15%. Data on standard quality control materials and spiked samples are shown in Table 1. The results obtained from spiked SJT samples and quality control material results indicated that the methods used were suitable for the determination of metal concentrations in the samples investigated.

The body size distribution shows that the size differences among the females and males captured, the largest female attaining 48 cm (806 g) and male 46 cm (875 g) of total body size (Table 2). There is a significant difference in the length and weight data between male and female fish ($p > 0.05$).

Trace element concentrations in the tissues of SJT are well documented on a global basis, with a focus on the non-essential elements Hg, Cd, Pb and As, as well as the essential ones, Cu, Zn and Fe for their role in the human body. However, few studies have investigated the wider range of trace elements present in the muscle tissues of SJT in Sri Lankan water as studied here. The trace metal concentrations of the muscle tissues of male and female SJT are presented in Table 3.

Table 1. Certified values, determined values, recovery (%) of quality control samples (T-07192 and 07194 QC) and spiked recovery values (n = 7)

Samples	Value	Metal						
		Hg (µg/kg)	Cd	As	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Pb (µg/kg)
T-07192QC	Assign value	95.68	5.62 mg/kg	13.36 mg/kg	-	-	-	-
	Determined value	94.47 ± 14.05	5.9 ± 1.02 mg/kg	11.0 ± 1.31 mg/kg	-	-	-	-
	Recovery (%)	98.73	101.40	82.93	-	-	-	-
T-07194QC	Assign value	141	4.99 µg/kg	1,063 µg/kg	-	-	-	-
	Determined value	155.2 ± 17.2	5.5 ± 0.7 µg/kg	1,011 ± 114 µg/kg	-	-	-	-
	Recovery (%)	110.07	110.22 µg/kg	95.1 µg/kg	-	-	-	-
Spiked sample	Recovery (%)	-	-	-	89.30	99.12	83.80	86.57
	LOQ (mg/kg)	0.07	0.006 µg/kg	0.34 µg/kg	0.01	0.15	0.43	0.52

Table 2. The variation of weight and length of fish

Description		Avg ± SD	Maximum	Minimum
Male	Weight (kg)	2.18 ± 0.56	3.15	1.10
	Length (cm)	47.2 ± 4.4	56.0	36.0
Female	Weight (kg)	2.31 ± 0.65	4.20	1.50
	Length (cm)	47.8 ± 3.4	56.0	43.0

Table 3. Mean concentrations of trace metals recorded in the muscle tissues of SJT (values expressed as mg/kg)

	Hg	Cd	As	Pb	Cu	Fe	Zn
Average (all)	0.13 ± 0.06	0.02 ± 0.02	0.85 ± 1.08	<0.52	4.68 ± 7.31	21.12 ± 9.02	6.50 ± 3.80
Female	0.14 ± 0.08	0.03 ± 0.02	0.85 ± 1.13	<0.52	3.75 ± 2.84	21.82 ± 7.48	8.11 ± 3.93
Male	0.12 ± 0.05	0.02 ± 0.01	0.85 ± 1.05	<0.52	5.45 ± 9.58	20.54 ± 10.25	5.15 ± 3.18

Mean concentrations (mg/kg) of the trace metals of SJT (Table 3) indicated that Fe was the highest accumulated metal in both sexes (21.82 and 20.54 mg/kg), followed by Zn in female (8.11 mg/kg) and Cu in male (5.45 mg/kg). The Pb (<0.52) and Cd (0.03 and 0.02 mg/kg) were the lowest in both sexes. The Hg levels obtained in this study did not differ greatly with the findings of Jessica, Michel, Matthieu, Richard, and Paco (2006); 0.19 mg/kg and Carlos et al. (2011); 0.15 mg/kg, but Matthews (1983) reported that the Hg concentration of SJT as 0.34 mg/kg and that is nearly one time higher than our values. The size of a fish is known as the determining factor of its trace metal burden, especially Hg (Jessica et al., 2006). However, this study was not able to identify significant relationships between the trace metal accumulation with the size (length as well as weight) of the fish. This could be due to SJT being a short-lived and relatively small fish when compared to other major tuna species, which exhibit good relationships with the body size and levels of trace metal accumulation.

Contradictory to the resulted Cd levels of this study, Jessica, Michel, Matthieu, Richard, and Paco (2007) have noted high levels of Cd in muscles of SJT. One of the prey items of SJT is cephalopods, which have been claimed that their tissues contain higher Cd levels that can be accumulated in top predators through food chains. The lower Cd levels resulted in this study may be due to the seasonal changes of prey items in the ocean.

The results obtained for Pb concentration in SJT by this study do not clearly agree with the results obtained by Jessica et al. (2007), they recorded that Pb in SJT is 0.07 mg/kg, but in our LOQ values it is higher than of this value (0.52 mg/kg). Lead is generally soluble in natural waters, therefore Pb in fish tissues comes from Pb-contaminated waters, food grown in polluted areas or Pb stored in the body of food such as squid (Jessica et al., 2007).

European Union (EU) has established maximum permitted levels for three trace metals i.e. Hg, Pb and Cd in seafood and Sri Lanka follows this regulation. According to the EU regulation 2073/2005, 1881/2006, 629/2008 and Sri Lanka export regulation (No. 1528/7), the maximum level of contaminants in target fish species in this study are Hg: 1.0, Cd: 0.1 and Pb: 0.03 mg/kg in wet weight basis. Based on the limits of non-essential trace metal levels (Hg, Cd and Pb) in above food safety regulation, the SJT is intended for human consumption (EU/EC, 2005, 2008; EU/EC-1881, 2006; MOFAR, 2007).

The main As species in marine and freshwater organisms are inorganic arsenic, arsenate and arsenite. High levels are frequently found in crab, in which the white meat generally contains more As than the brown meat. However, the majority of this As appears to be in the form of the less toxic organic As species, for example in shellfish, molluscs and seaweeds the predominant species is arsenosugars (Hajeb, Sloth, Shakibazadeh, Mahyudin, & Afsah-Hejri, 2014). Islam, Bang, Kyoung, Ahmed, and Jannat (2010) reported mean As concentrations as 1.80 and 1.03 mg/kg in the canned bluefin tuna (ventral) and longtail tuna from South Korea and that value is higher than this study. This may be the reason why the bluefin tuna and longtail tuna have higher body weight and life time than SJT.

The trend of the essential trace metal concentrations was Fe > Zn > Cu in female and Fe > Cu > Zn in male muscle of SJT. A higher Fe content in SJT is presumably related to the presence of the dark muscles in the body since dark muscles are characterized by higher Fe concentrations than light muscles. Contrary with the findings of Jessica et al. (2007), the present study has obtained higher Fe level as well as lower Zn and Cu levels. The trend of these reported essential trace metal concentrations

also differs from the present study. However, the standard deviations of Fe and Zn were high in both studies, which indicate that the resulted values for individual specimen have a wider distribution. Unlike this study, Jessica et al. (2007) have reported a lower standard deviation for Cu. Karunaratna and Attygalle (2010) have studied Fe, Zn and Cu separately in white as well as red muscles of SJT in Sri Lankan oceanic waters. The reported results of the trend of these essential trace metals are in line with the present study. However, the present data differ from the reported mean values for each trace metal as higher Fe and Cu as well as lower Zn in white muscle, whereas lower Fe, Zn and Cu values in red muscle.

The maximum limits (MLs) of non-essential trace metals differ from one seafood to another based on risk assessment. Sri Lanka follows the regulation established by the EU for MLs for Cd, Pb and total Hg in seafood and the Sri Lanka export regulation of fish. These MLs of Hg, Cd and Pb for SJT is 1.0, 0.1 and 0.2 mg/kg, respectively. None of the individual species exceeded the MLs for Hg and Cd, but the level was Pb was difficult to obtain because LOQ > MLs level.

4. Conclusion

SJT is a nutritionally valuable resource, especially in the case of protein content and the understanding of trace metal concentrations in fishes is significant in order to regulate the consumption of fish. Our results show that trace metal levels in the muscle samples taken from SJT caught from Sri Lankan waters were under the maximum permissible levels given by the EU and WHO. Hence, there is no pose to health risk by trace metal toxicity due to the consumption of SJT according to the obtained results of this study. The LOD of Pb in this study is higher than the EU and WHO regulation, hence authors unable to wrap up the conclusion regarding the Pb.

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Competing interests

The authors declare no competing interest.

Author details

B.K.K.K. Jinadasa¹

E-mail: jinadasa76@gmail.com

A.S. Mahaliyana²

E-mail: anjulasachintha@gmail.com

N.P.P. Liyanage²

E-mail: npsr@yahoo.com

ORCID ID: <http://orcid.org/0000-0002-3113-324X>

G.D.T.M. Jayasinghe¹

E-mail: thilini.madurangika123@gmail.com

¹ Analytical Chemistry Laboratory (ACL), Institute of Post-Harvest Technology (IPHT), National Aquatic Resources Research and Development Agency (NARA), Colombo 15, Sri Lanka.

² Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka.

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