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Metals in sardine and anchovy from Greek coastal areas: Public health risk and nutritional benefits assessment

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ABSTRACT

Concentrations of 26 metals and elements were measured in sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) sampled from 6 Greek coastal areas in order to assess public health risks and benefits. Nutritional benefits seem to outweigh the potential risks arising from fish metal content, since various parameters (Safety Standards, Estimated Daily Intake, Maximum Safe Consumption, Hazard Quotient, Metal Pollution Index, As Carcinogenic Risk, Mercury toxicity – Selenium benefits, Nutrient Reference Values) indicated mostly safe consumption of the studied species. Weekly consumption of 480.76 g of sardine and anchovy poses minor risks (due to increased levels of essential metals like Fe and Zn in some cases) but great benefits regarding intake of essential elements like Mg, Fe, Cu, Zn, Mo, Ca, P, Se. The traces of inorganic As detected were well below all safety limits. Hg toxicity symptoms are not likely to appear and Se benefits are not likely to be compromised.

1. Introduction

Human intake of both essential and toxic metals and elements occur via diet, water consumption and respiration, diet being the main pathway (Olmedo et al., 2013a; Nadal et al., 2008; Castro-Gonzalez and Mendez-Armenta, 2008). Fish and seafood have been reported to be the food group having the highest concentration of various metals (Nadal et al., 2008; Falco et al., 2006). Long term consumption of foodstuff polluted with certain metals may lead to their accumulation in vital organs with potential to disrupt critical biochemical processes (Renieri et al., 2014). Elevated levels of toxic metals such as As, Hg, Pb, Cd can damage the brain, kidneys, lung, liver and developing fetus, have hematological and immunological effects, cause skeletal damage, cardiovascular and neurological disorders, carcinogenesis, impairment of cognitive and behavioural development and chronic or acute diseases (Renieri et al., 2014; Afonso et al., 2013; Vieira et al., 2011; Castro-Gonzalez and Mendez-Armenta, 2008; Copat et al., 2013; USEPA/USFDA, 2017a; Copat et al., 2018). Even essential metals and elements such as Se, Zn, Mn may have toxic health effects at elevated levels (Nadal et al., 2008; Yildirim et al., 2009; Copat et al., 2018). Fish can also bioaccumulate other toxic pollutants such as organochlorines (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) (Bayarri et al., 2001; Guerranti et al., 2016; Stagakis et al., 2016; Perelló et al., 2015).

On the other hand, despite the risks involved, fish consumption is considered indispensable for a well-balanced diet since the benefits it provides to human health are of utmost importance. It has been argued that the benefits of a diet rich in seafood by far outweigh the risks (Lund, 2013; USFDA, 2009; FAO/WHO, 2011; Larsen et al., 2011). Fish have been acknowledged to be a rich source of polyunsaturated fatty acids (PUFAs), ω-6 linolenic acid (LA), ω-3 a-linolenic acid (ALA), eicosapentaenoic acid (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:6n-3) that provide energy, have anti-inflammatory and anticarcinogenic properties, contribute to normal neurodevelopment, better cognition and school performance in children, lower the risk of coronary heart disease, high blood pressure, artherosclerosis, stroke, rheumatoid arthritis, lung disease and psychiatric disorders (Cardoso et al., 2018; Kalogeropoulos et al., 2012; Renieri et al., 2014; Olmedo et al., 2013b; Vieira et al., 2011; Storelli and Barone, 2013; Castro-Gonzalez and Mendez-Armenta, 2008; Hoekstra et al., 2013; Lund, 2013; Larsen et al., 2011; Domingo et al., 2007). Furthermore, fish contain proteins of high biological value, carbohydrates, vitamins (A, D, E, B12), important amino acids, Se and other essential micro and

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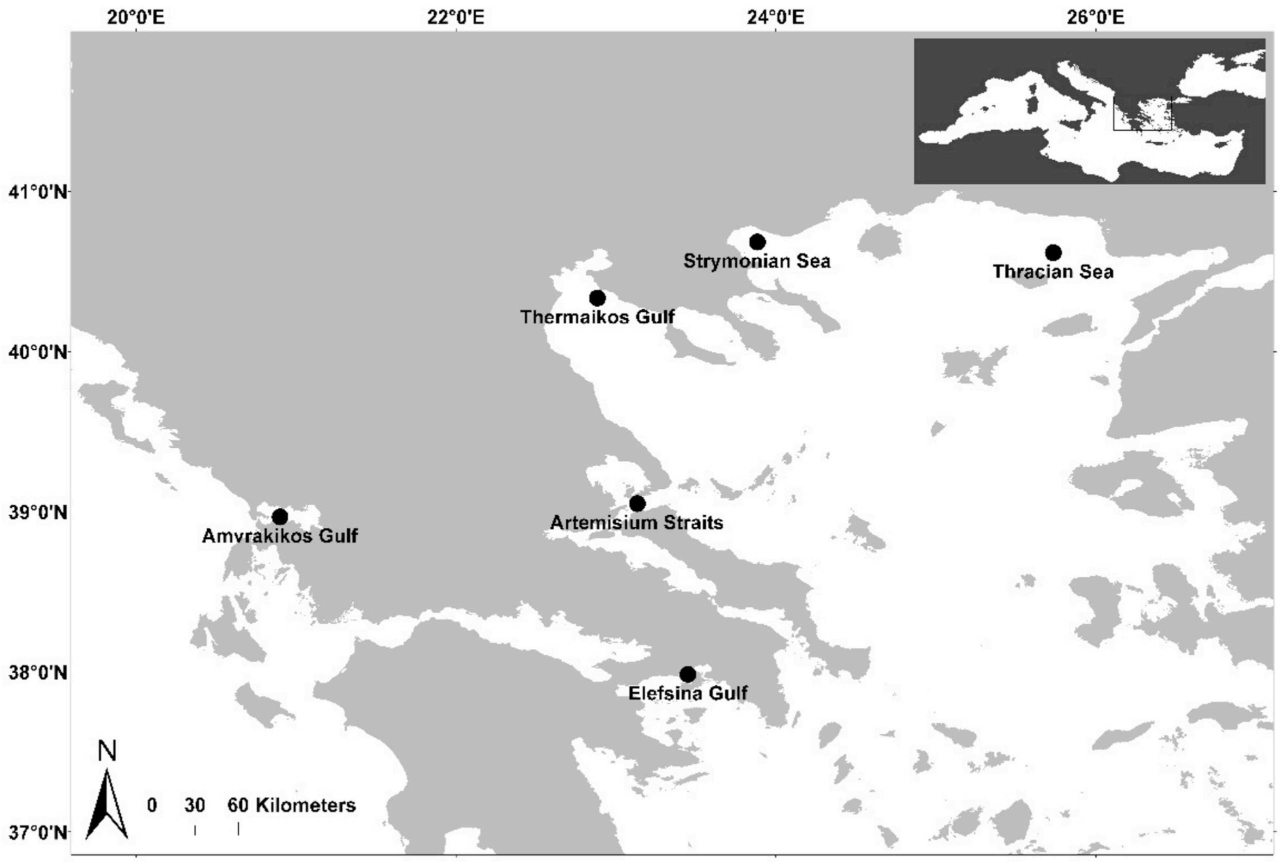

Fig. 1. Sampling sites in Greece, Eastern Mediterranean Sea.

macronutrients that play a vital role in human diet, sustainment of good health, disease prevention and health improvement (USEPA/USFDA, 2017a; Kalantzi et al., 2016; Kalogeropoulos et al., 2012; Renieri et al., 2014; Olmedo et al., 2013b; Vieira et al., 2011; Storelli and Barone, 2013; Castro-Gonzalez and Mendez-Armenta, 2008; Hoekstra et al., 2013; Lund, 2013; Larsen et al., 2011; Domingo et al., 2007). Fish also contain bioactive components such as taurine, phytosterols, antioxidants and phospholipids, that regulate cellular and molecular processes leading to enhanced physiological function (Larsen et al., 2011) and essential elements (e.g. Fe, Co, Cu, Zn, Ni, Mo, Cr, Mg, Se, V, P, Ca) that are important for skeletal structure, enzyme activation and acid-base equilibrium regulation (Afonso et al., 2013).

However, it has been suggested that the positive effect of ω-3 fatty acids can be reduced by toxic metals (Castro-Gonzalez and Mendez-Armenta, 2008) while health risks can be reduced by metabolic interactions between essential and toxic metals such as the interactions reported between Se and Hg, Pb, Cd and between Cd and Pb and essential elements like Zn, Fe, Ca (Afonso et al., 2013; Renieri et al., 2014; Copat et al., 2014; Castro-Gonzalez and Mendez-Armenta, 2008). Therefore, both risks and health benefits from fish consumption should be considered when assessments are conducted.

Specifically, when it comes to population consuming large quantities, monitoring in fish should be conducted on a regular basis regarding a wide range of species and sites (polluted and non-polluted), a wide range of potential pollutants (organic and metal), considering also essential nutrients, PUFAs, seasonal variations and the exposure of pregnant women and children while investigating as many assessment parameters as possible. Forming such a database would provide important feedback for the public policy that monitors fish consumption. From a regulatory point of view, as far as metals and elements are concerned, intake limits have been established by national and international authorities in order to protect public health (USEPA, 2017; European Union, 2011a, 2014, 2015) while daily reference intakes for essential elements have been established by the European Union (2011b) and recommendation regarding risks and benefits of fish and seafood consumption has been issued by the European Food Safety Authority (EFSA, 2015). The USEPA/USFDA have also established specific amounts of fish (according to species) that are recommended to be consumed by pregnant and breastfeeding women and children (USEPA/USFDA, 2017a).

The objective of this study is to assess the public health risks and benefits deriving from the total metal content (both toxic and essential metals and elements) of two of the most widely consumed fish species in Mediterranean countries: anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) (Kalogeropoulos et al., 2012; Olmedo et al., 2013b; Copat et al., 2012; Nadal et al., 2008; Galitsopoulou et al., 2012). In Greece, risk assessment studies have focused mainly on organic pollutants or the ones investigating metal pollutants have mainly focused on other species (e.g. Vassiliadou et al., 2015; Stagakis et al., 2016; Kalantzi et al., 2016, 2013; Giannakopoulou and Neofitou, 2014; Milatou et al., 2015) while to our knowledge, data regarding health risk due to metal pollutants in sardines and anchovies from Greek marine waters are scarce and refer to a limited number of metals (e.g. Kalogeropoulos et al., 2012; Galitsopoulou et al., 2012). This study has been an attempt to provide a complete screening of major and trace elements (Li, Na, Mg, P, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Pd, Cd, Cs, Ba, Hg, Tl, Pb, U) in the edible tissues of sardine and anchovy with a view to assessing the risks and benefits involved. Fish were sampled from several Greek coastal areas with varying pollution load, several assessment parameters were considered, assessment of inorganic As was conducted, the Hg—Se balance was explored and the exposure of pregnant women was discussed.

2. Materials and methods

2.1. Samples collection and pretreatment

Ninety samples of sardine (*S. pilchardus*) and ninety samples of anchovy (*E. encrasicolus*) (gonad stage 1) were collected from six coastal areas in Greece that have maximum stock density, receive different pressures due to anthropogenic activity and have been assessed to have different ecological status (Simboura et al., 2016; Pavlidou et al., 2015). These are Thermaikos Gulf, Elefsina Gulf, Thracian Sea, Strymonian Gulf, Artemisium Straits and Amvrakikos Gulf (Fig. 1). The sampling took place at the same time and date for both species at each site but varied slightly among sites (within one month: beginning of September till beginning of October). The fish were of commercial size: total length was115.7 ± 8.8 mm for sardine and 101.4 ± 11.0 mm for anchovy and body weight was 11.6 ± 2.8 g for sardine and 6.0 ± 1.9 g for anchovy. After fish were beheaded and gutted, edible parts (skin, muscle and spine) were removed and stored at −20 °C until laboratory analysis. Freeze-drying (freeze-drier Telstar Cryodos) and homogenization (Grindomix GM200 Retsch) followed. There were 15 individuals per species and site out of which three composites (consisting of five fish homogenized together) were created per species and site. Chemical analysis was conducted in triplicate for each composite (nine replicates per species and site). In order to assess the public health risk, all edible parts (skin, muscle and spine) were included in the analysis since it is quite common the whole body of sardine and anchovy to be consumed by humans. Such methodology has been previously reported in the literature (Falco et al., 2006; Nadal et al., 2008; Perugini et al., 2009; Copat et al., 2014; Zotos and Vouzanidou, 2012).

2.2. Chemical analysis

Concentrations of metals and other elements were determined using a modified version of the USEPA method 3052 (1996) for microwave-assisted acid digestion of siliceous and organically based matrices. Predigestion (0.2506 ± 0.0006 g per replicate and 5 ml HNO_3) was conducted in a sandbath (Combiplac Selecta) for an hour at 120 °C. 2 ml of H_2O_2 were added and digestion in a closed, high pressure, microwave system (Multiwave 3000, Anton Paar, Austria) was conducted for 75 min (up to 200 °C). Metal concentrations in the sample digests were measured with an Inductively Coupled Plasma – Mass Spectrometer (ICP–MS NexION300, PerkinElmer, Shelton, CT, U.S.) (USEPA method 6020A, 2007). Samples were diluted 600 times. Indium and Bismuth (10 μg L^{-1}) were added as internal standards to each sample and calibration standard. Metals and elements concentrations were expressed in wet weight (ww). The above-mentioned protocols are described in detail in Sofoulaki et al. (2018).

Quality control assurance was provided by analysing with every 6 samples digested one blank sample and one sample of internationally certified reference materials: DORM-4 and LUTS-1 certified by the Joint Research Centre of European Commission and BCR-668 certified by the National Research Council of Canada. Mean recovery of all elements in DORM-4 (fish protein) was 96.3 ± 9.0% (n = 7); in LUTS-1 (non-defatted lobster hepatopancreas) was 98.9 ± 8.6% (n = 6); and in BCR-668 (mussel tissue) was 105.6 ± 9.2% (n = 6). The limits of detection (LOD) were calculated by multiplying the standard deviation of the blanks (n = 19) by three and were: 0.02 (Li); 3.77 (Mg); 22.51 (P); 66.61 (Ca); 0.03 (V); 14.11 (Fe); 0.007 (Co); 0.05 (Ni); 2.68 (Cu); 7.53 (Zn); 0.05 (As); 0.09 (Se); 0.16 (Sr); 0.006 (Mo); 0.003 (Cd); 0.15 (Ba); 0.004 (Hg); 0.17 (Pb) 0.002 (U) mg/kg dry weight. If concentrations were below the detection limit, they were set equal to LOD/2.

2.3. Human health risk assessment and nutritional assessment analysis

In order to assess the human health risks and benefits of dietary metal exposure, several parameters were considered. These are described in detail below. Mean metal and element concentrations per species and site were used in all calculations.

It should be noted that in all risk assessment estimations (Safety Standards, Estimated Daily Intake, Maximum Safe Consumption, Hazard Quotient, Metal Pollution Index and As-Carcinogenic Risk) only the potentially toxic, inorganic form of As was taken into account. Initially, a content of 3% of the total As was assumed present in inorganic form (NSW FA, 2010; FSA, 2004; Copat et al., 2013, 2018; Cava-Montesinos et al., 2005). Afterwards, As speciation analysis was conducted (results were published in Kalantzi et al., 2017) in order to test this hypothesis and the risk deriving from the actual inorganic As detected was assessed using all the above mentioned parameters.

Regarding Hg, the worst case scenario was considered in all risk assessment estimations. Specifically, the total Hg content was assumed to be in its toxic organic methylated form (MeHg). The same assumption has been made by Nadal et al. (2008) while Olmedo et al. (2013b) detected more than 99.9% of total Hg in its MeHg form in some species. The European Food Safety Authority (EFSA, 2004) recommends considering more than 90% of Hg in its organic methylated form when conducting risk assessments.

2.3.1. Safety standards

Mean concentrations per species and site were compared with permitted limits for edible fish tissues established by different national or international authorities. These Safety Standards are 0.30 mg/kg ww for Pb, 0.25 mg/kg ww for Cd, 0.5 mg/kg ww for Hg, 30 mg/kg ww for Cu, 30 mg/kg ww for Zn, 1.0 mg/kg ww for Ni, 6.8 mg/kg ww for Se, 95 mg/kg ww for Ba, 10.2 mg/kg ww for Fe and 1.3 mg/kg ww for As (Kalantzi et al., 2013; European Union, 2011a, 2014, 2015).

2.3.2. Estimated daily intake (EDI)

Metals and elements Estimated Daily Intake (EDI; μg/kg bw/day) was calculated according to the following equation (Onsanit et al., 2010; Kalantzi et al., 2013; Copat et al., 2014):

$$EDI = [\ C \times AvC\ ]\ /\ bw$$

where C (μg/g ww of fish) is the mean metal concentration in the edible part of the fish (skin, flesh and bone) for each species per site, AvC is the average consumption of g fish per day and bw is the adult body weight of the general population. An AvC of 68.68 g/day of fish in Greece and an average bw of the general population of 70 kg were assumed (FAO, 2005–2012).

These EDI values were compared with Reference Doses (RfD) as established by the USEPA (2017). The RfD is an estimation (with an uncertainty up to one order of magnitude) of the daily exposure of the population due to ingestion which is unlikely to produce any (non-carcinogenic) effects, even in vulnerable population groups during a lifetime (USEPA, 2012). These Reference Doses are 2.0 μg/kg bw/d for Li, 5.0 μg/kg bw/d for V, 700 μg/kg bw/d for Fe, 0.3 μg/kg bw/d for Co, 20 μg/kg bw/d for Ni, 40 μg/kg bw/d for Cu, 300 μg/kg bw/d for Zn, 0.3 μg/kg bw/d for As (inorganic), 5.0 μg/kg bw/d for Se, 600 μg/kg bw/d for Sr, 5.0 μg/kg bw/d for Mo, 1.0 μg/kg bw/d for Cd, 200 μg/kg bw/d for Ba, 0.1 μg/kg bw/d for Hg (MeHg), 0.2 μg/kg bw/d for U (USEPA, 2017). Regarding Pb, since an RfD value was not set by USEPA in 2017, a previously established value by USEPA (2014) was used, that is 3.57 μg/kg bw/d.

2.3.3. Maximum Safe Consumption (MSC_A)

The Maximum Safe Consumption (MSC_A, kg fish ww/day) indicates a maximum level for safe daily consumption of fish due to the potential toxicity from an individual element. MSC_A was estimated for metals and elements that have established RfD following the equation (Metian et al., 2013; Kalantzi et al., 2016):

$$MSC_A = \frac{[bw\ x\ RfD]}{[C\ \ x\ 1000]}$$

where C (μg/g ww of fish) is the mean metal concentration in the edible part of the fish for each species per site, bw is the adult body weight of the general population (70 kg; FAO, 2005–2012) and RfD is the reference dose.

2.3.4. Hazard quotient (HQ)

The Hazard Quotient (HQ, dimensionless) was estimated for each metal and element that has an established RfD value, by dividing the EDI by the RfD. Values below 1 indicate that it is unlikely for even a sensitive population to experience adverse health effects. Values for an element higher than 1 indicate a higher probability for potential long term non-carcinogenic effects due to this element. For the overall health risk due to all metals and elements in an individual fish, the Total Hazard Quotient (THQ) was estimated per species and site as the sum of the respective HQs (Vieira et al., 2011; Copat et al., 2013; Storelli and Barone, 2013; Kalogeropoulos et al., 2012; Onsanit et al., 2010; Yang et al., 2013; Kalantzi et al., 2016). The contribution (%) of the Hazard Quotient (HQ) of each metal and element to the Total Hazard Quotient (THQ) per site and species was also estimated.

2.3.5. Metal pollution index (MPI)

The Metal Pollution Index (MPI, mg/kg ww) is an indication of the total elemental and metal pollution in an individual fish and can be used as a comparative means of different species or sites. In the present study the total elemental and metal content of the two species (Li, Na, Mg, P, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Pd, Cd, Cs, Ba, Hg, Tl, Pb, U) in each site is compared. It is estimated according to the following equation:

$$MPI = (C_1 \times C_2 \ldots \times C_n)^{1/n}$$

where C_i is the concentration of metal i in the sample (mean concentrations per species and sites were used), n is the number of metals and elements (Usero et al., 2005; Ibrahim and El-Regal, 2014; Kalantzi et al., 2016). The higher the MPI the greater the pollution level for a given site or species (Rodriguez-Barroso et al., 2009).

2.3.6. As Carcinogenic risk

The inorganic As is considered likely to cause carcinogenesis (Onsanit et al., 2010; FSA, 2009; Afonso et al., 2013). For the incremental probability of an individual developing cancer over a lifetime due to incremental As ingestion, the As Carcinogenic Risk (As-CR) was estimated according to the following equation:

$$As\text{-}CR = [\ EDI \times CSF\ ] / 1000$$

where As-CR is dimensionless, EDI (μg/kg bw/day) is the Estimated Daily Intake and CSF is the oral cancer slope factor for inorganic As, equal to 1.5 $(mg/kg/day)^{-1}$ as set by USEPA (2012). The exposure frequency was assumed to be 365 days per year and the exposure duration 70 years. The acceptable risk levels for carcinogens range from 10^{-4} to 10^{-6}. Exceeding these levels indicates a probability of an individual developing cancer greater than 1 in 10,000 and 1 in 1,000,000, respectively. Lower levels are considered to be safe with respect to carcinogenic risk (Vieira et al., 2011; Copat et al., 2013; Nadal et al., 2008; Kalantzi et al., 2016).

2.3.7. Mercury toxicity – selenium benefits

The Hg toxicity was assessed by determining the Se- Hg balance as Se is known to provide mitigation to Hg toxicity. Hence, the molar ratio of selenium to mercury and the selenium health benefit value (Se-HBV, mole) were estimated. Se molar excess (Se:Hg ratio exceeding unity) indicates fish consumption safety. Positive values of the Se-HBV indicate beneficial effects while negative indicate health risks. The magnitude of the values is proportional to the benefits or risks. Se-HBV was estimated using mean concentrations of Se and Hg per species and site according to the following equation (Olmedo et al., 2013a; Copat et al., 2014; Kalantzi et al., 2016; Kaneko and Ralston, 2007; Polak-Juszczak, 2015):

$$Se\text{-}HBV = [\ (\mu mol\ Se / kg\ fish) \times (Se / Hg)\ ] - [\ (\mu mol\ Hg / kg\ fish) \times (Hg / Se)\ ]$$

2.3.8. Nutritional value

In order to assess the nutritional benefits arising from the fish metal and elemental content, the mean concentrations of essential nutrients per species and site were compared with minimum dietary requirements that should be met for each element so that good health is maintained (Olmedo et al., 2013a; Kalantzi et al., 2013). Daily reference intakes for vitamins and minerals (Nutrient Reference Values, NRVs) have been established by the European Union (2011b). A foodstuff is considered to contain a significant amount of vitamins and minerals if 15% of the NRVs are supplied by 100 g of the foodstuff (European Union, 2011b).

3. Results

Metal and elemental concentrations in the edible parts of sardine and anchovy (skin, muscle and spine) from the studied sites are provided in Fig. 2. The estimations of the human risk and the respective nutritional benefits deriving from the metal and elemental content of the fish are provided in Table 1 – 4. The assessment results are presented below for each parameter investigated. Specifically, the assessment results of the risk from the actual inorganic As detected are presented in Table 2. According to Kalantzi et al. (2017), inorganic As(V) was detected only in traces in Artemisium Straits (0.009 mg/kg ww in sardine and 0.015 mg/kg ww in anchovy) while no As(III) was detected in any of the sites studied.

3.1. Safety standards

Mean concentrations per species and site of Pb, Cd, Hg, Cu, Ni, As, Se and Ba were well below the Safety Standards established while mean concentrations of Fe and Zn in some cases exceeded them. Mean Fe concentrations (14–31 mg/kg), were found for both species and all sites to be higher than the permitted limit of 10.2 mg/kg. Mean Zn concentrations in anchovy from Elefsina Gulf and Amvrakikos Gulf (32 and 48 mg/kg, respectively) exceeded the permitted limit of 30.0 mg/kg. Mean Zn concentrations in sardine from Elefsina Gulf and anchovy from Artemisium Straits and Thermaikos Gulf were similar to the abovementioned limit (29.32, 29.44 and 29.33 mg/kg, respectively), while the respective maximum concentrations exceeded the limit (ranging from 30.39 to 33.29 mg/kg). The detected As(V) is below the Safety Standard set for inorganic As (Table 2). Even if a content of 3% of the total As was assumed to be in its toxic inorganic form (that would be 0.13–0.81 mg/kg considering sardines and anchovies from all sites), the safety limit of 1.3 mg/kg would not be exceeded.

3.2. Estimated daily intake

The Estimated Daily Intake (EDI) of Li, V, Fe, Co, Ni, Mo, Cd, Ba, Cu, Zn, Se, Sr, Hg, Pb and U was found in all cases lower than the established Reference Doses (RfD) (Table 1). The EDI of the detected As (V) also remains well under the established RfD for As (Table 2). It is noteworthy that if a content of 3% of the total As was assumed to be in its toxic inorganic form, the EDI of both species from Elefsina Gulf would exceed the RfD of 0.3 μg/kg bw/day (0.49 for sardine and 0.79 μg/kg bw/day for anchovy; Table 1).

3.3. Maximum safe consumption

The Maximum Safe Consumption (MSC_A, kg fish ww/d) estimated,

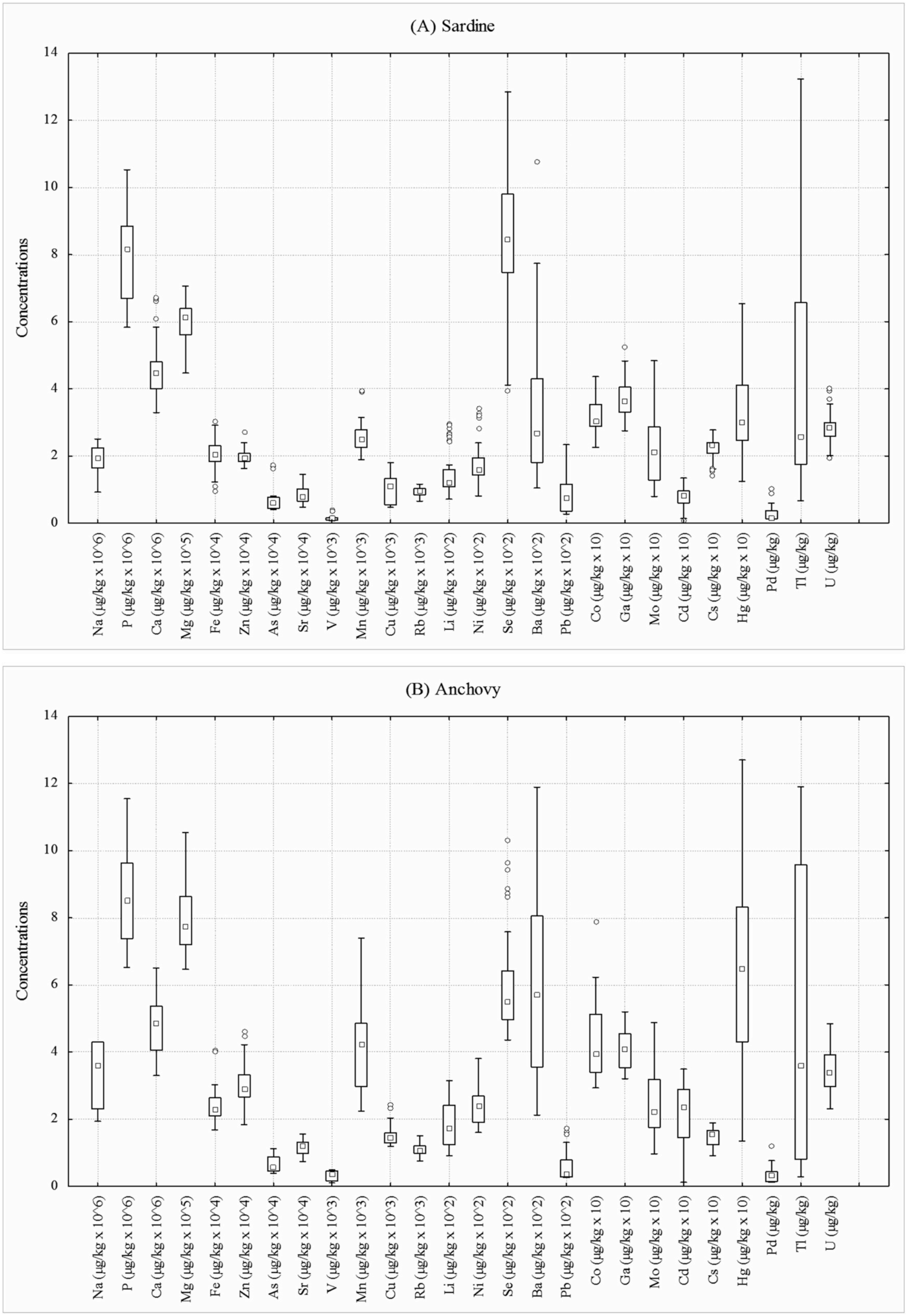

Fig. 2. Box-Whiskers plots of metal and elemental concentrations (wet weight) in edible parts of sardine and anchovy (skin, muscle and spine) from 6 sites in Greece (n = 90 individuals per species).

thus the maximum amount of fish (kg) a 70 kg person can safely consume in a day, was found to be high enough to ensure human health as far as Li, V, Fe, Co, Ni, Mo, Cd, Ba, Cu, Zn, Se, Sr, As, Pb and U are concerned (Table 1). However, Hg mean concentrations in some cases result in quite low values of Maximum Safe Consumption: more than 70–150 g of anchovy from 5 out of the 6 sites studied and more than

Table 1
Estimation of the human risk deriving from the consumption of sardine and anchovy due to their metal content. Fish were sampled from 6 sites in Greece (n = 15 per species and site).

Species	Sites	Hazard Quotient (HQ) ($\times 10^{-1}$)																THQ[a]	MPI[b]
		Li	V	Fe	Co	Ni	Cu	Zn	As (3%)	Se	Sr	Mo	Cd	Ba	Hg	Pb	U		
Sardine	**THE**	1.34	0.65	0.33	1.28	0.15	0.23	0.70	4.38	1.55	0.22	0.06	0.11	0.03	2.53	0.33	0.16	1.40	0.76
	STR	0.58	0.28	0.27	0.97	0.08	0.30	0.59	4.34	1.87	0.16	0.03	0.10	0.01	1.77	0.21	0.14	1.17	0.57
	THR	0.57	0.31	0.27	0.90	0.06	0.22	0.61	4.28	1.82	0.14	0.02	0.09	0.01	2.62	0.18	0.15	1.22	0.56
	ELE	0.40	0.16	0.37	1.19	0.11	0.38	0.96	16.42	2.27	0.11	0.04	0.02	0.01	5.79	0.55	0.13	2.89	0.64
	ART	0.92	0.30	0.31	1.04	0.09	0.22	0.62	6.16	1.51	0.13	0.05	0.08	0.02	3.59	< 0.09	0.15	1.53	0.59
	AMV	0.60	0.17	0.19	0.92	0.06	< 0.13	0.63	7.27	0.96	0.08	0.07	0.06	0.01	3.64	< 0.10	0.12	1.50	0.46
Anchovy	**THE**	1.38	3.68	0.31	1.41	0.13	0.35	0.96	4.71	0.97	0.20	0.04	0.30	0.04	6.17	0.23	0.20	2.11	0.89
	STR	0.67	0.70	0.26	1.03	0.09	0.31	0.66	4.16	0.98	0.15	0.03	0.21	0.03	3.29	< 0.11	0.14	1.28	0.65
	THR	0.71	0.82	0.33	1.14	0.09	0.34	0.87	4.51	1.00	0.15	0.03	0.24	0.02	4.61	< 0.08	0.13	1.51	0.69
	ELE	0.53	0.23	0.37	1.44	0.12	0.49	1.06	26.44	1.70	0.21	0.05	0.03	0.04	9.39	0.34	0.18	4.26	0.76
	ART	1.20	0.81	0.33	1.72	0.14	0.34	0.96	6.21	1.12	0.21	0.06	0.30	0.04	7.21	< 0.08	0.17	2.09	0.75
	AMV	1.00	0.33	0.43	1.64	0.12	0.40	1.56	9.12	1.26	0.20	0.08	0.17	0.01	7.28	< 0.13	0.18	2.39	0.77
		Estimated Daily Intake (EDI) (μg/kg bw/day) ($\times 10^{-1}$)																**As-CR[c] ($\times 10^{-4}$)**	
		Li	**V**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As (3%)**	**Se**	**Sr**	**Mo**	**Cd**	**Ba**	**Hg**	**Pb**	**U**		
Sardine	**THE**	2.68	3.24	228	0.38	2.95	9.17	209	1.31	7.73	133	0.28	0.11	6.28	0.25	1.16	0.03	2.0	
	STR	1.15	1.42	190	0.29	1.55	11.93	176	1.30	9.37	95	0.13	0.10	2.58	0.18	0.75	0.03	2.0	
	THR	1.14	1.55	186	0.27	1.23	8.78	182	1.28	9.12	83	0.09	0.09	2.90	0.26	0.63	0.03	1.9	
	ELE	0.80	0.81	261	0.36	2.20	15.40	288	4.92	11.34	67	0.22	0.02	1.74	0.58	1.97	0.03	7.4	
	ART	1.85	1.49	218	0.31	1.82	8.96	185	1.85	7.53	80	0.23	0.08	4.15	0.36	< 0.32	0.03	2.8	
	AMV	1.19	0.86	135	0.28	1.29	< 5.38	189	2.18	4.80	49	0.33	0.06	1.53	0.36	< 0.35	0.02	3.3	
Anchovy	**THE**	2.77	18.40	215	0.42	2.61	13.82	288	1.41	4.83	123	0.20	0.30	8.61	0.62	0.80	0.04	2.1	
	STR	1.33	3.50	185	0.31	1.85	12.22	199	1.25	4.90	90	0.17	0.21	5.13	0.33	< 0.39	0.03	1.9	
	THR	1.42	4.12	231	0.34	1.82	13.72	261	1.35	4.98	91	0.13	0.24	3.35	0.46	< 0.29	0.03	2.0	
	ELE	1.06	1.16	258	0.43	2.42	19.60	319	7.93	8.51	124	0.27	0.03	7.77	0.94	1.22	0.04	11.9	
	ART	2.39	4.07	229	0.52	2.90	13.42	289	1.86	5.62	128	0.28	0.30	7.25	0.72	< 0.27	0.03	2.8	
	AMV	2.00	1.63	301	0.49	2.38	15.81	469	2.74	6.32	119	0.42	0.17	2.87	0.73	< 0.45	0.04	4.1	
		Maximum Safe Consumption (MSC_A) (kg fish (wet weight)/day)																**Se:Hg[d]**	**Se-HBV[e]**
		Li	**V**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As(3%)**	**Se**	**Sr**	**Mo**	**Cd**	**Ba**	**Hg**	**Pb**	**U**		
Sardine	**THE**	0.51	1.06	2.11	0.54	4.66	3.00	0.99	0.16	0.44	3.10	12.1	6.51	21.9	0.27	2.11	4.3	77.6	774.6
	STR	1.19	2.42	2.52	0.71	8.86	2.30	1.17	0.16	0.37	4.33	26.5	6.96	53.3	0.39	3.27	4.9	134.7	1628.6
	THR	1.20	2.21	2.59	0.76	11.2	3.13	1.13	0.16	0.38	4.95	38.5	7.97	47.3	0.26	3.87	4.5	88.4	1041.3
	ELE	1.72	4.24	1.84	0.58	6.23	1.78	0.71	0.04	0.30	6.13	15.4	37.3	78.7	0.12	1.24	5.5	49.7	727.8
	ART	0.74	2.31	2.21	0.66	7.54	3.07	1.12	0.11	0.46	5.17	14.7	8.74	33.1	0.19	> 7.63	4.6	53.3	517.7
	AMV	1.15	4.00	3.56	0.75	10.7	> 5.11	1.09	0.09	0.72	8.43	10.3	10.7	89.6	0.19	> 7.09	5.8	33.4	207.0
Anchovy	**THE**	0.50	0.19	2.24	0.49	5.27	1.99	0.72	0.15	0.71	3.36	17.0	2.32	15.9	0.11	3.05	3.4	19.9	124.2
	STR	1.03	0.98	2.60	0.67	7.44	2.25	1.04	0.16	0.70	4.56	20.5	3.31	26.8	0.21	> 6.28	4.8	37.8	239.2
	THR	0.97	0.83	2.08	0.60	7.53	2.00	0.79	0.15	0.69	4.50	27.1	2.86	41.0	0.15	> 8.35	5.3	27.5	176.6
	ELE	1.29	2.97	1.87	0.48	5.66	1.40	0.65	0.03	0.40	3.31	13.0	26.3	17.7	0.07	2.01	3.9	23.0	252.7
	ART	0.57	0.84	2.10	0.40	4.74	2.05	0.71	0.11	0.61	3.23	12.2	2.28	19.0	0.10	> 9.10	4.0	19.8	143.8
	AMV	0.69	2.11	1.60	0.42	5.78	1.74	0.44	0.08	0.54	3.45	8.24	4.06	47.9	0.09	> 5.43	3.8	22.1	180.1

[a] THQ: total hazard quotient.
[b] MPI: metal pollution index (mg/kg ww).
[c] As-CR: carcinogenic risk of arsenic.
[d] Se:Hg: molar ratio.
[e] Se-HBV: selenium health benefit values (mole). Thermaikos Gulf (THE); Strymonian Gulf (STR); Thracian Sea (THR); Elefsina Gulf (ELE); Artemisium Straits (ART); Amvrakikos Gulf (AMV); bw: body weight. To estimate human risk due to arsenic, a content of 3% of the total As was assumed present in the inorganic form (NSW FA, 2010; FSA, 2004; Copat et al., 2013, 2018; Cava-Montesinos et al., 2005). If more than half of the values for the estimation of mean concentrations per species and site were below the detection limit, the risk assessment parameters estimated are marked with "<" or ">" accordingly.

120 g of sardine from Elefsina Gulf may pose health risks if consumed by a 70 kg person in a day (Table 1). The MSC_A of the detected As(V) is high enough to ensure public health (1.5–2.3 kg fish/day) (Table 2). However, if a content of 3% of the total As was inorganic, it would result in alarmingly low values of Maximum Safe Consumption: 80–160 g of fish (ww) in a day for 5 out of the 6 sites studied and even lower limits for Elefsina Gulf: health risks would not be excluded if more than 30 g of anchovy or 40 g of sardine were consumed in a day by a70 kg person (Table 1).

3.4. Hazard quotient

The HQ of Li, V, Fe, Co, Ni, Mo, Cd, Ba, Cu, Zn, Se, Sr, As, Hg, Pb and U was under the safety limit of 1 (Table 1), indicating that it is unlikely for even sensitive population to experience adverse health effects due to each of these elements individually. It should be noted that the HQ of Hg was very close to the limit for anchovy in Elefsina Gulf (0.94) (Table 1). The HQ of the detected As(V) remained well under unity (Table 2) while HQ of the initially assumed inorganic As (3% of the total As) exceeds the safety limit in both species from Elefsina Gulf (1.64 for sardine and 2.64 for anchovy) and approaches it in anchovy from Amvrakikos Gulf (0.91) (Table 1).

In all the sites studied the Total Hazard Quotient (THQ) of anchovy was found higher than the one of sardine (Table 1). The Total Hazard Quotient exceeds unity or approaches it in most cases if the detected As (V) is considered (THQ_2; Table 3) while it exceeds unity in all cases if As (3%) is considered (THQ_1; Table 3). This outcome was expected since HQs of all metals and elements are added for the THQ estimation and

Table 2
Estimation of the human risk deriving from the consumption of sardine and anchovy sampled from Artemisium Straits (n = 15 per species) due to their inorganic As(V) content.

	Sardine	Anchovy	Comparison to the limits set
As(V) content in fish (mg As(V)/kg wet weight) ([a])	0.009	0.015	< Safety Standard = 1.3 mg/kg
Estimated Daily Intake (EDI) (μg As(V)/kg bw/day)	0.009	0.014	< RfD = 0.3 μg/kg bw/day[b]
Hazard Quotient (HQ)	0.03	0.05	< 1
Maximum Safe Consumption (MSC_A) (kg fish/day)	2.298	1.460	ok
Carcinogenic Risk of As (As-CR)	0.000014	0.000022	< 10^{-4}

[a] According to Kalantzi et al. (2017); bw: body weight; RfD: Reference Dose.
[b] Set by USEPA (2017).

HQ values of As (3%) and Hg alone were quite high. Specifically, the contribution of the theoretically assumed inorganic As (3% of the total) and of Hg to the Total Hazard Quotient (THQ_1) ranged from 22.3% to 62% and 15.1–34.5%, respectively, while the other metals and elements contribution ranged from 0.1% to 17.5% (Table 3). It is remarkable that if the HQ of As (3%) and Hg are excluded from THQ_1, it will not exceed the safety limit (Table 3). Similarly, if the HQ of Hg is excluded from THQ_2 (for which the detected As(V) is considered) it will also not exceed the safety limit (Table 3).

3.5. Metal pollution index

Metal Pollution Indices (MPI), expressing the total elemental and metal pollution per species and site (Li, Na, Mg, P, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Pd, Cd, Cs, Ba, Hg, Tl, Pb, U), ranged between 0.46 mg/kg ww (for sardine in Amvrakikos Gulf) and 0.89 mg/kg ww (for anchovy in Thermaikos Gulf) (Table 1). All sites considered, MPI for anchovy was higher than that for sardine indicating higher total metal pollution for the former.

3.6. As carcinogenic risk

The As Carcinogenic Risk (As-CR) of the detected As(V) was below the highest established acceptable risk level of 10^{-4} (Table 2). It is noteworthy that if the theoretically assumed inorganic As (3% of the total) was taken into account, the As-CR would exceed the above-mentioned limit for both species and all the sites studied (Table 1). This would suggest that the incremental probability of developing cancer would not be totally excluded (greater than 1 in 10.000) if a person of 70 kg consumes 68.68 g of these fish per day, 365 days per year and 70 years long.

3.7. Molar ratio Se:Hg – selenium health benefit values (Se-HBV)

Se to Hg molar ratio, ranging between 19.8 and 134.7, demonstrated the existence of Se molar excess in both species from all the sites studied (Table 1). This suggests safe consumption as far as the Hg toxicity is concerned. Se mitigation results were also evident in Se Health Benefit Values (Se-HBV), which were not only positive but also ranged between 124.2 and 1628.6 (Table 1), indicating beneficial effects for human health arising from the consumption of sardines and anchovies from the studied sites. Average Se to Hg ratio values were 72.9 for sardine and 25 for anchovy, while average Se-HBV was 816.2 and 186.1, respectively, suggesting greater Se beneficial effects from sardine consumption compared to anchovy. Particularly high values were observed in fish from Strymonian Gulf: the highest Se to Hg ratio in both species (134.74 in sardine and 37.81 in anchovy), the highest Se-HBV in sardine (1628.64) and the second higher Se-HBV in anchovy (239.20). The highest Se-HBV for anchovy was found in Elefsina Gulf (252.70) (Table 1).

3.8. Nutritional value

The contribution of each essential element to the established Nutrient Reference Values, (NRVs) (%) was estimated per species and site (Table 4). Regarding Mo and Cu 2%–20% of these recommended daily intakes are covered while for Mg and Zn significant amounts (> 15% according to European Union, 2011b) are covered up to 48%. Significant amounts of Fe dietary requirements are also covered by sardines and anchovies from most of the sites (10–22%). Regarding Ca the contribution is even higher ranging from 45% to 76% of the

Table 3
Estimation of the contribution (%) of the Hazard Quotient (HQ) of each metal and element to the Total Hazard Quotient (THQ_1) per site and species and the THQ obtained if the HQ of As or of both As and Hg are excluded. THQ_1 was estimated assuming that 3% of the total As is present in inorganic form (NSW FA, 2010; FSA, 2004; Copat et al., 2013, 2018; Cava-Montesinos et al., 2005). THQ_2 was estimated taking into account the As(V) detected.

		Contribution of the HQ each element to the THQ_1 (%)																					
		Li	V	Fe	Co	Ni	Cu	Zn	As (3%)	As(V)[a]	Se	Sr	Mo	Cd	Ba	Hg	Pb	U	THQ_1	THQ_1 - HQ (As_3%)	THQ_1 - HQ (As_3% + Hg)	THQ_2	THQ_2 - HQ (Hg)
Sardine	**THE**	9.5	4.6	2.3	9.1	1.1	1.6	5.0	31.2	*bdl*	11.0	1.6	0.4	0.8	0.2	18.0	2.3	1.2	1.40	0.96	0.71	0.96	0.71
	STR	4.9	2.4	2.3	8.3	0.7	2.6	5.0	37.2	*bdl*	16.0	1.4	0.2	0.8	0.1	15.1	1.8	1.2	1.17	0.73	0.56	0.73	0.56
	THR	4.7	2.5	2.2	7.3	0.5	1.8	4.9	35.0	*bdl*	14.9	1.1	0.1	0.7	0.1	21.4	1.4	1.2	1.22	0.80	0.53	0.80	0.53
	ELE	1.4	0.6	1.3	4.1	0.4	1.3	3.3	56.8	*bdl*	7.8	0.4	0.2	0.1	0.0	20.0	1.9	0.4	2.89	1.25	0.67	1.25	0.67
	ART	6.0	1.9	2.0	6.8	0.6	1.5	4.0	40.3	3.2	9.9	0.9	0.3	0.5	0.1	23.5	0.6	1.0	1.53	0.91	0.55	0.94	0.58
	AMV	4.0	1.1	1.3	6.1	0.4	0.9	4.2	48.4	*bdl*	6.4	0.5	0.4	0.4	0.1	24.3	0.6	0.8	1.50	0.77	0.41	0.77	0.41
Anchovy	**THE**	6.6	17.5	1.5	6.7	0.6	1.6	4.6	22.3	*bdl*	4.6	1.0	0.2	1.4	0.2	29.3	1.1	0.9	2.11	1.64	1.02	1.64	1.02
	STR	5.2	5.5	2.1	8.0	0.7	2.4	5.2	32.5	*bdl*	7.6	1.2	0.3	1.6	0.2	25.7	0.9	1.1	1.28	0.87	0.54	0.87	0.54
	THR	4.7	5.5	2.2	7.6	0.6	2.3	5.8	29.9	*bdl*	6.6	1.0	0.2	1.6	0.1	30.6	0.5	0.9	1.51	1.06	0.59	1.06	0.59
	ELE	1.2	0.5	0.9	3.4	0.3	1.1	2.5	62.0	*bdl*	4.0	0.5	0.1	0.1	0.1	22.0	0.8	0.4	4.26	1.62	0.68	1.62	0.68
	ART	5.7	3.9	1.6	8.2	0.7	1.6	4.6	29.7	3.3	5.4	1.0	0.3	1.4	0.2	34.5	0.4	0.8	2.09	1.47	0.75	1.52	0.80
	AMV	4.2	1.4	1.8	6.9	0.5	1.7	6.5	38.1	*bdl*	5.3	0.8	0.3	0.7	0.1	30.4	0.5	0.8	2.39	1.48	0.75	1.48	0.75

Thermaikos Gulf (THE); Strymonian Gulf (STR); Thracian Sea (THR); Elefsina Gulf (ELE); Artemisium Straits (ART); Amvrakikos Gulf (AMV); *bdl*: below detection limit.
[a] The contribution (%) of As(V) refers to THQ_2.

Table 4
Estimation of the contribution (%) of the metal and elemental content of sardines and anchovies to the daily reference intakes (nutrient reference values, NRVs) that have been established for each element. Fish were sampled from 6 sites in Greece (n = 15 per species and site).

Species	Sites	Contribution to the nutrient reference values (NRVs) (%)							
		Mg	P	Ca	Fe	Cu	Zn	Se	Mo
Sardine	Thermaikos Gulf	16.8	138.7	76.4	16.6	9.3	21.3	143.3	5.8
	Strymonian Gulf	16.4	122.6	55.4	13.9	12.2	17.9	173.6	2.6
	Thracian Sea	16.0	102.6	51.9	13.5	9.0	18.5	169.1	1.8
	Elefsina Gulf	17.5	122.5	60.6	19.0	15.7	29.4	210.1	4.6
	Artemisium Straits	15.8	98.5	52.1	15.9	9.1	18.8	139.5	4.8
	Amvrakikos Gulf	12.3	88.4	47.2	9.8	5.5	19.2	88.9	6.8
Anchovy	Thermaikos Gulf	19.8	137.7	63.1	15.7	14.1	29.3	89.6	4.1
	Strymonian Gulf	19.3	104.3	45.2	13.4	12.5	20.3	90.8	3.4
	Thracian Sea	18.6	118.0	53.2	16.8	14.0	26.6	92.4	2.6
	Elefsina Gulf	25.6	148.4	68.2	18.8	20.0	32.5	157.6	5.4
	Artemisium Straits	22.1	99.4	57.7	16.7	13.7	29.4	104.2	5.8
	Amvrakikos Gulf	23.8	130.4	72.9	21.9	16.1	47.8	117.1	8.5
Nutrient reference values, NRVs (mg/100g) ([a])		375	700	800	14	1	10	0.055	0.05

[a] Daily reference intakes for minerals (nutrient reference values, NRVs) have been established by the European Union (2011b).

recommended daily intake. P and Se offered the maximum contributions: 88%–148% and 89%–210% of the respective Nutrient Reference Values. Consumption of anchovy seems to contribute more than sardine to the established dietary requirements of Mg, Fe, Cu, Zn, Mo, Ca, P for most of the studied sites while sardine seems to contribute more to Se dietary requirements (Table 4). Consumption of fish from Elefsina Gulf seems to contribute more to dietary requirements compared to the rest of the sites regarding Mg, Fe, Cu, Zn, Se (sardines) and Mg, P, Cu, Se (anchovies). Consumption of anchovies from Amvrakikos Gulf also seems to contribute more to the established dietary requirements of Ca, Fe, Zn, Mo compared to the rest of the sites (Table 4).

4. Discussion

4.1. Risk assessment

The determined risk assessment parameters indicated safety for the consumption of sardine and anchovy from the studied sites for the majority of the metals and elements examined (Li, V, Co, Ni, Mo, Cd, Ba, Cu, Se, Sr, Pb, U, As). Some minor concerns arise for Fe, Zn, Hg and the Total Hazard Quotient values. To the extent that comparisons can be made (since sampling sites were different), another risk assessment study in Greece also found no health risk for consumers from the consumption of raw and cooked sardine and anchovy and reported comparable concentrations of Cd (5.4–13.7 μg/kg), Hg (< 7.5–183 μg/kg), Pb (< 0.04–0.08 mg/kg), Cu (1.1–1.5 mg/kg), Fe (12–18 mg/kg) and Ni (< 0.08 mg/kg) (Kalogeropoulos et al., 2012). Similar concentrations of Cd (5–24 μg/kg) and Pb (0.094–0.257 mg/kg) were also observed in another study (Galitsopoulou et al., 2012) in sardines and anchovies caught in Greek coastal water bodies, although no risk assessment was conducted in that case.

4.1.1. Fe and Zn levels

In the present study, mean Fe concentrations in all cases and mean Zn concentrations in anchovy from Elefsina Gulf and Amvrakikos Gulf exceeded the safety standards set. On the other hand, Fe and Zn, when under the safety limits, are essential elements for which daily dietary requirements have been set (European Union, 2011b). High levels of Zn have been reported to mitigate Cu toxicity (Yuan et al., 2016). According to the nutritional assessment of the present study, consumption of sardine and anchovy from the sites where the limits were exceeded offers significant amount of these nutrients, covering 10–22% of Fe daily reference intake and 33% and 48% of Zn daily reference intake (from Elefsina Gulf and Amvrakikos Gulf, respectively).

4.1.2. As levels

The As speciation analysis conducted (Kalantzi et al., 2017) indicated that most of the As in the studied samples was in organic form (arsenobetaine). Organic As is considered to be of low or no toxicity and can be effectively excreted through urine (Onsanit et al., 2010; EFSA, 2009; USEPA, 1997; Afonso et al., 2013). Therefore, only risk deriving from inorganic As was assessed. Various parameters indicated that consumption of sardines and anchovies from the studied sites pose no carcinogenic or non-carcinogenic risk to human health due to their low inorganic As(V) content. It should also be noted that only when metabolised to the most toxic form of As(III) (Afonso et al., 2013; FSA, 2009), is As(V) considered toxic (FSA, 2009) and no As(III) was detected in samples of the present study (Kalantzi et al., 2017). These results are consistent with other studies suggesting that fish is not the major contributor to inorganic As intake (EFSA, 2014; Khoramnejadian and Fatemi, 2015). A recent study in Spain also reported that increased dietary inorganic As exposure was not associated with seafood consumption but with other products (rice products) (Signes-Pastor et al., 2017).

The theoretically assumed inorganic As content (3% of the total As) (NSW FA, 2010; FSA, 2004; Copat et al., 2013, 2018; Cava-Montesinos et al., 2005) proved to overestimate the potential risk. Even though, the Estimated Daily Intake (under this assumption) lies within the limits of the expected exposure to inorganic As via diet in E.U. (EFSA, 2009), some safety limits would be exceeded if this hypothesis was valid. When speciation analysis is not carried out, defining the inorganic As content seems to be a complex and controversial issue. Some studies have reported less than 0.5% inorganic As (Peshut et al., 2008) or less than 0.2% or 1.8% (Johnson and Roose, 2002) while others have reported less than 2% or 4% (USEPA, 1997), less than 5% (USEPA, 1997; Mania et al., 2015) or ranging from 1% to 5% (Peshut et al., 2008). For the present samples Kalantzi et al. showed by conducting As speciation analysis that inorganic As was 0.14% of the total in sardine and 0.24% of the total in anchovy. In some studies no inorganic As was detected (Schaeffer et al., 2005) while others considered the worst case scenario assuming that there is a 10% or 11% of the total As in inorganic form (Onsanit et al., 2010; Metian et al., 2013; Vieira et al., 2011; Kalantzi et al., 2016). The issue is further complicated by the fact that the inorganic to total As ratio is not constant and tends to decrease when total As increases while it can vary depending on the fish species (EFSA, 2009; EFSA, 2014). If the worst case scenario produces results indicating health risks and no data on the inorganic As content of the same species and site are available in literature, then speciation analysis needs to be carried out so that the real public health risk can be assessed.

4.1.3. Hg levels

Risks deriving from Hg toxicity cannot be excluded in some cases due to Maximum Safe Consumption low values. Also, Hazard Quotient values of Hg were close to the safety limit of unity contributing significantly to the Total Hazard Quotient high values. No other safety limits were exceeded as far as Hg is concerned. Investigation of Se−Hg balance suggests safe consumption due to Se molar excess (Se:Hg ratio exceeding unity). Moreover, beneficial effects are suggested by the high positive values of Se-HBV (124.2–1628.6).

Se mitigation against Hg toxicity has been demonstrated in various studies on several fish species since 1967 (Kaneko and Ralston, 2007; Copat et al., 2014; Ralston et al., 2016). It has been argued that Se can block Hg methylation or assist its demethylation (Raimundo et al., 2014) or that it can reduce Hg assimilation or bioaccumulation (Dang and Wang, 2011). According to Dang and Wang (2011) there can be interaction between Se and Hg(II) but not between Se and MeHg. In another study, Se did not activate Hg demethylation, which was attributed to potential low toxicity of MeHg (Raimundo et al., 2014). In the present study, the total Hg content was assumed to be in its toxic methylated form (MeHg). (EFSA, 2015; Nadal et al., 2008; Olmedo et al., 2013b; EFSA, 2004). Thus, further investigation is needed.

It has been argued that risk assessment based on MeHg exposure alone, without considering its interaction with Se, is inaccurate (Ralston et al., 2016). It has been suggested that MeHg toxicity cannot be indicated by Hg concentration but it is significantly (and inversely) correlated to Se concentration and Hg/Se molar ratio. High Hg/Se values indicated toxicity symptoms while low Hg/Se values were related to few or no symptoms (Ralston et al., 2008). Therefore, it has been argued that Hg/Se molar ratio is the most useful assessment criterion of MeHg toxicity (Ralston et al., 2008; Copat et al., 2014). In the current study Hg/Se values were quite low, in fact much lower than in the above-mentioned studies (Olmedo et al., 2013a; Kaneko and Ralston, 2007; Ralston et al., 2008; Copat et al., 2014): 0.01–0.03 for sardine and 0.03–0.05 for anchovy. Thus, it could be considered that Hg toxicity symptoms are not likely to occur. In a recent study (Cardoso et al., 2018), it was estimated that MeHg detrimental effects on IQ of children were counterbalanced by the beneficial impact of fish EPA and DHA content in a large number of fish including sardines.

The toxicity of MeHg has been suggested to target Se-dependent enzymes that offer antioxidant protection to the brain. When Se availability diminishes, the function of these enzymes is inhibited and Hg toxicity symptoms may appear. What was conceived as Se protection from Hg toxicity in the past, is actually Hg compromising the benefits Se offers by binding Se and preventing it from taking part in essential biological functions (Ralston et al., 2008, 2016; Copat et al., 2014; Afonso et al., 2015). Increased Se levels can compensate for MeHg toxic action and preserve normal synthesis and function of the Se-dependent enzymes (Ralston et al., 2008; Copat et al., 2014; Afonso et al., 2015). However, selenoenzymes activities in fetal brain are more sensitive than in adults' brain and thus can be irreversibly inhibited by MeHg. These activities can be preserved and pathological effects can be prevented by supplementary Se in diet (Ralston et al., 2016). In order not to overestimate the risks, it has also been reported that the bioaccessibility of MeHg should be considered (Jacobs et al., 2017; Afonso et al., 2015) since most of Hg remains bound to thiomolecules while in the body without binding cellular Se (Ralston et al., 2016). In the present study, there was Se molar excess in both species from all the sites (Se/Hg ranged from 19.82 to 134.74) while Se-HBV values were one to two orders of magnitude higher than the respective values in the above-mentioned studies (Olmedo et al., 2013a; Kaneko and Ralston, 2007; Ralston et al., 2008; Copat et al., 2014). Thus, it could be considered that, in the present study, health benefits Se offers are not likely to be compromised by Hg toxicity.

4.1.4. Total hazard quotient (THQ)

The THQ values found higher than 1 indicate that potential long term non-carcinogenic effects cannot be excluded due to the total elemental content of the fish (Vieira et al., 2011; Copat et al., 2013; Storelli and Barone, 2013; Kalogeropoulos et al., 2012; Onsanit et al., 2010; Yang et al., 2013; Kalantzi et al., 2016) if a 70 kg person consumes 68.68 g (FAO, 2005–2012) of sardine or anchovy from those sites on a daily basis. The HQ of Hg has significant contribution to these THQ high values but as has been pointed out, Hg toxicity symptoms are not likely to occur because of Hg- Se balance. If Hg toxicity was not taken into account, THQ values would fall below the safety limit of 1 indicating that adverse health effects due to the total elemental content of the fish would not be likely to appear even for sensitive populations.

4.2. Nutritional assessment

The nutritional assessment of sardine and anchovy highlighted that both species are rich in essential metals and elements (Mg, Fe, Cu, Zn, Mo, Ca, P, Se) in all the sites studied, regardless of the different anthropogenic pressures these sites receive (Simboura et al., 2016; Pavlidou et al., 2015) and the statistically significant differences found among these sites in the metal pollution load of sardines and anchovies (Sofoulaki et al., unpublished results). In most cases significant amounts (2–210%) of the recommended daily intakes (Nutrient Reference Values, NRVs) are covered. It is remarkable that out of the 26 metals and elements studied, it is the essential ones (P, Ca, Mg) that had the highest concentrations (1 order of magnitude compared to the rest) followed by Fe and Zn, which despite having a safety limit, are also essential for the maintenance of good health.

The large contribution (89%–210%) to the daily reference intake (Nutrient Reference Value) set for Se and the particularly high positive values of Se-HBV (124.2–1628.6) for both species and all the sites studied, indicate significant health benefits for consumers and are in good agreement with previous findings that pelagic fish can be considered as a source rich in Se (Kaneko and Ralston, 2007; Copat et al., 2014). Apart from its nutritional value, increased intake of Se has been associated with anticarcinogenic action and protective action against cardiovascular and neurological diseases (Larsen et al., 2011; Kaneko and Ralston, 2007). It has also been suggested to diminish the biological activity of metals like Cd, Ag, Tl and Pb (Renieri et al., 2014; Copat et al., 2014; Marval-León et al., 2014).

Apart from Se, all the nutrients found in sardine and anchovy, as well as the proteins, carbohydrates and vitamins they contain, play a significant role in human diet, disease prevention and health improvement (Kalantzi et al., 2016; Kalogeropoulos et al., 2012; Renieri et al., 2014; Olmedo et al., 2013b; Vieira et al., 2011; Storelli and Barone, 2013; Castro-Gonzalez and Mendez-Armenta, 2008; Hoekstra et al., 2013; Lund, 2013; Larsen et al., 2011; Domingo et al., 2007).

4.3. Overall assessment: combing risks and benefits

Risk and benefits assessment indicated higher total metal load in anchovy compared to sardine (based on MPI values) but also greater benefits from anchovy consumption regarding the essential elements Mg, Fe, Cu, Zn, Mo, Ca and P (based on contribution to the Nutrient Reference Values). On the other hand, consumption of sardine offers greater benefits as far as Se content is concerned (based on contribution to Se Nutrient Reference Value and Se-HBV). These differences in the risks and benefits involved are consistent with the statistical analysis conducted regarding the differences between the two species in their metal and elemental content (Sofoulaki et al., 2018).

For a thorough view of the risks and benefits deriving from the consumption of sardine and anchovy, apart from metals and nutrients, organic pollutants and fatty acids should be considered while the exposure of pregnant women and children should be examined. Furthermore, data of the seasonal variation of the potential pollutants would be meaningful in the framework of a study. As far as organic pollutants (PAHs, PCBs, PCDD/Fs etc) are concerned, sardines and anchovies have been reported to have higher concentrations compared to other species (Bayarri et al., 2001; Guerranti et al., 2016; Perelló et al., 2015) but they have been found not to exceed the relative safety limits both in Greek and other coastal areas (Stagakis et al., 2016; Vassiliadou et al., 2015; Perelló et al., 2015).

According to USEPA/USFDA (2017a) the concern about other contaminants is lower compared to the Hg content of the fish while according to EFSA (2015) the main sources of concern are MeHg and dioxin-like compounds. USEPA/USFDA issued an advice note in 2017 about eating fish and shellfish in order to encourage pregnant and breastfeeding women and children to consume fish. The recommended amount depends on the species that are classified according to their Hg content as best choices (less than 0.15 μg/g), good choices (more than 0.15 μg/g but less than 0.46 μg/g) or choices to avoid (more than 0.46 μg/g) (USEPA/USFDA, 2017a, 2017c). Previous advice notes were focused on setting maximum limits without encouraging the consumption of a minimum amount. As a result, most people and specifically pregnant women were reported to consume quite low amounts of fish (less than recommended) and thus miss nutritional benefits that are important for their child's growth and development (USEPA/USFDA, 2017a, 2017b). A study assessing the effects of Hg in blood of pregnant women on their birth outcomes in U.K. also concluded that consumption of two portions of fish per week by pregnant women should be encouraged in line with regulatory guidelines (Taylor et al., 2016) while another study stressed that for pregnant women the consumption of small and fatty species should not be limited to less than 200–350 g/week (Thomsen et al., 2018). Anchovy and sardine, in particular are included among the best choices a pregnant woman can make according to their Hg content: less than 0.15 μg/g (USEPA/USFDA, 2017a, 2017b), which is also the case for the fish of the current study (0.01–0.07 μg/g for sardine and 0.01–0.13 for anchovy).

Furthermore, sardine and anchovy are considered excellent sources of ω-3 fatty acids, DHA and EPA (Zotos and Vouzanidou, 2012; Gencbay and Turhan, 2016; Cardoso et al., 2018) which have anti-inflammatory and anticarcinogenic properties, contribute to normal neurodevelopment, lower the risk of coronary heart disease and offer various other health benefits (Olmedo et al., 2013b; Storelli and Barone, 2013; Castro-Gonzalez and Mendez-Armenta, 2008; Hoekstra et al., 2013; Lund, 2013; Larsen et al., 2011). Moreover, sardine and anchovy are rich sources of proteins (21.6–27.6% and 25.1–33.2%, respectively) (Sofoulaki et al., 2018) while the usual protein content of fish ranges from 15% to 18% and only a few cases of fish species having protein content up until 28% have been reported (Murray and Burt, 2001). The recommendation of consuming fish twice on weekly basis has been widely documented (Lund, 2013; Hoekstra et al., 2013; Afonso et al., 2013; Domingo et al., 2007) since the benefits of a diet rich in seafood have been suggested to outweigh the potential risks (Lund, 2013; USFDA, 2009; FAO/WHO, 2011; Larsen et al., 2011). Along similar lines, according to the results of the current study, weekly consumption of 480.76 g of sardine and anchovy (estimation based on the average daily consumption of 68.68 g of wild fish in Greece; FAO, 2005–2012) poses minor risks due to the increased levels of essential elements like Fe and Zn in some cases, but great benefits regarding the intake of essential elements like Mg, Fe, Cu, Zn, Mo, Ca, P, Se. This is consistent with the results of Hoekstra et al. (2013) who estimated that weekly consumption of 500 g of fish, despite the increased risk involved, would offer much higher benefits to Dutch population compared to the consumption made. Overall beneficial health effects for Danish population were also estimated if a part of red and processed meat consumption in their diet was substituted with fish (Thomsen et al., 2018). Similarly, the 2015–2020 Guidelines for Americans suggest increasing the amount of fish consumed and choosing a variety of species (USEPA/USFDA, 2017a).

However, caution is needed regarding consumption advisories; different species vary greatly both in their Hg content and in their PUFAs content (EFSA, 2015) and so does the consumption frequency of seafood in different countries and specifically the consumption frequency of each species. Thus, consumption advice and recommendations should be species-specific and country-specific (Jacobs et al., 2017; Domingo, 2016; EFSA, 2015) and Hg intake should be carefully monitored regarding pregnant women and children, especially in countries with high fish consumption (Cardoso et al., 2018). According to recent literature (Thomsen et al., 2018; Taylor et al., 2016; Jacobs et al., 2017), consumption of large predatory fish (especially during pregnancy) should be limited while consumption of small fatty fish should be encouraged. The recommended weekly amount of sardine and anchovy to be consumed by pregnant and breastfeeding women is 226–339 g (2–3 servings of 113 g per serving) while for children the recommended weekly amount of sardine and anchovy is 28.25–226 g depending on the child's age (USEPA/USFDA, 2017a, 2017c).

Finally, in order to estimate risks and benefits and guide consumers and health professionals on fish choices, frequency of meals and portion size, digital tools have been designed (Domingo, 2016; Vilavert et al., 2017) while it has been suggested that apart from physical health, other factors should also be considered in the risks and benefits balance such as socioeconomic and community factors, animal welfare and planetary health (Rideout and Kosatsky, 2017).

5. Conclusions

Various risk and benefit assessment parameters established by national and international authorities indicated mostly safe consumption of two of the most widely consumed fish species in Mediterranean countries. Specifically, weekly consumption of 480.76 g of sardine and anchovy from the sites studied (even the most polluted) poses minor risks (due to increased levels of Fe and Zn in some cases) but great benefits regarding the intake of nutrients like Mg, Fe, Cu, Zn, Mo, Ca, P, Se which are essential to human diet, disease prevention and health improvement. The traces of the potentially toxic inorganic As detected were well below all safety limits. The study of Se−Hg balance suggested that Hg toxicity symptoms are not likely to appear and Se benefits are not likely to be compromised. Therefore, sardine and anchovy from Greek coastal areas seem to be among the fish species for which the health benefits arising from their metal content outweigh the consumption risks.

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