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Heavy metal concentrations in edible muscle of whitecheek shark, *Carcharhinus dussumieri* (elasmobranchii, chondrichthyes) from the Persian Gulf: A food safety issue

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A B S T R A C T

Together with several health benefits, fish meat could lead to heavy metal intoxication of consumers. In this study, we discuss Zn, Cu, Pb, Hg and Cd concentrations in fillets of forty specimens of *Carcharhinus dussumieri*, analyzed with atomic adsorption spectroscopy (AAS). The potential human health risks due to consumption of C. *dussumieri* was assessed by estimating average daily intake (EDI) and target hazard quotient (THQ) of metals. The average concentrations of metals measured in this study were (ppm dry weight): Cu 7.49 $\pm$ 0.25; Zn 3.47 $\pm$ 0.26; Pb 0.12 $\pm$ 0.03; Hg 0.028 $\pm$ 0.02; Cd 0.11 $\pm$ 0.03. Our results showed that no metal exceeded the EC and FAO limits. Cu and Cd accumulate in muscles with a body length (age)-dependent manner. The exposure daily intake of all toxic metals analyzed was found lower than the PTDI provided by WHO and the THQ resulted lower than 1, suggesting no risk for human health derived from consumption.

1. Introduction

Aquatic environments are continuously threatened by inorganic contaminants including trace metals and metalloids (Sciacca and Oliveri Conti, 2009; Longo et al., 2013; Dadar et al., 2016), and organic contaminants (Conte et al., 2016; Oliveri Conti et al., 2012). Some metals show the ability to biomagnify through the aquatic food chain leading to levels of concern in top predators (Seixas et al., 2014; Ouédraogo et al., 2015).

Seafood is an important source of proteins and essential fatty acids necessary for a healthy human life (Oliveri Conti et al., 2015). Nevertheless, several studies have been highlight that seafood consumption is a major contributor to the uptake of heavy metals in the human body together with many other toxic substances such as algal bio-toxins and drugs (Adel et al., 2016; Oliveri Conti et al., 2015; Copat et al., 2012, 2014, 2015; Conte et al., 2015; Ferrante et al., 2010; Ferrante et al., 2013). Among metals, mercury (Hg), lead (Pb), cadmium (Cd), copper (Cu) and arsenic (As) have been frequently found to accumulate in fish fillet, with quantities above the legal limits (Bosch et al., 2016; Vandermeersch et al., 2015). Evidences of shark consumption have been available as early as the fourth century (FAO, 2016) and nowadays it represents a traditionally seafood of coastal areas.

For their predatory behavior, long life and high trophic levels, sharks accumulate higher metals concentrations than other marine fishes (Delshad et al., 2012).

The whitecheek shark, *Carcharhinus dussumieri*, is one of the most common sharks of the Persian Gulf. The species inhabits coastal waters affected by many urban and industrial wastewaters, which are sources of trace metals (Moore et al., 2015). This species is distributed in the northern Indian Ocean from the Persian Gulf to

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List of abbreviations

Mercury Hg
Lead Pb
Cadmium Cd
Copper Cu
Nickel Ni
Zinc Zn
Limit of detection LOD
Average Exposure Daily Intake EDIa
Target Hazard Quotient THQ
Exposure Daily Intake per meal size EDIm
Exposure frequency EF
Exposure duration for adults of 70 years ED
Oral Reference Dose (μg/g/day) RfDo
Averaging Time AT
Provisional Tolerable Daily Intake PTDI)
Meal sizeMS
Body Weight BW
Average Annual Ingestion Rate IR
Unites States – Environmental Protection Agency US-EPA
Vanadium Pentoxide V_2O_5
Potassium dichromate $K_2Cr_2O_7$)

India at depth of less than 100 m. White (2012) and, his meat is highly used for human consumption (Vannuccini, 2002; Delshad et al., 2012), representing a potential human health hazard for its content of certain metals (e.g. Hg, As, Pb etc.).

The main objectives of this study were:

- to analyze the total mercury (Hg), zinc (Zn), copper (Cu), nickel (Ni), lead (Pb) and cadmium (Cd) concentrations in muscle tissue of whitecheek sharks caught from the Iranian coastal waters of the Persian Gulf;
- to assess the relationship between body length and weight with metals levels in muscle;
- to estimate the potential human health risks derived from oral consumption of *C. dussumieri* by the average daily intake (EDI) and the target hazard quotient (THQ) for all metals studied.

2. Material and methods

2.1. Sampling and study area

Forty specimens of whitecheek shark (*Carcharhinus dussumieri*) (n = 20 male, n = 20 female) were collected between December and January 2014 by fishing trawler from Hurmozgan province. Sampling sites were located on the northern basin of Persian Gulf, between Larak and Lavan islands, in 4 coastal stations: A (26.55° N, 57.12° E), B (26.32° N, 56.55° E), C (26.20° N, 55.34° E) and D (26.09° N, 54.80° E) (Fig. 1). The sex of shark was recognized both by examining clasper in male shark and macroscopic examination. The whitecheek sharks were been transported to the central laboratory of Iranian Shrimp Research Center (Bushehr, Iran) and their total length (cm) and total weight (kg) were measured. Approximately 50 g of muscle from each individual shark were collected in sterile polythene bags and kept in the laboratory deep freezer (−80 °C) to prevent deterioration until analysis.

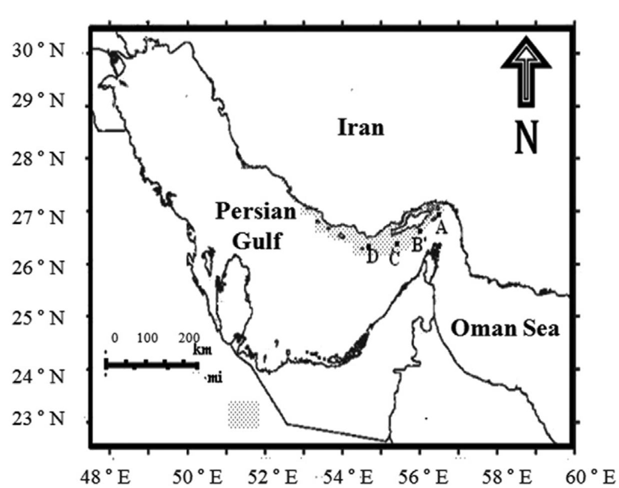

Fig. 1. Study area.

2.2. Analytical procedures

The procedure used to measure trace elements concentrations in shark samples was described previously (Dadar et al., 2014). Briefly, muscles of shark were been dried in an oven at 65 °C for a period of 48 h until a constant weight was obtained and ground separately. The average water content we found was of 76%. The tissue was measured using Walkey-Black titration and trace elements concentrations were been measured with the standard method with minor modification. Edible aliquots of muscle (0.3 g) were accurately weighed and digested by high pressure decomposition vessels according to the method described in our previous study (Dadar et al., 2014). Sample were mixed with 5 mL of 68% nitric acid (Suprapur; Romil Ltd., Cambridge, UK), 4 mL of 30% hydrogen peroxide (Suprapur; Merck, Darmstadt, Germany) and 1 mL concentrated perchloric acid (Suprapur; Merck, Darmstadt, Germany). For Hg digestion, 45 mg V_2O_5 was been added to the samples. Then they were diluted to 50 mL with 20 mL of distilled water and $K_2Cr_2O_7$ (2%). Digestion was been conducted on a hotplate, at 200 °C, for at least 4 h or until clear and all particles had turned white color. Digested samples were been filtered through a 0.45 μm membrane filter of nitrocellulose, diluted with high purity deionized water at a ratio of 1:5 and analyzed with flame atomic absorption spectrophotometry (Thermo M5 Series AA, Germany) equipped with a microcomputer-controlled acetylene flame. Samples were been analyzed in triplicate and, the results collected on a dry weight basis. Blanks were been processed in the same way of the samples. The overall recovery rates (mean ± SD) of Zn, Cu, Ni, Hg, Cd and Pb were 90 ± 3.3%, 95 ± 3.9%, 94 ± 9.6%, 90 ± 2.6%, 90 ± 12.4% and 88 ± 8.1%, respectively. The limit of detection (LOD) Zn, Cu, Ni, Hg, Cd and Pb were 0.17, 0.08, 0.02, 0.001, 0.006 and 0.005 μg/g, respectively.

2.3. Daily intake and risk assessment

The estimated daily intake per meal size (EDIm), per average annual ingestion rate of freshwater fish (EDIa), and target hazard quotient (THQ) were calculated according to the equation reported in previous reports (Copat et al., 2012; Oliveri Conti et al., 2012).

$$EDIm = MS \times C/BW$$

$$EDIa = IR \times C/BW$$

$$THQ = (EF \times ED \times MS\ kg \times C)/(RfDo \times BW \times AT)$$

where, MS is the adult meal size (expressed as 227 g in EDI and as 0.227 Kg in THQ); C is the metal concentration (expressed as μg/kg wet weight in EDI and as μg/g in THQ); BW is the body weight (adults, 70 Kg); IR is the average annual ingestion rate of pelagic fish for Iranian population (9 g), downloaded from FAOSTAT database; EF is the exposure frequency, or number of exposure events per year of exposure (365 days/year for people who eat fish seven times a week); ED is the exposure duration (adults, 70 years); RfDo is the oral reference dose (μg/g/day); AT is the averaging time (it is equal to EF x ED).

According to the US-EPA, EF, ED, MS, BW and AT are default data provided for consumption limits calculation in adult population. Values of the RfDo are provided by EPA's Integrated Risk Information System online database. If it was not available, we used the respective metal provisional tolerable daily intake (PTDI) for THQ calculation.

2.4. Statistical analysis

All data were analyzed using Statistical Package IBM SPSS 20.0. A Kruskal-Wallis non-parametric method was been applied to test metal distribution among sampling sites. A test t for independent samples was been applied to identify significant differences based on sex and a Spearman rank test was applied to identify any correlations between length and weight with metals concentrations.

3. Results and discussion

Metals concentration in edible portions of fish tissue can give an overview of environmental pollution and risk for consumer's health. It is known that fish accumulate different concentrations of contaminants based on their trophic levels, longevity, habitat as well as chemical characteristic of specific pollutant. Among fish species, shark is a predator organism, which could potentially accumulate high levels of pollutants, especially compounds which biomagnify up to the food chain contaminants such as Hg (Lopez et al., 2013; Teffer et al., 2014).

The average metal concentrations measured in this study, from highest to lowest were (ppm dry weight – d.w.) ($n = 40$): Cu 7.49 ± 0.25; Zn 3.47 ± 0.26; Pb 0.12 ± 0.03; Hg 0.028 ± 0.02; Cd 0.11 ± 0.03. We tested metals distribution based on sampling sites with Kruskal-Wallis non-parametric test. It revealed no differences except for Hg concentration which was found lower in C site ($p < 0.05$) (Table 1, Fig. 2).

Table 1
Descriptive statistics of metals (ppm dw) based on sampling sites.

Metals	Mean	Median	Dev. Std.	Mean	Median	Dev. Std.
Sampling sites		A			B	
Zn	3.62	3.57	0.33	3.45	3.44	0.20
Cu	7.40	7.32	0.25	7.51	7.46	0.31
Pb	0.12	0.13	0.03	0.12	0.12	0.03
Hg	0.03	0.03	0.02	0.02	0.02	0.02
Cd	0.11	0.11	0.03	0.10	0.10	0.03
Sampling Sites		C			D	
Zn	3.40	3.37	0.22	3.41	3.37	0.23
Cu	7.52	7.50	0.23	7.53	7.52	0.21
Pb	0.10	0.11	0.03	0.13	0.13	0.04
Hg	0.02	0.01	0.01	0.04	0.04	0.02
Cd	0.12	0.12	0.02	0.10	0.10	0.03

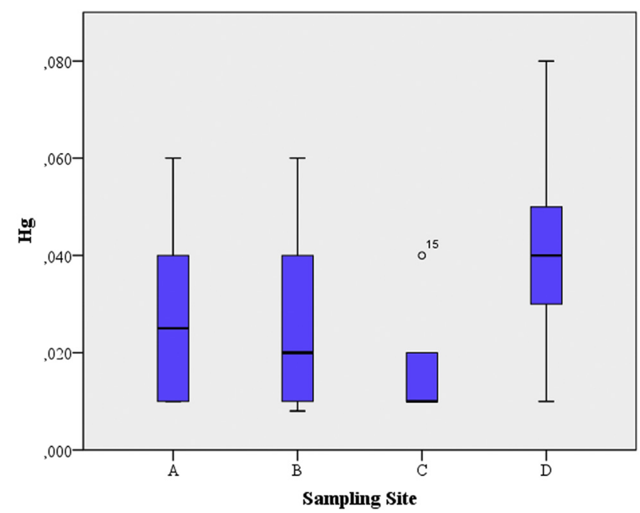

Fig. 2. Box plot distribution of Hg base on sampling site.

Zn and Cu are essential metals in humans as well as in animals. Their biological roles are associated to their participation in the structures and functions of several enzymes-Zn dependent (e.g. the superoxide dismutase with known antioxidant activity). Conversely, if adsorbed to concentrations levels higher than nutrient requirements, they could lead to chronic systemic effects becoming toxic to aquatic life. The role of Cu in the inflammation process has not been clearly defined (Michalska-Mosiej et al., 2016). International regulations does not set concentrations threshold in edible tissue for these metals, but the World Health Organization suggests a PTDI (μg/Kg/day) of 500 for Cu and 300–1000 for Zn (JECFA, 2016). Comparing our results with literature data (Table 2) we found high levels of Cu (Bosch et al., 2016; Moore et al., 2015; Olmedo et al., 2013a; Domi et al., 2005; Frías-Espericueta et al., 2014a, b) even if the daily intake and the THQ calculated are lower the level of risk (Table 3). In addition, Cu concentrations were found proportional with length ($p < 0.05$) (Fig. 3). Conversely, we found Zn concentrations lower than literature data (Bosch et al., 2016; Moore et al., 2015; Olmedo et al., 2013a, 2013b; Domi et al., 2005; Frías-Espericueta et al., 2014a) as well as very small amounts of ingestion doses, largely lowering the level of risk for human health (Table 3).

Endo reports that Zn and Cu in sharks decrease during the growth stage and, then increase with a concomitant increase in Cd burden after maturation (Endo et al., 2016). In biological systems, as confirmed by Kasperczyk et al. (2016), they could have antagonistic relationship. Zn competes with Cu for binding to metallothionein (MT), thereby reducing its absorption (Michalska-Mosiej et al., 2016). Probably a minor basal level of Zn can justify a not balanced ratio between Zn and Cu that, in adult shark, can cause an increase of Cu concentration versus a Zn deficiency in mature age.

Among toxic metals, we analyzed Pb, Hg and Cd, which can cause health risks in consumers through ingestion of contaminated foods. Cd is toxic to the kidney and it is persistent for its long biological half-life in human (Copat et al., 2015). Pb is associated to damage of central nervous system, leading to decrements of intelligence quotients in children. As regards the toxicity of Hg, especially methylmercury, it adversely affects central nervous system, its main target organ, particularly during foetal

Table 2
Comparison of trace elements in shark'species sampled worldwide. na: not available; mdl: method detection limit.

References	Units	Hg	Cu	Zn	Pb	Cd	Species	Study area
Current study ppm	ww	*0.01*	*1.797*	*0.832*	*0.029*	*0.027*	*Carcharhinus dussumieri*	*Persian Gulf*
Bosch et al., 2016	ww	0.041	0.311	4.384	0.961	0.038	*Mustelus mustelus*	Langebaan Lagoon. South Africa
Branco et al., 2004	ww	0.16–1.84	na	na	na	na	*Prionace glauca*	Northeast Atlantic
Endo et al., 2015	ww	0.86	na	na	na	na	*Galeocerdo cuvier*	Ishigaki Island. Japan
Moore et al., 2015	ww	4.37	0.74	13.66	0.06	<mdl	*Carcharhinus leiodon*	Arabian Gulf
Delshad et al., 2012	ww	0.73	na	na	na	na	*Carcharhinus dussumieri.*	Persian Gulf
Endo et al., 2008	ww	0.78	<mdl	4.72	na	<mdl	*Galeocerdo cuvier*	Ishigaki Island. Japan
Teffer et al., 2014	ww	2.65	na	na	na	na	*Isurus oxyrinchus*	New England waters
Teffer et al., 2014	ww	0.87	na	na	na	na	*Alopias vulpinus*	New England waters
Cresson et al., 2014	ww	1.43	na	na	na	na	*Scyliorhinus canicula*	Northwestern Mediterranean
Cresson et al., 2014	ww	0.98	na	na	na	na	*Galeus melastomus*	Northwestern Mediterranean
Olmedo et al., 2013a	ww	na	0.142	1.952	na	na	*Prionace glauca*	Basque Country. Spain
Olmedo et al., 2013a	ww	na	0.449	3.421	na	na	*Galeus melastomus*	Valencia. Spain
Olmedo et al., 2013b	ww	0.35	na	na	0.004	0.003	*Prionace glauca*	Basque Country. Spain
Olmedo et al., 2013b	ww	0.698	na	na	0.004	0.002	*Galeus melastomus*	Valencia. Spain
Current study ppm	*dw*	*0.028*	*7.49*	*3.47*	*0.12*	*0.11*	*Carcharhinus dussumieri*	*Persian Gulf*
Domi et al., 2005	dw	1.1	1.1	16.5	na	0.4	*Galeorhinus galeus*	Celtic Sea
Domi et al., 2005	dw	2.1	1.2	14.5	na	0.7	*Galeus melastomus*	Celtic Sea
Domi et al., 2005	dw	0.2	<0.3	10.1	na	<0.16	*Squalus acanthias*	Celtic Sea
Gilbert et al., 2015	dw	9	1	13	na	< mdl	*Carcharhinus* spp.	south-eastern Australian
McKinney et al., 2016	dw	0.1–10	na	na	na	na	283 species	South Africa
Frías-Espericueta et al., 2014a	dw	na	1.19	16.96	4.96	0.03	*Rhizoprionodon longurio*	Mexican Pacific coastal waters
Frías-Espericueta et al., 2014b	dw	na	0.89–2.27	12.7–29.7	0.03–0.08	0.18–0.44	marketed Shark	Mexico

Table 3
Exposure daily intake (μg/Kg/day) average (EDAa) and per meal (EDIm) and Target Hazard Quotient (THQ) per meal, calculated for each metal.

Metals	PTDI (μg/Kg/day)	RfDo (mg/Kg/day)	EDIa (μg/Kg/day)	EDIm (μg/Kg/day)	THQm
Zn	300–1000	300	0,107	2,7	9,00E–03
Cu	500	n.d.	0,231	5829	1,17E–02
Pb	3570	n.d.	0,004	0093	2,62E–02
Hg	0,571	0,0001	0,001	0022	2,18E–01
Cd	0,828	1000	3,41E-03	8,60E-02	8,60E–02

development. To the general population, the dietary intake is the main exposure pathway (Renieri et al., 2014; McKinney et al., 2016).

In Europe, the fillet of fish and seafood must be appropriate to the human consumption according to the standard limits of Commission Regulation (EC) No 1881/2006.

The European Food Safety Authority (EFSA) in the Commission Regulation (EC) (EC No.1881/2006) set the maximum levels for Pb, Hg and Cd in fish muscle in 0.30, 0.1 and 0.1 ppm w.w., respectively. The Codex Alimentarius Commission (FAO; 2003) sets maximum levels for Pb, Hg and Cd of 0.2, 0.50 and 0.1 ppm w.w. respectively, and we found values below these limits in all specimens of *C. dussumieri* analyzed (Table 1), with daily ingestion rates lower of the level of risk for human health (Table 2).

Respect to the literature data there is a great variability of results depending by the area of origin of the shark species and by the interspecific variability. Pb range from 0.004 ppm w.w. in *Prionace glauca* (Olmedo et al., 2013a) to 1.17 ppm ww (corresponding to 4.96 ppm d.w.) in *Rhizoprionodon longurio* (Frías-Espericueta et al., 2014a). Cd concentrations are found frequently lower than the method's detection limit and the detectable concentrations ranged from 0.002 (ppm w.w.) in *Galeus melastomus* (Olmedo et al., 2013a) to 0.106 ppm w.w. (correspond to 0.44 d.w.) in Mexican marketed sharks (Frías-Espericueta et al., 2014b).

Furthermore, we also found that Cd concentrations increased proportionally with weight ($p < 0.01$) (Fig. 4) and body length ($p < 0.05$) (Figs. 4 and 5), as also reported by Endo et al. (2016). Cd concentrations tend to reflect the feeding preferences and

Table 4
Descriptive statistics of metals based on sex.

Sex	Males			Females		
Metals	Mean	Median	Dev. Std.	Mean	Median	Dev. Std.
Zn	3.47	3.42	0.28	3.46	3.42	0.24
Cu	7.51	7.53	0.24	7.47	7.43	0.26
Pb	0.13	0.12	0.04	0.11	0.11	0.03
Hg	0.02	0.02	0.01	0.04	0.04	0.02
Cd	0.11	0.12	0.03	0.11	0.11	0.03

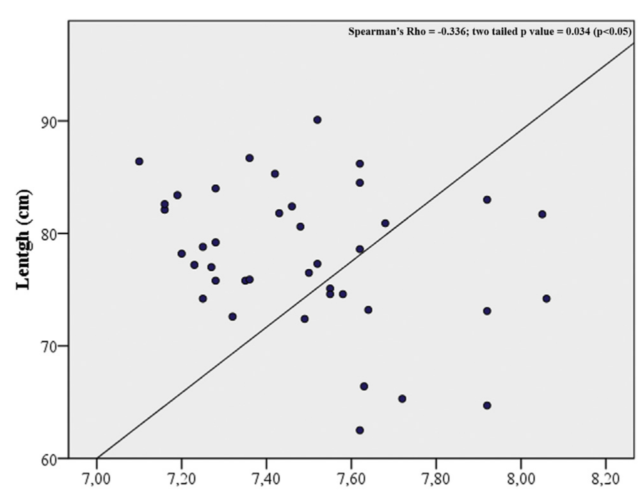

Fig. 3. Cu content vs length of sharks.

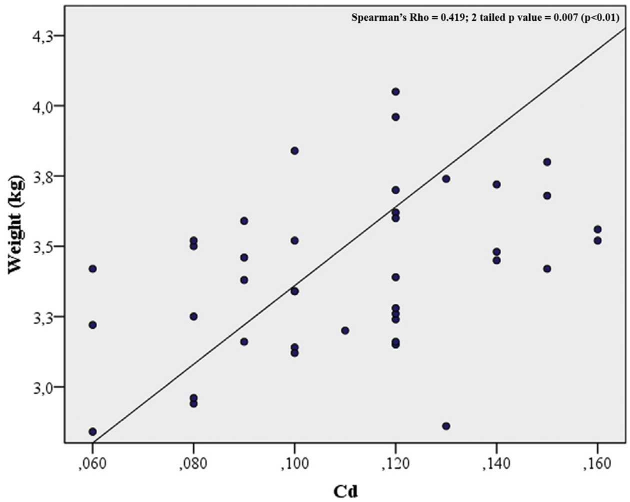

Fig. 4. Cd content vs weight of sharks.

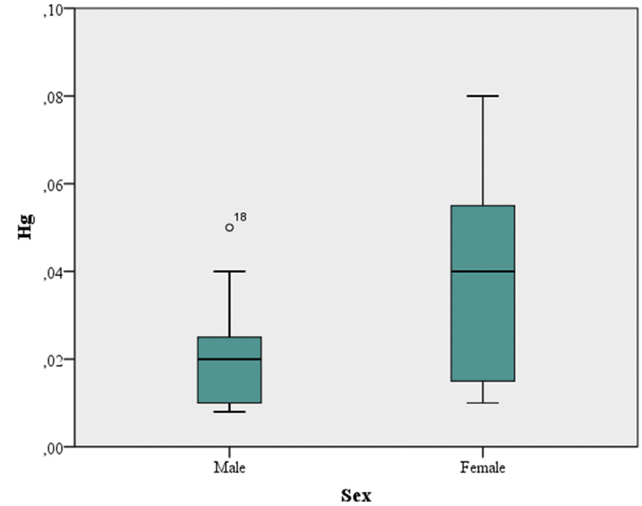

Fig. 6. Box plot distribution of Hg base on sex.

inhabiting area. Whitecheek sharks may preferentially eat Cd-contaminated species such as cephalopods and crustaceans; so, we can explain this relatively high concentration by the feeding preference and by the slow growth rate of whitecheek sharks or metabolic rate (Delshad et al., 2012). In fact, sharks with a slow growth rate show a minor content of Hg and higher Cd than the sharks' species with a fast growth rate.

Overall, our results of Pb and Cd fall within the general range found by other authors with concentrations (ppm d.w.) of 0.12 ± 0.034 and 0.11 ± 0.027 respectively. Conversely, Hg content was been found significantly lower than the general literature data available for several sharks' species sampled worldwide (Table 1) (Endo et al., 2015; Moore et al., 2015; Teffer et al., 2014; Cresson et al., 2014; Olmedo et al., 2013a; Domi et al., 2005; McKinney et al., 2016; Gilbert et al., 2015) and in the same study area (Delshad et al., 2012). Furthermore, the exposure daily intake of all toxic metals analyzed resulted lower than the PTDI provided by WHO and the THQ resulted lower than 1, suggesting no risk for human health derived from consumption.

It known that marine predators, particularly long-lived species such as sharks, accumulate high levels of Hg via the food chain, and the hepatic Hg of adult specimen concentration is greater than the Hg concentration in the muscle due to the formation of Hg—Se complexes (Endo et al., 2002; Karimi et al., 2013). Besides, in several shark species such as *Squalus acanthias* and *Mustelus manazo*, Hg concentrations increased markedly after maturation (Endo et al., 2009, 2013). Whitecheek shark reaches maturity after 70 cm of length, and our specimens had a median length of 77.5 (76.15 for male and 81.15 for female) and a median weight of 3.42 Kg (3.36 for male and 3.52 for female). As said before, we found lower levels of Hg respect to literature data as also reported by Lopez et al. (2013), perhaps because these specimens had just reached sexual maturity. Furthermore, test t for independent samples was been applied to identify any different distribution of metals based on sex. It revealed significant higher concentrations in females only for Hg ($p < 0.01$) (Table 4, Fig. 6), who have also higher weight and length medians respect to males as reported by Endo et al. (2016).

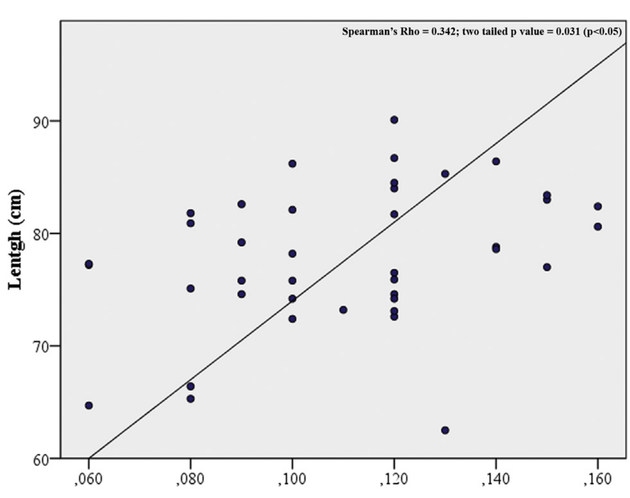

Fig. 5. Cd content vs length of sharks.

4. Conclusions

People living in Persian Gulf coasts consume the whitecheek shark and therefore, its consumption could result in a major exposure to metals. Our results suggest that consumption of this species does not represents risks for health of local consumers.

Nevertheless, the abiotic environmental characterization coupled with the analysis of biological tissue more active metabolically would support a better understanding of our results. So, next studies we will deep metals concentrations in the livers of shark as well as the metals content in sediments and seawaters from the same study area.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2016.09.002.

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