

# Potential Health Risks of Heavy Metals to the Turkish and Greek Populations Via Consumption of Spiny Dogfish and Thornback Ray from the Sea of Marmara

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## Abstract

Spiny dogfish and thornback ray caught from Turkish waters in the northern part of the Sea of Marmara are mainly exported to Greece for human consumption. Therefore, assessing the health risks from heavy metals via consumption of these species is of importance for the Turkish and Greek consumers. The mean levels of heavy metal concentrations were below the guidelines. The weekly intakes through these species were generally lower than the limits for both communities. However, the percent of the provisional tolerable weekly intake of Hg was above 100% for the spiny dogfish, indicating a potential risk only for the Greek consumers. In general, the target hazard quotients (THQs) and total THQs of heavy metals (TTHQs) were below 1. On the other hand, for the Greek population, THQ of Hg and TTHQ indicated a potential risk due to spiny dogfish consumption. The Hazard Index (HI) was above 1 for both populations, but the HI value of the Greek population was more than three times that of the Turks. The total diet THQ (TDHQ) of Hg also indicated a potential health risk for the Greek population. Differences in consumption rates should be taken into consideration when assessing health risks for various populations.

Keywords: heavy metals, fish, target hazard quotients (THQs), hazard index (HI), provisional tolerable weekly intake (PTWI)

## Introduction

Fish is a necessary component of a balanced diet, supplying high-quality proteins, essential amino acids, and omega-3 polyunsaturated fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It is also a good source of vitamins and essential trace elements. Hence fish consumption is recommended for people of all ages (Domingo, 2007; Pieniak, Verbeke, Olsen, Hansen, & Brunso, 2010).

But, as a result of developing industry, transportation, agriculture, urbanization and other human activities, concentrations of heavy metals have increased in the aquatic environments. Toxic heavy metals have been found to accumulate especially in the muscles of predator fish species such as sharks and rays, which then gets transferred via the food chain, and may ultimately pose a significant risk to human health (Gorur, Keser, Akcay, & Dizman, 2012).



Consequently, vital organ functions may be damaged due to the chronic assimilation of heavy metals and even some type of cancers may be induced. Then Alzheimer's disease, Parkinson's disease, muscular dystrophy and multiple sclerosis are the other possible results of heavy metal exposure (Amirah, Afiza, Faizal, Nurliyana & Laili, 2013). Mercury, lead, cadmium, and arsenic have no known roles in biological systems, and they are also the most dangerous metals having adverse health effects (Bilandzic, Dokic & Sedak, 2011). Although copper and zinc are essential metals, but they too can be harmful to human health above the permitted limits (Celik & Oehlenschlager, 2004; Ikem & Egiebor, 2005).

Chondrichthyans such as sharks and rays are among the top predators, having a long life span, and also reported to accumulate high levels of trace metals (Cornish *et al.*, 2007; Eronat & Ozaydin, 2015). Thereby, pregnant women are advised to avoid high intake of sharks, considering the risk of heavy metals in edible tissues (Jarup, 2003). The rays on the other hand are basically demersal species and they may contain high levels of metals as a result of direct contact between gills and the sediment (Cronin *et al.*, 1998). Further, the dietary habit of rays is the other point of concern since they mainly feed on benthic organisms (Mormede & Davies, 2001). Thereby, metal accumulation rates can be higher in sharks and rays than in marine teleosts, and thus there is a growing concern over the heavy metal accumulation in the edible tissues of these species (Eisler, 2009).

Today, various shark species are being consumed especially in coastal regions (Adel *et al.*, 2016). With the spiny dogfish (*Squalus acanthias*) being the mostly consumed species and the European Union representing the main market of spiny dogfish, consuming about 65% of the global catch in 2001 (Fowler *et al.*, 2004). As a result of the increased landings of sharks for human consumption in Europe, so a more detailed information is needed on toxic metal concentrations in shark tissues (Storelli, Cuttone & Marcotrigiano, 2011). As for the rays, the economic potential of *Raja clavata* in Turkish waters have been noticed (Eronat & Ozaydin, 2015; Yeldan, Avşar & Manaşırılı, 2008); even though they are non-target species for Turkish fisherman, but the amount of catch has been reported as 299 tons in 2013 (Fishery Statistics, 2013). Turkey further has been reported as an important country for the Chondrichthyan fisheries in the Mediterranean/Black Sea region (Vannuccini, 1999). The Turkish fishermen who formerly caught sharks and rays as by-catch now have started turning towards exporting these species (Fowler, Raymakers & Grimm, 2004), and Greece in turn became the main importer (Vannuccini, 1999; Istanbul Exporters' Association General Secretariat, 2014).

The Sea of Marmara is regarded as one of the most important fishery grounds for the Turkish fishery sector (Fishery Statistics, 2013). But being located between the Black Sea and the Aegean Sea, the maritime traffic is very intensive. It is also surrounded by a highly populated residential area, and contaminated with heavy metals as a result of industrial, agricultural and municipal activities (Balkıs & Algan, 2005; Keskin *et al.*, 2007). Then *Squalus acanthias* and *Raja clavata* are among the fish species caught from the Sea of Marmara (Bök *et al.*, 2011). However, there is still scarce information on the heavy metal concentrations in their edible tissues. But since they are exported fishery products, therefore assessing the health risks is of utmost interests both for domestic and international markets.

It is noteworthy that some recent methods have been successfully used for the purpose of risk assessment (Amirah *et al.*, 2013). The term PTWI has been used to emphasize the importance of limiting periodical intake for contaminants



(Herrman &Younes, 1999). The THQ method on the other hand could also be used to assess possible health risk associated with heavy metals in fish.

The purpose of this study was to determine the heavy metal concentrations in *Squalus acanthias* and *Raja clavata* collected from the Sea of Marmara, and to examine possible health risks associated with their consumption by Turkish and Greek populations. Taking into account the different consumption rates ratios of these populations, it was aimed to determine possible differences in the risk levels, even if they consume the same type of fish.

## Materials and Methods

In this study, 47 thornback rays (*Raja clavata*, Linnaeus, 1758) and 38 spiny dogfish (*Squalus acanthias*, Linnaeus, 1758) were caught using gillnet from the Northern Marmara Sea (Figure 1) during 2013. The biometric parameters of thornback ray (total length:  $38.22 \pm 4.33$  cm; weight:  $1.94 \pm 0.58$  kg) and spiny dogfish (total length:  $80.88 \pm 13.07$  cm; weight:  $6.47 \pm 1.15$  kg) were measured. Then 10 g of muscle samples were dissected from each individual fish. Samples were ground and frozen in plastic cups until the day of analysis. No metal equipment was used during sampling and analysis.

For each sample, 0.3-0.5 g (wet weight) of fish muscle was weighed in a Teflon digestion vessel, then 7 ml of 65% nitric acid ( $\text{HNO}_3$ ) and 1 ml 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were added. The sample in the vessel was then subjected to a microwave digestion using the Ethos D (Type Ethos plus 1) microwave lab station as indicated below: Step 1: 25–200°C for 10 min at 1,000 W and Step 2: 200°C for 10 min at 1,000 W. The digests volume was finally made up with deionized water to 25 ml in acid washed standard flasks. The concentrations of copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) were measured by Thermo electron X7 inductively coupled plasma mass spectrometry (ICP-MS; model X series, UK). The instrument detection limits were set at 20 ppt for Cu, 100 ppt for Zn, 25 ppt for As, 2 ppt for Cd, 50 ppt for Hg and 10 ppt for Pb. The ICP-MS operating conditions were: nebulizer gas flow 0.91 L/min, radio frequency 1,200 W, lens voltage 1.6 V, cool gas 13.0 L/min, auxiliary gas 0.70 L/min. Calibration standards (High-Purity Standards, Charleston, SC, USA) were diluted and used to calibrate the ICP-MS (EPA, 1994). Catalogue numbers were ( $1000 \pm 3 \mu\text{g/ml}$  in 2%  $\text{HNO}_3$ ) Cu: 100014-1, Zn: 100068-1, As: 10003-1, Cd: 10008-1, Hg: 100033-1 and Pb: 100028-1. Certified reference material (catalogue no. SRM 2976, Gaithersburg, MD) was analyzed to validate the method for accuracy (Table 1). For statistical analyses, statistical package SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used. The analyses were carried out in triplicate, and the significance level was chosen as 0.05. Differences in heavy metal concentrations of thornback ray and spiny dogfish were determined by using the t-test.

The mean concentrations of each element were multiplied by the amount of weekly consumed fish to determine the estimated weekly intake (EWI) values. The amount of weekly fish consumption in Turkey is 148 g; while it is 568 g in Greece (Speedy 2003). The provisional tolerable weekly intake (PTWI) values of heavy metals were shown in Table 4, and they were multiplied by the average human weight, 70 kg (Kumar, Verma, Naskar, Chakraborty & Shah, 2013). Then the percent PTWI was calculated.



The Target Hazard Quotient (THQ) was used to determine health risks through thornback ray and spiny dogfish consumption. The THQ value was determined by the following equation (Chien *et al.*, 2002; Han *et al.*, 1998; Storelli 2008):

$$\text{THQ} = [(\text{EF} \times \text{ED} \times \text{FIR} \times \text{C}) / (\text{R}_{\text{FD}} \times \text{W}_{\text{AB}} \times \text{T}_{\text{A}})] \times 10^{-3}$$

In this formula,  $E_{\text{F}}$  is the exposure frequency (365 days/year),  $E_{\text{D}}$  is the exposure duration (70 years, according to Bennett, Kastenberg & McKone, 1999),  $F_{\text{IR}}$  is the food ingestion rate (21.09 g/day for Turkish, and 81.1 g/day for Greek consumers, according to Speedy, 2003),  $C$  is the metal concentration (mg/kg),  $R_{\text{FD}}$  is the oral reference dose (mg/kg),  $W_{\text{AB}}$  is the average body weight (70 kg, according to Kumar *et al.*, 2013),  $T_{\text{A}}$  is the average exposure time for noncarcinogens (365 days/year  $\times E_{\text{D}}$ ). The oral reference doses ( $R_{\text{FD}}$ ) have been presented in Table 2.

The Total Target Hazard Quotient (TTHQ) of heavy metals for individual fish was calculated using the following formula (Chien *et al.*, 2002; Zheng *et al.*, 2007; Storelli, 2008):

$$\text{TTHQ (individual fish)} = \text{THQ (toxicant 1)} + \text{THQ (toxicant 2)} + \text{THQ (toxicant } n)$$

The Hazard Index (HI) to human population due to Cu, Zn, Cd, Hg and Pb exposure through spiny dogfish and thornback ray consumption was calculated as follows (Kumar *et al.*, 2013; Zheng *et al.*, 2007):

$$\text{HI} = \text{TTHQ (spiny dogfish)} + \text{TTHQ (thornback ray)}$$

The Total Diet THQs of each metal (TDHQ) were also calculated using the following formula of Saha & Zaman, 2013:

$$\text{TDHQ (individual toxicant)} = \text{THQ (foodstuff 1)} + \text{THQ (foodstuff 2)} + \text{THQ (foodstuff } n)$$

With, the food stuffs in the present study being the thornback ray and spiny dogfish.

## Results and Discussion

The mean concentrations and ranges of Cu, Zn, As, Cd, Hg and Pb in thornback rays and spiny dogfish have been presented in Table 3. The EDI and PTWI values of thornback rays and spiny dogfish were presented in Table 4 while THQ values were shown in Table 5. Since Turkey exports a significant part of thornback ray and spiny dogfish catch to Greece, these values were calculated both for the Turkish and Greek consumers.

### Metal Concentrations in Thornback Rays and Spiny Dogfish

The maximum level of Cu in fish permitted by FAO (1983) and Maff (1995) is 30mg/kg. However, Turkish Food Codex (2002) have limited this metal as 20 mg/kg. Available literature have generally reported low concentrations of Cu in various species of sharks and rays. For instance, the mean concentrations of Cu in *Mustelus mustelus* from the Mediterranean Sea (Storelli *et al.*, 2011), *Chiloscyllium plagiosum* from Southern waters of Hong Kong (Cornish *et al.*, 2007) and *Scyliorhinus caniculus* from the Northeast Atlantic (Celik & Oehlenschlaeger, 2004) were reported as being 0.71 mg/kg, 0.15 mg/kg and 0.51 mg/kg, respectively. But Cu has not been detected in tiger sharks (*Galeocerdo cuvier*), from the coast of Ishigaki Island, (Endo, Hisamichi, Haraguchi, Kato & Ohta, 2008). Then Guerin *et al.* (2011) reported that Cu concentrations in catsharks and rays as being 0.421 and 0.174mg/kg, respectively. In this study, the reported mean concentrations of Cu in thornback rays (7.09 mg/kg) and in spiny dogfish (4.22 mg/kg) were below the permissible limits, but then in some of the rays the Cu concentration was up to 50.93 mg/kg and in spiny dogfish up



to 22.59 mg/kg. Likewise, Vas (1991) also reported Cu concentrations between 0.02–68.0 mg/kg in the shark tissues and diet was considered as the main reason for the different Cu levels in the muscle tissues.

Zinc is an essential micronutrient for human metabolism, but its excessive intake may be harmful to human health. Therefore, its maximum level has been limited to 50 mg/kg in fish (Maff, 1995; Turkish Food Codex, 2002). In this study, the Zn content of all the samples analyzed were found to be below this value. Likewise, Zn concentrations in various species of sharks (Celik & Oehlenschlager, 2004; Cornish *et al.*, 2007; Endo *et al.*, 2008; Storelli *et al.*, 2011) and rays (Jones, Mercurio & Olivier, 2000; Guerin *et al.*, 2011) from different waters have reported of values below 50 mg/kg.

Arsenic concentration in cat shark (*Galeus melastomus*) from Andalusia (Southern Spain) was reported to be 0.340 mg/kg by Olmedo *et al.* (2013). However, the average As concentration in smooth hound (*Mustelus mustelus*) shark from Langebaan Lagoon, South Africa was 28.31 mg/kg according to Bosch, O'Neill, Sigge, Kerwath and Hoffmann (2016). Thus it can be seen that there is a variation in the amount of As, in various species caught from different regions. Likewise, in this study the mean concentrations of As in thornback rays and spiny dogfish were found to be 10.08 mg/kg and 30.23 mg/kg, respectively. But on the other hand it is difficult to judge the potential health risks related to As concentration in fish, because some of the statements and guidelines takes into account the total arsenic concentration, while others are related to the inorganic form. Thus, there is no clarity in these norms (De Gieter *et al.*, 2002). Likewise, Francesconi (2007) reported typical concentrations of arsenic in fish which were between 1-20 mg/kg but did not consider any maximum permissible concentration for arsenic. Similarly, the total As concentrations in dogfish and ray have been reported to be above 20 mg/kg by De Gieter *et al.* (2002); whereby, the mean As concentration in spiny dogfish was above 30 mg/kg, and significantly higher ( $p < 0.05$ ) than that of the thornback rays. Therefore, as a result of their feeding habits, sharks may accumulate a large amount of arsenic in their tissues and they may even be used as environmental pollution indicators (Barrera-García *et al.*, 2012).

Then the maximum allowed concentration of Cd in fish is 0.05 mg/kg (EC, 2014; FAO, 1983; Turkish Food Codex, 2011), and in this case the thornback ray samples were found to contain Cd below this value. Even though some spiny dogfish had Cd concentration slightly above this limit, but the overall mean concentrations were below 0.05 mg/kg in this particular study. Likewise, low amounts of Cd have generally been reported in sharks (Eisler, 2009).

Mercury content in marine animals has been regarded as an important public health concern, and high levels of Hg in the muscle tissues may also cause recalls of shark from the market. Then considering the risk of adverse effects of methyl mercury exposure on the fetus, it is thus advised that pregnant women do not consume shark more than once per month (De Boeck *et al.*, 2010; NSW, 2001). Furthermore, earlier studies have reported the mean Hg concentrations in *Squalus acanthias*, *S. megalops* and *S. mitsukurii* from Australia (Pethybridge, Cossa & Butler, 2010), and *Mustelus mustelus* from the Mediterranean Sea (Storelli *et al.*, 2011) being above 1 mg/kg. Then Marcovecchio, Moreno and Perez (1991) reported Hg contents of various shark species from the Bahia Blanca Estuary, Argentina to be between 0.79–2.26 mg/kg. Likewise, Endo *et al.* (2008) reported Hg concentration in the muscles of various shark species to have been between 0.73-3.65 mg/kg. However, in the present study Hg concentrations of all samples were lower than the permitted limit of 1 mg/kg (EC, 2006; Turkish Food Codex, 2011). In the case of *Raja fyllae* the maximum Hg concentration has been reported to be 0.41 mg/kg (Mormede & Davies, 2001).



With regard to Pb, the maximum permitted concentration is 0.3 mg/kg (EC, 2006; Turkish Food Codex, 2011) for fish; and in this study, the mean concentrations of Pb were found to be below this value in both species.

### **Comparative Analysis of Dietary Intake of Heavy Metal Contaminated Fishes Among the Populations**

It is a common method to evaluate possible health risks associated with consumption of heavy metal contaminated fishes. But the amounts of fish consumed may be very different in various populations of the world, and the risks arising from the heavy metals in fish might also be very different. Therefore, additional information is needed to determine possible risks of various populations from the perspective of their fish consumption rates (Olmedo *et al.*, 2013).

A significant part of shark and ray species, caught from Turkish waters, are exported to Greece (Vannuccini, 1999). Turkish consumer tends to eat less fish in their diet (7.7 kg/capita/year), but the consumption rate among the Greek population (29.6 kg/capita/year) is significantly higher (Speedy, 2003). As a result of the difference in the consumption rates of these two populations, potential health risks may also be different, even if they consume the same fish. Therefore, EWI and percent PTWI values were studied both for Turkish and Greek populations; whereby, the effect of consumption rates on the possible health risks for different populations was thus determined.

The established provisional tolerable weekly intake values ( $\mu\text{g}/\text{kg}$  body weight) were suggested as 3500 for Cu, 7000 for Zn, 7 for Cd, 25 for Pb (FAO/WHO, 2004), and 1.6 for Hg (FAO/WHO, 2011). In this study, the average adult body weight was considered as 70 kg (Turkmen, Turkmen, Tepe, Tore & Ates, 2009). Then, PTWI for an adult person was calculated and compared with EWI values (Table 4). For both communities, heavy metal intakes through spiny dogfish and thornback ray consumption were generally lower than the tolerable weekly intake values. Only the percent PTWI of Hg was above 100% for the spiny dogfish, showing a potential risk for the Greek consumer. Likewise, Olmedo *et al.* (2013) reported no risk of heavy metals in fish, including blue shark and cat shark for the average consumers. But at the same time they highlighted the possibility of health risk for heavy consumers on account of excessive shark consumption. Storelli *et al.* (2011) studied the associated risk for humans due to the consumption of *Mustelus mustelus* from the Mediterranean Sea. The reported estimated weekly intakes of Cd and Pb were much lower than the specified values. Ruelas-Inzunza & Páez-Osuna (2007) studied some toxic metals in two species of sharks (*C. leucas* and *S. lewini*) from two coastal lagoons in the Eastern Gulf of California. The reported findings were below the established PTWI and there was no presumed health risk associated with shark consumption.

With regard to arsenic, only 2% of the As in fish is the toxic inorganic form, and an important part of the As in fish is mostly organic and at the same time non-toxic (EFSA, 2009). It has also been reported that the PTWI of 15  $\mu\text{g}/\text{kg}$  b.w. is no longer appropriate and withdrawn by the JECFA (2011). Similarly, Olmedo *et al.* (2013) studied the As concentrations in sharks, but did not calculate the PTWI percentages, due to the same reason.

### **Potential health risk from thornback ray and spiny dogfish consumption**

The health risks through spiny dogfish and thornback ray consumption by Turkish and Greek consumers were determined based on THQ values. The THQ values above 1 indicated that health risk associated with heavy metals exposure is a potential matter of concern (Zheng *et al.*, 2007). In this study, THQ values were generally found to be

below 1. This result shows that consumers would not encounter significant health risks from the intake of individual metals through these species. The intakes of Zn, Cu, Pb, Hg and Cd through the dietary route due to the consumption of whitecheek shark were studied by Adel *et al.* (2016). The THQ values of all these metals were reported to be below 1, which indicates no associated human health risk. Han *et al.* (1998) estimated THQs for metals through consumption of seafood in Taiwan and reported THQ values below 1 for various seafood containing Cd and Zn. The THQ of heavy metals through seafood consumption have also been reported to have been below 1 for the population from Huludao City, China (Zheng *et al.*, 2007).

Thus, it should be noted that the species, region and consumption habits are important factors to be considered when evaluating potential risks for the consumer. Heavy metal concentrations in *Sphyrna lewini* and *Caraharinus porosus* from Trinidad and Tobago were studied and THQ values were calculated by Mohammed and Mohammed (2017). They reported that the THQ values for heavy metals were above 1 for both species, showing the estimated exposure is potentially of concern. In the context of the present study, THQ of Hg was found to be 2.53 due to the spiny dogfish consumption by the Greek population (Table 5). So what can be seen is that although the mean concentrations of Hg were below recommended limits, THQ value of this metal was above 1 for the Greek consumer. Thus, it can be concluded that the elevated consumption may increase the risk, and estimated exposure could be considered as a potential concern. Similarly, THQ value of Hg was reported to have been above 1 in thornback ray, but again the mean concentration of this metal was 0.87 mg/kg (Storelli, 2008).

Then in this study, although the average arsenic concentrations seem high, but these values show the total arsenic content in muscles. This may not necessarily be a cause of worry, since it is a known fact that a significant part of the total arsenic is in non-toxic, organic form (EFSA, 2009); and even the proposed RfDo by the US EPA is for the organic arsenic only. Therefore, it would be inaccurate to calculate THQ for arsenic. Likewise, Bandowe *et al.* (2014) reported high concentrations of arsenic in the fish samples, but they also did not calculate THQ values for arsenic and at the same time reported no threat to the health of the local population.

#### **Assessment of Potential Health Risk from The Combined Effects of Metals**

It is known that exposure to more than one contaminant may cause cumulative effects (Hallenbeck, 1993). Therefore, TTHQs of heavy metals due to consumption of each species were determined. TTHQ value was determined as 2.81 for the Greek consumer, due to the consumption of spiny dogfish. This was mainly ascribed to mercury since its THQ was 2.53 (Table 5). Similarly, Storelli (2008) considered THQ of Hg (1.04) as the main cause of TTHQ value (1.06) in thornback ray. The TDHQ of Hg was also determined as 3.19, showing a potential health risk for the Greek consumer (Table 5).

Hazard Index (HI) on the other hand shows the sum of hazard quotients for multiple foodstuffs and/or exposure pathways. In this study, HIs for the Turkish and Greek consumers were found to be 1.06 and 3.54, respectively. A potential health risk was determined for both populations, but HI value was higher for the Greek consumer due to the higher consumption rates in this country. As suggested by Bandowe *et al.* (2014), health risks related to the dietary intake of heavy metals may be different for various populations; and the amount of fish consumed affect this result. Likewise, Chien *et al.* (2002) evaluated the public health risks associated with contaminants from oysters in Taiwan

and reported higher exposure of fishermen than the general population. The increased levels of seafood consumption also resulted in higher TTHQ and THQ values, in similar studies conducted by Han *et al.* (1998) and Yi, Yang and Zhang (2011).

The THQ-based risk assessment method was recently shown to be useful in indicating risk level associated with heavy metal exposure (Yi *et al.*, 2011). But, it should be remembered that only known threats are considered in this method, and the risks related to other pollutants and mutual effects of different metals, such as selenium, were ignored (Wang, Xu, Sun, Liu & Li, 2013).

### Conclusions

The present study aimed at assessing the potential health risks of heavy metals to the Turkish and Greek populations via consumption of spiny dogfish and thornback ray from the Sea of Marmara; and even though the mean concentrations of heavy metals were below the permitted limits, the percent PTWI of Hg was above 100%, showing a potential risk for the Greek consumer due to the consumption of spiny dogfish. Likewise, THQ and TTHQ values were found to be above 1 for the Greek consumers, due to the consumption of this species. Further, the TDHQ of Hg also indicated a potential health risk for the Greek consumer. A potential health risk assessment was performed using the Hazard Index (HI) for both populations. But here again it was found to be much higher for the Greek consumer. Our results revealed that there may be various health risks for different populations according to their consumption behaviors, so the consumption amounts should be taken into consideration when assessing potential risks associated with various populations. As well as monitoring programs, health risk assessment is also crucial for the heavy metal contents in seafood, especially for the species marketed internationally for human consumption.

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**Table 1** Certified, observed values (mg.kg-1) and recoveries (%) of trace metal concentrations in standard reference material

	Certified value	Observed value	Recovery (%)
Cu	4.02	4.79	119.15
Zn	137.0	138.2	100.9
As	13.30	13.91	104.59
Cd	0.82	0.87	106.10
Hg	61.00	67.81	111.16
Pb	1.19	1.28	107.56

**Table 2** The oral reference doses (R<sub>FD</sub>) for heavy metals (mg.kg-1, day) (US EPA, 2009)

R <sub>FD</sub>	Cu	Zn	Cd	Hg	Pb
(mg.kg-1, day)	$4 \times 10^{-2}$	$3 \times 10^{-1}$	$1 \times 10^{-3}$	$1.6 \times 10^{-4}$	$4 \times 10^{-3}$

**Table 3** Range and mean concentrations (mg.kg-1 wet weight) of heavy metal for each species

Metal		Thornback ray	Spiny dogfish
Cu	min.-max.	0.83-50.93	0.90-22.59
	mean ± SD	7.09 ± 9.41 <sup>a</sup>	4.22±4.57 <sup>a</sup>
Zn	min.-max.	5.88-29.13	6.66-49.38



	mean ± SD	13.22 ±4.72 <sup>a</sup>	18.32 ±10.48 <sup>b</sup>
As	min.-max.	3.79-21.24	3.98-86.62
	mean ± SD	10.08 ±4.45 <sup>a</sup>	30.23 ±22.04 <sup>b</sup>
Cd	min.-max.	0.00-0.01	0.00-0.07
	mean ± SD	0.00±0.00 <sup>a</sup>	0.01±0.01 <sup>b</sup>
Hg	min.-max.	0.02-0.54	0.04-0.81
	mean ± SD	0.07±0.10 <sup>a</sup>	0.35±0.17 <sup>b</sup>
Pb	min.-max.	0.05-1.83	0.04-1.21
	mean ± SD	0.28±0.40 <sup>a</sup>	0.27±0.26 <sup>a</sup>

<sup>a,b</sup>: Different letters in the same row show statistical differences ( $P < 0.05$ ).

**Table 4** The estimated weekly intakes (EWI) and percent PTWI's for ray and shark, consumed by Turkish and Greek adults

Metals	PTWI*	PTWI**	Thornback ray				Spiny dogfish			
			Turkish consumer		Greek consumer		Turkish consumer		Greek consumer	
			EWI	Percent PTWI	EWI	Percent PTWI	EWI	Percent PTWI	EWI	Percent PTWI
Cu	3500 <sup>a</sup>	245000	1049.32	0.43	4027.12	1.64	624.56	0.25	2396.96	0.98
Zn	7000 <sup>a</sup>	490000	1956.56	0.40	7508.96	1.53	2711.36	0.55	10405.76	2.12
Cd	7 <sup>a</sup>	490	0.00	0.00	0.00	0.00	1.48	0.30	5.68	1.16
Hg	1.6 <sup>b</sup>	112	10.36	9.25	39.76	35.50	51.80	46.25	198.80	177.50
Pb	25 <sup>a</sup>	1750	41.44	2.37	159.04	9.09	39.96	2.28	153.36	8.76

\* Provisional Tolerable Weekly Intake in  $\mu\text{g}/\text{kg}$  body weight

\*\* PTWI for a 70 kg adult ( $\mu\text{g}/\text{week}$  per person)

EWI= Estimated Weekly Intake ( $\mu\text{g}/\text{week}$  per person)

<sup>a</sup> FAO/WHO, 2004

<sup>b</sup> FAO/WHO (2011)

**Table 5** Estimated Target Hazard Quotients (THQs) for metals caused by consuming shark and ray by different populations

Metal	Turkish consumer			Greek Consumer		
	Thornback ray	Spiny dogfish	TDHQ	Thornback ray	Spiny dogfish	TDHQ
Cu	0.05	0.21	0.26	0.03	0.12	0.15
Zn	0.01	0.05	0.06	0.02	0.07	0.09
Cd	0.00	0.00	0.00	0.00	0.01	0.01
Hg	0.13	0.51	0.64	0.66	2.53	3.19
Pb	0.02	0.08	0.10	0.02	0.08	0.10
TTHQ	0.21	0.85	1.06(HI)	0.73	2.81	3.54(HI)

TDHQ=The Total Diet THQ of each metal

TTHQ= Total THQ of heavy metals due to the consumption of each species

HI= Hazard Index, the sum of TTHQs



Figure 1.