Assessment of mercury and cadmium via seafood consumption in Italy: estimated dietary intake (EWI) and target hazard quotient (THQ)

Grazia Barone, Arianna Storelli, Rita Garofalo, Vito Pietro Busco, Nicoletta Cristiana Quaglia, Giuseppe Centrone, Maria Maddalena Storelli*

Biosciences, Biotechnologies and Biopharmaceutical Department - University of Bari - Strada Prov. le per Casamassima Km 3, 70010 Valenzano (Ba), Italy

* Corresponding author. Tel.: +39 80 5443865; fax: +39 80 5443863.

E-mail address: mariamaddalena.storelli@uniba.it (M.M. Storelli)

Abstract

Hg and Cd were quantified in fish, cephalopods and crustaceans from Italian supermarkets. Sample compliance with European dietary standards as well as human health risks according to Provisional Tolerable Weekly Intake and the methodology of Target Hazard Quotient were evaluated. Differences in metal concentrations among organisms were observed. Both element levels were under European legal limits, except for some fish having Hg and Cd contents exceeding or equal to critical values. Estimated weekly intakes (Hg: fish = 0.07-1.44 μg kg^{-1} bw/week; cephalopods = 0.05-0.15 μg kg^{-1} bw/week; crustaceans = 0.04-0.08 μg kg^{-1} bw/week; Cd: fish = 0.04-0.32 μg kg^{-1} bw/week; cephalopods = 0.07-0.27 μg kg^{-1} bw/week; crustaceans = 0.05-0.11 μg kg^{-1} bw/week) as well as target hazard quotient values (THQ<1) were within safe limits. Although there seems to be no important risks associated to seafood consumption, Hg exposure was in some cases close to safety margins and thus levels of this metal should be under frequent surveillance.

Keywords: mercury, cadmium, seafood, dietary intake, PTWI, THQ

1. Introduction

Seafood is an essential component of the world population's basic diet being an important source of proteins, minerals, vitamins and unsaturated essential fatty acids (PUFAs), especially omega-3 PUFAs, which are renowned globally for greatly reducing cholesterol levels and maintaining healthy human hearts, brains, joints and immune systems (Daviglus, Sheeshka, & Murkin, 2002). Despite this scenario, fishery products are a commodity of potential health concern as they can be contaminated with a wide range of environmentally persistent chemicals. One group of contaminants accumulated by marine organisms is constituted by heavy metals and, on toxicity scale, Hg and Cd are amongst the most toxic even in trace amounts. Major human health effects that could strongly be correlated with Cd toxicity are irreversible damage to most cell types due to the inhibition of cell respiration and some key enzyme systems (Santos, Trigueiro, Lemos, Fortunato, & Cardoso, 2013). Specific target systems include the bones, spleen, endocrine glands, liver and above all kidneys where it can cause severe damage (Navas-Acien et al., 2009; Thomas, Hodgson, Nieuwenhujisen, & Jarup, 2009). Mercury exposure may, instead, produce neurotoxin effects and motor function impairment (UNEP, 2002), harm the cardiovascular system (Virtanen, Rissanen, Voutilainen, & Tuomainen, 2007) and cause genetic damage (Grotto et al., 2009). Food safety agencies throughout the world conduct surveillance to ensure that human exposure to toxicants is sufficiently low to not affect health and provide guidelines on the intake of the trace elements by human as well as, hygienic limits for human consumption regularly updated according to advances in scientific knowledge. The Joint FAO/WHO Expert Committee on Food Additives (JEFCA) had established the Provisional Tolerable Weekly Intake (PTWI) of 5 μg kg^{-1} bw/week for total Hg (WHO, 1978) of which no more than 1.6 μg kg^{-1} bw/week should be in the form of methylmercury (EFSA, 2004). Recently, the European Food Safety Authority revised its former opinion on methylmercury proposing a Tolerable Weekly Intake of 1.3 μg kg^{-1} bw/week lower than the former one (EFSA, 2012) on the basis of multiple epidemiological studies. For cadmium, the JEFCA had set a Provisional Tolerable Monthly Intake (PTMI) of 25 μg kg^{-1} bw corresponding to a weekly intake of 5.8 μg kg^{-1} bw/week (JEFCA, 2010). Next, the Panel on Contaminants in the Food Chain issued an opinion in which they recommended that the PTWI should be reduced to a Tolerable Weekly Intake (TWI) of 2.5 μg kg^{-1} bw in order to ensure a high level of protection of all consumers, including exposed and vulnerable subgroups of the population (EFSA, 2009). Also the hygienic standards relatively to marketing are often updated, as happened very recently for Cd whose limits have been further diversified depending on the species (Official Journal of the European Union, 2014). Despite of the fact that the concentrations of these toxicants in seafood have been assessed in a large number of studies (Copat et al., 2013; Santos et al., 2013; Vieira, Morais, Ramos, Delerue-Matos, & Oliveira, 2011), this new legislative scenario imposes further research work. Therefore, this study provides and updates information on the content of mercury (Hg) and cadmium (Cd) in seafood including fish, cephalopods and crustaceans randomly collected from supermarkets in Southern Italy in order to ascertain whether the concentrations of these elements are compliant with European dietary standards. Moreover, possible alert regarding human health hazards either calculating the weekly intake and comparing it with the Provisional Tolerable Weekly Intake (PTWIs) (EFSA, 2004, 2009) or through the methodology of target hazard quotient (THQ) were evaluated.

2. Material and methods

2.1. Sample collection

Different fishery products including fish [*Raja oxyrhincus* (long-nose skate), *Solea vulgaris* (commom sole), *Conger conger* (European conger eel), *Helicolenus dactylopterus* (blackbelly rosefish), *Mugil cephalus* (flathead grey mullet), *Boops boops* (bogue), *Xiphias gladius* (swordfish), *Thunnus thynnus*, (Atlantic bluefin tuna), *Engraulis encrasicolus* (European anchovy), *Sardina pilchardus* (European pilchard), *Scomber scombrus* (Atlantic mackerel),] cephalopod molluscs [*Illex coindetii* (Southern shortfin squid), *Loligo vulgaris* (European squid), *Sepia officinalis* (common cuttlefish), *Sepia orbignyana* (pink cuttlefish), *Sepia elegans* (elegant cuttlefish), *Octopus vulgaris* (common octopus), *Octopus salutii* (spider octopus)] and crustaceans [*Parapenaeus longirostris* (rose shrimp), *Penaeus kerathurus* (grooved shrimp), *Aristeus antennatus* (red shrimp), *Squilla mantis* (mantis shrimps), *Nephrops norvegicus* (Norway lobster)] were randomly collected from supermarkets in Southern Italy during 2012-2013. The number of specimens differed depending on the species: 30-40 specimens of similar size for fish, slices from 10 specimens for swordfish and Atlantic bluefin tuna and 40-50 specimens for cephalopod molluscs and crustaceans. Within of each species, specimens were pooled and edible parts were taken, homogenized and kept in a deep freeze at -20 °C until chemical analysis.

2.2. Chemical analyses

The extractive analytical procedures and the instrumental conditions to determine metal concentrations have been described in detail elsewhere (Storelli, 2008). Briefly, for Cd, aliquots (1-2 g) of the homogenised samples were digested in a quartz Erlenmeyer flask with 11 ml of a mixture of HNO_3-$HClO_4$ (8:3) using a hotplate heated to 150 ^{0}C. Additional aliquots of nitric acid were added until a completely colourless solution was obtained. After evaporation the residue was dissolved in 2 ml of water, and finally, the volume was made up to 25 ml with deionised water. For Hg, the samples were weighted into a conical flask and digested in 10 ml of H_2SO_4-HNO_3 (1:1). The flask was heated under reflux conditions until a completely colourless solution was obtained. After cooling, the resultant solution was diluted to a known volume (100 ml) with deionised water according to the method recommended by Official Italian Agencies (GUCE, 1994). The content of toxic metals was determined by atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000). Cd was analyzed by graphite furnace technique and Hg by a hydride system (HGV-1) after reduction by $NaBH_4$.

2.3. Quality control and assurance

Reference tissue (Tort-2 Lobster Hepatopancreas, National Research Council of Canada, Ottawa, Ontario, Canada) was treated and analysed in the same way as the samples. Results (Hg: 0.28 ± 0.03; Cd: 26.2 ± 2.4 μg g^{-1} dry weight) were in good agreement with the certified values (Hg: 0.27 ± 0.06; Cd: 26.7 ± 0.60 μg g^{-1} dry weight) and the standard deviation were low, proving good repeatability of the methods. The results for standard reference material displayed recoveries of the elements ranging from 91% to 104% (n = 3). The limit of detection (LOD) (Hg: 5 ng g^{-1} wet weight; Cd: 10 ng g^{-1} wet weight) is defined as the concentration corresponding to three times the standard deviation of blanks and the standard of quantification (LOQ_s) are the following: Hg: 13 ng g^{-1} wet weight; Cd: 0.38 ng g^{-1} wet weight. Two samples blank were analyzed together with each sample batch. Metal concentrations in blanks were below the detection limits in all the analyses. Blanks and calibration standard solutions were similarly analyzed as the digested sample solution, and calibration curves constructed. Analyses were duplicated to check the reproducibility of the results. Relative standard deviations among replicates were always less than 10%. Recovery tests were performed for the investigated metals in selected samples by spiking analysed samples with aliquots of the metal standards and then carrying out digestion. The recovery percentages ranged from 96 to 99%. Throughout the manuscript metal concentrations are presented as μg g^{-1} wet weight.

2.4. Health risk assessment

2.4.1. Estimated weekly intake (EWI)

Dietary intake of Hg and Cd through seafood consumption was calculated using the following equation:

$$EWI = (C \times IR)/BW$$

where C represents the element concentration in seafood, IR the daily ingestion rate (g/day) of seafood (fish: 126 g/week; cephalopods: 63 g/week; crustaceans: 49 g/week) (FAO, 2011) and BW the body weight (70 kg). The estimated weekly intakes were compared with the Provisional Tolerable Weekly Intake (PTWI) of Hg [5 μg kg^{-1} bw/week (WHO, 1978) and 1.3 μg kg^{-1} bw/week for MeHg (EFSA, 2012) and a Tolerable Weekly Intake (TWI) of 2.5 μg/kg body weight established by EFSA (2009) for Cd.

2.4.2. Target hazard quotient (THQ)

The methodology for estimation of non-carcinogenic risk target hazard quotient (THQ) and carcinogenic risk (TR) was available in USEPA Region III Risk-based Concentration table (US EPA, 2014) and it is described by the following equation:

$$THQ = [(EF \times ED \times FIR \times C/ RFD \times BW \times AT)] \times 10^{-3}$$

where EF is exposure frequency (365 days/year); ED is the exposure duration (82 years) (Istat, 2013), equivalent to the average lifetime; FIR is the food ingestion rate (fish: 126 g/week; cephalopods: 63 g/week; crustaceans: 49 g/week) (FAO, 2011); C is the metal concentration in fish (μg g^{-1}); RFD is the oral reference dose (Hg = 1.0×10^{-4} μg g^{-1}/day, Cd = 1.0×10^{-3} μg g^{-1}/day) (US EPA, 2014); BW is the body weight (70 kg), and AT is the averaging exposure time for non-carcinogens (365 days/year x ED).

2.5. Statistical analysis

Analysis of variance (Kruskal-Wallis test) was used to test hypothesis about differences in the levels of contaminant accumulation and to determine whether there were concentration differences in the accumulation pattern as a function of the different group of seafood. Statistical analysis was performed with XLSTAT.4.05 (Windows, 2010). Statistically significant differences were expressed for $P \leq 0.05$.

3. Results and discussion

3.1. Metals in fishery products

Concentrations of Hg and Cd in the fishery products are summarized in Table 1. The series that describe interspecies statistical comparison for metal levels are the following: fish>cephalopods=crustaceans ($P<0.03$) for Hg and cephalopods>crustaceans>fish ($P<0.01$) for Cd. In fish, Hg ranging from 0.04 μg g^{-1} wet weight to 0.80 μg g^{-1} wet weight (mean: 0.40 μg g^{-1} wet weight) was the dominating element, whereas Cd occurred at lower levels varying from 0.02 μg g^{-1} wet weight to 0.18 μg g^{-1} wet weight (0.07 μg g^{-1} wet weight) ($P<0.004$). The statistical comparison also revealed that Hg concentrations were different among fish species. The highest levels were detected in swordfish and bluefin tuna with values of 0.80 μg g^{-1} wet weight and 0.74 μg g^{-1} wet weight, respectively and the lowest in anchovies (0.06 μg g^{-1} wet weight) and sardines (0.04 μg g^{-1} wet weight). The other fish species that were surface and mid water (Atlantic mackerel: 0.31 μg g^{-1} wet weight) to bottom level, predators either on small to intermediate sized fish or crustaceans and molluscs (European conger eel: 0.70 μg g^{-1} wet weight; blackbelly rosefish: 0.68 μg g^{-1} wet weight; long-nose skate: 0.62 μg g^{-1} wet weight; common sole: 0.18 μg g^{-1} wet weight; bogue: 0.15 μg g^{-1} wet weight; flathead grey mullet: 0.08 μg g^{-1} wet weight) showed levels intermediate. Accumulation of metals in the marine organisms depends primarily on their content in the aquatic ambient, foraging method, growth cycle, age, trophic position and tendency of metals to undergo biomagnification in the food chain. That is, the fish that are high on the trophic level might be expected to concentrate higher levels of bioaccumulative metals as mercury (Jakimska, Konieczka, Skora, & Namiesnk, 2011) and the same was found in this study for swordfish and bluefin tuna, typical high-order marine predators. In the same way, in accordance with feeding spectra, anchovies and sardines feeding mainly of water plants and plankton (Karachle & Stergiou, 2014) contained significantly lower levels of Hg ($P=0.05$) than fish as European conger eel, long-nose skate, common sole and blackbelly rosefish, whose diet consisting largely of fishes (Stergiou & Karpouzi,

2002) reflected a major metal content. Moreover, the low levels observed in anchovies and sardines could also be habitat effect being they surface feeders and thus exposed to lower contamination levels than demersal and benthic counterparts (Storelli, Barone, Storelli, & Marcotrigiano, 2006). The exception to this trend was flathead grey mullet which, being a bottom living fish, did not exhibit high levels of Hg probably as a result of variation in feeding habits during the life cycle, having a nutritional regime consisting of benthic organisms and detritus in juvenile stage and a diet comprising mainly algae in adult fish (Soyinka, 2008). Concerning Cd, data in literature have demonstrate that this metal in fish preferentially accumulates in internal organs such as liver and kidney, and but not in muscle where the concentrations are usually low (Castro-Gonzalez & Méndez-Armenta, 2008; Storelli, Giacominelli-Stuffler, Storelli, & Marcotrigiano, 2005; Viera et al., 2011). In accordance with this picture, our values were relatively low, ranging from a minimum value of 0.02 μg g^{-1} wet weight to a maximum of 0.18 μg g^{-1} wet weight (mean: 0.07 μg g^{-1} wet weight) encountered in blackbelly rosefish and swordfish, respectively. There is evidence that a major route of metal accumulation in marine organisms is via diet. For example, high amounts of Cd in marine species are usually ascribed to diet in which cephalopods predominate (Storelli & Marcotrigiano, 2004), but evidence for biomagnification up the food chain is inconsistent (Castro-Gonzales & Mendez-Armenta, 2008; Falcó, Llobet, Bocio, & Domingo, 2006; Storelli & Marcotrigiano, 2004). These assumptions justify either the trend of Cd accumulation, with fish having the lowest levels respect to cephalopods and crustaceans or highest Cd concentrations encountered in swordfish and bluefin tuna, organisms with a dietary preference for cephalopods and fish (Stergiou & Karpouzi, 2002) ($P<0.02$). Concerning cephalopods, Hg concentrations varied from 0.05 μg g^{-1} wet weight to 0.17 μg g^{-1} wet weight (mean: 0.12 μg g^{-1} wet weight). The results for mollusc species differed ($P<0.04$) being higher in benthic organisms such as cuttlefish (0.11-0.17 μg g^{-1} wet weight) and octopus (spider octopus: 0.10μg g^{-1} wet weight; common octopus: 0.15 μg g^{-1} wet weight) than squids (European squid: 0.05 μg g^{-1} wet weight; Southern shortfin squid: 0.06 μg g^{-1} wet weight). This variability reflects what observed in fish, confirming the importance of habitat in the uptake of this metal. Also Cd concentrations exhibited a high variability in the species tested (0.07-0.26 μg g^{-1} wet weight; mean: 0.15 μg g^{-1} wet weight) ($P<0.04$), with the lowest concentrations in loliginid squid, *Loligo vulgaris* (0.07 μg g^{-1} wet weight) and the highest in the benthic species, *Sepia officinalis* (0.26 μg g^{-1} wet weight), while the other organisms exhibited levels intermediate ranging from 0.09 μg g^{-1} wet weight to 0.20 μg g^{-1} wet weight. The lack of uniformity for Cd is not surprising, but in accordance with the differences in physiological characteristics of each species. For example, the lysosomal system of loliginids is less developed than in other cephalopod species and these squids could be physiologically limited to storing and detoxifying Cd via binding to insoluble compounds (Pierce, Stowasser, Hastie, & Bustamante, 2008). Alternatively, squids may also have developed mechanisms favouring the excretion of Cd (Bustamante, Cosson, Gallien, Caurant, & Miramand, 2002), so a lower accumulation occurs. However, the interaction of numerous other factors, including diet, size, sex and maturity stage, etc., can also influence the extent of this metal accumulation in cephalopod molluscs (Astorga Espana, Rodríguez-Rodríguez, & Díaz-Romero, 2007; Bustamante, Lahaye, Durnez, Churlaud, & Caurant, 2006; Ichihashi, Nakamura, Kannan, Tsumura, & Yamasaki, 2001; Pierce et al., 2008). Shrimps are promoted as a "low-mercury" seafood (Burger, Stern, & Gochfeld, 2005) and our results (0.06-0.12 μg g^{-1} wet weight; mean: 0.08 μg g^{-1} wet weight) confirm this assumption. Although Norway lobster tended to have the highest concentrations (0.12 μg g^{-1} wet weight), between-species variation in Hg concentration was not significant ($P>0.05$). Conversely, Cd accumulation varied significantly ($P=0.04$) among the examined crustacean species ranging from a minimum of 0.07 μg g^{-1} wet weight to a maximum value of 0.15 μg g^{-1} wet weight (mean: 0.10 μg g^{-1} wet weight) in rose shrimp and in grooved shrimp, respectively. The differences in accumulation between crustacean species are not well known. However, the metal load can be strongly affected by features of the biology of the organism i.e. the crustacean's morphology, its physiology, its mode of feeding and its ecophysiological adaptations to the physicochemistry of the environment (Rainbow, Fialkowski, & Smith, 1998).

3.2. Comparison with literature data

In recent years, a number of studies evaluating the Hg and Cd concentrations in the edible parts of different fishery products have been performed. However, bacause metal concentrations in the tissues of aquatic fauna vary widely depending on where the animal is caught, it seemed interesting to gather data from recent research activities able to improve our knowledge about the occurrence of these metals in organisms from the Mediterranean Sea. As can be seen in Table 2, Hg amounts encountered in this study for anchovy are lower than those reported in literature for the same species from different marine areas including Adriatic Sea (Desideri, Meli, & Roselli, 2010; Storelli, 2008), Sicily Channel in the South Western Mediterranean Sea (Copat, Bella, Castaing, Fallico, Sciacca, & Ferrante, 2012), Tyrrhenian Sea (Brambilla et al., 2013; Copat et al., 2012) and Aegean Sea (Kalogeropoulos, Karavoltsos, Sakellari, Avramidou, Dassenakis, & Scoullos, 2012), while comparable values are reported for *Sardine pilchardus* from different marine areas of the Mediterranean Sea (Bonsignore et al., 2013; Brambilla et al., 2013; Copat et al., 2012; Kalogeropoulos et al., 2012; Storelli, 2008). Except some cases, also Hg data obtained for flathead grey mullet (Brambilla et al., 2013; Storelli et al., 2006), bogue (Bonsignore et al., 2013; Brambilla et al., 2013), common sole (Desideri et al., 2010) and Atlantic mackerel (Brambilla et al., 2013; Desideri et al., 2010; Perugini, Visciano, Manera, Zaccaroni, Olivieri, & Amorena, 2014) are consistent with those reported for different Mediterranean sea locations. Concerning the other fish species, when Hg concentrations are compared with published data substantial variations are noted (Brambilla et al., 2013; Desideri et al., 2010; Storelli, 2008; Storelli & Barone, 2013). An explanation of the contradictory results reported in literature could be attributed to different length/size classes of investigated samples. There is, in fact, a large body data showing that mercury tissue concentrations increase with age/size of marine fauna (Branco, Vale, Canario, & dos Santos, 2007), especially for large predators as swordfish and Atlantic bluefin tuna. They belonging to high trophic levels and exhibiting a predatory behaviour have, in fact, a high potential for Hg accumulation. With respect to Cd, our results are lower than those determined in anchovy from Tyrrhenian Sea (Copat et al., 2012) and in sardine from Aegean Sea (Kalogeropoulos et al., 2012), while values of the same order of magnitude are published for common sole (Desideri et al, 2010), flathead grey mullet (Tepe, 2009; Ylmaz, 2009), Atlantic mackerel (Bilandzic, Đokić, & Sedak, 2011; Desideri et al., 2010; Perugini et al., 2014; Turkemen, Turkemen, Tepe, Tore, & Ates, 2009), swordfish (Desideri et al., 2010), rajee spp., European conger eel and blackbelly rosefish (Storelli, 2008; Storelli and Barone, 2013) from different sea locations of the Mediterranean Sea. For cephalopods, data from the open literatures show that both metal concentrations vary, with values of Hg and Cd extremely higher in common cuttlefish (Duysak, Ersoy, & Dural, 2013) and common octopus from Ionian Sea (Bonsignore et al., 2013) and in spider octopus from Adriatic Sea (Storelli, 2008). Few data are available on the metal content in crustaceans for Mediterranean Sea, and therefore research on this topic remains necessary basic work. However, the Hg and Cd levels found in this study are, in general, lower than those reported by different Authors for crustaceans from Adriatic Sea (Desideri et al., 2010; Storelli, 2008) and from Algeria along the Mediterranean coasts (Ismahene & El Hadi, 2012).

3.3. Compliance with European dietary standards

Protecting human health by reducing exposure to toxic chemicals is nowadays a priority objective in developed countries. A practical implication of this has been the imposition of new and more restrictive regulations. As depicted in Table 1, Hg limit for fishery products is 0.5 µg g^{-1} wet weight, except for certain larger predatory species having a consumption limit of 1.0 µg g^{-1} wet weight (Official Journal of the European Union, 2011), while in the case of Cd, the new guideline values adopted by the European Commission vary from 0.05 µg g^{-1} wet weight to 1.0 µg g^{-1} wet weight, according to different fishery products (Official Journal of the European Union, 2014). The comparison of the results obtained with these maximum permissible levels revealed that Hg concentrations were lower than hygienic limits for human consumption in all species, except for European conger eel (0.70 µg g^{-1} wet weight). However, it can be observed that levels of this metal in swordfish (0.80 µg g^{-1} wet weight), bluefin tuna (0.74 µg g^{-1} wet weight), blackbelly rosefish (0.68 µg g^{-1} wet weight), long-nose skate (0.62 µg g^{-1} wet weight) and Atlantic mackerel (0.31 µg g^{-1} wet weight) specimens were rather close to the maximum permitted, a point that raises concern about the safety consumption. With respect to Cd, the concentrations in all species were below safety levels for human consumption, except for Atlantic bluefin tuna (0.12 µg g^{-1} wet weight) and long-nose skate (0.06 µg g^{-1} wet weight) having values exceeding the permissible limit and for bogue (0.05 µg g^{-1} wet weight) exhibiting concentrations equal to critical value. However, it must be noted that also for this element in some fish species as swordfish (0.18 µg g^{-1} wet weight), European conger eel, flathead grey mullet and common sole (0.04 µg g^{-1} wet weight) the concentrations were close to the European legislative measures.

3.4. Assessment of potential public health risks: Estimated weekly intake (EWI) and estimated target hazard quotient (THQ)

When considering the metal content in marine organisms, suitable for human consumption, the most important aspect is their toxicity to humans (Turan, Dural, Oksuz, & Öztürk, 2009). Different approaches for estimation of human health risks of the metals in food have been proposed. The most widely applied is comparison with the Provisional Tolerable Weekly Intakes (PTWI) representing the amount of substance that can be ingested over a lifetime without appreciative risk. An other way of determining risk is the target hazard quotient (THQ) value proposed by US EPA (2014). It is an integrated risk index that compares the ingested amount of a contaminant with a standard reference dose. A THQ less than one signifies that the level of exposure is lower than the reference dose, which assumes that a daily exposure at this level is not likely to cause any negative health effects during a lifetime in a human population. As illustrated in Figure 1 the dietary exposure calculation for Hg relatively to consumption of fish, cephalopods and crustaceans varied largely being in the ranges of 0.07-1.44 µg kg^{-1} bw/week, 0.05-0.15 µg kg^{-1} bw/week and 0.04-0.08 µg kg^{-1} bw/week, respectively. Among fish, the highest values were calculated for European conger eel (1.26 μg kg^{-1} bw/week), blackbelly rosefish (1.22 μg kg^{-1} bw/week) and long-nose skate (1.12 μg kg^{-1} bw/week) and top-level predators (high-order predators), swordfish (1.44 μg kg^{-1} bw/week) and Atlantic bluefin tuna (1.33 μg kg^{-1} bw/week) constituting from 22.4% to 28.8% of the PTWI. Although the present estimations were consistent with the recommended PTWI, emphasis should be placed on the fact that the toxicity of mercury is related to the chemical form present in matrix, with organic species such as methylmercury being the most toxic ones. It essentially account for the majority of mercury found in marine organism muscle tissue (70-100%) either fish (Kuballa, Moellers, Schoeberl, & Lachenmeier, 2011) or molluscs (Kehrig, Costa, Moreira, & Malm, 2006) and crustaceans (Julshamn, Valdersnes, Duinker, Nedreaas, Sundet, & Maage, 2015). Considering the worst case hypothesis (100% methylmercury), the consumer would exceed the PTWI defined for methylmercury (1.3 μg kg^{-1} bw/week) solely through the consumption of swordfish and Atlantic bluefin tuna, although the dietary exposure calculation for European conger eel and blackbelly rosefish was very close to the critical value. Regarding THQ (Fig. 2), Hg values were lower than 1 for all species tested (fish: 0.04-0.80; cephalopods: 0.03-0.09; crustaceans: 0.02-0.05), although values close to one were associated with the consumption of swordfish and Atlantic bluefin tuna. For Cd, weekly exposure calculation showed values very below the established limit for all fishery products (fish: 0.04-0.32 μg kg^{-1} bw/week; cephalopods: 0.07-0.27 μg kg^{-1} bw/week; crustaceans: 0.05-0.11 μg kg^{-1} bw/week), A similar scenario was encountered with the target hazard quotient (THQ) (fish: 0.01-0.05; cephalopods: 0.01-0.04; crustaceans: 0.01-0.02), whose values less than one, indicated that the health risk associated with Cd exposure was negligible.

4. Conclusions

In the present study samples of seafood including fish, cephalopods and crustaceans were collected and analyzed for Hg and Cd content. Analytical data showed that there were significant differences in metal levels among the different species tested. The series describing interspecies differences are the following: fish>cephalopods=crustaceans for Hg and cephalopods>crustaceans>fish for Cd. In general, levels particularly high of Hg and Cd, above the limits set by European Community regulations, are observed in top predators and benthic carnivorous fish. However, Cd dietary exposure does not seem to pose any risk to the consumer while Hg toxicological evaluation suggests that although there seems to be no important hazards associated to consumption of these seafood, exposure was in some cases close to the safety margins and thus Hg level in seafood should be under frequent surveillance. This assumption is more true if one considers that the estimated intake does not include the contribution of other foods that may constitute further contamination sources to which population is also subjected. Consequently, Hg levels particularly high in swordfish, Atlantic bluefin tuna and European conger eel, suggests caution in their consumption, especially in regular fish consumers and particular population segments as pregnant and lactating women and young children.

References

Astorga-España, M. S., Rodríguez-Rodríguez, E. M., & Díaz-Romero, C. (2007). Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). *Journal of Food Composition and Analysis, 20*, 273–279.

Bilandzic, N., Đokić, M., & Sedak, M. (2011). Metal content determination in four fish species from the Adriatic Sea. *Food Chemistry,* 124, 1005-1010.

Bonsignore, M., Salvagio Manta, D., Oliveri, E., Sprovieri, M., Basilone, G., Bonanno, A., et al. (2013). Mercury in fishes from Augusta Bay (Southern Italy): Risk assessment and health implication. *Food and Chemical Toxicology, 56*, 184-194.

Brambilla, G., Abete, M. C., Binato, G., Ciaravalle, E., Cossu, M., Dellatte, E., et al. (2013). Mercury occurrence in Italian seafood from the Mediterranean Sea and possible intake scenarios of the Italian coastal population. *Regulatory Toxicology and Pharmacology, 65*, 268-277.

Branco, V., Vale, C., Canario, J., & dos Santos, M. N. (2007). Mercury and selenium in blue shark (*Prionace glauca*, L. 1758) and swordfish (*Xiphias gladius*, L. 1758) from two areas of the Atlantic Ocean. *Environmental Pollution, 150*, 373-380.

Burger, J., Stern, A., & Gochfeld, M. (2005). Mercury in Commercial Fish: Optimizing Individual Choices to Reduce Risk. *Environmental Health*

Perspectives, 113, 266–271.

Bustamante, P., Cosson, R. P., Gallien, I., Caurant, F., & Miramand, P. (2002). Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. *Marine Environmental Research, 53*, 227–241.

Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., & Caurant, F. (2006). Total and organic Hg concentrations in cephalopods from the North East Atlantic waters: influence of geographical origin and feeding ecology. *Science of the Total Environment, 368*, 585–596.

Castro-Gonzalez, M. I., & Méndez-Armenta, M. (2008). Heavy metals: implications associated to fish consumption. *Environmental Toxicology and Pharmacology, 26*, 263-71.

Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S., et al. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Composition advisories. *Food and Chemical Toxicology, 53*, 33-37.

Copat, C., Bella, F., Castaing, M., Fallico, R., Sciacca, S., & Ferrante, M. (2012). Heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers. *Bulletin of Environmental Contamination and Toxicology, 88,* 78-83.

Daviglus, M., Sheeshka, J., & Murkin, E. (2002). Health benefits from eating fish. *Comments on Toxicology, 8*, 345-374.

Desideri, D., Meli, M. A., & Roselli, C. (2010). A biomonitoring study: ^{210}Po and heavy metals in marine organisms from the Adriatic Sea (Italy). *Journal of Radioanalytical and Nuclear Chemistry, 285,* 373-382.

Duysak, O., Ersoy, B., & Dural, M. (2013). Metal concentrations in different tissues of cuttlefish (*Sepia officinalis*) in Iskenderun Bay, Northeastern Mediterranean. *Turkish Journal of Fisheries Aquatic Sciences, 13,* 205-210.

EFSA (European Food Safety Authority). (2004). Opinion of the scientific panel on contaminants in food chain on a request from the commission related to mercury and methylmercury in food. *EFSA Journal, 34*, 1-14.

EFSA (European Food Safety Authority). (2009). Scientific opinion of the panel on contaminants in the food chain. Cadmium in food. *EFSA Journal, 980*, 1-139.

EFSA (European Food Safety Authority). (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal, 10*, 2985, 1-241.

Falcó, G., Llobet, J. M., Bocio, A., & Domingo, J. L. (2006). Daily intake of arsenic cadmium, mercury, and lead by consumption of edible marine species. *Journal of Agricultural and Food Chemistry, 54*, 6106-6112.

FAO (Food and Agriculture Organization). (2011). FAOSTAT Food Supply: Livestock and Fish Primary Equivalent. URL http://faostat.fao.org/site/6107DesktopDefault.aspx?PageID=610. Accessed 29.09.14.

Galitsopoulou, A., Georgantelis, D., & Kontominas, M. (2012). The influence of industrial-scale canning on cadmium and lead levels in sardines and anchovies from commercial fishing centres of the Mediterranean Sea. *Food Additives and Contaminants, 5*, 75-81.

Grotto, D., Barcelos, G. R., Valentini, J., Antunes, L. M., Angeli, J. P., Garcia, S. C., et al. (2009). Low levels of methylmercury induce DNA damage in rats protective effects of selenium. *Archives of Toxicology, 283*, 249-254.

GUCE (Gazzetta Ufficiale della Repubblica Italiana). (1994). Metodi di analisi per la ricerca di residui di metalli pesanti e arsenico. No. 21 of 27/01/1994.

Ichihashi, H., Nakamura, Y., Kannan, K., Tsumura, A., & Yamasaki, S. (2001). Multi-elemental concentration in tissues of Japanese common squid (*Todarodes pacificus*). *Archives of Environmental Contamination and Toxicology, 41,* 483–490.

Ismahene, G., & El Hadi, K. M. (2012). Assessment of heavy metal concentrations (Lead, Cadmium and Zinc) in three Crustacean species fished for in two region of eastern Algeria. *Annals of Biological Research, 3*, 2838-2842.

Istat. (2013). Demografia. Struttura della popolazione residente. URL http://www.istat.it/it/archivio/demografia. Accessed 05.10.14.

Jakimska, A., Konieczka, P., Skora, K., & Namiesnk, J. (2011). Bioaccumilation of metals in tissues marine animals, Part II: Metal concentrations in animal tissues. *Polish Journal of Environmental Studies, 5,* 1127-1146.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). (2010). Summary report of the seventy-third meeting of JECFA. Geneva, 8–17 June 2010. JECFA/73/SC: 1-17.

Julshamn, K., Valdersnes, S., Duinker, A., Nedreaas, K., Sundet, J. H., & Maage, A. (2015). Heavy metals and POPs in red king crab from the Barents Sea. *Food Chemistry, 167,* 409-417.

Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Avramidou, S., Dassenakis, M., & Scoullos, M. (2012). Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish. *Food and Chemical Toxicology, 50,* 3702-3708.

Karachle, P. K., & Stergiou, K. I. (2014). Feeding and ecomorphology of three clupeoids in the North Aegean Sea. *Mediterranean Marine Science, 15*, 9-26.

Kehrig,, H. A., Costa, M., Moreira, I., & Malm, O. (2006). Total and Methyl Mercury in Different Species of Molluscs from Two Estuaries in Rio de Janeiro State. *Journal of the Brazilian Chemical Society, 17*, 1409-1418.

Kuballa, T., Moellers, M., Schoeberl, K., & Lachenmeier, D. W. (2011). Survey of methylmercury in fish and seafood from the southwestern German market. *European Food Research and Technology, 232*, 737-742.

Navas-Acien, A., Tellez-Plaza, M., Guallaz, E., Muntner, P., Sibergeld, E., Jaar, R., et al. (2009). Blood cadmium and lead and chromic kidney desease in US adults: a joint analysis. *American Journal of Epidemiology, 170*, 1154.1164.

Official Journal of the European Union. (2011). Commission Regulation (EU) No 420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. L *111*, 3-6.

Official Journal of the European Union. (2014). Commission Regulation (EU) No 488/2014 of 12 May 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in foodstuffs. L *138*, 75-79.

Perugini, M., Visciano, P., Manera, M., Zaccaroni, A., Olivieri, V., & Amorena, M. (2014). Heavy metal (As, Cd, Hg, Pb, Cu, Zn, Se) concentrations in muscle and bone of four commercial fish caught in the central Adriatic Sea, Italy. *Environmental Monitoring and Assessment, 186*, 2205–2213.

Pierce, G. J., Stowasser, G., Hastie, L. C., & Bustamante, P. (2008). Geographic, seasonal and ontogenetic variation in cadmium and mercury concentrations in squid (Cephalopoda: Teuthoidea) from UK waters *Ecotoxicology and Environmental Safety, 70,* 422–432.

Rainbow, P. S., Fialkowski, W., & Smith, B. D. (1998). The sandhopper *Talitrus saltator* as a trace metal biomonitor in the Gulf of Gdansk, Poland. *Marine Pollution Bulletin, 36*, 193–200.

Santos, L. F. P., Trigueiro, I. N. S., Lemos, V. A., Fortunato, D. M. N., & Cardoso, R. C. V. (2013). Assessment of cadmium and lead in commercially important seafood from Säo Francisco do Conde, Bahia, Brazil. *Food Control, 33,* 193-199.

Soyinka, O. O. (2008). The feeding ecology of *Mugil cephalus* (Linnaeus) from a high brackish tropical lagoon in South-west, Nigeria. *African Journal of Biotechnology, 7*, 4192-4198.

Stergiou, K. I., & Karpouzi, V. S. (2002). Feeding habits and trophic levels of Mediterranean fish. *Reviews in Fish Biology and Fisheries, 11*, 217-254.

Storelli, M. M, & Barone, G. (2013). Toxic Metals (Hg, Pb, and Cd) in Commercially Important Demersal Fish from Mediterranean Sea: Contamination Levels and Dietary Exposure Assessment. *Journal of Food Science, 78*; T362-T366.

Storelli, M. M. (2008). Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). *Food Chemical and Toxicology, 46*, 2782-2788.

Storelli, M. M., & Marcotrigiano, G. O. (2004). Content of mercury and cadmium in fish (*Thunnus alalunga*) and cephalopods (*Eledone moschata*) from the south-eastern Mediterranean Sea. *Food Additives and Contaminants, 21*, 1051-1056.

Storelli, M. M., Barone, G., Storelli, A., & Marcotrigiano; G. O. (2006). Trace metals in tissues of Mugilidis (*Mugil auratus*, *Mugil capito* and *Mugil labrosus*) from the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology, 77*, 43-50.

Storelli, M. M., Giacominelli-Stuffler, R., Storelli, A., & Marcotrigiano, G. O. (2005). Accumulation of mercury, cadmium, lead and arsenic in swordfish and bluefin tuna from the Mediterranean Sea: a comparative study. *Marine Pollution Bulletin, 50*, 1004-1007.

Tepe, Y. (2009). Metal concentrations in eight fish species from Aegean and Mediterranean Seas. *Environmental Monitoring and Assessment*, *159*, 501-509.

Thomas, L. D., Hodgson, S., Nieuwenhujisen, M., & Jarup, I. (2009). Early kidney damage in a population exposed to cadmium and other heavy metals. *Environmental Health Perspectives, 117*, 181-184.

Turan, C., Dural, M., Oksuz, A., & Öztürk, B. (2009). Levels of heavy metals in some commercial fish species captured from the Black Sea and Mediterranean Coast of Turkey. *Bulletin of Environmental Contamination and Toxicology, 82*, 601-604.

Turkemen, M., Turkemen, A., Tepe, Y., Tore, Y., & Ates, A. (2009). Determination of metals in fish species from Aegean and Mediterranean seas. *Food Chemistry, 113*, 233-237.

UNEP (United Nations Environment Programme). (2002). Global mercury assessment. Geneva: United Nations Environment Programme chemicals. pp. 270.

US EPA (United States Environmental Protection Agency). (2014). Risk-Based Concentrations Table. URL http://www.epa.gov/reg3hwmd/risk/human/index.htm. Accessed 11.10.14.

Vieira, C., Morais, S., Ramos, S., Delerue-Matos, C., & Oliveira, M. B. P. P. (2011). Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption. *Food and Chemical Toxicology*, *49*, 923-932.

Virtanen, J. K., Rissanen, T. H., Voutilainen, S., & Tuomainen, T. P. (2007). Mercury as a risk factor for cardiovascular diseases. *The Journal of*

Nutritional Biochemistry, 18, 75-85.

WHO (World Health Organization). (1978). Mercury. In: Safety evaluation of certain food additives and contaminants. Twenty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Food Additives: Series Number 13. Geneva (Switzerland): WHO.

Yilmaz, F., (2009). The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three Economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreichromis niloticus*) inhabiting Köycegiz Lake-Mugla (Turkey). *Turkish Journal of Science and Technology, 4*, 7-15.

Figure Captions

Fig. 1.

Fig. 2.