

Highlights

- Concentration of trace elements (TEs) in six marine species was analyzed.
- Liver had higher TEs concentration (except As) compared to muscle tissue.
- Relationships between size and TEs level in muscle and liver were investigated.
- Higher Se:Hg molar ratio and HBV_{Se} were found in liver than muscle.
- As, Cd and Hg exhibited higher health risks through seafood consumption.

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Original Research Article

Occurrence of trace elements (TEs) in seafood from the North Persian Gulf: Implications for human health

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ABSTRACT

In the present study the concentrations of 12 trace elements (TEs): As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn were quantified in muscle and, where possible, liver tissues, of six commercially important marine species including fish (*Euryglossa orientalis, Sardinella longiceps, Carcharhinus dussumieri*), crustacean (*Portunus armatus, Thenus orientalis*) and mollusc (*Sepia pharanois*) collected from the North Persian Gulf. Arsenic, copper, iron, and zinc were observed to be the most dominant TEs in muscle and liver tissues of all the species. In comparing with the maximum permissible limits (MPL) set by international organizations for seafood including FAO (1983), EC (2007), FAO/WHO (2007), and USEPA (2011), the mean concentrations of As, Cd in all the species and Mn, Zn, and Hg in some species exceeded their MPL limits. The relationships between TEs concentration and biometric indices (body length and weight) varied markedly among the TEs within both species and tissues. Estimated daily intake (EDI) results in comparison with values of the tolerable daily intake (TDI) confirmed that consumption of *T. orientalis, C. dussumieri* and *S. pharanois* from the sampled locations exceed their maximum values for As, Cd, Hg, and Se established for children.

1. Introduction

Marine ecosystems are exposed to many different types of pollutants that come in various forms and disrupt their functioning. The most commonly cited are toxic chemicals, solid waste, excessive inputs of nutrient and sediment (Wilhelmsson et al., 2013). Of the long list of hazardous substances, a particular focus has been given to trace elements (TEs) (Richir and Gobert, 2016). In addition to natural sources including continental runoff (Worakhunpiset, 2018), shelf inputs and atmospheric deposition from volcanic events and wildfire (Chouvelon et al., 2019), TEs can enter the marine environment from anthropogenic emission sources such as oil spills, marine transportation, sewage discharge, aquaculture, industry, agricultural and urban activities as well as mining and smelting (Pal and Maiti, 2019). Trace elements occur in sediments as dissolved, bound to suspended particulate solids, or accumulated and hence can potentially be incorporated into biological processes (Jiang et al., 2019). Trace or micro elements can be classified

into two groups on the basis of their metabolic role and their regulation by organisms (Bouchoucha et al., 2019). Elements such as Zn, Fe, Se and Cu are categorized as essential because they play vital roles in the organism's metabolism (enzymatic cofactors, metabolism of glucose and protein, immune function, components of metal-enzyme complexes and metalloenzymes) (Koller and Saleh, 2018). Whereas, TEs such as Hg, Pb and Cd are classified as non-essential and may cause extreme toxicity in marine biota even in low concentrations and their significant adverse effects are magnified by their potential tendency to accumulate in marine organism's tissue (Suami et al., 2018). The concentrations of TEs vary significantly among marine species and their organs (Keshavarzi et al., 2018). Generally, bioaccumulation of TEs in marine organisms is affected by their concentration and speciation, exposure duration, exposure route (Abida et al., 2009), geographical location (Ternengo et al., 2018), environmental conditions (water temperature and flow rate, seasonal variation, pH, total suspended solid, water hardness, salinity, dissolved oxygen and ammonia), and intrinsic biological factors

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(species, gender, age, body size, reproductive cycle, growth rate, swimming pattern, feeding behaviour) (Stanek et al., 2017).

Marine species can bioaccumulate TEs in their bodies from water, sediment, and food (Maurya et al., 2019). Furthermore, marine organisms such as fish is a suitable bioindicator of TEs contamination in water (Boutahar et al., 2019). TEs in tissues of marine species can be representative of manifold times of exposure than their corresponding waterborne contents (Ezemonye et al., 2019). Sediments are also considered to be an important factor in TEs accumulations in marine organisms. Direct exposure of contaminated sediments may increase TEs concentration in bottom dwelling and bottom feeding marine organisms (Soltani et al., 2019). Among marine species inhabiting sediments, those ingest sediments or filter feed can have particularly elevated body burdens of TEs (Lavilla et al., 2010).

Seafood is an important part of the human diet and is currently considered as a source of proteins, essential minerals, vitamins, and unsaturated fatty acids including omega-3 fatty acids (FAO/WHO, 2011). But consumption of seafood is also considered as a major route of human exposure to TEs (Sofoulaki et al., 2019). Several adverse effects of TEs on human health have been reported (Renieri et al., 2019) which include serious threats such as nephrotoxicity, hepatic damage, neurological and cardiovascular diseases, and even death (Genchi et al., 2017). In the recent decades, the concentration of TEs in marine species have been widely studied in different parts of the world (Marengo et al., 2018). Most of these studies have focused mainly on TEs in the edible tissues (e.g. muscle). Analysis of TEs concentrations in liver tissues is also a good indicator of exposure due to its storage, metabolism and detoxification ability of TEs (Shah and Altindağ, 2005).

Persian Gulf provides diverse coastal and marine ecosystems including mangrove forests, coral reefs, seagrass habitats, and intertidal mud and sand flats (Naser and Grillo, 2017). This marginal and semi-enclosed area is a unique habitat for many biota species including fish, birds, crustaceans, bivalves, etc. However, these ecosystems are under increasing pressure from anthropogenic activities that are associated with the rapid economic, social and industrial developments in the Gulf nations. Besides pollution through riverine inputs, the study area has been notoriously exposed to various additional environmental stresses including oil and gas production, municipal, petrochemical and agricultural activities, marine transportation, and sewage discharges (Abbasi et al., 2019). The Persian Gulf is considered as one of the most affected ecosystem by human activities in the world (Halpern et al., 2008). Delshab et al. (2017) reported elevated concentrations of Cu, Zn, Ni, Mn, and Hg in surface sediments collected near petrochemical and oil refinery units in Asaluye County (Bushehr Province, Persian Gulf). Abbasi et al. (2019) showed that As, Cu, and Pb were enriched more in coastal sediments of Bushehr at stations close to urban areas and where ships and boats were used. Aghadadashi et al. (2019) observed that As and Hg significantly enriched in surface sediment around Pars special economic energy zone (Bushehr Province, Persian Gulf).

Local residents in this area consume more seafood than other parts of Iran with the average ingestion rate of $68.5 \text{ g capita}^{-1} \text{ day}^{-1}$ (equal to 25 kg annually) in 2018 for the Bushehr province as reported by The Bushehr Fisheries Organization. Most of the fish caught in the area is consumed mainly by local citizens of Bushehr (population 298,945), Borazjan (110,600), Genaveh (73,500), Kangan (60,200), and Asalouyeh (60,000). All six investigated species are representative of the North Persian Gulf and are commercially important. All the species are commonly consumed by local residents as well as in other parts of Iran. According to Bushehr Fisheries Organization, 1918 tons of Sardinella longiceps, 1437 tons of Thenus orientalis, 653 tons of Sepia pharanois, 251 tons of Euryglossa orientalis and 140 tons of Portunus armatus are consumed or exported annually. Euryglossa orientalis is the main species which is consume by local people due to its good taste and low price. These fishery products are also exported to other countries, mostly to the Gulf nations and China.

The specific objectives of the present study were to: (i) investigate

the distribution of TEs including As, Cd, Co, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Se, V, and Zn in both muscle and liver tissues of six marine species (three fishes, two crustaceans and one mollusc) from the North Persian Gulf including Indian oil sardine *Sardinella longiceps* (Valencienns, 1947): Clupeidae, Whitecheek Shark *Carcharhinus dussumieri* (Valenciennes, 1839): Carcharhinidae, and sole fish *Euryglossa orientalis* (Bloch and Schneider, 1801): Soleidae, flathead lobster *Thenus orientalis* (Lund, 1793): Scyllaridae, blue swimmer crab *Portunus armatus* (Linnaeus, 1758): Portunidae, and pharaoh cuttlefish *Sepia pharanois* (Ehrenberg, 1831): Sepiidae; (ii) determine whether TEs concentration in the targeted species exceeds their maximum permissible levels for human consumption; (iii) compare TEs concentration in liver and muscle tissues of the investigated marine species; (iv) evaluate Se:Hg molar ratios and Se health benefit value in the studied species; and finally (v) assess the potential health risk associated with seafood consumption.

2. Materials and methods

2.1. Study area

The Persian Gulf, with an average depth of 35 m and a total area of approximately 240,000 km² (Barth and Khan, 2008), is located in south of Iran. This is an important fishing area for inhabitants. Based on an internal report of Bushehr Fisheries Organization (2019), there are nearly 1481 fishing boat, 535 fishing trawler and 21 fishing ships in the area. 8440 fishermen and 15000 staff are directly and indirectly involved in the fishing industry, respectively. More recently, many aquaculture farms including shrimp ponds and offshore fish farms with an area of approximately 15120 ha have been established. Agricultural activities in Bushehr province is mostly based on date palm trees (Naderizadeh et al., 2016). Other important commercial crops in the area include leafy and other vegetables especially tomatoes. The area is recipient of many point sources of pollution including several petrochemical plants and gas stations, oil wells and industrial waste, as well as untreated wastewaters. This gulf contains nearly 60 % of the world's proven oil reserves and 30 % of the world's oil production (Goldenberg and Rushe, 2012). One of the important oil and gas facilities (Pars Special Energy Economic Zone, PSEEZ) consists of 10 refineries and 7 petrochemical complexes as well as a mix of light and heavy industries are located in Asaluyeh County, Bushehr province for the utilization of oil and gas resources in the Persian Gulf (Aghadadashi et al., 2019). TEs are commonly used in these industrial activities (Alipour et al., 2014). In addition, rapid development and industrialization, discharges of untreated sewage, agricultural practices, land-based activities, shipping and transport, overfishing, and invading alien species impose further environmental pressure (Bayani, 2016). Moreover, some TEs are derived to the Persian Gulf from natural resources including crustal materials (Karbassi and Bayati, 2005) and atmospheric dust deposition (Yigiterhan et al., 2020). The water circulation is counter-clockwise, driven by wind-stress, surface buoyancy fluxes, fresh water runoff, water exchange through the Strait of Hormuz and tides (Khazaali et al., 2016). The slow movement of water with an approximate north-westward flow of speed between 3 to 10 cm $\rm s^{-1}$ along the Iranian coast (Kämpf and Sadrinasab, 2006) with poor flushing characteristics and the semi-enclosed nature of the Gulf, contributes to the accumulation of pollutants (Sereshk and Bakhtiari, 2014).

2.2. Sample collection and preparation

In total, 152 individual samples of six commercial marine species: Sardinella longiceps (Indian oil sardine), Carcharhinus dussumieri (Whitecheek Shark), Thenus orientalis (flathead lobster), Sepia pharanois (cuttlefish), Portunus armatus (blue swimmer crab), and Euryglossa orientalis (sole fish) were collected over a period of three months (December 2018 to February 2019) using appropriate fishing gears with the assistance of local fishermen. The samples were collected at four main fresh fish landing areas on the North Persian Gulf: Genaveh and Busher cities, Kangan district and Asaluyeh County in the area of nearly 15713 km² (Fig. 1). The collected samples were immediately preserved in an ice chest and transferred to the laboratory for identification and were kept frozen at -20 °C until further analysis. For the identification of fish, crustaceans and molluscs, the methods outlined in Randall (1995) and Carpenter et al. (1997), respectively were followed. Fish nomenclature in the region is based on as described in Eagderi et al. (2019). After biometric measurements, the thawed samples were dissected for their liver and muscle tissues using stainless steel scalpel and scissors washed by deionized water to prevent cross-contamination. The epaxial muscle on the dorsal surface of fish (Rajeshkumar and Li, 2018) was removed for further analysis. Approximately 15 g of wet composite samples were lyophilized in a freeze-dryer (Model Zirbus technology GmbH VaCo5, Germany). The samples were ground and homogenized into fine powder using ceramic mortar and pestle and then stored in pre-treated plastic containers which were rinsed three times with deionized water to avoid cross contamination until their transfers to the University of Liège (Belgium) and their corresponding analysis.

2.3. Biometric measurements and trophic level

At the laboratory, frozen samples were pre-washed with deionized water to remove adhered particles, then partially thawed and measured for biometric parameters (weight and total length). All six investigated species are commercially important. P. armatus with the trophic level of 3 (Johnston et al., 2020) and T. orientalis with the trophic level of 3 (Johnston and Yellowlees, 1998) live on soft bottom of mud or sand (Clarke and Ryan, 2004), and feed on variety of molluscans, debris, crustaceans, and echinoderms (Josileen, 2011). The trophic level is the position that an organism occupies in a food chain or nutritive series ranging from 1 for primary producers to 5 for marine mammals and humans (Pavluk and Bij de Vaate, 2013). E. orientalis with the trophic level of 3 (Khan and Hoda, 1993) inhabits shallow sand and mud bottoms in coastal waters and is a scavenger and predator of bottom-dwelling invertebrates, especially small crustaceans (Sommer et al., 1996). Among the targeted species, S. longiceps occupies the lowest trophic level of 2.4 (Froese and Pauly, 2007), and feeds mainly on phytoplankton (especially diatoms) and small crustaceans (Sommer et al., 1996). C. dussumieri, a carnivore with a trophic level of 4 (Cortés,

1999), lives in marine and reef-associated environments and is predator of fish, cephalopods, and crustaceans (Last et al., 2009). *S. pharanois,* with the widest distribution in the Indo-Pacific zone, is cephalopod lives in neritic with a benthic living form (Nabhitabhata and Nilaphat, 1999). They are migratory species, with the trophic level of 3.6 (Ohkouchi et al., 2013), and feasts on a variety of smaller fish, crustaceans, and occasionally other cuttlefish (Nair et al., 1993).

2.4. Chemical analysis

In the present study, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, and Zn concentrations were determined in muscle (n = 152, 30 composite samples) and liver tissues (n = 102, 21 composite samples). The freezedried, homogenized samples, 0.2 g were weighed and transferred into acid-washed Teflon digestion vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc. Sorisole, Italy). Approximately 2 mL of 60 % nitric acid (HNO₃) and 1 mL of 30 % hydrogen peroxide (H₂O₂) were added to the vessels as reagents (suprapur grade, Merck, Darmstadt, Germany). According to the method described by Richir and Gobert (2014) and Gobert et al. (2017), samples were analyzed by inductively coupled plasma mass spectrometry using dynamic reaction cell technology (ICP-MS ELAN DRC II, PerkinElmer ®, Wellesley, United States). Dried samples (~2 mg) were weighed in quartz boats (preheated to 400 °C for 5 min to remove any mercury) prior to the analysis (0.001 mg balance precision). The Total Hg (T-Hg) content was determined using atomic absorption spectrometry at 254 nm, in a Direct Mercury Analyser (DMA 80 Milestone, Minnesota, USA) according to the US EPA standard method 7473.

2.5. Analysis reliability of TEs

Analytical quality control was ensured using Certified Reference Materials (CRM), DORM-4, and DOLT 5 (Table S1, supplementary information). These CRMs from the National Research Council Canada were used to assess the accuracy of the Hg concentration: DOLT-3 (dogfish liver) and DORM-2 (dogfish muscle). For each TE, detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution (Currie, 1999). The detection limits in mg kg⁻¹ dry weight were: As = 0.0145; Cd = 0.0089; Co = 0.0078; Cr = 0.0504; Cu = 0.3079; Fe = 0.2164; Mn = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ni = 0.0161; Pb = 0.0145; Cd = 0.0078; Ci = 0.0078;



Fig. 1. Study area map showing the main fishery areas of the Persian Gulf.

0.0206; Se = 0.0985; and Zn = 0.3285. For Hg, quality assurance assessment was monitored using replicates, standards (T-Hg 100 ng g⁻¹), blanks (HCl 1%) and Certified Reference Materials (DORM-2, muscle = 4640 \pm 260 ng g⁻¹ dw; DOLT3, muscle = 3370 \pm 140 ng g⁻¹ dw) at the beginning and the end of each series. The percentage recovery for DORM-2 and DOLT3 ranged between 96 % and 106 % showing optimal run of the analyses.

2.6. Human health risk assessment

In the present study, several methods were used to evaluate the human health risk potentially arising from seafood consumption: (1) comparisons of TEs concentration in the studied seafood muscles (wet weight) with the maximum permissible limit (MPL) defined by international organizations; (2) comparison of the estimated daily intake (EDI) values of TEs with the tolerable daily intake (TDI) values (JECFA, 2010; USEPA, 2013); (3) determination of metal pollution index (MPI) proposed by Usero et al. (1997); (4) estimation of the risk/benefit value associated with Se and Hg levels in the targeted seafood, including Se:Hg molar ratio (Azad et al., 2019) and selenium health benefit value (HBV_{Se}) (Ralston et al., 2016); and (5) evaluation of non-carcinogenic effects of the TEs using target hazard quotient (THO) (USEPA, 1989) and hazard index (HI) (Zhao et al., 2018); and (6) estimation of the lifetime cancer risk (CR) for carcinogenic Cd, Pb and inorganic As (USEPA, 2006). A detailed health risk assessment methodology is described in the Supplementary Information (SI).

2.7. Statistical analysis

Statistical analysis tests were applied using Microsoft Excel 2019, IBM SPSS statistics software (version 22.0 for windows) and R software (version 6.2). The mean and standard deviation of TEs concentration in the studied species were calculated. The Kolmogorov-Smirnov and Shapiro-Wilk tests were employed to test distribution of the datasets. Possible significant differences (p < 0.05) in TEs concentration among species were assessed by Kruskal–Wallis test. The Mann-Whitney *U* test was performed to examine the statistical significance of the differences in the TEs concentration between muscles and liver tissue samples. The effect of species' length and weight on TEs concentration was studied using Multiple Linear Regression analysis (MLR) and Bayesian Multiple Linear Regression (BMLR) approach. The details of MLR and BMLR are summarized in the Supplementary Information. The Spearman correlation coefficient (two-tailed test) was utilized to examine the relationship

Table 1

Summary of biometric measurements an	d trophic leve	el of the six e	examined species.
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between Se:Hg molar ratio and biota specie's Hg content and size. Values of p < 0.01 were designated as being statistically significant.

3. Results

3.1. Biometric measurements and TEs concentration

The summary of the biometric indices as well as biological background information and trophic level of six studied marine species are presented in Table 1. The overall body length (BL) ranged from 13 to 91 cm and the weight varied between 19-3250 g. Concentration of TEs in muscle and liver tissues of the six marine species on a wet weight basis (mg kg $^{-1}$ ww) are given in Table 2. Arsenic, iron, zinc, and copper were the dominant TEs found in all species, whereas Cr and Pb were found in negligible concentrations. Among all of the species, the highest concentration of As was found in the muscle tissue of T. orientalis (42.7 mg kg⁻¹ ww) followed by S. pharanois (37.5 mg kg⁻¹ ww). The highest Cd content in muscle tissues was 2.87 mg kg⁻¹ ww (mean of 1.27 mg kg⁻¹ ww) in T. orientalis with 6-423 times greater muscle concentration compared to muscle of other studied species. The highest mean contents of Cu (9.35 mg kg⁻¹ ww) and Zn (23.6 mg kg⁻¹ ww) were measured in P. aramatus muscle. Liver tissues had significantly higher TEs concentration than the muscle with the exception of As in S. pharanois and As, Cr, Ni, and Pb in C. dussumieri. The maximum concentrations of Cd (85.0 mg kg⁻¹ ww), Co (12.4 mg kg⁻¹ ww), Cu (256.6 mg kg⁻¹ ww), and Zn (518.7 mg kg⁻¹ ww) were observed in the liver of *S. pharanois*. The Hg concentration in the analyzed muscle tissues varied between 0.012 mg kg^{-1} ww (S. longiceps) to 1.78 mg kg^{-1} ww (C. dussumieri). Concentration of Hg in liver tissues ranged from 0.010 mg kg⁻¹ ww (S. longiceps) and 1.23 mg kg⁻¹ ww (*C. dussumieri*). The highest concentrations of Fe and Mn were also determined in liver of S. longiceps which varied between 139.7–1097 mg kg^{-1} and 1.69–17.5 mg kg^{-1} ww, respectively (Table 2).

3.2. Influence of body size on TEs concentration in muscle and liver tissues

The effects of two factors X_1 = (length), and X_2 = (weight) on TEs muscle and liver burden as well as MPI were investigated. The results of BMLR are summarized in Table 3. In addition, relationships between the TEs concentrations (mg kg⁻¹ dw) and body length are illustrated in Figs. S1 and S2 (supplementary information). In the muscle tissues, Hg concentration was influenced by weight of the marine species (posterior

5		1			1					
	Species	Family	Number	Body length	Body length (cm)		g)	Trophic level	Feeding habit	Habitat
				Range	$\begin{array}{l} \text{Mean} \pm \\ \text{SD} \end{array}$	Range	$\text{Mean}\pm\text{SD}$			
Crustaceous	Portunus armatus	Portunidae	34	13.0-16.1	$\begin{array}{c} 14.5 \pm \\ 0.95 \end{array}$	180.7-313.0	$\begin{array}{c} \textbf{224.8} \pm \\ \textbf{35.7} \end{array}$	3 ^a	Omnivore	Benthic
	Thenus orientalis	Scyllaridae	16	14.3-28.0	21.3 ± 4.3	53.5-297.3	$\begin{array}{c} 177.1 \pm \\ 81.4 \end{array}$	3 ^b	Omnivore	Benthic
Fish	Euryglossa orientalis	Soleidae	30	16.0-41.1	$\textbf{27.6} \pm \textbf{9.4}$	64.0–1378	$\begin{array}{c} 539.9 \pm \\ 518.8 \end{array}$	3 ^c	Detrivore	Benthic
	Sardinella longiceps	Clupeidae	32	16.9-20.6	$\begin{array}{c} 18.4 \pm \\ 0.97 \end{array}$	47.4–79.0	61.1 ± 8.2	2.4 ^d	Omnivore	pelagic- neritic
	Carcharhinus dussumieri	Carcharhinidae	19	30.3-91.0	$\begin{array}{c} 49.1 \pm \\ 15.4 \end{array}$	19.0-3250	656.1 ± 755.1	4 ^e	Carnivore	Pelagic
Mollusc	Sepia pharanois	Sepidae	21	19.1-45.3	$\textbf{30.4} \pm \textbf{9.5}$	104.5-1808	$\begin{array}{c} 706.3 \pm \\ 638.7 \end{array}$	3.6 ^f	Carnivore	Benthic

^a Johnston et al. (2020).

^b Johnston and Yellowlees (1998).

^c Khan and Hoda (1993).

^d Froese and Pauly (2007).

^e Cortés (1999).

^f Ohkouchi et al. (2013).

Table 2

Summary statistics of TES Concentration (mg kg⁻¹ ww) in muscle and liver tissues of the six marine species captured from the Persian Gulf and the maximum permissible level (MPL, mg kg⁻¹ ww) in seafood. SD: standard deviation. Bold values represent > MPL.

Туре	Species	Organ	Statistics	As	Cd	Со	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn
Crustacean	P. armatus	Muscle	Mean \pm	16.6 ±2.9	0.11 ± 0.05	0.037 ±	0.021 ±	$\textbf{9.35}\pm\textbf{2.02}$	$\textbf{5.23} \pm \textbf{1.51}$	0.080 ±	$0.32 \pm$	$\textbf{0.10} \pm \textbf{0.01}$	$\textbf{0.02}\pm\textbf{0.01}$	0.50 ±	23.6 ±2.1
	p = 24a 4b		5D Min	197	0.06	0.008	0.004	6 16	9.91	0.015	0.07	0.00	0.01	0.05	22.0
	II = 34, 4		Max	10.0	0.00	0.03	0.015	11 03	7.0	0.000	0.20	0.09	0.01	0.45	22.0
	T orientalis	Muscle	Mean +	25 1 +11 9	1.27 ± 0.9	0.03 ± 0.01	0.025	451 ± 265	2.29 ± 1.0	0.093	1 23 +0 39	0.11 + 0.05	0.03 + 0.02 +	0.57	20.0 21.1+9.6
	1. orientatio	musere	SD	20.1111.9	1.27 ± 0.9	0.00 ± 0.01	0.00 ±	1.01 ± 2.00	2.2) ± 1.0	0.015	1.20±0.09	0.12 ± 0.00	0.003	0.20	21.11.2.0
	n = 16, 5		Min	16.0	0.54	0.02	0.02	0.63	1.53	0.022	0.88	0.07	0.016	0.50	10.6
	- , -		Max	42.7	2.87	0.05	0.04	7.28	4.01	0.064	1.82	0.18	0.024	0.99	36.1
Fish	E. orientalis	Muscle	Mean \pm	12.7±5.4	$0.003 \pm$	$0.005 \pm$	$0.06 \pm$	0.25 ± 0.08	2.94 ± 0.2	0.071 \pm	$0.12 \pm$	0.031 \pm	0.015 \pm	$0.54 \pm$	$\textbf{4.06} \pm \textbf{0.40}$
			SD		0.002	0.005	0.05			0.014	0.05	0.003	0.002	0.09	
	n = 30, 5		Min	7.5	0.001	0.002	0.03	0.18	2.72	0.049	0.06	0.028	0.014	0.43	3.61
			Max	20.4	0.005	0.012	0.14	0.37	3.18	0.083	0.20	0.034	0.018	0.67	4.61
	E. orientalis	Liver	Mean \pm	20.9 ± 10.2	$1.14{\pm}0.4$	$\textbf{0.15} \pm \textbf{0.04}$	$0.02~\pm$	93.7±27.6	334.2±88.9	0.146 \pm	1.03 ± 0.14	$\textbf{0.06} \pm \textbf{0.01}$	0.026 \pm	$\textbf{4.23} \pm$	42.0 ±6.1
			SD				0.1			0.034			0.002	1.67	
	n = 30, 5		Min	5.3	0.5	0.10	0.01	55.3	228.0	0.115	0.90	0.04	0.023	2.59	33.8
			Max	32.6	1.58	0.21	0.03	130.3	474.2	0.199	1.23	0.07	0.029	6.41	50.0
	S. longiceps	Muscle	Mean \pm	1.1 ± 0.1	0.06 ± 0.02	0.023 \pm	0.02 \pm	0.60 ± 0.09	12.8 ± 2.4	$0.025~\pm$	0.62 \pm	$\textbf{0.06} \pm \textbf{0.01}$	$\textbf{0.08} \pm \textbf{0.04}$	0.21 \pm	5.89 ± 1.51
			SD			0.002	0.01			0.026	0.16			0.04	
	n = 32, 5		Min	1.0	0.04	0.021	0.02	0.46	10.4	0.012	0.43	0.04	0.05	0.16	4.26
			Max	1.3	0.1	0.026	0.03	0.70	15.4	0.072	0.86	0.08	0.15	0.27	8.36
	S. longiceps	Liver	Mean \pm	1.8 ± 0.1	0.86 ±0.1	0.29 ± 0.16	$1.33 \pm$	2.60 ± 1.01	565.0 ±373.1	$0.014~\pm$	8.21 ±6.10	1.70 ± 1.19	0.39 ±0.17	$1.15 \pm$	11.2 ± 3.4
			SD				0.94			0.003				0.39	
	n = 32, 5		Min	1.7	0.74	0.10	0.22	1.36	139.7	0.010	1.69	0.38	0.14	0.53	6.90
			Max	2.0	1.02	0.53	2.60	3.80	1097	0.018	17.5	3.39	0.59	1.59	15.5
	C. dussumieri	Muscle	Mean ± SD	18.8 ±3.9	0.04 ± 0.08	$\begin{array}{c} 0.002 \pm \\ 0.002 \end{array}$	$\begin{array}{c} 0.03 \pm \\ 0.02 \end{array}$	0.23 ± 0.05	4.0 ± 3.3	0.977 ±0.589	0.13 ± 0.1	0.06 ± 0.08	0.016 ± 0.004	0.95 ± 0.47	2.55 ± 0.88
	n = 19, 6		Min	14.0	0.001	0.001	0.01	0.18	1.86	0.176	0.05	0.03	0.012	0.43	2.05
			Max	23.5	0.21	0.005	0.06	0.31	10.7	1.78	0.33	0.22	0.022	1.81	4.33
	C. dussumieri	Liver	Mean \pm	9.8 ±4.1	1.09±2.17	$0.012 \pm$	0.013 \pm	$\textbf{0.79} \pm \textbf{0.23}$	$\textbf{70.7} \pm \textbf{27.89}$	0.57 ±0.50	$0.36 \pm$	$0.023 \pm$	$0.011 \pm$	$3.18 \pm$	3.27 ± 1.67
			SD			0.014	0.004				0.14	0.004	0.004	1.73	
	n = 19, 6		Min	6.0	0.02	0.003	0.011	0.60	25.8	0.04	0.19	0.016	0.004	1.18	1.97
			Max	16.8	5.51	0.039	0.021	1.23	110.6	1.23	0.52	0.028	0.013	5.90	6.34
Mollusc	S. pharanois	Muscle	Mean ±	24.9±8.5	0.19 ± 0.2	0.035 ±	$0.028 \pm$	3.02 ± 2.86	1.07 ± 0.41	$0.067 \pm$	0.10 ±	0.06 ± 0.02	0.021 ±	$0.37 \pm$	12.6 ± 0.8
	01 5		SD	14.4	0.01	0.027	0.013	0.00	0.50	0.019	0.04	0.04	0.003	0.10	11.0
	n = 21, 5		Min	14.4	0.01	0.006	0.013	0.66	0.56	0.048	0.05	0.04	0.018	0.21	11.9
	C pharapois	Liver	Moon	37.5 157110	0.51 E6.6 17.6	0.074	0.043	7.00 1E6 7162.2	1.39	0.092	0.15	0.09	0.025	0.45	13.9
	3. priarations	LIVEI		13.7±1.9	50.0 ±17.0	3.39 ±4.20	$0.03 \pm$	130.7±03.3	114.3±30.1	$0.070 \pm$	$0.73 \pm$	0.20 ± 0.12	0.20 ±0.08	4.01 ±	244.0±100.0
	n - 21 = 5		Min	12.2	41.7	2 /2	0.02	82.3	39.7	0.027	0.20	0.12	0.17	3.07	122.7
	n = 21, 3		May	18.1	85.0	2.43	0.04	256.6	179 4	0.055	0.95	0.12	0.17	5.07	518 7
MPL (w.	FAO, 1983		Wax	1.0	0.5	0.5	0.00	230.0	175.4	0.105	0.95	0.45	0.5	3.95	30.0
,	EC. 2007				0.5	1.0		10.0		1.5			0.2 - 0.3		
	FAO/WHO 20	007			0.5	2.0	2.0	30.0	100.0	0.5	1.0			9.6	
	USEPA, 2000,	2011		1.3	2.0		2.0	2010				70.0-80.0	0.5	5.0	20.0

^a Number of specimen of each species. ^b Number of composite sample.

Table 3

The results of Bayesian Multiple Linear Regression (BMLR) method to study the effects of length and weight on TEs concentration in muscle and liver tissues of six marine species. b_i: estimated coefficient, Pos: posterior, and SD: standard deviation. Values in bold-face font indicates probability higher than 0.5.

	Body size variables	Muscle			Liver						
		Pos Mean of b _i	Pos SD of \boldsymbol{b}_i	Pos Probability of b _i	Pos Mean of b _i	Pos SD of \boldsymbol{b}_i	Pos Probability of b _i				
As	Length	0.2898	0.4955	0.3656	0.0574	0.2602	0.1787				
	Weight	0.0008	0.0109	0.2140	0.0018	0.0078	0.1800				
Cd	Length	-0.0032	0.0140	0.1592	6.9144	1.5665	0.9972				
	Weight	-0.0001	0.0005	0.1628	-0.1417	0.0467	0.9834				
Со	Length	-0.0001	0.0007	0.3078	0.6123	0.2491	0.9678				
	Weight	0.0001	0.0000	0.6338	-0.0095	0.0072	0.7910				
Cr	Length	-0.0001	0.0005	0.1123	-0.0434	0.0476	0.5928				
	Weight	0.0001	0.0000	0.1183	0.0001	0.0010	0.3064				
Cu	Length	-0.1481	0.1914	0.4949	3.6252	5.6541	0.4242				
	Weight	-0.0007	0.0042	0.2661	-0.0484	0.1313	0.2934				
Fe	Length	-0.3087	0.2568	0.7139	-42.0243	20.8849	0.9395				
	Weight	-0.0012	0.0059	0.3238	0.4154	0.5637	0.5673				
Hg	Length	0.0051	0.0175	0.3154	0.0022	0.0138	0.3374				
	Weight	0.0020	0.0007	0.9578	0.0016	0.0005	0.9804				
Mn	Length	-0.0330	0.0267	0.7273	-0.2747	0.2959	0.6029				
	Weight	-0.0002	0.0006	0.3391	0.0002	0.0060	0.3072				
Ni	Length	-0.0015	0.0024	0.4329	-0.0451	0.0551	0.5378				
	Weight	-0.0001	0.0001	0.4857	-0.0002	0.0012	0.3119				
Pb	Length	-0.0004	0.0010	0.2643	-0.0002	0.0057	0.1720				
	Weight	0.0001	0.0000	0.2456	-0.0001	0.0002	0.2256				
Se	Length	0.0009	0.0064	0.1198	0.1132	0.1420	0.5332				
	Weight	0.0001	0.0002	0.1148	-0.0010	0.0031	0.3153				
Zn	Length	-0.1712	0.3583	0.2950	22.9656	10.7896	0.9440				
	Weight	-0.0022	0.0091	0.2170	-0.3407	0.3024	0.7305				
MPI	Length	-0.0056	0.0080	0.4779	0.0891	0.1288	0.4428				
	Weight	-0.0001	0.0002	0.4211	-0.0021	0.0035	0.3967				

probability or pos probability: 0.96). The concentration of TEs in the muscle tissue are related to body length and weight in a negative way in the following decreasing trend Fe > Zn > Cu > Mn > Cd > Ni > Pb > Co-Cr, and Zn > Fe > Cu > Mn > Ni > Cd > Co-Cr-Pb-Se, respectively (Table 3). Total body length of species effected Mn and Fe concentrations in muscle tissues more than other TEs, with the probability of 0.73 and 0.71, respectively with the negative pos (posterior) mean of b_i (estimated coefficients) indicating a decline in their concentrations with increasing length. Concentrations of Fe and Mn in muscle tissue of C. dussumieri were found to decrease with increasing body length (Fig S1, supplementary information). In muscle of C. dussumieri Fe concentration varied between 11.65 mg kg⁻¹ dw and 51.6 mg kg⁻¹ dw with the highest and lowest total length, respectively (Fig. S1, supplementary information). Similarly, increase in total length of C. dussumieri was matched by a decrease in Mn concentration. The maximum Mn (1.6 mg kg^{-1} dw) was measured in a sample with the smallest total length (31.38 cm) compared to 0.24 mg kg⁻¹ dw observed in the largest total length sample (91 cm). However, in the liver tissue of C. dussumieri, the Fe and Mn contents increased with the body length of the fish (Fig. S2, supplementary information).

In the liver tissues, highest positive effect of body length was observed in Cd concentration (pos probability: 0.99) followed by Co (pos probability: 0.97) and Zn (pos probability: 0.94). Body length negatively affected Fe and Mn concentrations in liver tissues (pos probability: 0.94 and pos probability: 0.60, respectively). In liver tissue of S. pharanois maximum (408.9 mg kg $^{-1}$ dw) and minimum (210.7 mg kg $^{-1}$ dw) concentration of Cd and were measured in individuals with highest and lowest body length of 89.8 cm and 39.6 cm, respectively (Fig. S2, supplementary information). In the liver tissue of S. pharanois, the maximum Zn (2494 mg kg⁻¹ dw) was determined in a sample with highest body length, while the lowest Zn (589.9 mg kg⁻¹ dw) was recorded in the smallest sample. Body length effected positively the concentration of TEs in liver tissues based on Pos Mean of $B_{\rm i}$ in the following decreasing trend of Zn > Cd > Cu > Co > Se, while negatively affected the TEs concentration in the order of Fe > Mn > Ni > Cr > Pb (Table 3). Concentration of TEs in the liver tissues decreased with increasing body weight in the following order of Fe > As > Hg > Mn and

increased in the order of Zn > Cd > Cu > Co > Se > Ni > Pb > Cr. In the liver tissue, body weight had the highest effect on Hg concentration with the probability of 0.98.

3.3. Se:Hg molar ratio calculation and Se health benefit value

The calculated Se:Hg molar ratio and HBV_{Se} are presented in Fig. 2 and Table S3 (supplementary information). The mean values of Se:Hg molar ratios were generally above 1 in all studied species. The mean \pm SD values varied widely in the muscle tissues from 6.26 \pm 9.85 (C. dussumieri) to 40.3 \pm 11.9 (T. orientalis) and in the liver tissues from 46.4 \pm 52.4 (C. dussumieri) to 216.2 \pm 77.9 (S. longiceps). In the liver tissues, the highest Se:Hg molar ratio (410.1) was fond in S. pharanois while the lowest (5.56) was recorded in C. dussumieri with the largest body size. Lower muscular and liver Hg:Se ratio values in C. dussumieri compared to the other species demonstrate that the large specimens of top-predators fish present the highest Hg concentrations. On average, the Se:Hg molar ratios were higher in the liver than muscle tissues in all studied species (p < 0.05). The relationships between Se:Hg molar ratio, body size (length and weight), and Hg concentration for each species are shown in Table S3 (supplementary information). The mean Se:Hg molar ratio was significantly and negatively correlated with mean Hg concentrations, length and weight for all the species (Table S3, supplementary information).

In the muscle tissue, HBV_{Se} varied between -0.72 in *C. dussumieri* and 0.66 in *T. orientalis* and in the liver tissue ranged from 1.15 (*S. longiceps*) to 4.61 (*S. pharanois*). No negative HBV_{Se} values were found except for the largest species, *C. dussumieri*, that showed elevated Hg concentration. This species also had the lowest Se:Hg molar ratio. The liver tissues had higher HBV_{Se} values compared to the muscle tissues. The liver of *S. pharanois* had the highest value (4.61), followed by the liver of *E. orientalis* (4.23). The highest Se concentration of the liver tissues was measured in *S. pharanois*, resulting in the highest positive HBV_{Se} value among the species.



Fig. 2. Box plot of Se:Hg molar ratios in A) muscle; and B) liver of six studied marine species.

3.4. Human health risk assessment

To examine the TEs concentration in the different marine species, the metal pollution index (MPI) was used. According to Jamil et al. (2014), MPI values in the tissues < 2 means that not impacted, between 2–5 indicate very low pollution, between 5–10 mean low pollution, and between 10–20 show medium pollution (Table S4, supplementary information). The average MPI of the investigated TEs for both liver and muscle tissues are shown in Fig. 3. The MPI presented higher results for the liver of *S. pharanois*, indicating a greater TEs concentration in the samples. The liver order of TEs concentration based on MPI was *S. pharanois* (18.45, medium pollution) > *S. longiceps* (8.12, low pollution) > *E. orientallis* (6.87, low pollution) > *C. dussumieri* (1.76, not impacted). Highest MPI values in the muscle tissues were observed in *T. orientalis* (2.41, very low pollution), followed by *P. aramatus* (2.04, very low pollution)."

Results of the human health risk assessment including estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR) of TEs from seafood consumption by the local inhabitants (adults and children) are presented in Table 4 and Fig. S3 (supplementary information). The risk factors were also calculated for liver of *C. dussumieri* (Whitecheek Shark) since it has been consumed by local people and in other Southeast Asian countries as food. The EDI

values were compared with the respective tolerable daily intake (TDI) values from the data of Joint FAO/ WHO Expert Committee on Food Additives. The highest EDIs of As (2.6E + 00 mg kg⁻¹ bw day⁻¹) was measured for *T. orientalis* in children. The highest EDIs of Cd (4.4E + 00 mg kg⁻¹ bw day⁻¹) and Se (1.1E + 01 mg kg⁻¹ bw day⁻¹) in children were through consumption of *T. orientalis* and liver of *C. dussumieri*, respectively. The EDI value for Hg in muscle (3.3E + 00 mg kg⁻¹ bw day⁻¹) and liver (2.0E + 00 mg kg⁻¹ bw day⁻¹) of *C. dussumieri* exceed their TDI values of 1.6 for children. EDI values for the rest of TEs were well below the corresponding TDI values and revealed no risk for human consumption.

The THQ values for the studied TEs in all of the marine species were < 1, with the exception of As, Cd, Hg, and Se. The highest THQ values were obtained for Hg in adult (6.0E + 00) and in children (2.1E + 01) for muscle of *C. dussumieri*. The next higher THQs was calculated for As in adult (2.5E + 00) and in children (8.6E + 00) for *T. orientalis* and 2.4E + 00 in adult and 8.5E + 00 in children for *S. pharanois* (Table 4). Moreover, the THQ values of other TEs were < 1 in all species indicating no health risk to consumers from those TEs in the studied marine species. The HI values for all the species ranged from 4.6E-01 to 8.1E + 00 in adult and 1.6E + 00-2.8E + 01 in children. Maximum and minimum calculated HI were measured in muscle tissues of *C. dussumieri* and *S. longiceps*, respectively. Mercury, As and Cd were the major



Fig. 3. Metal pollution index (MPI) in muscle and liver tissues of six marine species. Degree of pollution is applied according to Jamil et al. (2014).

Groups	Species		Age Groups	PTEs												
*	•		0	As ^a	Cd	Со	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Zn	HI
		TDI ^b		2.14	0.8	1.4 ^c	5 ^c	500	800	1.6	140	5	3.6	5	300	
		RfD ^d		0.0003	0.001	0.03	0.003	0.04	0.7	0.00016	0.14	0.02	0.004	0.005	0.3	
Crustacean	P. armatus	EDI	Adult	4.9E-01	1.1E-01	3.6E-02	2.1E-02	9.2E+00	5.1E + 00	7.8E-02	3.1E-01	9.8E-02	2.0E-02	4.9E-01	2.3E+01	
			Children	1.7E+00	3.9E-01	1.3E-01	7.2E-02	3.2E + 01	1.8E + 01	2.7E-01	1.1E + 00	3.4E-01	7.1E-02	1.7E + 00	8.1E+01	
		THQ	Adult	1.6E + 00	1.1E-01	1.2E-03	6.8E-03	2.3E-01	7.3E-03	4.9E-01	2.2E-03	4.9E-03	5.1E-03	9.8E-02	7.7E-02	2.7E+00
			Children	5.7E+00	3.9E-01	4.9E-02	2.4E-02	8.0E-01	2.6E-02	1.7E+00	7.7E-03	1.7E-02	1.8E-02	3.4E-01	2.7E-01	9.3E+00
	T. orientalis	EDI	Adult	7.4E-01	1.2E + 00	2.8E-02	2.7E-02	4.4E + 00	2.2E + 00	4.3E-02	1.2E + 00	1.2E-01	1.9E-02	6.5E-01	2.1E + 01	
			Children	2.6E + 00	4.4E + 00	9.8E-02	9.3E-02	1.5E+01	7.9E+00	1.5E-01	4.2E+00	4.0E-01	6.8E-02	2.3E + 00	7.2E+01	
		THQ	Adult	2.5E + 00	1.2E+00	9.3E-04	8.9E-03	1.1E-01	3.2E-03	2.7E-01	8.6E-03	5.8E-03	4.8E-03	1.3E-01	6.9E-02	4.3E+00
			Children	8.6E + 00	4.4E + 00	3.8E-02	3.1E-02	3.9E-01	1.1E-02	9.5E-01	3.0E-02	2.0E-02	1.7E-02	4.6E-01	2.4E-01	1.5E+01
Fish	E. orientalis	EDI	Adult	3.7E-01	2.5E-03	5.0E-03	5.6E-02	2.4E-01	2.9E+00	7.0E-02	1.1E-01	3.0E-02	1.5E-02	5.3E-01	4.0E+00	
			Children	1.3E+00	8.8E-03	1.8E-02	1.9E-01	8.4E-01	1.0E + 01	2.4E-01	4.0E-01	1.1E-01	5.3E-02	1.8E + 00	1.4E+01	
		THQ	Adult	1.2E+00	2.5E-03	1.7E-04	1.9E-02	6.0E-03	4.1E-03	4.4E-01	8.1E-04	1.5E-03	3.8E-03	1.1E-01	1.3E-02	1.8E+00
			Children	4.3E+00	8.8E-03	6.8E-03	6.5E-02	2.1E-02	1.4E-02	1.5E+00	2.8E-03	5.3E-03	1.3E-02	3.7E-01	4.6E-02	6.4E+00
	S. longiceps	EDI	Adult	3.2E-02	6.3E-02	2.3E-02	2.3E-02	5.9E-01	1.3E + 01	2.5E-02	6.0E-01	6.0E-02	8.3E-02	2.1E-01	5.8E+00	
			Children	1.1E-01	2.2E-01	8.0E-02	8.0E-02	2.1E+00	4.4E+01	8.7E-02	2.1E + 00	2.1E-01	2.9E-01	7.4E-01	2.0E + 01	
		THQ	Adult	1.1E-01	6.3E-02	7.6E-04	7.6E-03	1.5E-02	1.8E-02	1.5E-01	4.3E-03	3.0E-03	2.1E-02	4.2E-02	1.9E-02	4.6E-01
			Children	3.8E-01	2.2E-01	3.1E-02	2.7E-02	5.1E-02	6.3E-02	5.4E-01	1.5E-02	1.0E-02	7.3E-02	1.5E-01	6.7E-02	1.6E + 00
	C. dussumieri	EDI	Adult	5.5E-01	4.1E-02	2.3E-03	2.8E-02	2.2E-01	3.9E+00	9.6E-01	1.3E-01	5.8E-02	1.6E-02	9.3E-01	2.5E + 00	
	(muscle)		Children	1.9E+00	1.4E-01	8.1E-03	9.9E-02	7.8E-01	1.4E + 01	3.3E + 00	4.4E-01	2.0E-01	5.5E-02	3.3E + 00	8.7E+00	
		THQ	Adult	1.8E+00	4.1E-02	7.7E-05	9.4E-03	5.6E-03	5.6E-03	6.0E + 00	9.0E-04	2.9E-03	3.9E-03	1.9E-01	8.3E-03	8.1E + 00
			Children	6.5E+00	1.4E-01	3.1E-03	3.3E-02	1.9E-02	2.0E-02	2.1E + 01	3.2E-03	1.0E-02	1.4E-02	6.5E-01	2.9E-02	2.8E + 01
	C. dussumieri	EDI	Adult	2.9E-01	1.1E+00	1.2E-02	1.3E-02	7.8E-01	6.9E+01	5.6E-01	3.5E-01	2.2E-02	1.1E-02	3.1E + 00	3.2E + 00	
	(liver)		Children	1.0E + 00	3.7E+00	4.1E-02	4.6E-02	2.7E + 00	2.4E + 02	2.0E + 00	1.2E + 00	7.9E-02	3.8E-02	1.1E + 01	1.1E + 01	
		THQ	Adult	9.6E-01	1.1E+00	3.9E-04	4.4E-03	1.9E-02	9.9E-02	3.5E+00	2.5E-03	1.1E-03	2.7E-03	6.2E-01	1.1E-02	6.3E+00
			Children	3.3E + 00	3.7E+00	1.6E-02	1.5E-02	6.8E-02	3.5E-01	1.2E+01	8.7E-03	3.9E-03	9.4E-03	2.2E+00	3.7E-02	2.2E+01
Mollusc	S. pharanois	EDI	Adult	7.3E-01	1.9E-01	3.4E-02	2.7E-02	3.0E + 00	1.1E + 00	6.6E-02	9.5E-02	5.7E-02	2.1E-02	3.6E-01	1.2E + 01	
			Children	2.6E + 00	6.5E-01	1.2E-01	9.4E-02	1.0E + 01	3.7E+00	2.3E-01	3.3E-01	2.0E-01	7.2E-02	1.3E+00	4.3E+01	
		THQ	Adult	2.4E + 00	1.9E-01	1.1E-03	9.0E-03	7.4E-02	1.5E-03	4.1E-01	6.8E-04	2.8E-03	5.1E-03	7.1E-02	4.1E-02	3.2E+00
			Children	8.5E + 00	6.5E-01	4.6E-02	3.1E-02	2.6E-01	5.3E-03	1.4E+00	2.4E-03	9.9E-03	1.8E-02	2.5E-01	1.4E-01	1.1E + 01

The estimated daily intakes (FDI) target hazard quotient (THO)	and hazard index (HI) of TEs in six marine species from the Persian Gulf EDI value	res > TDI and THO and HI values > 1 are shown in hold
The estimated daily makes (EDI), target hazard quotient (ThQ)	and hazard index (11) of TES in Six marine species from the Persian Guit. EDI var	100 and 1100 and 11000 and 110000 and 1100000 and 100000 and 1000000 and 10000000 and 10000000 and 10000000 and 1000000 and 10000

^a 3% of total As concentration (mg kg⁻¹ ww) in muscle tissues (see in Table 2) was assumed as inorganic As used for EDI, THQ and HI assessment. ^b TDI (Tolerable Daily Intake, $\mu g kg^{-1}$ bw day⁻¹), Joint FAO/WHO Expert Committee on Food Additive (JECFA).

^c Baars et al. (2001).
 ^d RfD (Reference Dose, mg kg⁻¹ bw day⁻¹) as published by the USEPA (USEPA IRIS, 2017).

Table 4

contributors for HI, accounting for 41.8 % and 39.6 %, respectively, while Mn (0.07 %) and Ni (0.08 %) contributed the least to the HI. The HI values were > 1 for all studied species, suggesting that people would experience significant health risk effect due to consumption of seafood from the study area.

The average CR values for As, Cd, Ni, and Pb were 1.54×10^{-3} , 5.5×10^{-3} , 2.41×10^{-4} and 5.04×10^{-7} , respectively (Fig. S3, supplementary information). CR values obtained for As, Cd and Ni were not within the acceptable range of 10^{-4} to 10^{-6} . The CRs for As and Cd were much higher than the acceptable value in most of the species. Therefore, the carcinogenic risk of As and Cd in seafood should be given more attention. The carcinogenic risks for Ni in some of the species were slightly higher than the acceptable value, whereas the CR value of Pb was in the acceptable range.

4. Discussion

4.1. Biometric characteristics and TEs concentration

The widest BL variability was found in C. dussumieri with the heaviest weight and the highest length values, while P. armatus showed small BL variation. C. dussumieri had the highest trophic level (4). On the contrary, small fish like *S.longiceps* were those with a low trophic level (2.4). Among the species *C. dussumieri* and *S. pharanois* are pelagic and benthic carnivore species, respectively. The maximum TEs concentration including As, Cd, Mn, and Ni was measured in the muscle tissue of T. orientalis (crustacean) compared to muscle tissues of the other species (p < 0.05). This high TEs concentration is probably due to the difference in habitats as T. orientalis is considered as benthic organisms which encounter more TEs concentration due to their proximity to bottom sediments (Soltani et al., 2019). Crustaceans, such as lobster, are benthic organisms with a burying behaviour that reside within the sediment, resulting more ingestion/absorption of TEs from the sediment (Zhao et al., 2012). Furthermore, crustaceans are omnivores and important scavengers and tend to accumulate more contaminants along the aquatic food chain (Ip et al., 2005). Younis et al. (2015) believed that among the marine organisms, crustaceans naturally have high concentration of TEs in their body tissues.

A preferential As concentration in the muscle compared to the liver was observed in *S. pharanois* and *C. dussumieri* (p < 0.05). These findings are consistent with findings on elasmobranch fish from the Mediterranean Sea (Storelli and Marcotrigiano, 2004), Pontic shad from the Danube River in Serbia (Visnjic-Jeftic et al., 2010), seamount fish from Azores region, Portugal (Raimundo et al., 2015) and subarctic fish from Yellowknife Bay in Canada (Chetelat et al., 2019). Amlund et al. (2006) revealed that As bioaccumulates in fish muscle tissue mainly in the form of arsenobetaine due to its relatively slow and incomplete elimination. Francesconi (2010) believed that As partition between muscle and liver of marine species is probably related to differences in the bioavailability of its elemental forms. Bouchoucha et al. (2019) reported that the relatively low As concentrations in the liver of some marine species might be connected to efficient hepatic metabolic function. Marine species differ in their arsenic uptake/elimination rates, which may have contributed to the different ratios of liver to muscle As content among the species (Zhang et al., 2016). The difference in TEs concentration in different organs or tissues of marine species is primarily due to the various physiological roles carried out in each organ (Perera et al., 2016).

The Mann-Whitney *U* test indicated significant differences (p < 0.05) between concentration of TEs in liver and muscle tissues of all the investigated species except As, Cr and Ni. The higher concentration of nonessential elements such as Cd was present in the liver tissues of *S. pharanois, S. longiceps* and *C. dussumieri*. As expected, the liver tissues contained higher TEs concentration (except As in muscle of *C. dussumieri* and *S. pharanois*) than the muscle tissues. Similar results have been reported by other studies in different types of fish which indicated that the

liver is the primary TEs storage site. Garnero et al. (2018) reported elevated TEs concentration in the liver tissues of Hoplias malabaricus, Oligosarcus jenynsii, Rhamdia quelen, Bryconamericus iheringii, Astyanax fasciatus and Odontesthes bonariensis compared to their muscle tissues. Keshavarzi et al. (2018) measured higher TEs concentration in the liver tissues of Anodontostoma chacunda, Belangerii and Cynoglossurs arel. This tissue-specific concentration of TEs in liver can be described by the activity of metallothioneins (MT), sulfur-rich proteins in the metabolic processes of TEs which have the capacity to naturally bind certain TEs (Görür et al., 2012) through the thiol group of its cysteine residues (Ploetz et al., 2007). The liver also plays a significant role in detoxification, particularly of environmental toxins, including TEs (Hechtman, 2018). A higher concentration of essential TEs in the liver indicates their roles in blood cells and haemoglobin synthesis (Fe), bile secretion (Zn), enzymatic and cellular metabolic demands (Cu) in marine species (Görür et al., 2012). The presence of MT natural proteins in the hepatic tissue has a greater tendency to bind with Fe, Cu, Zn and Cd.

4.2. Relationship between body size and TEs concentration

Total Hg concentrations in the muscle and liver tissues of most of the targeted species were observed to generally increase with the body size. Given their higher trophic position and larger body sizes (Table 1), it was expected that *C. dussumieri* would exhibit higher Hg concentrations compared to other marine species examined in this study. Mercury is known to bioaccumulate in fish, and thus relatively high concentrations can be expected to be attained in top predators (Lescord et al., 2018; Bergés-Tiznado et al., 2019). Several factors such as longer exposure time as a result of greater longevity of sharks as compared to other fish, lower Hg excretion due to slow metabolic rate (Huckabee et al., 1979), and an increase in Hg content due to biomagnification in sharks (Bergés-Tiznado et al., 2015a; Ruelas-Inzunza et al., 2020) and other top predator fish (Bergés-Tiznado et al., 2015b, 2019) could result in higher Hg levels top predators.

Nicolaus et al. (2016) reported significantly positive relationships between Hg concentration and shark length in both red and white muscle tissues. In this study, As, Fe, Mn, Ni, and Zn in muscle tissues of S. longiceps were found to increase with body size while the opposite trend was found in the liver tissues. Differently, concentration of Cr, Cu, Fe, Mn, Ni, and Zn in C. dussumieri and As, Cd, Co, Cu, Mn, Pb, and Se in S. pharanois were found to decrease in the muscle tissues in relation to body size. On the contrary, the reverse pattern was observed in the liver tissues. Szefer et al. (2003) found an increase in the concentration of Cu in liver tissues of the European perch (Perca fluviatilis) relative to fish growth whereas no effect was noticeable in the muscle Cu levels. Moreno-Sierra et al. (2016) reported for essential TEs, that only Zn levels in the liver and muscle tissues were significantly correlated with the length and weight, and for non-essential TEs, Cd levels in the liver were correlated with the length and weight in sailfish Istiophorus platypterus from the Eastern Pacific. Baptista et al. (2019) reported that certain tissues might grow at a faster rate than specific TEs are incorporated, leading to the dilution of elemental contents as fish increase in size. For the essential TEs, it is assumed that their content in tissues is homeostatically controlled, resulting in positive or negative relationship (Bajc et al., 2016). Kojadinovic et al. (2007) stated that positive or negative correlations of TEs in fish tissues may be related to the elemental regulation which is affected by metabolic activity, environmental conditions and physiological needs. Accumulation of TEs in the bodies is controlled by absorption, elimination and detoxification, which are highly dependent on the rate of metabolism that varies with age (Schuster et al., 2019). Several factors including faster short-term uptake by smaller individuals (Liang et al., 1999) lower percentage of fat tissue resulting in higher relative dilution effect of the lipid content (Farkas et al., 2003) and higher metabolic rate of young specimens (Parker, 2011), imply a higher potential uptake of TEs in smaller than larger marine species resulting in negative slopes in the size-concentration

relationships.

4.3. Se:Hg molar ratios and Se health benefit value (HBV_{Se})

The wide variation in Se:Hg molar ratios were observed among the examined species which are driven by significant differences in Hg and Se concentrations (P < 0.01). The Se:Hg molar ratios differed between the examined muscle and liver tissues, reflecting the distribution patterns of Hg and Se. The Se:Hg molar ratio values in the liver tissues of the different marine species were several times higher than the values noted in the muscles. A similar pattern was observed in the shark S. lewini (Bergés-Tiznado et al., 2015a) and the sailfish I. platypterus (Bergés-Tiznado et al., 2015b) in the Gulf of California. Reasons for greater interspecies and intraspecies variability in the ratios demonstrate the fact that Se as a trace element is homeostatically controlled, but its concentration still varies somewhat, whereas Hg is biomagnified through the aquatic food web in relation to increasing body size and age (Ralston et al., 2016). All marine species samples collected from the study area had Se:Hg molar ratios higher than 1, therefore, potentially they were probably protected by the Se against Hg toxicity. Recent studies have revealed that despite the molar ratio approach being contentious, but values surplus of Se:Hg > 1 might be sufficient to prevent Hg toxicity in the species and consumers (Niane et al., 2015). This ratio thus should be an important consideration for risk assessment (Cusack et al., 2017). Selenium can limit toxicity induced by Hg through its antioxidant properties and through formation of inert Se-Hg complexes, by competing with Hg for binding sites (Cuvin-Aralar and Furness, 1991). Lemire et al. (2010) proved that higher Se:Hg molar ratio level can relatively protect species against Hg toxicity in some organs or tissues. In the present study, lowest Se:Hg molar ratios in liver and muscle tissues were found in the large, top level predator, represented by the carnivorous pelagic shark fish, C. dussumieri, mainly due to the highest Hg concentrations in its tissues. These factors suggest that Se:Hg ratios in large predator species should be low, which is hypothesized to confer the least protective effect against Hg toxicity (Burger and Gochfeld, 2013). Across the targeted marine species the Se:Hg molar ratio decreased with increasing mean Hg concentrations. Burger et al. (2014) reported that marine species with the highest Hg contents had the lowest Se:Hg molar ratios.

The HBV_{Se} reflects the Se surplus or deficit in a marine species compared to its Hg content, providing a more reliable index for assessing Hg exposure risks (Ralston et al., 2016). All the studied species presented a positive mean HBV_{Se} value due to their relatively high Se and low Hg contents except for C. dussumieri (shark). The negative HBVSe found for C. dussumieri may be due to its excessive Hg level (0.98 mg kg^{-1} ww, Table 2) rather than a poor Se content (0.95 mg kg⁻¹ ww, Table 2). Furthermore, Se:Hg molar ratio in C. dussumieri is the lowest among the investigated muscle tissues. Ralston et al. (2019) and Ruelas-Inzunza et al. (2020) also reported a negative HBV_{Se} due to elevated Hg content and largest body size. The examined liver tissues displayed the higher HBV_{Se} values than muscle tissues. Looi et al. (2016) observed higher values in liver in relation to muscle tissues. In the present study the application of HBV_{Se} suggests that Se concentration in all examined species (except C. dussumieri) could be sufficient to moderate and protect them and their consumers with its antagonistic effect in relation to the potential Hg toxicity.

4.4. Human health risk assessment

The TEs concentration in different marine species could be evaluated by the MPI to compare the total TEs concentration in various marine species (Sharma et al., 2008). Higher TEs concentration in the marine species tissues results in higher MPI values which represent potential adverse health effect through the consumption of contaminated seafood. Due to considerably higher TEs concentration, the higher MPI values corresponded to liver tissues (Fig. 3). This was in agreement with measurements obtained by Omar et al. (2015), which found higher MPI values in liver than muscle tissues. Trace elements are not uniformly distributed in the body of marine species and accumulate significantly in metabolically active tissues like liver (Tiwari et al., 2014) while muscle shows the least TEs concentration that is mostly due to its low levels of union binding proteins and enzymatic activities (Yeşilbudak and Erdem, 2014). It was observed that C. dussumieri with the largest length and the greatest weight recorded a lower MPI values both in liver and muscle tissues which is consistent with earlier studies (Kwaansa-Ansah et al., 2019). The hypothesis that species in the upper marine web food position how more TEs was not found in the present study because the higher TEs concentration were measured in the muscle of omnivorous species. Bawuro et al. (2018) reported higher TEs concentration in omnivore than carnivore species. Thus, feeding habit, type of habitat, TEs accumulation capacity, and organisms type are important factors, affecting the MPI results of marine species (Hao et al., 2013). The levels of As and Cd in muscle tissues of all the studied species and in the liver of C. dussumieri, which is consumed by locally as well as Asian people in general, and Mn and Zn in T. orientalis and P. aramatus exceeded their recommended maximum levels established by FAO (1983), EC (2007), FAO/WHO (2007) and USEPA (2011). The As concentrations in 100 % of the species except S. longiceps (60 %), Cd concentrations in 100 % of T. orientalis, 20 % of S. pharanois, 33.4 % of the liver tissue of C. dussumieri, Cu concentrations in %50 of P. aramatus, Fe concentrations in 20 % of the liver tissue of C. dussumieri, Mn concentrations in 60 % of T. orientalis exceed their recommended limits. The concentration of Zn in 100 % of P. aramatus and 16.7 % and 60 % of T. orientalis surpass the FAO (1983) and USEPA (2011) limits, respectively. Furthermore, the mean concentration of Hg in liver (50 %) and muscle (66.7 %) tissues of C. dussumieri, as a carnivore, were above the limits recommended by FAO/WHO (2007). Concentration of rest of the TEs were within the acceptable limits. The EDI results indicated that the inhabitants are exposed to high doses of As, Cd, Hg, and Se from the consumption of muscle tissues of T. orientalis and S. pharanois and liver and muscle tissues of C. dussumieri. Consumption of the other species does not seem to pose any health risk, as the obtained EDI values are below TDI suggested by the joint FAO/WHO Expert Committee on Food Additives (JECFA). The THQs >1 for As, Cd, Hg, and Se in some species for both adult and children indicate a potential health risk of the TEs exposure through consumption of some of these marine species. Of these, C. dussumieri stood out with elevated THQs for Hg, followed by T. orientalis and S. pharanois for As. The calculated HI values for TEs were higher than 1 in all the studied species except adult in S. longiceps. Mercury, As and Cd were the major risk contributors with the maximum HI values in C. dussumieri and T. orientalis.

The EDI, THQ and HI values were higher for children; thus, caution must be taken since perennial intake of these contaminated seafood is likely to cause potential adverse health effects, arising mainly from As, Cd, Hg, and Se exposure. Children are particularly vulnerable to the potential health risk stemming from chemical pollutants exposure, since they consume more food per unit body weight than adults (USEPA, 2017). Thus, intakes of these toxic TEs through food could be higher for children in the study area. CR values of studied marine species in the Persian Gulf followed the order of Cd > As > Ni > Pb (Fig. S3) for both adult and children. Similar results have been observed by Korkmaz et al. (2018) and Traina et al. (2019). Therefore, the potential health risk for the inhabitants due to TEs exposure through seafood consumption should not be ignored.

5. Conclusion

The concentration of TEs in muscle and liver tissues measured in this study provide baseline information on their contents and distribution in six commercially important marine species from the Persian Gulf. The concentrations of As, Cd, Mn, Zn and Hg were found higher than their global prescribed safe limits. Higher TEs concentration were detected in

the liver tissues compared to the muscle tissue. The TEs distribution showed different trends in the muscle and livers tissues. Concentrations of Fe, Cu, and Zn in the muscles and Fe and Mn in the liver tissues, showed a negative relationship with the marine species' body size. In contrast, Hg in the muscle and Cd, Cu and Zn in the liver tissues showed a positive relationship. Concentration of TEs the targeted species exhibited species/tissues dependent distribution patterns. The TEs variation and sequestration between the muscle and liver tissues is not uniform during species' lifetime, probably because of physiological (age, body size, diet, metabolic and growth rates) and detoxification processes. The lowest Se:Hg molar ratio and negative HBV_{Se} value were observed in the large top-predator, C. dussumieri due to its excessive Hg content. In terms of potential human health risks, consumption of T. orientalis and C. dussumieri species pose potential risk mainly from As, Cd and Hg. Accordingly, the cancerogenic risk due to the long-term seafood consumption in the study area is of particular concern. Thus, constant and long-term monitoring of TEs pollution in the marine ecosystem of the Persian Gulf is highly recommended, and a variety of edible marine species should be investigated to provide a comprehensive health risk assessment.

Author contributions

All authors discussed the results and contributed to the final manuscript.

CRediT authorship contribution statement

Naghmeh Soltani: Investigation, Writing - original draft. Michel Marengo: Writing - review & editing. Behnam Keshavarzi: Supervision, Conceptualization. Farid Moore: Conceptualization, Resources. Peter S. Hooda: Writing - review & editing. Mohammad Reza Mahmoudi: Formal analysis. Sylvie Gobert: Writing - review & editing, Resources, Validation.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jfca.2020.103754.

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Journal of Food Composition and Analysis 97 (2021) 103754

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N. Soltani et al.

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N. Soltani et al.

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