Bioaccumulation of cadmium in muscle and liver tissues of juvenile Yellowfin tuna (Thunnus albacares) from the Indian Ocean.

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Abstract

The present study evaluated the cadmium (Cd) levels and temporal variation of Cd in dark muscle, white muscle, and liver of juvenile *Thunnus albacares*. 72 individuals (Standard length: 50 -67cm; weight: 0.8 kg - 2.5 kg) were collected from the selected landing sites in Sri Lanka during the period between April 2021 to May 2022. Total Cd levels were analyzed using an inductivity-coupled Coupled Plasma Mass Spectrophotometer. The mean Cd levels (mean ± SD mg/kg dry weight) in different tissues varied with significantly higher levels in the liver (13.62 ± 0.98, $p < 0.05$), compared to dark muscle (0.52 ± 0.05), and white muscle (0.42 ± 0.04). Cd levels in liver tissues were positively correlated ($p < 0.05$) with the fish weight. The highest Cd levels in liver tissue and dark muscle were reported in October 2021 (26.35 ± 3.46, 0.93 ± 0.10 mg/kg d.w. respectively) while in white muscle, the highest Cd level was found in November (0.60 ± 0.07 mg/kg d.w.). The Cd levels reported in dark muscles, white muscles, and liver tissues were significantly higher ($p < 0.05$) during 2nd inter-monsoon than in the other monsoonal regimes. The measured Cd levels (mg/kg wet weight) in white and dark muscles, were well below the maximum permissible level (0.2 mg/kg wet weight) set by WHO/FAO, but in the liver tissues of all samples were above the level. Accordingly, the edible flesh (white and dark muscles) of *T. albacares* from the Indian Ocean can be considered safe for human consumption whereas the liver tissues are unsafe. A human with a body weight of 60 kg can consume white muscles up to 4.667 kg per week without exceeding the Provisional Tolerable Weekly Intake defined by WHO/FAO.

Introduction

In recent decades, oceanic pollution due to anthropogenic sources has substantially increased. Due to rapid industrial development, levels of many pollutants have increased in all environmental compartments (Chouvelon et al. 2017). Aquatic animals, including fish, are exposed to a range of contaminants some of which are bioaccumulating in their tissues. Accordingly, particular concern has been devoted to monitoring toxic contaminants in fish which are the most common source of protein from seafood (Chiesa et al. 2016).

The Indian Ocean is surrounded by several countries with different population densities and industrial activities. It is therefore a potential repository for numerous anthropogenically generated environmental contaminants, including trace metals. Heavy metals in the ocean can also originate from natural sources and some natural processes, including wind-blown dust, may further increase the heavy metal burden (Chen et al. 2018). Cadmium (Cd) is a non-essential metal with high toxicity and persistence in the human body, and it is one of the most dangerous toxic metals affecting humans (Torres et al. 2016). Cd toxicity is associated with reproductive, hepatic, renal, and pulmonary dysfunction while excessive exposure may result in adverse effects including cancer (Varol et al. 2017). Cadmium biogeochemical transfer, bioaccumulation, and biomagnification through food webs are influenced by both biotic and abiotic factors (Chouvelon et al. 2017). The European Commission has established a maximum permissible limit for cadmium in fish muscle as 0.1µg per gram, which is 10 times lower than the corresponding limit set for mercury (European Food Safety Authority 2009).

Dietary intake is one of the major pathways of metal exposure for humans. Cd can also enter the human body through occupational or environmental exposure including inhalation, and exposure to soil, dust, and air (Muczynska et al. 2021). Cadmium becomes dispersed between the water phase and sediments, while aquatic organisms can readily take up dissolved Cd through the dietary pathways and bioaccumulate in their bodies (Chiesa et al. 2016). Fishes take up Cd through food ingestion or direct absorption through the skin or gills (Mehana et al. 2020), and uptake depends on environmental conditions such as the quantity of Cd, its chemical form, and water quality (Alizada et al. 2020). Cd levels in fish vary with tissue type, age, sex, maturity stage, diet, and trophic level (Chiesa et al. 2016). Fish have been extensively used as a bioindicator of metal pollution (Gunningham et al. 2019).

Elevated levels of Cd in some tuna species have been reported from the Indian Ocean and other oceans (Besada et al. 2006; Chouvelon et al. 2017; Chen et al. 2018). *Thunnus albacares* (yellowfin tuna) is a long-lived, migratory pelagic species that occupies high trophic levels in tropical waters (Besada et al. 2006). Because of their high trophic position and high metabolic rate, *T. albacares* may accumulate heavy metals including Cd in their tissues and become vulnerable to toxicity (Kojadinovic et al. 2007).

Sri Lanka is an important tuna-exporting country in the Indian Ocean region, and intense commercial fishing effort focuses on large yellowfin tuna for the export market, while juvenile catches are mainly channeled to the domestic market (Ministry of Fisheries 2013, 2021). Cd levels in different tuna species including *T. albacares* from Sri Lanka have been reported previously (Jinadasa et al. 2010, 2015, 2019). However, no published data are available on tissue level and temporal variations of Cd in juvenile *T. albacares.* The objectives of this research were to report the level of Cd in white and dark muscles and liver of *T. albacares*, to analyze monthly and seasonal variations, and to assess if Cd levels exceed the maximum permissible level established by WHO/FAO and the European Commission. The results are critical to understanding the permissible dietary intake of edible flesh, including dark and white muscles and liver tissues of juvenile *T. albacares*.

Material and methods

Sampling preparation

A total of 72 samples of juvenile yellowfin tuna (standard length: 50-67cm; Body weight: 0.8 kg -2.5kg) from catches of longline vessels operated in South and West offshore waters around Sri Lanka (Indian Ocean) were collected from Negombo fish landing center (7^0 12’N, 79^0 49’E, n = 12) and Tangalle harbor (5^0 58’ 39”N, 80^0 44’ 2”E, n = 60) during April 2021 to May 2022.

Sample preparation for Cd analysis was done in the laboratory at the University of Jaffna, and the standard length (cm) and weight (kg) of each specimen were measured. Liver tissue (approximately 0.005–0.008 kg) and dark and white skeletal muscles (0.02–0.03 kg) from the dorso-caudal region were obtained. Each tissue sample was dried in Petri dishes until constant weight at 60–65 ^oC using a hot air sterilizer. Dried samples were homogenized using a grinder (Preethi; India), covered in aluminum foil, and stored within a desiccator until further analysis (UNEP/FAO/IAEA/IOC 1984).

Reagents and materials

Analytical reagent grade, concentrated nitric acid (HNO_3), hydrochloric acid (HCl), and 30% (w/v) hydrogen peroxide (H_2O_2) were used for the digestion process. De-ionized water (Milli-Q Water Purification System) was used for cleaning and dilution. All the glassware was soaked in 20% (v/v) HNO_3 for 24 hours and rinsed with de-ionized water prior to use.

Sample digestion and elemental measurements

The United States Environmental Protection Authority (USEPA) method (3050B) of acid digestion was used with slight modifications. Dried/ grounded muscle and liver tissues (1.00 g each) were weighed (OHAUS, USA) and were transferred separately to Kjeldhal tubes (VELP- SCIENTIFICA). Then 10 mL of conc. HNO_3 was added into each tube and heated at 95 ^{o}C ± 5 ^{o}C and refluxed for 10–15 minutes, without boiling, using a heating digester (DKL, China). The samples were allowed to cool, added 5 mL of conc. HNO_3, and refluxed for 30 minutes. Then the solutions were evaporated to approx. 5 mL, without boiling, using a vapor recovery system.

After cooling, 2 mL of distilled water and 3 mL of 30% H_2O_2 were added into each tube, which was subsequently warmed to start the peroxide reaction. Then, 10 mL conc. HCl was added to the acid-peroxide digestate that was heated at 95 ^{o}C ± 5 ^{o}C, refluxed for 10–15 minutes, and filtered (Whatman No.41). Filtrate was diluted until 50 mL with deionized water and stored.

The analysis of Cd was performed by an Inductivity Coupled Plasma Mass Spectrophotometer (ICP-MS) at the Industrial Technology Institute (ITI) in Colombo, Sri Lanka. All results (means ± SD, three replicates) were expressed in terms of mg kg^{-1} dry weight. The muscle/liver ratios were calculated using the mean Cd and expressed on a dry weight basis.

Assessment of maximum allowable weekly intake

Dietary safety was evaluated according to the maximum permissible levels of Cd in fresh fish set by the European Commission and the Joint FAO/ WHO Expert Committee on Food Additives. Maximum allowable weekly intake (PTWI) was calculated using a provisional tolerable weekly intake value for Cd (Schulz et al. 2017). To compare the Cd levels with international standards, data were converted to a wet weight basis using a converting factor of 0.3, assuming the moisture content in the *T. albacares* tissues is 70% (Karunarathna and Attygalle 2010).

Statistical analysis

Data and statistical analysis were performed with R commander 4.0.3. software. Data were subjected to the test of normality by Q-Q plot and Shapiro Wilk test and homogeneity of variances by Levene’s test.

Extreme values that were identified in the box plots were statistically tested with Grubb's test to exclude outliers before calculating the mean Cd levels. Due to the noncompliance with parametric assumptions, the Kruskal Wallis (Post hoc Dunn test a Bonferroni correction) test performed using tissue type as a fixed factor to assess the significant difference in Cd concentrations between three different tissues and used to evaluate the hypothesis about Cd levels among different monsoon regimes that influence offshore seas around Sri Lanka according to Karunathilaka et al., (2017) namely first inter-monsoon, southwest monsoon, second inter-monsoon, and northeast monsoon. Pearson product-moment correlation matrix was used to examine the relationship between Cd concentration in different tissues and weight. All hypotheses were tested at $p < 0.05$ significant level.

Quality assurance and validation parameters

Quality control procedures included measured recovery percentage, limit of detection, quantification, and repeatability including reagent blanks and certified reference materials. Two blanks with digesting reagents were inserted in each digesting process to detect any alien contaminants. The accuracy of the elemental measurement with the recovery of analytes was evaluated by using three replicates with standard reference materials of ERM-BB422 fish muscle from the European Commission, Joint Research Centre Institute for Reference Materials and Measurements (IRMM), and canned crab meat (T/07279QC) from Food Analysis Performance Assessment Scheme (FAPAS, UK).

The validity of the ICP-MS analytical procedure was verified by the Limit of Detection (LOD) and recovery results of fish muscle (ERM-BB422) and canned crab meat (T/07279QC) as shown in Table 1. The recovery percentages (94.67%, 95.28%) with good repeatability confirmed the efficacy of the analytical method to monitor the cadmium levels. The LOD value for Cd was 0.001 mg/L for three replicates.

Table 1
Verification of standard reference material ERM-BB422 fish muscle, canned crab meat (T/07279QC*)*
(mg/kg dry weight) obtained in the analysis

*Quality Control material	Certified value (mg/kg)	Experimental value (mg/kg)	Recovery (%)
ERM-BB422	0.0075	0.0071 ± 0.0004	94.67
T/07279QC	0.106	0.101 ± 0.005	95.28

*The number of replicates for certified reference material was six.

Results

Tissue variation of Cd in juvenile T. albacares

The Cd levels in dark and white muscle tissues of *T. albacares* ranged from 0.05 to 0.67 mg kg^{-1} dry weight (d.w.), and 0.05 to 0.63 mg kg^{-1} d.w., respectively. Liver Cd levels were in the range of 1.39 to 40.95 mg kg^{-1} d.w. (Table 2). Maximum Cd level was observed in liver tissue (13.62 ± 6.20 mg kg^{-1} d.w.), followed by dark muscles (0.52 ± 0.23 mg kg^{-1} d.w.) and white muscles (0.42 ± 0.16 mg kg^{-1} d.w.) (Table 2). The ratios of Cd levels (liver/muscle) were 32.4 for the white muscle and 26.2 for the dark muscle. Cd levels were significantly different between tissues (Kruskal Wallis: H = 142.95, df = 2, p-values < 0.05), where liver Cd level was significantly greater than Cd levels in both dark muscle and white muscle (Dunn test with a Bonferroni correction: Z= -10.18, p-value < 0.05 and Z = 10.27, p-value < 0.05 respectively) (Table 2).

Table 2
Mean Cd levels (± SD) (mg/kg d.w.) in tissues of juvenile *Thunnus albacares*

Tissue type	Sample size (n)	*Mean Cd (mg/kg d.w.)
White muscle	60	0.42 ± 0.16^a
Dark muscle	72	0.52 ± 0.23^a
Liver	72	13.62 ± 6.20^b

*Limit of detection (LOD) was 0.001 mg/L for Cd.

[a,b] Different superscript letters indicate significant differences (Dunn test, $p < 0.05$) between tissue types.

A strong significant positive correlation was found between Cd levels in liver tissues and fish weight in juvenile *Thunnus albacares* (Pearson correlation, r = 0.573, P < 0.05) (Table 3).

Table 3
Pearson product-moment correlation between Cd levels in different tissues and fish weight of juvenile *Thunnus albacares*

	Dark muscle	**Liver**	**White muscle**	**Weight**
Dark muscle	-	$p < 0.05$	$p < 0.05$	
Liver	**0.479***	-		$p < 0.05$
White	**0.464***	0.171	-	
weight	0.199	**0.573***	0.268	-

*The bold values indicate correlation is significant at 0.05 level.

Monthly variation of Cd levels in juvenile Thunnus albacares

Cd in liver tissue, dark muscle, and white muscle discernibly varied among months within the study period (Fig. 1, 2, 3). The highest levels of Cd in liver tissue and dark muscle were found in October 2021 (26.35 ± 3.46, 0.93 ± 0.10 mg/kg d.w. respectively) while in white muscle, the highest level was reported in November (0.60 ± 0.07 mg/kg d.w.). The lowest Cd levels in dark muscles and liver tissues were found in

July 2021 (0.08 ± 0.007, 2.59 ± 0.007 mg/kg d.w. respectively) while the lowest Cd level in white muscles was reported in December 2021 (0.05 ± 0.001 mg/kg d.w.) with much lower values below the LOD reported in April 2022. Cd levels in liver and dark muscles showed an increasing trend from July to October 2021 whereas Cd levels in white muscles showed an increasing trend from September to November 2021. Discernible decreasing trends of Cd levels were observed in dark muscles, white muscles, and liver tissues from October to December, November to December, and October to January respectively. Cd in dark muscle and white muscle showed much more fluctuation over the months than liver Cd levels.

Seasonal variation of Cd in tissues

Seasonal variations of Cd in different tissues are depicted in Table 4 showing the Cd levels in the four seasons namely, first inter-monsoon (March-April), southwest monsoon (May-September), second inter-monsoon (October-November), and northeast monsoon (December-February) (Karunathilaka et al. 2017). The most discernible changes in Cd levels among seasons were found in dark muscles, white muscles, and liver tissues during the 1st inter-monsoon (0.23 ± 0.11, 0.26 ± 0.09, and 10.77 ± 5.31 mg/kg d.w. respectively) and 2nd inter-monsoon (0.63 ± 0.04, 0.52 ± 0.08, and 21.78 ± 2.25 mg/kg d.w. respectively). The highest Cd levels in dark muscles, white muscles, and liver tissues were found in 2nd inter-monsoon regime. For liver tissues, dark muscles, and white muscles, significant Cd concentration differences were attributed among the four different monsoon regimes (Wilcoxon rank sum test: W = 13.3, W = 13.07 and W = 32.7, $p < 0.05$ respectively).

Table 4
Seasonal variation of mean (± SD) Cd levels (mg/kg dry weight) in dark muscle, white muscle, and liver tissues in juvenile *Thunnus albacares*. Monsoon regimes indicate first-inter monsoon; March-April, Second-inter monsoon; October-November, Southwest monsoon; May-September and Northeast monsoon; December-February (Karunathilaka et al. 2017)

Tissue type	N	Monsoon regimes	*Cd levels (mg/kg d.w)
Dark muscle	12	1st Inter-monsoon	0.23 ± 0.11^{a}
	30	Southwest monsoon	0.33 ± 0.17^{ab}
	12	2nd Inter-monsoon	0.63 ± 0.04^{b}
	18	Northeast monsoon	0.42 ± 0.21^{ab}
White muscle	12	1st Inter-monsoon	0.26 ± 0.09^{a}
	30	Southwest monsoon	0.19 ± 0.14^{a}
	12	2nd Inter-monsoon	0.52 ± 0.08^{b}
	18	Northeast monsoon	0.43 ± 0.14^{b}
Liver	12	1st Inter-monsoon	10.77 ± 5.31^{a}
	30	Southwest monsoon	11.63 ± 6.2^{a}
	12	2nd Inter-monsoon	21.78 ± 2.25^{b}
	18	Northeast monsoon	9.73 ± 3.63^{a}

* Significant differences between monsoon seasons are indicated by non-shared superscript letters within a given tissue type ($p < 0.05$, Kruskal Wallis and Dunn test)

Health risk assessment

According to the regulations of Maximum Permissible limits (MPLs) set by FAO/WHO for the *T. albacares* edible tissues, the mean Cd levels of dark and white muscles were below the MPLs during four monsoon regimes (Table 5). According to the European Commission (EU) regulation, mean Cd levels found in dark muscles and white muscles during 2nd Inter-monsoon and Northeast monsoon exceeded the permitted level. Cd levels recorded for liver tissues from all individuals analyzed in this study well exceeded the limit set by both FAO/WHO and EU (European Food Safety Authority 2009; Vracko et al. 2007).

Table 5
Mean Cd levels (mg/kg wet weight basis) in juvenile *Thunnus albacares* in different monsoon regimes. Values in bold font show the Cd level above permissible limits in edible tuna tissues established by the European Union (E), and FAO/WHO (F)

Monsoon regimes*	Cd levels in dark muscles	Cd levels in white muscles	Cd levels in liver tissues
1st IM	0.05 ± 0.01	0.06 ± 0.01	**2.91 ± 1.30[e,f]**
SW	0.07 ± 0.03	0.04 ± 0.01	**3.47 ± 2.10[e,f]**
2nd IM	**0.17 ± 0.03[e]**	**0.13 ± 0.02[e]**	**6.28 ± 0.90[e,f]**
NE	**0.11 ± 0.06[e]**	**0.10 ± 0.06[e]**	**2.63 ± 0.98[e,f]**

[e]European Union regulation The MAL is 0.1 mg/kg for Cd for *Thunnus* species

[f]FAO/WHO 0.2 mg/kg for Cd

*IM = Inter monsoon, SW = Southwest monsoon, NE = Northeast monsoon

Discussion

The present study reported Cd levels in three tissue types of juvenile *Thunnus albacares* collected from waters around Sri Lanka in the Indian Ocean. Tissue levels of Cd had not been reported in juvenile *T. albacares* prior to this work and hence the findings are important to assess the health risks of Cd for consumers. Further, the lack of previously published data on Cd levels in dark and white muscle of juvenile *T. albacares* prevents a comprehensive comparison of present results. Increased input of Cd arising from industrial development in the countries bordering the Indian Ocean has been suggested (Chen et al. 2018) and therefore, *T. albacares* in the Indian Ocean in general may accumulate higher Cd levels compared to other oceans. The variation of Cd levels in tissues can be integrated with natural, geographical, and biological factors. Miedico et al. (2020) reported comparatively lower Cd level (median: 0.0132 mg kg^{-1} wet weight) in muscle tissues of *T. albacares* caught in Italy. Results obtained by Besada et al. (2006) found that Cd level (median: 0.004 mg kg^{-1} wet weight) in muscle tissues of *T. albacares* (size ranged from 96–145 cm) caught from the Atlantic Ocean also lower than the Cd levels (1st IM: 0.06 ± 0.0, SW: 0.04 ± 0.01, 2nd IM: 0.13 ± 0.02, NE: 0.10 ± 0.06 mg kg^{-1} wet weight) reported in the present study. The geographic variation in Cd levels of the fish may be related to the available waterborne Cd levels within its distribution range. Population growth, and industrial development associated with the Indian Ocean region are some prominent factors that may induce Cd contamination in the Indian Ocean (Chen et al. 2018). According to the United States Geological Survey, India is the top ten Cd-producing country with an annual Cd production of 600 metric tons (Schulz et al. 2017). Apart from natural sources, mining is a major contributor to the increase in trace metals in the Indian Ocean region (Chen et al. 2018). In addition, coal burning, use of mineral fertilizers, domestic effluents, and industrial wastewater released from bordering nations without treatment are important sources of Cd (Chen et al. 2018), and it will be important to study large-scale geographical variation in Cd loads in fish.

Although the mean Cd levels in the dark muscle of *T. albacares* were apparently slightly higher than that in the white muscle, there was no significant difference in Cd between the two muscle types. No previously published data are available in this regard for the same species. However, slightly higher Hg levels in the dark muscles than the white muscles were reported for *T. albacares* by Bosch et al. (2016) which suggested that, this variation could be caused by differences in muscle function. The dark muscles of *T. albacares* are developed with strong muscle fibers to produce thrust for fast and continuous swimming (Bosch et al. 2016). High muscle activity and fiber development in the dark muscles may provide more protein-binding sites for Cd accumulation ((Bosch et al. 2016). Hence, this can contribute to the slight differences in Cd levels between the dark and white muscles of *T. albacares* in the present study.

Cd levels (mg/kg wet weight) in the dark and white muscles of juvenile *T. albacares* (0.8 -2.5kg) reported in this study are apparently higher than values reported by previous studies for adults from the Indian Ocean. Jinadasa et.al. (2019) reported a Cd level of 0.017 mg kg^{-1} in muscle tissues of adult *T. albacares* (mean weight: 45.9 kg) caught from the Indian Ocean around Sri Lanka. The mean Cd levels of muscle tissue of the present also exceeded the values reported for flesh/muscles (0.02–0.03 mg kg^{-1}) of *T. albacares* (weight range from 35–55 kg) from Lakshadweep Island, India (Farejiya and Dikshit, 2016). Cd levels in both muscle tissues in this study were above the values reported in adult *T. albacares* from the Mozambique Channel and Reunion Island (0.25 ± 0.21mg kg^{-1} d.w., and 0.23 ± 0.20 mg kg^{-1} d.w. respectively) (Kojadinovic et al. 2007).

Higher Cd levels from juvenile *T. albacares* may be linked to their diet composition. Previous studies have reported high Cd levels in cephalopods which are reported as a common food item for *T. albacares*, especially for juveniles (Das et al. 2000). Therefore, higher Cd levels in muscles of juvenile *T. albacares* in the present study are likely to be linked to their cephalopod diet. Verification in this regard is however needed.

Significantly higher Cd levels were recorded in the liver tissues of *T. albacares* than in the muscle tissues. These findings are consistent with previous studies conducted by several authors (Farejiya and Dikshit 2016; Kojadinovic et al. 2007). A similar pattern was observed for *T. albacares* collected from the Red Sea, Egypt by Moselhy et al. (2014). Kojadinovic et al. (2007) reported higher Cd levels in liver tissues of adult *T. albacares* caught around the Mozambique Channel and Reunion Island in the western Indian Ocean (138 ± 60 mg kg^{-1} d.w. and 126 ± 130 mg kg^{-1} d.w. respectively) than the mean values measured in the present study. The ratios of Cd concentration between liver and muscle tissues (dark and white) (L/M ratio) reveal the relative distribution of Cd between different tissue types. Apparently, higher L/M ratios were observed for white muscle (32.4) than for dark muscle (26.2) owing to slightly higher Cd levels found in the dark muscles of *T. albacares*. L/M ratio generally depends on the chemical properties of trace metals, species, and tissue-specific variation (Gaspic et al. 2002). The liver/muscle Cd-ratios have been calculated for marine fishes from different trophic levels. For instance, L/M ratios for fish in lower trophic levels such as hake (2.72) and red mullet (3.95) were much lower than those for *T. albacares* (Gaspic et al. 2002). Higher L/M ratios reported in present studies and elsewhere indicate a greater Cd bioaccumulation in liver tissues in top predatory species like *T. albacares*. Cd bioaccumulation in liver tissues is linked with the Cd filtered from the digestive tract (Alizada et al. 2020).

The liver is the target organ for metal bioaccumulation (Moselhy et al. 2014). Higher liver Cd levels found in the liver tissues than in either of the muscle tissues of juvenile *T. albacares* were in agreement with this. Higher concentrations of Cd in liver tissues of *T. albacares* corroborate a high intake of cadmium from direct sources (dietary) or indirect sources. Furthermore, cadmium is readily accumulated in liver tissues due to metallothionine formation (Gaspic et al. 2002). Metallothionines can displace essential metals such as Pb with Cd ions in the liver tissues (Moselhy et al. 2014). The present study found a significant positive correlation between Cd concentrations in liver tissues and the weight of the fish. This might be due to the increased induction of cadmium-binding protein in the liver with increasing specimen size (Ancora et al. 2020).

There is no literature available for monthly variation of Cd level in *T. albacares* in the Indian Ocean. Monthly variation of Cd in muscle and liver tissues could be attributed to the availability of Cd in the seawater and the differences in the rate of Cd uptake by *T. albacares* (Srichandan et al. 2016). Comparatively high Cd concentration in the Indian Ocean water was reported in November (0.01 ppb) 2011, due to the Cd from industrial effluents and sewage entering the coastal water during inter-monsoon (Srichandan et al. 2016). Peak Cd levels in all three tissues in the present study were reported through October and November 2022 indicating a greater availability of Cd during 2nd inter-monsoon in agreement with Srichandan et al. (2016) observation. Metals loads in fishes might fluctuate due to a combination of ecological factors, biological factors, and the persistence of residual contaminants from anthropogenic sources associated with monsoonal changes (Digoarachchi et al. 2022; Srichandan et al. 2016; Zhang et al. 2023).

The present study also investigated seasonal variation in tissue Cd levels, and the results indicated a higher Cd load during the 2nd inter-monsoon than in the other monsoon regimes. This might be due to the high rainfall experienced in many regions of the country during the 2nd inter-monsoonal season rather than the region-specific rainfall pattern during the monsoon season in Sri Lanka (Karunathilaka et al. 2017), leading to discharges of industrial effluents, and sewage into the ocean with river influx (Srichandan et al. 2016). Consumers should be aware of seasonal variations in Cd level especially the peak seasons to avoid intake of higher Cd load through their diet. Similarly, greater attention should be given to the long-term monitoring of heavy metals in marine fishes of high consumer preference.

Generally, the target size of *T. albacares* in the Sri Lankan domestic market is less than 2 kg, while the larger sizes are considered preferably for the export market. For Cd levels in edible muscle tissues, the most prominent aspect is the evaluation of toxicity for human consumption. European Food Safety Authority has established a provisional tolerable weekly intake (PTWI) for cadmium at 0.007 mg kg^{-1}

body weight (bw)/week (European Food Safety Authority 2009). Based on the PTWI value, we calculated the maximum allowable weekly intake of white muscles of *T. albaacres* to be 4.667 kg for an average adult of 60 kg body weight. The estimated daily intake (666.7 g/day) based on PTWI value is much greater than the per capita fish consumption (43 g/day) in Sri Lanka reported by Jinadasa et al (2019). Accordingly, the inference is that the Cd level of edible tissues may not exceed the safety margins when the fish is consumed at a similar daily intake.

Based on the maximum permissible levels in edible, dark, and white muscles of juvenile *T. albacares* we suggest that the fish is within the secure confine for human consumption during 1st inter-monsoon and southwest monsoon regimes. Consumers however should limit the consumption of juvenile *T. albacares* during 2nd inter-monsoon and northeast monsoon as Cd levels exceeded the maximum permissible levels set by the European Union. Cd levels in liver tissues exceeded safety levels set by both the European Union and FAO/WHO throughout all monsoon periods. Therefore, the results confirmed that people should avoid the consumption of the liver of juvenile *T. albacares* to be safe from Cd toxicity.

Conclusion

This study characterized and gathered the first data on the variation of Cd levels in muscle and liver tissues of juvenile *T. albacares* in Sri Lanka monthly as well as seasonally. Liver tissues had significantly higher Cd levels than the white and dark muscle tissues. A significant positive correlation between Cd levels in liver tissues and fish weight was found. Cd levels in all three tissue types exhibited temporal variations, with higher Cd levels in juvenile *T. albacares* during the 2nd inter-monsoon than that is found in other monsoon regimes over 13 months from April 2021 to May 2022. The Cd levels in white and dark muscle tissues of *T. albacares* are generally lower than the maximum permissible level (MPLs) set by FAO/WHO during four monsoon regimes, whereas higher levels than the MPLs set by the European Commission were reported during 2nd inter-monsoon and northeast monsoon. Based on the maximum allowable weekly intake value and average Cd levels in dark and white muscles, it can be concluded that the consumption of juvenile *T. albacares* tuna is still safe for consumers. Cd levels in liver tissues of juvenile *T. albacares* were greater than MPL throughout the study period and therefore, its human consumption should be discouraged.

Statements & Declarations

Declarations

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Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Authors' Contribution

All authors contributed significantly to the study's conception and design. Material preparation, data collection, and analysis were performed by Dhanushka Dilini Jayaweera. The first draft of the manuscript was written by Dhanushka Dilini Jayaweera. The first draft was reviewed, commented and edited by Sivashanthini Kuganathan, Suneetha Gunawickrama, and Anita Evenset. This study was supervised by Sivashanthini Kuganathan, Suneetha Gunawickrama, and Anita Evenset. All authors read and approved the final manuscript.

Availability of data and material

All data generated during this study are included and presented in this paper.

Code availability (software application or custom code)

Not applicable

Compliance with Ethical Standards

Ethics approval

It is declared that the study involved no human participants.

Consent to participate

The authors declare that they all consented to participate in the research work.

Consent for publication

The authors declare that they all consented to publish this research work.

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Figures

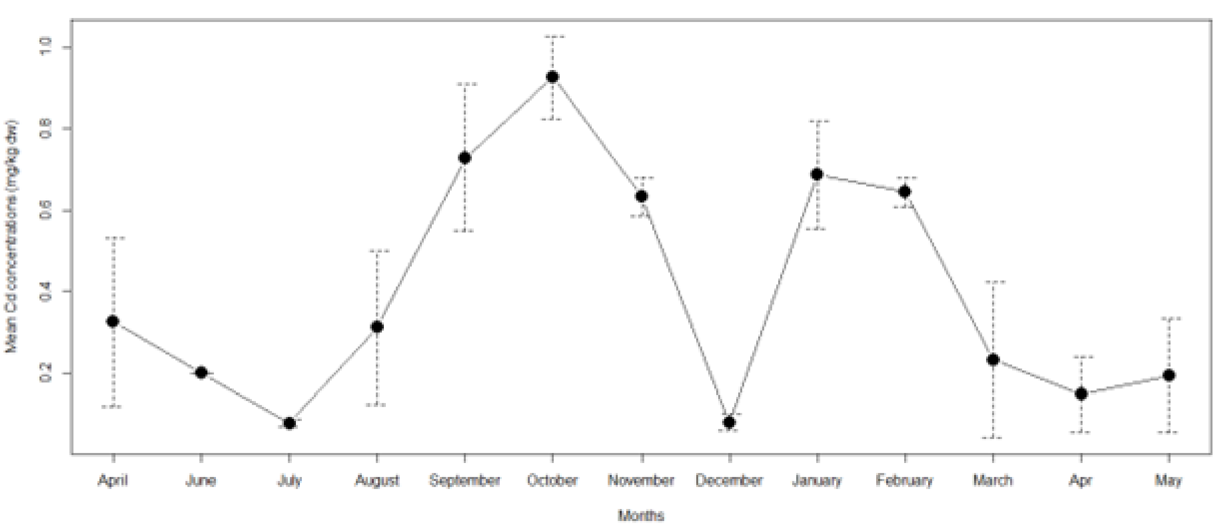

Figure 1

Monthly variations of the mean (± SD) Cd levels (mg/kg dry weight) in the dark muscle of juvenile *Thunnus albacares* from April 2021 to May 2022

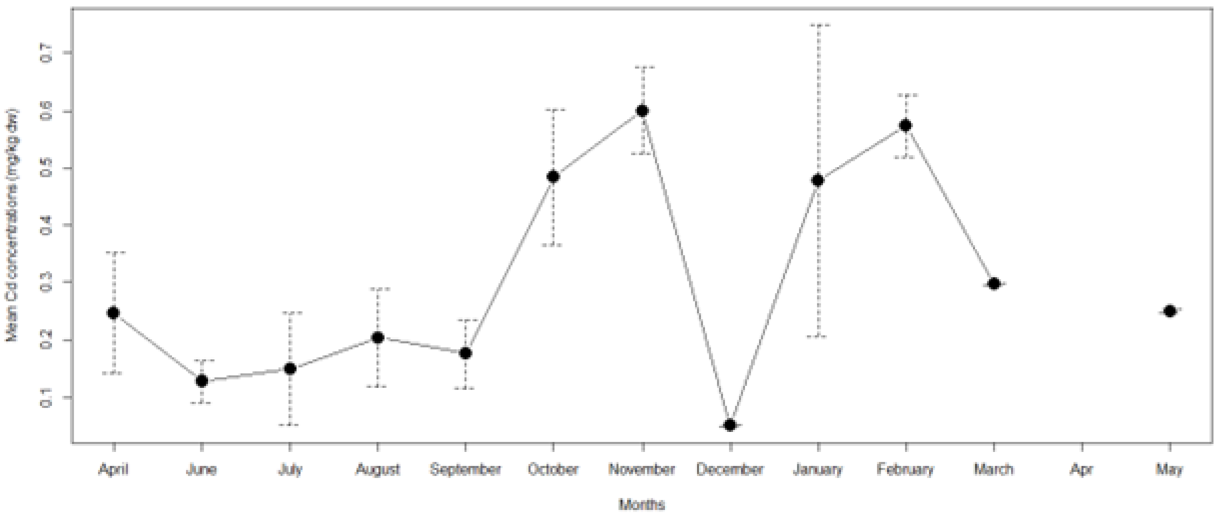

Figure 2

Monthly variations of mean (± SD) Cd levels (mg/kg dry weight) in white muscle of juvenile *Thunnus albacares* from April 2021 to May 2022 (For April 2022 all values were below the limit of detection, LOD 0.001 mg/L).

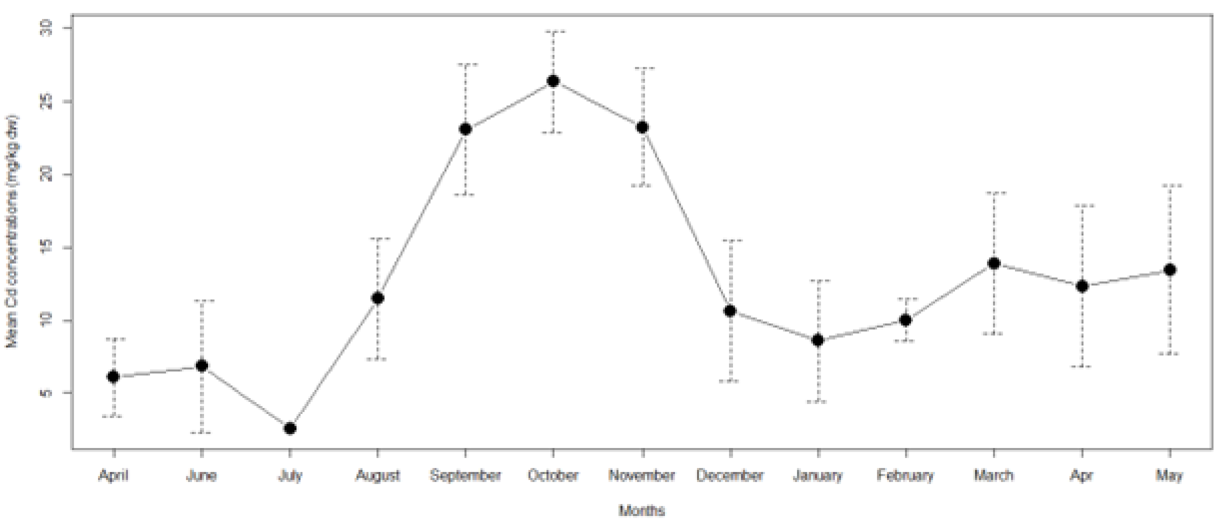

Figure 3

Monthly variations of mean (± SD) Cd levels (mg/kg dry weight) in liver tissues of juvenile *Thunnus albacares* from April 2021 to May 2022

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