



Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers



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ABSTRACT

Although fish intake has potential health benefits, the presence of metal contamination in seafood has raised public health concerns. In this study, levels of mercury, cadmium, lead, tin and arsenic have been determined in fresh, canned and frozen fish and shellfish products and compared with the maximum levels currently in force. In a further step, potential human health risks for the consumers were assessed. A total of 485 samples of the 43 most frequently consumed fish and shellfish species in Andalusia (Southern Spain) were analyzed for their toxic elements content. High mercury concentrations were found in some predatory species (blue shark, cat shark, swordfish and tuna), although they were below the regulatory maximum levels. In the case of cadmium, bivalve mollusks such as canned clams and mussels presented higher concentrations than fish, but almost none of the samples analyzed exceeded the maximum levels. Lead concentrations were almost negligible with the exception of frozen common sole, which showed median levels above the legal limit. Tin levels in canned products were far below the maximum regulatory limit, indicating that no significant tin was transferred from the can. Arsenic concentrations were higher in crustaceans such as fresh and frozen shrimps. The risk assessment performed indicated that fish and shellfish products were safe for the average consumer, although a potential risk cannot be dismissed for regular or excessive consumers of particular fish species, such as tuna, swordfish, blue shark and cat shark (for mercury) and common sole (for lead).

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1. Introduction

Fish has been acknowledged as an integral component of a well-balanced diet, providing a healthy source of energy, high-quality proteins, vitamins and a wide range of other important nutrients (Pieniak et al., 2010). Moreover, fish is a significant source of omega-3 polyunsaturated fatty acids (PUFAs) whose benefits lowering the risk of coronary heart disease and contributing to normal neurodevelopment in children have been widely recognized (Mozaffarian and Wu, 2011; Swanson et al., 2012).

In contrast to the potential health benefits of dietary fish intake, the chemical pollutants contained in these products have emerged as an issue of concern, particularly for frequent fish consumers (Domingo, 2007; Dórea, 2008; Martorell et al., 2011). In this regard, heavy metals contamination is a worldwide-recognized public health hazard because

these pollutants are widespread in the environment, including marine ecosystems, from either natural or anthropogenic sources (Lozano et al., 2010). As a consequence, they can be accumulated by marine organisms through exposure to metals present in water and sediments or in the food chain. Thus, diet comprises the main route of exposure to these elements in the general population (Kim and Lee, 2010).

Some of these elements such as mercury, arsenic, cadmium, lead and tin have no known role in biological systems. They are natural trace components of the aquatic environment, but their levels have increased due to industrial, agricultural and mining activities. Even low metal concentrations may threaten the health of aquatic and terrestrial organisms, man included (Sarmiento et al., 2011). Mercury is an element of special concern because its inorganic form is biologically transformed in aquatic environments into methylmercury (MeHg), a lipophilic organic compound that bioaccumulates and biomagnifies as it moves up the aquatic food chain (Carrasco et al., 2011; Gewurtz et al., 2011; Jaeger et al., 2009). As a result, human populations with a traditionally elevated dietary intake have the highest potential exposure to MeHg and are at an increased risk for developing neurotoxic effects. This is a particularly important issue for children, pregnant women and breast-feeding mothers (Jedrychowski et al., 2007; Ramón et al., 2008, 2011).

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Spain is a country with a relatively high fish consumption in some regions (Welch et al., 2002) with Andalusia (a Southern region of Spain) being a representative example of this situation, as its population not only consumes but also provides fish for the rest of Spain and even Europe. Although fish has always been perceived as a healthy and nutritive food (Serra-Majem et al., 2007), a recent report of the Spanish Food Safety and Nutrition Agency (AESAN) has raised public concern as it claimed that some heavy metals (mercury and cadmium) levels in certain fish species make them unsuitable for children and pregnant women consumption (AESAN, 2011a,b). This debate concerning the benefits and risks of consuming fish has resulted in confusion among consumers who demand further information on this issue.

Despite the diversity of results found in the scientific literature, no study has addressed so far the biomonitoring of a number of metal elements in a wide variety of fish and shellfish species consumed in Andalusia. Accordingly, there is a need for additional information in order to achieve a better risk assessment from fish and shellfish consumption in Andalusia, a geographical area with an increased touristic attractive. This article assessed the widest variety of fish and shellfish species ever analyzed for toxic elements in Andalusia and in the rest of Spain where fresh, canned and frozen products have been considered. The specific objectives of this study were (1) to determine levels of 4 heavy metals (Hg, Pb and Cd) and one metalloid (As) in samples of fresh, canned and frozen fish and shellfish species currently consumed in Andalusia, (2) to check the possible tin (Sn) transfer into canned food by analyzing this metal in canned fish samples, (3) to determine the amount of MeHg in samples showing the highest concentration of total mercury, (4) to compare the results obtained with those from other studies and with the maximum levels (MLs) set by the European Commission Regulation and (5) to assess the potential human health risks from fish and shellfish consumption in the target area.

2. Material and methods

2.1. Biological samples

A total of 485 samples from fresh, frozen and canned fish and shellfish products were collected for this study (see species distribution in Tables 2 and 3) between years 2009 and 2010. The origin of the fresh and frozen samples was the central market of Granada (Southern Spain) and the canned species were purchased in some of the major supermarkets in the city. A random sampling was carried out using as inclusion criteria: (1) frequent consumption in Andalusia (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2008) and (2) sample collection was conducted during the same season. The exclusion criteria were: (1) uneven size or lengths with respect to the species average and (2) non-fresh samples or expired canned products. All samples, with the exception of canned products, were stored in a freezer at $-32\text{ }^{\circ}\text{C}$ before analysis that was performed in less than 9 weeks.

2.2. Reagents and material

Atomic absorption spectrometry standard solutions for Hg, Cd, Pb, Sn and As (Titrisol grades from Merck) were used to build up calibration curves. They were prepared from a stock solution of 1000 mg/L for each metal by successive dilutions. Aqueous solutions of reagents and standards were prepared using a Milli-RO 12 plus Milli-Q purification system for water (Millipore, Bedford, MA). Stock standard solutions of 1000 mg/L methylmercury (Alfa Aesar) and 10 mg/L mercury (SPEX CertiPrep, United Kingdom) were used for mercury speciation analysis.

All the chemicals used were of analytical reagent grade. High-quality concentrated (65% w/v) nitric acid (Panreac), (96% w/v) sulfuric acid (Panreac), (37% w/v) hydrochloric acid (Panreac), sodium

borohydride (Panreac), sodium hydroxide (Sigma-Aldrich, Steinheim, Germany), ascorbic acid (Panreac), potassium iodide (Panreac), ammonium dihydrogen phosphate (Merck), magnesium nitrate (Merck), palladium nitrate (Merck), Triton X-100 (Merck), potassium permanganate (Merck), silicone antifoaming agent (Merck), (25% w/w) tetramethylammonium hydroxide (Alfa Aesar), methanol (Sigma-Aldrich), L-cysteine (Sigma-Aldrich), ammonium acetate (Sigma-Aldrich) and 2-mercaptoethanol (Sigma-Aldrich) were used.

Volumetric polyethylene material was used. Syringes (10 mL), syringe filters (with $0.2\text{ }\mu\text{m}$ pore) and 2 mL capped glass vials were required for HPLC sample preparation. The glass material was cleaned by soaking in 20% v/v HNO_3 for 24 h. It was finally rinsed with Milli-Q® water and dried in a polypropylene container.

2.3. Sample preparation

Approximately 0.7 g of edible fish/shellfish samples was microwave-digested for 30 min in a closed quartz vessel with 2 mL of H_2O , 4 mL of HNO_3 and 0.5 mL of HCl. The microwave oven was programmed at 1400 W and 80 bar as power and pressure limits, respectively (ramp time 10 min; hold time 10 min; cooling time 20 min). The digested solution was then transferred to a decontaminated tube for its later analysis. Quartz vessels were vigorously cleaned, soaked for 24 h in 15% HNO_3 , thoroughly rinsed with Milli-Q® water before use and dried at $80\text{ }^{\circ}\text{C}$ for about 2 h.

A simpler, faster and less aggressive digestion process was needed for methylmercury determination in order not to alter mercury species. Approximately 0.25 g of wet fish sample was cut in 4 small pieces and then transferred to a glass flask which was closed after addition of 5 mL of 25% w/w tetramethylammonium hydroxide (TMAH). The whole sample was dissolved after 3 h at room temperature without the need of agitation. It was then filtered to a capped chromatography vial that was analyzed in the same day.

2.4. Instrumentation

An AAAnalyst 800 atomic absorption spectrometer (Perkin Elmer, Norwalk, USA) equipped with a graphite furnace and an AS-800 autosampler, Zeeman background correction system and graphite tubes with integrated L'vov platform, was used for determining Pb, Cd and Sn.

Arsenic was measured with direct flow injection through hydride generation system (Perkin-Elmer FIAS-100) coupled to the AAAnalyst 800 atomic absorption spectrometer. Total mercury was determined in a Perkin Elmer 560 atomic absorption spectrometer (Perkin Elmer, Norwalk, USA) equipped with power supply lamp system and MHS-10 mercury hydride system.

For methylmercury speciation, a PlasmaQuad PQ ExCell inductively coupled plasma-mass spectrometer (ICP-MS, Vg Elemental) was used together with a high-performance liquid chromatograph (HPLC, Thermo Surveyor) equipped with an autosampler and a Hypersil BDS C18 column (length: 10 cm; internal diameter 4.6 mm; particles diameter: $3\text{ }\mu\text{m}$).

Fish and shellfish samples were subjected to a microwave-assisted digestion procedure (Multiwave 3000, Anton Parr).

2.5. Analytical procedures

- Arsenic (As): The arsenic contained in standard solutions (calibration curve 0, 0.5, 1.5 and $2.5\text{ }\mu\text{g/L}$) or digested fish samples was reduced to As^{3+} prior to analysis with a mixture of potassium iodide and ascorbic acid. One milliliter of concentrated HCl and 1 mL of 5% (w/v) KI-ascorbic acid were added to 1 mL of digested sample. After 45 min at room temperature, the mixture was diluted to 10 mL with water. The reducing agent was an aqueous solution of 0.2% (w/v) NaBH_4 in a 0.05% (w/v) NaOH solution freshly prepared and

filtered. Standard addition was required and cell temperature was set at 900 °C. An electrodeless discharge lamp was used.

- **Mercury (Hg):** The mercury standard calibration plot (0, 2.5, 5, and 10 µg/L) was prepared in 10 mL of acid mixture containing 1.5% HNO₃ and 1.5% H₂SO₄. Nine milliliters of acid mixture were added to 1 mL of digested sample. Mercury was determined using an aqueous solution of 3% (w/v) NaBH₄ in a 1% (w/v) NaOH solution freshly prepared and filtered as reducing agent. One to two drops of silicone antifoaming was dispensed into a reaction flask before introducing any solution. All solutions were stabilized by adding 500 µL of 5% KMnO₄ solution before starting the determination. An electrodeless discharge lamp was used.
- **Cadmium (Cd):** A calibration curve (0, 1, 3, and 5 µg/L) was prepared in 0.2% HNO₃ and samples were diluted 1:4. Aliquots of 20 µL of digested samples were introduced directly into a graphite furnace with an equal volume of matrix modifier (a mixture of 3.3% Pd and 0.03% Mg as nitrates in 0.2% HNO₃). An electrodeless discharge lamp was used.
- **Lead (Pb):** A calibration curve (0, 50, 100 and 200 µg/L) was prepared in 0.2% HNO₃ and samples were diluted 1:4. Aliquots of 10 µL of digested samples were introduced directly into the graphite furnace with an equal volume of matrix modifier (10 g/L of NH₄H₂PO₄ prepared in 0.2% (v/v) nitric acid and 0.1% Triton X-100). A hollow cathode lamp was used.
- **Tin (Sn):** A calibration curve (0, 10, 25, 50, and 100 µg/L) was prepared in 1 M HCl and samples were diluted 1:4. Aliquots of 20 µL of digested samples were introduced directly into the graphite furnace with an equal volume of matrix modifier (10 g/L of NH₄H₂PO₄ prepared in 0.2% [v/v] nitric acid and 0.1% Triton X-100). An electrodeless discharge lamp was used.
- **Methylmercury (MeHg):** A calibration curve was performed with inorganic mercury (0, 1, 2, 5, and 10 µg/L) and methylmercury (0, 10, 20, 50, and 100 µg/L) in 25% tetramethylammonium hydroxide (TMAH). A digested sample volume of 10 µL was injected into the HPLC through a 1 mL/min flow of mobile phase (0.40% [w/v] n-cysteine, 0.01% [v/v] 2-mercaptoethanol, 0.46% [w/v] ammonium acetate and 5% methanol).

2.6. Validation of analytical methods

Analytical methods for the determination of Hg, Cd, Pb and As concentrations reported elsewhere for other biological matrices (Gil et al., 2006; Olmedo et al., 2010) were validated for this study by using appropriate certified reference material (Fish Muscle European Reference Material, ERM BB422) subjected to a microwave-assisted acid digestion procedure. Table 1 shows the validation parameters for the analytical procedures. The methodology for tin (Sn) determination in canned products described by Boutakhrit et al. (2011) was validated in the same way (Table 1), although in our study NH₄⁺ was used as matrix modifier. Because of the lack of certified reference values for Pb and Sn in the reference material used, recovery studies were used instead of accuracy assessment. For the methylmercury

speciation analysis, the extraction at room temperature methodology described by Clémens et al. (2011) was mainly followed with some parameters of the analytical method being checked using a certified reference material (BCR® Tuna Fish 463).

2.7. Data treatment

Levels of metals in the different species studied were represented by median rather than average values because of their non-normal distribution. For calculations, when the level of an element was under the limit of detection (LOD), the concentration was assumed to be half of the respective detection limit and if more than 60% of samples were below LOD, lower (LB) and upper (UB) bounds were estimated by replacing those values with 0 and LOD, respectively according to WHO (1995). A scaling of the toxic elements analyzed was performed by using the sums of their median levels. All statistical analyses were performed using IBM-SPSS 19.0 (Chicago, IL, USA).

2.8. Risk assessment

The assessment of dietary metal exposure was estimated using mean concentrations for the fish and shellfish species studied rather than median levels because their values are higher and thus a more cautious approach can be assumed for risk assessment purposes. The rate of fish and shellfish consumption was taken from the Spanish National Survey on Dietary Intake – ENIDE – (AESAN, 2011c). The estimated dietary intakes were compared with the current provisional tolerable weekly intakes (PTWI) for Hg, Cd, Pb and Sn. Recently, the Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA, 2009a), considered inorganic As as probably genotoxic and definitely carcinogenic, so that an experimental benchmark response of 1% extra risk was selected as a reference. A range of benchmark dose lower confidence limit (BMDL₀₁) values between 0.3 and 8 µg/kg body weight/day were identified for several types of cancer. Accordingly, the As risk assessment was performed following EFSA (2009a) guidelines for the exposure to substances with genotoxic and carcinogenic properties by using the Margin of Exposure (MOE). MOE is defined as the ratio between the BMDL₀₁ and the estimated daily dietary exposure to inorganic arsenic. In this respect, high MOE values are desirable. Similarly, for the Pb risk assessment, BMDL₁₀ has also been considered according to EFSA (2010) recommendations. In all cases, the contribution of fish and shellfish to the total daily intake for each element studied was considered.

3. Results and discussion

3.1. Methylmercury (MeHg)

Speciation analyses were carried out to determine the percentage of methylmercury (MeHg, the most toxic form of mercury) to total mercury. Target species were those showing the highest levels of total mercury (see Section 3.2), namely fresh blue shark, cat shark,

Table 1

Summary of results for the characteristic parameters of the instrumental method for the determination of Hg, Cd, Pb, As and Sn in fish samples.

	Certified ^a values (mg/kg)	Measured values (mg/kg)	LOD (mg/kg ww)	LOQ (mg/kg ww)	Recovery ^b (%)	Precision (%)		Linear range (µg/L)
						Minimal	Reproducibility	
Hg	0.601 ± 0.030	0.596	1.07 × 10 ⁻⁵	3.56 × 10 ⁻⁵	96.86	1.98	2.55	20
Cd	0.0075 ± 0.0018	0.0085	1.58 × 10 ⁻⁴	5.28 × 10 ⁻⁴	104.64	2.62	1.17	7
Pb	–	–	4.35 × 10 ⁻³	1.45 × 10 ⁻²	96.57	3.21	0.85	200
As	12.7 ± 0.7	12.11	1.59 × 10 ⁻⁴	5.31 × 10 ⁻⁴	97.87	3.51	4.20	4
Sn	–	–	1.36 × 10 ⁻³	4.53 × 10 ⁻³	96.34	2.14	2.28	400

LOD: limit of detection, LOQ: limit of quantification.

^a The Certified Reference Material used was Fish Muscle European Reference Material (ERM) BB422.

^b Samples were spiked with Hg, Cd, Pb, As and Sn by adding a standard solution with different concentrations.

swordfish and tuna and canned tuna. Despite Hg^{2+} showing a much higher peak signal than MeHg for the same concentration (Fig. 1a), no Hg^{2+} peak was detected for any of the fish samples analyzed (Fig. 1b). Assuming that the limit of detection (LOD) for Hg^{2+} was set at $0.5 \mu\text{g/L}$, it could be inferred that more than 99.9% of total mercury found in the predatory fish species analyzed (fresh blue shark, cat shark, swordfish, tuna and canned tuna) was in the form of MeHg. That figure is consistent with the 75–100% reported for fresh tuna species (Storelli et al., 2002), the 89–100% found in canned tuna (Burger and Gochfeld, 2004) and the 95–98% observed for fresh swordfish (Hight and Cheng, 2006). For this reason, EFSA guidelines (2004) recommended considering that more than 90% of total mercury contained in fish is actually methylmercury. Thus, according to EFSA and our results, it is assumed that the total mercury concentration found in the fish species analyzed is actually methylmercury.

3.2. Mercury (Hg)

Tables 2 and 3 show metal levels (median and 5th and 95th percentiles) of the fresh, canned and frozen products studied. The highest concentrations of mercury were found in predatory species (Tables 2 and 3) namely, fresh, cat shark (0.698 mg/kg ww), swordfish (0.540 mg/kg ww), tuna (0.470 mg/kg), blue shark (0.350 mg/kg ww) and canned tuna (0.222 mg/kg ww) as consistently reported in the scientific literature. For instance, Martorell et al. (2011) found very similar Hg levels in fresh (0.554 mg/kg ww) and canned tuna (0.222 mg/kg) as well as Storelli et al. (2012) in fresh tuna (0.530 mg/kg ww) and swordfish (0.800 mg/kg ww). Burger and Gochfeld (2006) also found comparable levels (0.340 mg/kg ww and 1.400 mg/kg ww , respectively) in these species. Other species with remarkable levels were fresh anglerfish (0.158 mg/kg ww), perch (0.128 mg/kg ww) and scad (0.107 mg/kg ww) as shown in Table 2. Storelli and Marcotrigiano (2000) found higher mercury levels for anglerfish ($0.61\text{--}2.22 \text{ mg/kg ww}$); however, perch mercury levels

in our study fell within those found by Machiwa, 2005 ($0.031\text{--}0.684 \text{ mg/kg ww}$). In contrast, bivalve species, such as fresh and canned clams and mussels, showed the lowest levels of mercury since they are at the base of the food chain and their life cycle is short (Gutierrez et al., 2006).

Table 4 shows metal levels (median, 5th and 95th percentiles), number and percentage of fish samples analyzed below the limit of detection (LOD) and above the maximum level (ML) set by the EC Regulation for each metal in different foodstuffs. Mercury concentrations were generally below ML, with only 1.24% of samples exceeding that level. Most of samples showing mercury content above ML belonged to the predatory fish species category (3.3.2) while all of the 129 samples below LOD (26.59% of the total fish samples studied) were included in category 3.3.1 (rest of the fishery products and muscle meat of fish). Accordingly, category 3.3.2 showed much higher mercury levels (0.237 mg/kg ww) than category 3.3.1 (0.002 mg/kg ww).

3.3. Cadmium (Cd)

With regard to cadmium, bivalve mollusks such as mussels (0.110 mg/kg ww) and clams (0.041 mg/kg ww) presented the highest levels for fresh products (Table 2). Falcó et al. (2006) also found the highest cadmium concentration ranges in these species ($0.02\text{--}0.20$ and $0.03\text{--}0.22 \text{ mg/kg ww}$ for mussels and clams, respectively). On the other hand, the species showing the lowest Cd concentrations were European hake, salmon, red mullet, megrim and blue whiting. For the rest of species analyzed, cadmium concentration was rather low, which is in contrast to Storelli et al. (2012) who found remarkable cadmium levels in cuttlefish and swordfish (0.85 and 0.25 mg/kg ww , respectively) caught in Italy, and Tuzen (2009) who reported higher levels in fresh anchovy and scad (0.27 and 0.32 mg/kg ww , respectively) caught in Turkey. Nevertheless, the highest Cd concentrations were found in canned fish and shellfish such as clams (0.244 mg/kg ww), mussels (0.208 mg/kg ww), squids (0.107 mg/kg ww), octopus (0.095 mg/kg ww) and anchovies

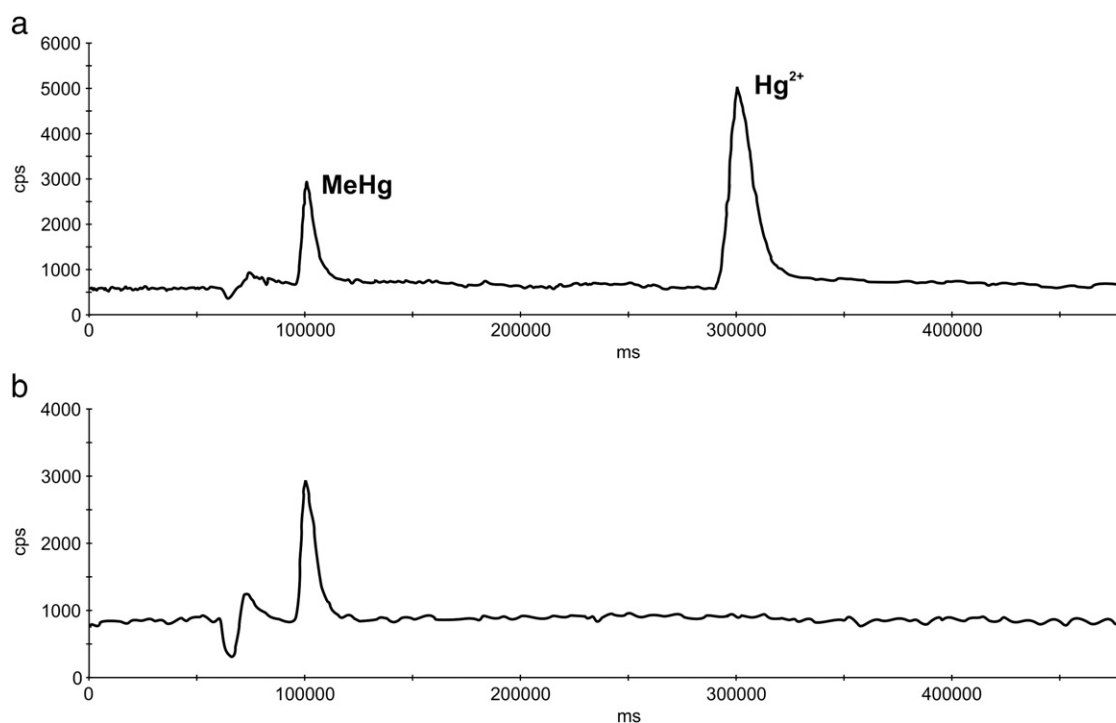


Fig. 1. Chromatogram of an equal concentration ($20 \mu\text{g/L}$) MeHg solution (peak in $100,000 \text{ ms}$) and Hg^{2+} (peak in $300,000 \text{ ms}$); cps: counts per second (a). Chromatogram of one of the fish samples analyzed where no peak of Hg^{2+} was observed (b).

Table 2

Hg, Cd, Pb and As concentrations (mg/kg wet weight) in the edible part of fresh fish and shellfish products analyzed.

Common name	Scientific name	Origin	Hg	Cd	Pb	As
Fresh fish (n = 11)			Median (P5–P95)	Median (P5–P95)	Median (P5–P95)	Median (P5–P95)
Anchovy	<i>Engraulis encrasicolus</i>	Catalonia, Spain	0.018 (0.000–0.078)	0.001 (0.000–0.004)	0.004 (0.004–0.231)	0.188 (0.129–0.378)
Anglerfish	<i>Lophius piscatorius</i>	Mauritania	0.158 (0.073–0.379)	0.001 (0.001–0.003)	0.004 (0.004–0.383)	0.043 (0.030–0.077)
Blue shark	<i>Prionace glauca</i>	Basque Country, Spain	0.350 (0.238–0.963)	0.003 (0.000–0.008)	0.004 (0.004–0.316)	0.144 (0.090–0.381)
Blue whiting	<i>Micromesistius poutassou</i>	Galicia, Spain	0.048 (0.023–0.090)	0.000 (0.000–0.002)	0.004 (0.004–0.004)	0.134 (0.099–0.217)
Cat shark	<i>Galeus melastomus</i>	Valencia, Spain	0.698 (0.153–1.406)	0.002 (0.001–0.007)	0.004 (0.004–0.004)	0.340 (0.183–0.450)
Clam	<i>Venus gallina</i>	Italy	0.003 (0.000–0.039)	0.041 (0.028–0.131)	0.004 (0.004–0.274)	0.307 (0.189–0.415)
Common sole	<i>Solea vulgaris</i>	Andalusia (Atlantic Coast), Spain	0.019 (0.000–0.044)	0.001 (0.000–0.067)	0.052 (0.004–0.464)	0.233 (0.150–0.402)
Cuttlefish	<i>Sepia officinalis</i>	Andalusia (Atlantic Coast), Spain	0.032 (0.010–0.067)	0.000 (0.000–0.012)	0.117 (0.020–0.695)	0.172 (0.092–0.430)
European hake	<i>Merluccius merluccius</i>	Valencia, Spain	0.051 (0.007–0.112)	0.000 (0.000–0.000)	0.094 (0.004–0.225)	0.165 (0.082–0.282)
European seabass	<i>Dicentrarchus labrax</i>	Murcia, Spain	0.079 (0.020–0.121)	0.002 (0.001–0.006)	0.004 (0.004–0.004)	0.025 (0.007–0.043)
Gilt-head bream	<i>Sparus aurata</i>	Murcia, Spain	0.037 (0.000–0.111)	0.001 (0.000–0.014)	0.004 (0.004–0.035)	0.103 (0.028–0.281)
Hake	<i>Merluccius gayi</i>	Mauritania	0.070 (0.043–0.535)	0.007 (0.001–0.016)	0.004 (0.004–0.004)	0.048 (0.017–0.070)
Mackerel	<i>Scomber scombrus</i>	Andalusia (Mediterranean Coast), Spain	0.022 (0.005–0.049)	0.001 (0.000–0.005)	0.004 (0.004–0.004)	0.190 (0.096–0.292)
Megrim	<i>Lepidorhombus boscii</i>	Andalusia (Atlantic Coast), Spain	0.091 (0.031–0.179)	0.000 (0.000–0.000)	0.004 (0.004–0.040)	0.066 (0.015–0.094)
Mussel	<i>Mytilus edulis</i>	Galicia, Spain	0.000 (0.000–0.000)	0.110 (0.079–0.251)	0.004 (0.004–0.025)	0.222 (0.131–0.381)
Pangasius	<i>Pangasius hypophthalmus</i>	Vietnam	0.000 (0.000–0.000)	0.002 (0.000–0.007)	0.004 (0.004–0.439)	0.021 (0.002–0.030)
Perch	<i>Lates niloticus</i>	Tanzania	0.128 (0.056–0.158)	0.002 (0.001–0.007)	0.004 (0.004–0.136)	0.002 (0.000–0.017)
Red mullet	<i>Mullus surmuletus</i>	Andalusia (Atlantic Coast), Spain	0.067 (0.041–0.139)	0.000 (0.000–0.002)	0.004 (0.004–0.012)	0.427 (0.230–0.729)
Salmon	<i>Salmo salar</i>	Norway	0.000 (0.000–0.004)	0.000 (0.000–0.000)	0.004 (0.004–0.188)	0.047 (0.019–0.114)
Sardine	<i>Sardina pilchardus</i>	Andalusia (Mediterranean Coast), Spain	0.034 (0.009–0.067)	0.002 (0.000–0.003)	0.004 (0.004–0.034)	0.561 (0.169–0.959)
Scad	<i>Trachurus trachurus</i>	Valencia, Spain	0.107 (0.063–0.299)	0.001 (0.000–0.004)	0.004 (0.004–0.211)	0.243 (0.080–0.347)
Shrimp	<i>Parapenaeus longirostris</i>	Andalusia (Atlantic Coast), Spain	0.017 (0.000–0.057)	0.029 (0.014–0.063)	0.004 (0.004–0.055)	0.739 (0.388–0.884)
Squid	<i>Dosidicus gigas</i>	Argentina	0.000 (0.000–0.028)	0.010 (0.006–0.021)	0.004 (0.004–0.352)	0.018 (0.005–0.062)
Swordfish	<i>Xiphias gladius</i>	Galicia, Spain	0.540 (0.177–1.227)	0.009 (0.002–0.060)	0.004 (0.004–0.064)	0.096 (0.010–0.910)
Tuna	<i>Thunnus thynnus</i>	Canary Islands, Spain	0.470 (0.298–0.779)	0.008 (0.000–0.127)	0.004 (0.004–0.004)	0.033 (0.018–0.592)

n: number of samples, P5: 5th percentile, P95: 95th percentile.

(0.094 mg/kg ww) as described in Table 3. This finding could be accounted for the raised concentration derived from the canning process as reported by AESAN (2008).

EC Regulation set several categories with different ML for fish meats and shellfish (Table 4). Cadmium concentrations in the present study were below these ML in practically all cases and only 0.62% of the samples (3 of them) exceeded this limit. Samples below LOD (15.05% of total) fell mainly in the 3.2.5 category for which the ML is lower. As expected, foodstuff categories with higher ML such as 3.2.10 (bivalve mollusks) or 3.2.11 (cephalopods) presented the highest cadmium concentrations, 0.108 mg/kg ww and 0.061 mg/kg ww, respectively.

3.4. Lead (Pb)

Tables 2 and 3 show that lead levels of a significant number of shellfish and fish species were below the LOD (0.004 mg/kg ww). The only fresh products showing levels above the LOD were cuttlefish (0.117 mg/kg ww), European hake (0.094 mg/kg ww) and common sole (0.052 mg/kg ww). These levels are very similar to those recently found by Storelli et al. (2012) in the same species: 0.14 mg/kg ww, 0.11 mg/kg ww and 0.09 mg/kg ww, respectively. The highest Pb levels corresponded to canned bivalve mollusks, particularly cockles and mussels (0.548 and 0.202 mg/kg ww, respectively) as shown in

Table 3

Hg, Cd, Pb, As and Sn concentrations (mg/kg wet weight) in the edible part of canned and frozen fish and shellfish products analyzed.

Common name	Scientific name	Origin	Hg	Cd	Pb	As	Sn
Canned fish (n = 12)			Median (P5–P95)	Median (P5–P95)	Median (P5–P95)	Median (P5–P95)	Median (P5–P95)
Albacore tuna	<i>Thunnus alalunga</i>	Spain	0.000 (0.000–0.000)	0.008 (0.004–0.014)	0.004 (0.004–0.004)	0.033 (0.007–0.082)	0.001 (0.001–0.010)
Anchovy	<i>Engraulis encrasicolus</i>	Spain	0.049 (0.024–0.163)	0.094 (0.016–0.227)	0.077 (0.010–0.199)	0.159 (0.089–0.251)	0.001 (0.001–0.001)
Clam	<i>Protothaca thaca</i>	Chile	0.000 (0.000–0.000)	0.244 (0.033–0.594)	0.004 (0.004–0.347)	0.289 (0.198–0.634)	0.001 (0.001–0.194)
Cockle	<i>Cerastoderma edule</i>	United Kingdom	0.023 (0.008–0.043)	0.043 (0.020–0.079)	0.548 (0.269–0.971)	0.608 (0.244–1.138)	0.001 (0.001–0.033)
Frigate	<i>Auxis thazard</i>	Andalusia, Spain	0.000 (0.000–0.000)	0.006 (0.001–0.057)	0.004 (0.004–0.046)	0.015 (0.005–0.058)	0.001 (0.001–0.037)
Mackerel	<i>Scomber scombrus</i>	Spain	0.020 (0.000–0.039)	0.018 (0.003–0.046)	0.004 (0.004–0.004)	0.052 (0.033–0.201)	0.001 (0.001–0.003)
Mussel	<i>Mytilus galloprovincialis</i>	Spain	0.000 (0.000–0.000)	0.208 (0.117–0.343)	0.202 (0.042–1.303)	0.372 (0.243–0.536)	0.008 (0.001–0.155)
Octopus	<i>Octopus vulgaris</i>	Spain	0.000 (0.000–0.000)	0.095 (0.042–0.249)	0.004 (0.004–0.004)	0.095 (0.069–0.264)	0.014 (0.001–0.068)
Sardine	<i>Sardina pilchardus</i>	Spain	0.000 (0.000–0.012)	0.002 (0.001–0.067)	0.004 (0.004–0.026)	0.086 (0.015–0.185)	0.007 (0.001–0.114)
Small sardine	<i>S. pilchardus</i>	Spain	0.000 (0.000–0.035)	0.014 (0.005–0.039)	0.004 (0.004–0.029)	0.119 (0.083–0.288)	0.001 (0.001–0.065)
Squid	<i>Loligo vulgaris</i>	Spain	0.000 (0.000–0.000)	0.107 (0.072–0.270)	0.004 (0.004–0.004)	0.038 (0.015–0.093)	0.001 (0.001–0.756)
Tuna	<i>Thunnus albacares</i>	Spain	0.222 (0.035–0.894)	0.046 (0.020–0.170)	0.004 (0.004–0.385)	0.005 (0.000–0.044)	0.024 (0.001–0.481)
Frozen fish (n = 11)							
Codfish	<i>Gadus morhua</i>	Atlantic Northeast	0.000 (0.000–0.000)	0.002 (0.001–0.025)	0.004 (0.004–0.004)	0.036 (0.012–0.076)	
Common Sole	<i>Solea vulgaris</i>	Morocco	0.000 (0.000–0.000)	0.003 (0.001–0.017)	0.544 (0.022–7.726)	0.059 (0.039–0.126)	
Hake	<i>Merluccius gayi</i>	Mauritania	0.000 (0.000–0.000)	0.001 (0.000–0.002)	0.086 (0.004–1.066)	0.028 (0.011–0.045)	
Prawn	<i>Litopenaeus vannamei</i>	Panama	0.000 (0.000–0.000)	0.001 (0.000–0.190)	0.033 (0.004–1.366)	0.045 (0.018–0.286)	
Shrimp	<i>Parapenaeus longirostris</i>	Tunisia	0.070 (0.000–0.131)	0.010 (0.005–0.100)	0.004 (0.004–0.773)	0.509 (0.113–0.723)	
Squid	<i>L. vulgaris</i>	Argentina	0.000 (0.000–0.000)	0.073 (0.026–0.184)	0.004 (0.004–0.134)	0.044 (0.025–0.078)	

n: number of samples, P5: 5th percentile, P95: 95th percentile.

Table 4
Comparison between metal levels found in the fish and shellfish samples analyzed and the legal categories for each metal according the European Commission (Regulation EC No. 1881/2006 amended by EC No. 629/2008 and EC No. 420/2011).

Foodstuffs (maximum levels, ML)	n	<LOD (%)	P5	Median	P95	>ML (%)
Hg						
3.3.1. Fishery products and muscle meat of fish excluding species listed in category 3.3.2. (ML: 0.50 mg/kg)	384	129 (33.59%)	0.000	0.002	0.113	1 (0.26%)
Muscle meat of fish listed in category 3.3.2. (ML: 1.0 mg/kg)	101	0 (0.00%)	0.000	0.237	0.974	5 (4.95%)
Total	485	129 (26.59%)	0.000	0.017	0.469	6 (1.24%)
Cd						
3.2.5. Muscle meat of fish excluding species listed in categories 3.2.6, 3.2.7 and 3.2.8 (ML: 0.05 mg/kg)	187	59 (31.55%)	0.000	0.001	0.008	1 (0.53%)
Muscle meat of fish listed in category 3.2.6. (ML: 0.10 mg/kg)	104	3 (2.88%)	0.000	0.005	0.061	2 (1.92%)
Muscle meat of fish listed in category 3.2.7. (ML: 0.20 mg/kg)	12	0 (0.00%)	0.002	0.006	0.058	0 (0.00%)
Muscle meat of fish listed in category 3.2.8. (ML: 0.30 mg/kg)	34	3 (8.82%)	0.000	0.009	0.183	0 (0.00%)
3.2.9. Crustaceans (ML: 0.5 mg/kg)	33	1 (3.03%)	0.001	0.014	0.099	0 (0.00%)
3.2.10. Bivalve mollusks (ML: 1.0 mg/kg)	58	0 (0.00%)	0.027	0.108	0.419	0 (0.00%)
3.2.11. Cephalopods (ML: 1.0 mg/kg)	57	7 (12.28%)	0.000	0.061	0.241	0 (0.00%)
Total	485	73 (15.05%)	0.000	0.004	0.205	3 (0.62%)
Pb						
3.1.5. Muscle meat of fish (ML: 0.30 mg/kg)	337	261 (77.45%)	LB 0.000 UB 0.004	LB 0.000 UB 0.004	LB 0.409 UB 0.409	20 (5.93%)
3.1.6. Crustaceans (ML: 0.50 mg/kg)	33	20 (60.61%)	LB 0.000 UB 0.004	LB 0.000 UB 0.004	LB 0.900 UB 0.900	5 (15.15%)
3.1.7. Bivalve mollusks (ML: 1.5 mg/kg)	58	21 (36.21%)	LB 0.000 UB 0.004	LB 0.049 UB 0.049	LB 0.958 UB 0.958	0 (0.00%)
3.1.8. Cephalopods (ML: 1.0 mg/kg)	57	44 (77.19%)	LB 0.000 UB 0.004	LB 0.000 UB 0.004	LB 0.486 UB 0.486	0 (0.00%)
Total	485	346 (71.34%)	LB 0.000 UB 0.004	LB 0.000 UB 0.004	LB 0.567 UB 0.567	25 (5.15%)
Sn						
3.4.1. Canned foods other than beverages (ML: 200 mg/kg)	144	97 (67.36%)	LB 0.000 UB 0.001	LB 0.000 UB 0.001	LB 0.152 UB 0.152	0 (0.00%)

<LOD (%): number of samples and percentage under limit of detection.

>ML (%): number of samples and percentage over the maximum legal limit.

n: number of samples, P5: 5th percentile, P95: 95th percentile.

LB: lower bound, replacing values under limit of detection with 0.

UB: upper bound, replacing values under limit of detection with the limit of detection.

Table 3. It has been reported that cockles (*Cerastoderma edule*) have a capacity to accumulate lead from polluted environmental sources (Figueira et al., 2011) since cockles samples from Portugal were found to show remarkable lead concentrations, ranging from 1.16 to 5.20 mg/kg ww, far above ML set by the EC Regulation for cockles (1.5 mg/kg ww). By contrast, canned mussels from the Canary Islands showed fairly low Pb levels ranging from 0.006 to 0.008 mg/kg ww (Gutierrez et al., 2004). Frozen common sole (*Solea vulgaris*) was the only fish species showing median Pb concentration above the ML set by EC Regulations (0.544 mg/kg ww, Table 3). However, this particular species shows low levels in the literature, for example, Usero et al. (2004) found concentrations ranging from 0.03 to 0.05 mg/kg ww in common sole from the Southern Atlantic coast of Spain and Henry et al. (2004) found very low Pb levels in similar flatfish species from the French Atlantic Coast such as dab (0.001–0.120 mg/kg ww), flounder (0.008–0.050 mg/kg ww) and plaice (0.010–0.100 mg/kg ww). Since our frozen common sole was caught in the coast of Morocco, the differences found in these studies might be accounted for geographic reasons.

More than 70% of samples analyzed showed Pb levels below LOD (Table 4), hence lower and upper bounds were used as described in

Material and methods. Despite these low concentrations, twenty fish and five crustacean samples (5.15% of the total samples studied) exceeded the ML set by the EC for both foodstuffs. This percentage of samples was the highest found among all the toxic elements analyzed. Bivalve mollusks were the only category not showing a minimal lower and upper bound (LB and UB: 0.049 mg/kg ww).

3.5. Tin (Sn)

The presence of tin in canned food has been acknowledged as an important issue for both human health and quality assessment (Blunden and Wallace, 2003). A high tin content indicates migration of tin from the container to food as a result of inexistent or poor lacquering (Mol, 2011). In the present study, the highest tin concentrations were observed in canned tuna (0.024 mg/kg ww), octopus (0.014 mg/kg ww), mussels (0.008 mg/kg ww) and sardines (0.007 mg/kg ww) as shown in Table 3. All canned fish and shellfish samples analyzed presented tin levels far below the ML set by EC Regulation (200 mg/kg) as seen in Table 4. Furthermore, as most of canned samples analyzed (67.36%) were below LOD, there was a

need to use lower and upper bounds as described in [Material and methods](#) (see [Table 4](#)).

3.6. Arsenic (As)

No maximum levels are currently set by the EC for As. As shown in [Table 2](#), fresh products with higher As levels were shrimp (0.739 mg/kg ww), sardine (0.561 mg/kg ww) and red mullet (0.427 mg/kg ww). Other species worth noting were cat shark (0.340 mg/kg ww), clam (0.307 mg/kg ww), scad (0.243 mg/kg ww),

common sole (0.233 mg/kg ww) and mussel (0.222 mg/kg ww). The canned and frozen species studied had comparable arsenic concentration ranges to fresh products, with canned cockles (0.608 mg/kg ww), mussels (0.372 mg/kg ww), clams (0.289 mg/kg ww) and frozen shrimp (0.509 mg/kg ww) showing the highest levels ([Table 3](#)). In spite of these findings, the As concentration observed for fish and shellfish species is lower than those previously reported for common species. Thus, [Falcó et al. \(2006\)](#) and [Martinez-Gómez et al. \(2012\)](#) found arsenic levels in red mullet ranging from 15.39 to 17.77 mg/kg ww and 19.8–6.9 mg/kg ww respectively, whereas in our study arsenic

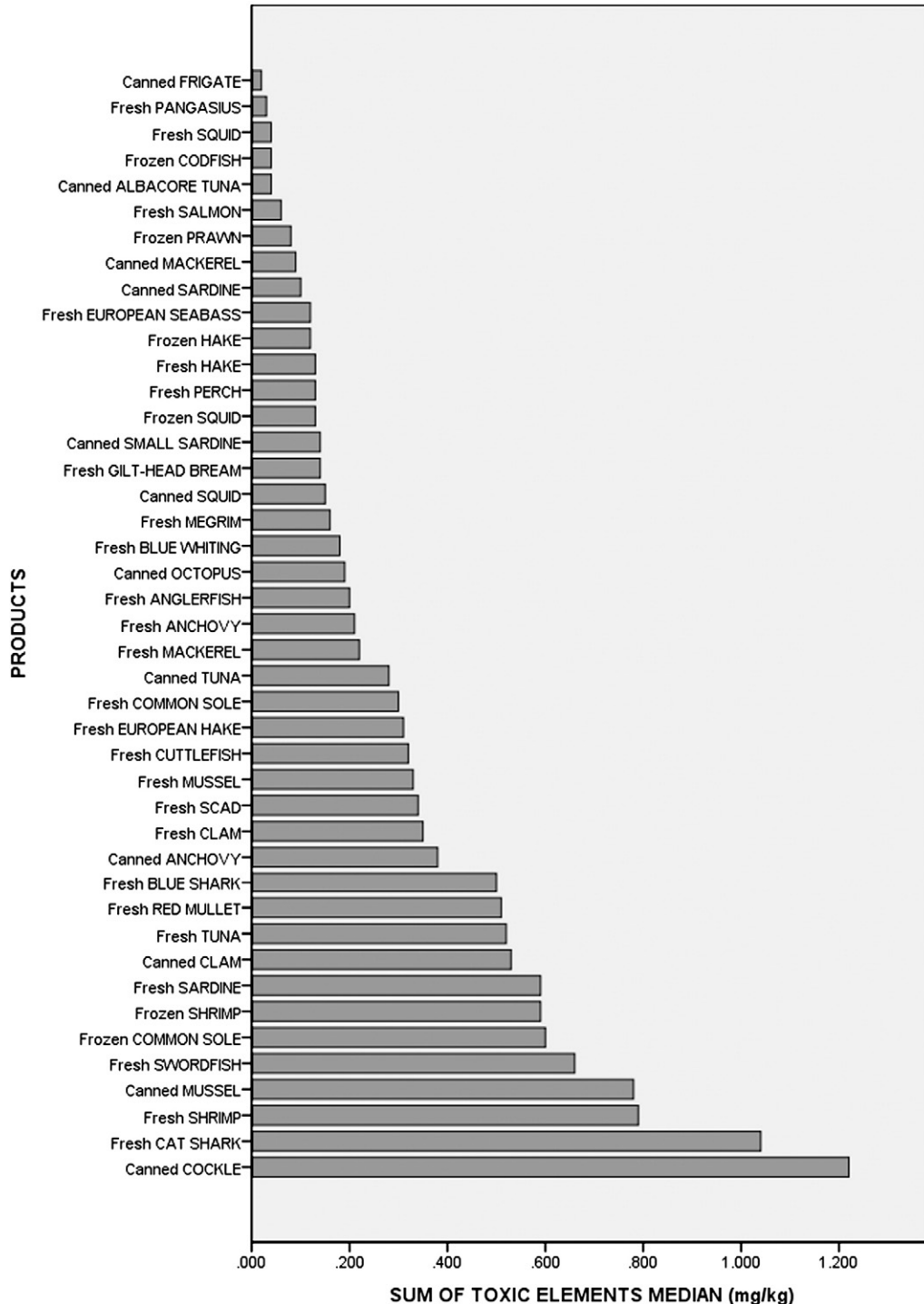


Fig. 2. Scaling graph showing the sum of the median concentrations for Hg, Cd, Pb and As found for every species studied (mg/kg wet weight). Fish and shellfish species are ranked according to their lower to higher metal levels considered as a whole.

level found in this species was 0.427 mg/kg ww as shown in Table 2. Furthermore, the Catalan Food Safety Agency (ACSA, 2010) reported 16.58 mg/kg ww in red mullet and 6.09 mg/kg ww in common sole, concentrations which stands in glaring contrast to our results. Nevertheless, our data were in the same order of magnitude as those reported by the European Scientific Cooperation Report (EC, 2004) for other European countries such as Finland (0.017–1.0 mg/kg ww), Germany (0.694–1.409 mg/kg ww) and Greece (0.019–0.022 mg/kg ww).

3.7. Scale grouped data

A scaling graph (Fig. 2) was performed to rank fish and shellfish species according to the sum of their median levels of Hg, Cd, Pb and As (since all these elements have been determined in every sample studied). It can be seen that the species with the highest toxic metal content are cat shark and cockles, while frigate, pangasius and codfish are those showing the lowest metal concentrations. The higher levels of metal compounds found in cat shark and swordfish may be explained by their position on the top level of the food chain, which allows for lipophilic metal compounds accumulation. On the other hand, given that cockles and mussels are filtering organisms, this property may account for their higher levels of metal compounds (Figueira et al, 2011).

3.8. Risk assessment

We have calculated the weekly intake of Hg, Cd, Pb, As and Sn for an average person weighting 60 kg by taking into consideration the metal concentrations found in this study and fish and shellfish consumption rates reported in ENIDE survey (AESAN, 2011c). Since metal levels failed to show a normal distribution (mean values were strongly influenced by extreme concentrations found in some samples), the assessment of dietary metal exposure was estimated using mean concentrations rather than median levels because they were higher. Thus, a more cautious viewpoint was assumed for risk assessment purposes. The calculated intakes for each metal compound were further compared with their corresponding PTWI set by regulatory agencies (EFSA and JECFA) (Table 5).

The calculated mercury intake was approximately 63.63 µg/week (9.1 µg/day). This value was higher than that found by Rubio et al. (2008) in the Canary Islands (5.69 µg/day), lower than that depicted by Martí-Cid et al. (2008) in Catalonia for male adults (11.5 µg MeHg/day) and similar to the intakes reported in other studies also performed in Spain (Falcó et al. (2006), who found 9.89 µg/day in Catalonia) and in neighboring countries, such as France (Noël et al. (2003) reported 9 µg/day). These Hg ingestion rates are among the weekly intake range (1.3–92.0 µg/week) observed by EFSA (2004). The calculated weekly mercury intake in our study represents 66.5% of the PTWI for Hg set by JECFA (2004) and 81.5% of the new PTWI reported in the recent EFSA guidelines (2012a) for a person weighing 60 kg. According to this intake, and considering fish and shellfish as the major contributors of Hg exposure in the diet, the consumption of these products could be considered safe although the PTWI might be exceeded by regular or excessive consumers of the predatory fish species above mentioned. This supports the AESAN's recommendation for particularly vulnerable population groups, such as children

and pregnant women, to limit the weekly intake of these fish (AESAN, 2011a).

Concerning cadmium, the calculated weekly intake of this metal is 0.29 µg/kg/week. For an average person weighting 60 kg this intake represents 17.57 µg/week, which amounts to approximately 12% of PTWI (EFSA, 2009b) (Table 5). However, fish and shellfish only represent a fraction of the total cadmium intake in the diet, which has been estimated to be between 17.3 and 33.9% by AESAN. Hence, Cd intake due to fish and shellfish might range from 0.22 to 0.44 µg/kg/week. Thus, the calculated weekly Cd intake reported in this study (0.29 µg/kg/week) falls within the normal intake range (AESAN, 2011d) and is very close to the normal cadmium intake from fish consumption estimated by EFSA (2012b), ranging from 0.16 to 0.27 µg/kg/week. Therefore, it is assumed that Cd intake from fish and shellfish does not pose a health risk for consumers.

With regard to lead, a PTWI of 25 µg/kg of body weight was set by JECFA (1993), which is equivalent to the intake of 1500 µg Pb/week for a 60 kg individual. Taking into account Pb concentrations found in the current study and data for fish and shellfish consumption from the ENIDE survey (AESAN, 2011c), the calculated Pb intake represents 75.95 µg/week, which amounts to 5% of the PTWI as seen in Table 5. According to a total diet study made by the Catalan Food Safety Agency (ACSA, 2010), fish and shellfish account for 12.4% of total Pb intake for the Catalonia population (a region located in the northeast of Spain's Mediterranean coast). By assuming this percentage for calculation purposes, the total Pb intake from the fish products studied would represent 612.5 µg/week, which amounts to 41% of the PTWI for Pb. Accordingly, the intake of Pb from the species analyzed in the current study would not represent a major health threat. Nevertheless, if this intake is compared to the BMDL₁₀ for toxic lead effects on kidney (EFSA, 2010), a low but still possible risk of renal damage could not be ruled out.

Finally, the calculated weekly tin intake from the canned fish species analyzed is negligible as it only represents 0.002% of the PTWI for Sn set by JECFA (1988) as shown in Table 5. From these results it might be concluded that the migration of Sn from can to the content is negligible, indicating a proper internal lacquering for the canned products. Similarly, no "health risk" was found for the arsenic levels contained in the fish species studied. Although all analyses carried out determined total arsenic (both organic and inorganic), it is well-known that most As found in fish and shellfish is organic As, the less toxic form. According to EFSA (2009a), only 2% and 3.5% of the arsenic contained in fish and shellfish products, respectively, could be considered as toxic inorganic arsenic. By assuming these percentages, the estimated intake for inorganic As would represent 0.42 µg/day (2.94 µg/week) which is far below the most restrictive BMDL₀₁ for carcinogenic effects of inorganic arsenic (JECFA, 2010). Taking into account that fish and shellfish provide a reduced amount of inorganic arsenic to diet and that the arsenic concentrations found in this study were very low, it is assumed that the total As intake from the fish species analyzed would not be of health concern.

4. Conclusions

In summary, levels of the toxic elements analyzed were broadly comparable to those found in similar national and international studies. For those metals with ML set by EC Regulation for different fish and shellfish products (Hg, Cd, Pb and Sn), median concentrations found in almost all the species analyzed were below those limits, with only a few samples exceeding the ML. Hence, the percentage of non compliance with the EC Regulation was marginal, ranging from 0% (Sn) to 5.15% (Pb) of the total samples analyzed. In general, and considering fish and shellfish species as a whole, we can conclude that the ingestion of the toxic elements studied from fish and shellfish does not present any health risk for the average consumer. Nevertheless, it should not be dismissed that a regular or excessive consumption of certain fish species, e.g. tuna, swordfish, blue shark and cat shark (in

Table 5
Estimated amounts of toxic elements ingested and their respective PTWI percentages.

Metal	Weekly intake (µg/week)	PTWI (µg/week)	% PTWI
Hg	63.63	96	66.281
Cd	17.57	150	11.713
Pb	75.95	1500	5.063
Sn	15.40	840,000	0.002

PTWI: provisional tolerable weekly intake.

the case of mercury) and common sole (for lead) might exceed the recommended weekly intakes (PTWI) or the benchmark dose lower confidence limit (BMDL) for a certain toxic effect. However, this would not necessarily entail a noticeable risk for heavy consumers.

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