İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2020; 35(1): 6-12 • DOI: https://doi.org/10.26650/ASE2019604880

Research Article

Determination of Trace/Toxic Mineral Risk Levels for Different Aged Consumers of Three Fish Species Caught in the Marmara Sea

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Cite this article as: Erkan, N., Kaplan, M., Özden, Ö. (2020). Determination of trace/toxic mineral risk levels for different aged consumers of three fish species caught in the Marmara Sea. *Aquatic Sciences and Engineering, 35*(1), 6–12.

ABSTRACT

The Marmara Sea is a semi closed fishing area in Turkey and has valuable fish species such as the common sole, black scorpionfish and horse mackerel. Domestic waste, generated by the population of over 25 million living in the coastal zone as well as industrial facilities in the region are the main causes of pollution in the Marmara Sea. In the current study, the levels of toxic metals (arsenic, cadmium, mercury, and lead) in samples of three fish tissue were analysed over a year-long period and compared with international results. The health risks for different age groups caused by the consumption of these fish were estimated. The results revealed that regular weekly consumption of sole and horse mackerel caught from the Marmara Sea posed health risks for child and youth populations. These fish species should be consumed more carefully due to potential arsenic-related hazards and carcinogenic risks for vulnerable consumer groups.

Keywords: Trace toxic mineral, HI, THQ, Health risk, The Marmara Sea, Common sole, Black scorpionfish, Horse mackerel

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Submitted:
09.08.2019

Revision Requested:
13.09.2019

Last Revision Received:
25.09.2019

Accepted:
11.10.2019

Online published:
28.11.2019

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INTRODUCTION

Metals are one of the basic components of nature, rapid industrialization, urbanization, mining, related changes in land use and associated enhanced terrestrial runoff (to seas) with anthropogenic activities which can significantly increase their content (Bat et al. 2012; Saha et al. 2016). Biological material from aquatic sources are most easily contaminated by environmental pollution, especially due to their location in the nutritional chain. Therefore, contaminated seafoods is an important contributory factor in the transfer of environmental pollutants to consumers. Because of this reason consumption of fisheries' products is one of the main steps in the transfer of environmental pollutants to consumers. A lot of nutrient components and elements found in seafood are crucial for sustaining human life, but metals such as mercury, cadmium and lead do not play a known role in biological systems, and dietary intakes of these metals - even at very low concentrations - may cause toxic effects (Storelli et al. 2012). The trace toxic minerals from sea pollutants accumulating in fish tissues are the major risk factor and therefore need to be monitored for the protection of human health. The metal toxicity associated with fish consumption depends mainly on the metal content, how often it is consumed, and on the age of the consumers (Safahieh et al. 2011). Consumption of trace toxic minerals/contaminated fishes may result in a weakening of the endocrine system in children and adults, and these compounds have neurotoxic and nephrotoxic effects (Herreros et al. 2008).

Another important hazard rooted in environmental pollution is arsenic and its presence in seafood is an important source of arsenic exposure in humans, and according to the European Commission Scientific Cooperation Project

(SCOOP) this ratio is may be 50% higher than other foods. Fish and shellfish are usually contaminated with arsenobetain, while arseno-lipids are found in fatty marine fish. Moreover, the inorganic form of arsenic is more toxic than organic arsenic. These compounds are genotoxic and generally have a neurotoxic effect, in the central nervous system of developing foetuses, infants and growing children (Mania et al. 2015; Koesmawatia et al. 2015).

Common sole (*Solea solea*) and black scorpionfish (*Scorpaena porcus*) are demersal fish species which do not leave their habitats, whereas horse mackerel (*Trachurus trachurus*) is a seasonal migratory pelagic fish in the Marmara sea. In 2017, the total production quantity of Turkey's sea fish catch was reported as 269676.4 metric tons (Turkish Statistical Institute (TUIK), 2018). The total catch was 486.4 tons for common sole, 306 tons for black scorpionfish, and 8065.6 tons for horse mackerel in Turkey. These fish species mainly live in the Marmara Sea where pollution is one of the major challenges. The domestic waste of densely populated cities (Istanbul and İzmit with a total population of nineteen million people) in the northeastern region of the Marmara Sea, maritime traffic and the waste of heavy industry plants in the İzmit Gulf are the main causes of pollution.

Fish are good indicators for the long term monitoring of metal accumulation in the marine environment. The trace toxic minerals in fish produce toxic effects at high concentrations (Keskin et al. 2007; Tepe et al. 2008; Türkmen and Ciminli, 2007). The possible health risks to consumers may vary depending on the age groups and the amount of fish consumed. In recent years, the target hazard ratio (THQ) and/or total target hazard area (TTHQ) have been used to assess health risks (Copat et al. 2013).

The data on trace toxic minerals levels in common sole, black scorpionfish and horse mackerel from Turkey are very limited and little information is available in the literature to quantify the health risks related with the consumption of contaminated fish from the Marmara Sea.

Therefore the main aims of the study are twofold: firstly, to determine the levels of arsenic cadmium, mercury and lead in selected fish species samples over a one-year period, and to compare the results with the limits set by International Organisations. Secondly, to assess the health risks of trace toxic minerals to consumers of different age groups.

MATERIALS AND METHODS

Samples

The common sole (*Solea solea*), black scorpionfish (*Scorpaena porcus*) and horse mackerel (*Trachurus trachurus*) samples (caught from the Marmara Sea) were purchased from a local fish market in Istanbul. Fish samples were collected on a monthly basis throughout the fishing season. The maximum, minimum and average of all values were presented and evaluated. Each species (10 samples for common sole, 20 samples for black scorpionfish and 100 samples for horse mackerel in each sampling) was gutted, filleted and muscle tissue was minced for analysis. The minced samples were then placed in individual polythene bags and kept frozen prior to analysis. Determination of arsenic (As), cadmium (Cd), total mercury (Hg), and lead (Pb) amounts in each fish species were analysed according to the Method 3051A (US Environmental protection Agency (US EPA) 2007) method.

Toxic metals analysis

Fish samples were accurately weighed (approximately 0.3 g) and digested in a closed-vessel microwave digestion system (ETHOS 1, Milestone, Italy) using 6 mL nitric acid (65% v/v) and 4 mL hydrogen peroxide (30% v/v) (Merck, Darmstadt, Germany) at 200°C for 20 min. After cooling to room temperature (20-23°C), the solution was quantitatively transferred into a 25 mL volumetric flask and diluted to volume with ultra-pure water obtained from the Milli-Q system (Millipore, Bedford, MA, USA) and stored at 4°C prior to analysis.

The toxic metal contents of the fishes (three sub-samples of each material) were analysed using a Thermo Scientific iCAP Qc ICP-MS instrument (Thermo Fisher Scientific GmbH, Germany) equipped with an Elemental Scientific SC-2DX auto sampler (Omaha, NE, USA). The ICP-MS sample introduction system consists of a PFA concentric nebulizer coupled with a peltier-cooled cyclonic spray chamber. The iCAP Q interface consists of a pair of standard Ni sample and skimmer cones. The ICP-MS was operated in kinetic energy discrimination mode, using helium as the collision cell gas with a flow rate of 4.5mL min^{-1}. The following operating parameters were also set: RF power, 1,550 W; plasma gas flow rate, 14 L min^{-1}; auxiliary gas flow rate, 0.89 L min^{-1}; carrier gas flow rate, 0.91 L min^{-1}; spray chamber temperature, 2.70°C.

Arsenic, ^{75}As; cadmium, ^{111}Cd; mercury, ^{202}Hg; and lead, ^{208}Pb were the isotopes monitored. Quantitative analysis of the samples was performed using a five-point calibration curve (0.5, 1.0, 2.5, 5.0, 10.0 μg/L) constructed for each isotope. To cover the mass range of isotopes a mixture of internal standard elements (^{6}Li, ^{45}Sc, ^{73}Ge, ^{103}Rh, ^{111}In, ^{159}Tb, ^{175}Lu and ^{209}Bi) at 2 μg/L concentration level were used. The response factors of both higher and lower mass internal standards were used to correct the concentration of each isotope monitored.

The toxic metal content results were given as μg/g in wet weight (w.w.) of fish samples and the accuracy of the analytical method was monitored by analysing certified reference materials from mussel tissue (Catalogue No. ERM-CE278k).

Estimation of potential public health risks

Nine different age-categories (US Environmental protection Agency (US EPA) 2011) were used for estimating health risks: **A1** 1-3 years children, **A2** 4-6 years children, **A3** 7-10 years children, **A4** 11-14 years adolescents, **A5** 15-19 years adolescents, **A6** 20-24 years adults, **A7** 25-54 years adults, **A8** 55–64 years adults and **A9** >65 years seniors. Body weights (kg) were 14 kg for A1, 21 kg for A2, 32 kg for A3, 51 kg for A4, 67 kg for A5, 72 kg for A6, 77 kg for A7, 77 kg for A8 and 72 kg for A9. The weekly fish consumption value was obtained by measuring one portion as 150 g/week/person for A4, A5, A6, A7, A8, A9 and 50 g/week/person for A1, A2, A3. The risk assessments of the fish samples for the different groups were made according to the methods and calculations given by Erkan and Özden (2017) (Table 1).

Table 1. Risk Assessments calculation methods

Risk Assessment Name	Formula	Explanation
Estimated Weekly Intake (EWI)	$\frac{WFC \times C}{BW}$	**WFC:** Weekly Consumption Values (g/week/person) **C:** Concentration of contaminant (µg/g) **BW:** Body Weight (kg)
Hazard Index (HI)	$\frac{CWICC}{PTWI}$	**CWICC:** Calculated Weekly Intake for Certain Contaminant **PTWI:** Provisional tolerable weekly intake (µg/week/kg bw) in European Food Safety Authority (EFSA) (2009a) for As, in EFSA (2009b) for Cd, in EFSA (2019) for MeHg and in EFSA (2010) for Pb
Target Hazard Quotient (THQ)	$\left(\frac{EF \times ED \times FIR \times C}{RFD \times BW \times TA}\right) \times 0.001$	**EF**: Exposure Frequency (365 days/year) **ED**: Exposure Duration (years) for different age groups. **FIR**: Food Ingestion Rate (/person/day) for children and adult age groups **C**: Concentration of contaminant (µg/g) **RFD**: Oral Reference Dose (µg/g/day for As, Cd, Hg, Pb) (Qin et al., 2015) **BW**: Body Weight (kg) **TA**: Averaging exposure time for non -carcinogens (365 days/ year x ED)

Table 2. The concentration of trace toxic minerals in three fish species of the Marmara Sea.

Fish/ Season	As (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Sole -Spring	1.103	<0.001	0.038	0.061
Sole -Summer	5.244	<0.001	0.122	0.046
Sole-Autumn	3.796	<0.001	0.060	0.042
Sole-Winter	20.450	<0.001	0.055	0.020
Season average	**7,648 ±7.539**	**<0.001**	**0.069 ±0.032**	**0.042 ±0.015**
European Commission (EC)'s upper limits (mg/kg)	**-***	**-***	**-***	**-***
Horse mackerel -Spring	13.30	<0.001	0.170	0.052
Horse mackerel -Summer	2.147	0.004	0.021	0.056
Horse mackerel -Autumn	1.217	0.004	0.010	0.037
Horse mackerel -Winter	2.740	0.005	0.024	0.047
Season average	**4.852 ±4.909**	**0.004 ±0.000**	**0.056 ±0.066**	**0.048 ±0.007**
EC's upper limits (mg/kg)	**-***	**0.10**	**0.50**	**0.30**
Scorpion fish -Spring	4.301	<0.001	0.025	0.071
Scorpion fish -Summer	1.153	<0.001	0.094	0.029
Scorpion fish -Autumn	3.314	<0.001	0.100	0.033
Scorpion fish -Winter	2.319	<0.001	0.138	0.099
Season average	**2.772 ±1.168**	**<0.001**	**0.089 ±0.041**	**0.058 ±0.029**
EC's upper limits (mg/kg)	**-***	**0.50**	**0.50**	**0.30**

• No limits in EC

Total THQ (TTHQ) of heavy metals for seafood is the sum of the following composition: **TTHQ (individual seafood) = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant n)**.

The EWI, HI and THQ calculations of this study were conducted using 100% Hg (EFSA, 2012) and 10% As (Qin et al., 2015).

RESULTS AND DISCUSION

The concentration of toxic metals determined in the fish tissues are presented in Table 2. Arsenic concentration was found to be the highest of all the toxic metals measured in the three fish species studied. Arsenic toxicity depends on the form of arsenic: inorganic arsenic in drinking water is much more toxic than organic arsenic in seafood. The trivalent form of inorganic arsenic types is also more toxic. Arsenobetaine and arsenosugars is the major form of arsenic in marine fish and most other seafood. Arsenolipid is the form of arsenic present in fish oils and fatty fish tissue likely to be present in other seafood. According to the Turkish Food Codex (2011) there is no reported limit value for arsenic in fish and fish products. Similarly, Falcó et al. (2006) and Martorell et al. (2011) have reported high arsenic concentrations of 4.55 μg/g and 11.614 μg/g of fresh weight in muscle tissues of sole fish from the Catalonia Region of Spain. Unlike our results, Erkan et al. (2009) and Özden et al. (2010) reported lower amounts of arsenic content in scorpion fish (0.189-0.244 μg/g) and sole

Table 3. Trace toxic minerals levels in literature for the three fish species.

Sample area	Fish	As (μg/g)	Cd (μg/g)	Hg (μg/g)	Pb (μg/g)	Literature
Trabzon (Turkey) local fish market	*Trachurus trachurus*	0.63	3.58	-	<LOD	Aydın and Tokalıoğlu, 2015
Coastal Waters of the Black Sea,	*Trachurus trachurus*	-	0.043-0.048	-	0.17-0.23	Bat et al. 2012
	Solea solea	-	0.020-0.023	-	0.03-0.08	
Iskenderun Bay	*Solea lascaris*	-	0.04	NE	0.39	Renieri et al. 2014
Aegean Sea	*Scorpaena porcus*	-	0.8	NE	0.66	
Marmara Sea	*Scorpaena porcus*	0.189	0.001	0.672	0.007	Erkan et al. 2009
	Scorpaena scrofa	0.244	0.011	0.405	0.032	
Varna (Bulgarian) local fish market	*Trachurus mediterraneus ponticus)*	-	0.045	-	0.166	Stoyanova et al. 2015
Black Sea	*Trachurus trachurus*	-	0.045-0.052	-	0.363-0.638	Yaman et al. 2013
Iskenderun Bay	*Trachurus mediterraneus*	-	-	-	1.037	Yilmaz, 2003
Istanbul (Turkey) local fish market	*Solea solea*	0.34	0.05	0.56	0.39	Özden and Erkan, 2016
	Trachurus trachurus	0.25	0.04	0.29	0.17	
Istanbul (Turkey) local fish market	*Trachurus trachurus*	0,026-0.644	0.001-0.016	0.37-1.282	0.015-0.807	Özden, 2010
Marmara Sea	*Trachurus mediterraneus*		0.011	0.035	0.063	Keskin et al. 2007
	Solea solea		0.022	0.329	0.133	
Granada (Spain) local fish market	*Trachurus trachurus*	0.032	0.253	0.146	0.672	Rivas et al. 2014
	Trachurus mediterraneus	0.043	0.141	0.204	0.814	
Marmara Sea (Yalova)	*Solea solea*	-	0.02	-	0.17	Türkmen, 2011
North Eagean Sea (Çanakkale)		-	0.07		0.25	
Eagean Sea (İzmir		-	0.26	-	0.48	
Meditreanean (Mersin Bay)		-	0.38	-	0.37	
Bulgarian Black Sea coast	Thrachurus mediterraneus ponticus	0.73	0.008	0.16	0.06	Makedonski et al. 2017
Istanbul (Turkey) local fish market	*Solea solea*	0.153-0.820	<0.001-0.233	0.135-1.858	0.006- 0.912	Özden et al. 2010

NE: not estimated.

(0.153-0.820 μg/g). Other researchers reported lower arsenic levels of 1.73 μg/g (Falcó et al. 2006), 3.141 μg/g (Martorell et al. 2011), 0.026-0.644 μg/g (Özden, 2010), 0.032-0.043 μg/g (Rivas et al. 2014), 0.63 μg/g (Aydın and Tokalıoğlu 2015) and 0.73 μg/g (Makadonski et al. 2017) in horse mackerel.

The mean concentration of Cd detected in sole and scorpion fish was <0.001 μg/g and higher mean values of 0.004 μg/g wet wt. was found in horse mackerel. The Cd content of sole, ranging from <0.001-0.233 μg/g and of horse mackerel ranging from 0.141 to 0.253 μg/g, was reported by Özden et al. (2010) and Rivas et al. (2014). However, this value was higher (for horse mackerel 3.58 μg/g, for scorpion fish 0.8 μg/g, for sole 0.38 μg/g than that reported by other authors (Aydın and Tokalıoğlu, 2015; Renieri et al. 2014; Türkmen, 2011).

The average amounts of mercury in the tissues of the investigated species were 0.069±0.032 μg/g w/w. (ranging from 0.038–0.122), 0.056±0.066 μg/g (0.010-0.170) and 0.089±0.041 μg/g (0.025–0.138) for sole, horse mackerel and scorpion fish, respectively.

The results obtained in Hg content analysis of the tissue samples are similar to the results of Keskin et al. (2007) in common sole (0.329 μg/g); Erkan et al. (2009) in scorpion fish (0.405-0.672 μg/g); Rivas et al. (2014) in horse mackerel (0.146-0.204 μg/g).

The average lead content was found to be 0.042, 0.048 and 0.58 μg/g for common sole, horse mackerel and scorpionfish respectively. In this study, Scorpionfish contained the highest lead concentration.

The lead content of sole was reported by Guérin et al. (2011) as 0.011 μg/g. Bat et al. (2012) and Türkmen (2011) reported higher lead cocentrations of 0.03-0.08 μg/g and 0.17-0.48 μg/g in sole, respectively. In another study, even higher lead concentrations of 0.672-0.814 μg/g were determined in pink salmon (Rivas et al. 2014).

The trace metal limits for the European Communities are for Hg 0.5 mg/kg (μg/g) in fishery products, for Pb 0.3 mg/kg (μg/g) in fish, for Cd 0.05 mg/kg (μg/g) in lean fish, 0.1 mg/kg (μg/g) in wedge sole, eel, horse mackerel, sardine, and anchovy (European Commission (EC) No 1881/2006). Unlike mercury, lead and

Table 4. HI and THQ risk factors for people of different ages and kilograms in three fish species from Marmara Sea.

		As		Cd		Hg		Pb		S	
		HI	THQ	HI	THQ	HI	THQ	HI	THQ	HI	THQ
Sole	A_1	0.364	1.300	<0.001	<0.001	0.379	0.352	0.012	0.014	0.757	1.666
	A_2	0.243	0.867	<0.001	<0.001	0.253	0.235	0.008	0.010	0.504	1.110
	A_3	0.159	0.569	<0.001	<0.001	0.166	0.154	0.005	0.006	0.331	0.728
	A_4	0.098	1.051	<0.001	<0.001	0.102	0.284	0.003	0.012	0.203	1.346
	A_5	0.076	0.815	<0.001	<0.001	0.079	0.221	0.003	0.010	0.157	1.044
	A_6	0.071	0.759	<0.001	<0.001	0.074	0.205	0.002	0.008	0.146	0.972
	A_7	0.066	0.710	<0.001	<0.001	0.069	0.192	0.002	0.008	0.137	0.908
	A_8	0.066	0.710	<0.001	<0.001	0.069	0.192	0.002	0.008	0.137	0.909
	A_9	0.071	0.759	<0.001	<0.001	0.074	0.205	0.002	0.008	0.146	0.972
Horse mackerel	A_1	0.231	0.825	0.011	0.002	0.308	0.286	0.014	0.016	0.564	1.129
	A_2	0.154	0.550	0.008	0.001	0.205	0.190	0.009	0.011	0.376	0.753
	A_3	0.101	0.361	0.005	0.001	0.135	0.125	0.006	0.007	0.247	0.494
	A_4	0.062	0.667	0.003	0.002	0.083	0.231	0.004	0.013	0.152	0.912
	A_5	0.048	0.517	0.002	0.001	0.064	0.179	0.003	0.010	0.118	0.708
	A_6	0.045	0.481	0.002	0.001	0.060	0.167	0.003	0.010	0.110	0.659
	A_7	0.042	0.450	0.002	0.000	0.056	0.156	0.002	0.009	0.103	0.615
	A_8	0.042	0.450	0.002	0.001	0.056	0.156	0.002	0.009	0.103	0.616
	A_9	0.045	0.481	0.002	0.001	0.060	0.167	0.003	0.010	0.110	0.659
Scorpion fish	A_1	0.132	0.471	<0.001	<0.001	0.489	0.454	0.017	0.020	0.639	0.944
	A_2	0.088	0.314	<0.001	<0.001	0.326	0.303	0.011	0.013	0.426	0.629
	A_3	0.058	0.206	<0.001	<0.001	0.214	0.199	0.007	0.009	0.279	0.413
	A_4	0.036	0.381	<0.001	<0.001	0.132	0.367	0.004	0.016	0.171	0.763
	A_5	0.028	0.296	<0.001	<0.001	0.102	0.285	0.003	0.012	0.133	0.592
	A_6	0.026	0.275	<0.001	<0.001	0.095	0.265	0.003	0.012	0.124	0.551
	A_7	0.024	0.257	<0.001	<0.001	0.089	0.248	0.003	0.011	0.115	0.515
	A_8	0.024	0.257	<0.001	<0.001	0.089	0.248	0.003	0.011	0.115	0.515
	A_9	0.026	0.275	<0.001	<0.001	0.095	0.265	0.003	0.012	0.124	0.551

HI ≥ 1, unacceptable, THQ ≥ 1, unacceptable cadmium levels were considerably below the legal limits for all three fish species studied.

The hazard index, the ratio of the weekly intake value to the PTWI value for As, Cd, Hg, and Pb calculated for different aged categories of people, was found to be well below the value of 1 (HI<1) in all three fish species (Table 4). According to the hazard index value, consumption of these fish species for the nine different age groups does not pose any health risk. Concentrations of the contaminants in food, may cause risks to the consumer in long-term consumption even if they do not exceed legal limit values. In this process, the body weight of the consumer group and the amount of food consumed are effective in the formation of health risk. Therefore, any health risk has been estimated by making THQ calculations in recent years. The THQ value being equal to or higher than 1 indicates that there is a potential health risk. In this study, the THQ values calculated for the heavy metals in selected fish species showed that arsenic-derived health risks could occur for certain groups of individuals. The total THQ values from consumption of sole have been found above 1 for the following age groups; A1 (1-3 years old children), A2 (4-6 years old children), A4 (11-14 years adolescents) and A5 (15-19 years adolescents) (Table 4). Also, the total THQ was calculated higher than 1 for the first group consuming horse mackerel. Similar to our findings, Vieira et al. 2011 calculated the THQ value for different age groups in sardine, chub and horse mackerel collected from Portuguese waters (Northeast and Eastern Central Atlantic Ocean) during one year. They reported metal concentrations below the tolerable limits adopted by the European Commission Regulation and the United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO). However, their recommendation is that the fish investigated should be consumed more carefully due to possible hazard and carcinogenic risks from arsenic. In another study, Martorell et al. (2011) reported no risk for As, Cd, Hg, and Pb for individuals of different ages and kilograms in seafood products in Catalonia.

CONCLUSIONS

The THQ results calculated for different age groups has revealed that, due to their high arsenic content, regular weekly consumption of sole and horse mackerel from the Marmara Sea poses a health risk for the young age groups - 4-19 years old. Although there is no health risk calculated for Scorpion fish and the toxic metals studied, it is important to establish local research and toxic metal monitoring policies in the Marmara Sea fishing areas. As a result, it is recommended that periodic monitoring studies be conducted to evaluate the relationship between exposure to these toxic metals and fish and seafood consumption. It is also important that the results should be shared with the community and necessary warnings should be made in a timely manner.

Conflict of interests: The authors declare that they have no conflict of interest.

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