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Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories

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ABSTRACT

The present study evaluate concentrations of arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni), vanadium (V) and zinc (Zn) in fish and shellfish from the Gulf of Catania. Heavy metal analysis was carried on with an ICP-MS, and consumption rates advisory for minimizing chronic systemic and non cancer endpoints in child and adults have been estimated. Among metals investigated, only Cd and Pb have a limit set by European Community for human consumption, and the thresholds were not been exceeded in analyzed species. The As, toxic in its inorganic form, have not a regulatory limit yet, but the Environmental Protection Agency and the World Health Organization provide a reference dose, a cancer slope factor and a tolerable intake, applicable in the risk factors assessment. Arsenic target hazard quotient (THQ) values, suggest that human should minimizing meals/week of analyzed species to avoid deleterious effect during lifetime, furthermore, with As cancer risk assessment, for most of the fish, the risk for cancer is greater than the acceptable lifetime risk of 10^{-5}. Our results give important finding about the consumption limits on certain metals, especially for As, all for minimizing potential health risks in population.

1. Introduction

The environmental monitoring projects have always been playing an important role to provide scientific knowledge in assessing health and sustainability of ecosystems (Copat et al., 2012a,b; Tigano et al., 2009; Tomasello et al., 2012). In recent years, in the scientific community, the interest in knowing if there are risks to human health is growing, derived from consumption exposure to individual contaminants or groups of contaminants occurring in the environment (Chien et al., 2002; Conti et al., 2012; Copat et al., 2012c; Domingo et al., 2007; Falco et al., 2006; Ferrante et al., 2010; Llobet et al., 2003, 2006, 2007; Marti-Cid et al., 2008; Spada et al., 2011; Storelli, 2008; Turyk et al., 2012). Though different nutritional benefits come from fish consumption, mainly due to the high quality protein content (fish provides 17% of the total protein of animal origin and 6% of the total protein intake by humans), vitamins and essential nutrients (Adeyeye, 2002; Ersoy and Celik, 2010; Zalloua et al., 2007), to date, the balance between benefits and risks due to ingestion of chemical contaminants has been poorly characterized (Domingo et al., 2007).

For this reason, in recent years the fish consumption advisories for the population has become of great concern, as though sometimes contaminants exceed the legal limits set by European Regulations for food (EC, 2006), they do not always represent a risk for human health.

Due to anthropogenic and natural emissions, toxic heavy metals have long been recognized as one of the most important pollutants in ecosystems, because they can be readily assimilated and bioaccumulated in organisms, determining a potential risk to human health by consuming contaminated food.

In this study, we analyzed arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), manganese (Mn), nickel (Ni), vanadium (V) and zinc (Zn) in edible tissues from local seafood, and related risk-based consumption limits for adults and children.

Among the analyzed metals, the European Community has set a limit only for Pb and Cd (EC, 2006) in seafood (Pb: 0.3 mg/kg w.w. for all analyzed fish and 1.5 mg/kg w.w. for *Donax trunculus*; Cd: 0.1 mg/kg w.w. for *Engraulis encrasicolus* and *Trachurus trachurus*, 0.3 mg/kg w.w. for *Scomber scombrus*, *Mullus barbatus* and *Arnoglossus laterna*, 1 mg/kg w.w. for *D. trunculus*); instead, the World Health Organization, the Environmental Protection Agency and other international organizations have suggested tolerable intake, reference dose and health risk factors applicable to a large number of pollutants.

To estimate the potential risk for human health derived from ingesting contaminated seafood we have evaluated: the daily intake per meal comparing it with the provisional tolerable daily

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intake (PTDI) recommended by Joint FAO/WHO Expert Committee on Food Additive online database (JECFA, 2009); the target hazard quotient (THQ) (US-EPA, 1989), in order to evaluate possible alert regarding adverse effects; the cancer risk for As (within an acceptable lifetime risk – ARL – of 10^{-5}); the number of eligible meals per week in order to minimize chronic systemic effects (US-EPA, 2000).

2. Materials and methods

2.1. Fish and shellfish collection

A total of 150 specimens belonging to five different fish species and 250 g of edible part of *D. trunculus* (Linneaus, 1758) (Sand Dwelling Mussel), were purchased during February–March 2012 from local fishermen in the Catania fish market. The chosen fish species are *A. laterna* (Walbaum, 1792) (Mediterranean scaldfish, $n = 30$), *M. barbatus* (Linneaus, 1758) (Red mullet, $n = 30$), *E. encrasicolus* (Linneaus, 1758) (European anchovy, $n = 30$), *T. trachurus* (Linneaus, 1758) (Atlantic horse mackerel, $n = 30$) and *S. scombrus* (Linneaus, 1758) (Atlantic mackerel, $n = 30$). Fish and shellfish were transported to the laboratory and stored at −80 °C until analysis.

2.2. Heavy metals analysis

From each individual fish, two aliquots of 0.5 g of muscle tissue were taken, from which were extracted and quantified separately the metals of interest. Regarding the species *D. trunculus*, for each specimen the edible part was separated, collecting in this way a sample of 250 g which was homogenized and treated as a single sample. From this 30 aliquots of 0.5 g were analyzed. The samples were mineralized in an Ethos TC microwave system (Milestone S.r.l., Italy) equipped with pressurized vessels (N. 12), using a heated mixture of strong acids. A digestion solution was prepared with 6 ml of 65% nitric acid (HNO_3) (Carlo Erba) and 2 ml of 30% peroxide hydrogen (H_2O_2-Carlo Erba) over a 50 min operation cycle at 200 °C. After mineralization, the vessels were opened if a temperature < 25 °C was reached, then the content was decanted in falcon tubes and ultra-pure water (Merck) was added to the samples up to 30 ml; for quantification of metals an ICP-MS Elan-DRC-e (Perkin–Elmer, USA) was used.

Analytical blanks were run in the same way as the samples, and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono element certified reference solution ICP Standard (Merck).

Standard reference material Lake Superior fish 1946 NIST was used to validate analysis.

Mean recoveries for As, Cd, Cu and Zn were 97.4%, 94.6%, 110% and 90.4% respectively of the certified values.

Cr, Pb, Ni, Mn and V reference values were not given in the analysis certificate of the standard reference material used, thus we spiked 10 real samples in twice with 5 μg/l of each analyte to validate analysis, and the percentage of recovery is given in Table 1.

The method detection limits (MDL) estimated with 3σ of the procedure blanks were (mg/kg w.w.): As 0.013, Cd 0.0003, Cr 0.003, Pb 0.0001, Mn 0.055, Ni 0.007, V 0.002, and Zn 0.109.

The limits of as 0.13, Cd 0.003, Cr 0.03, Pb 0.001, Mn 0.55, Ni 0.07, V 0.02, and Zn 1.09 quantification (LOQ) estimated with the MDL 10 were (mg/kg w.w.).

2.3. Statistical analysis

Statistical analysis was carried out with Student's *t*-test for paired samples, applying $p < 0.05$ as the minimum level of significance, using the statistical software package SYSTAT, version 9 (Systat Inc., Evanston, IL, USA).

Table 1
Recovery percentage of 10 spiked (5 μg/l) real samples performed in twice and recovery means.

	Cr (%)	Pb (%)	Ni (%)	Mn (%)	V (%)
1	95	105	104	81	87
2	93	109	98	101	91
3	105	90	85	106	96
4	87	96	96	99	106
5	89	98	84	82	102
6	108	111	106	96	116
7	105	108	110	95	91
8	91	103	119	98	97
9	97	112	97	106	96
10	88	117	90	115	106
Mean	95.8	105	99	98	99

2.4. Risk-based consumption limits

Although there are several online databases that provide information on the European population average ingestion rate for fish and shellfish (for ex. Pan-European Food Data Bank, Food and Agricultural Administration Statistical Databases, Italian National Institute of Statistics ISTAT), we choose Environmental Protection Agency Methodology, based on the estimation of risk-based consumption limits expressed in terms of real meals.

All consumption limits and risk factors were calculated assuming for adults a meal size of 227 g and a body weight (BW) of 70 kg (US-EPA, 2000), and for children of six years old a meal size of 114 g and a BW of 16 kg (Mansilla-Rivera and Rodriguez-Sierra, 2011).

Furthermore, all As consumption limits calculations were made assuming that the toxic inorganic arsenic was 3% of the total (FSA, 2004).

The estimated daily intake per meal size of seafood (EDIm) was made according to the equation reported in a previous report (Copat et al., 2012c)

$$EDIm = (MS \times C)/BW$$

where MS is the meal size, *C* is the metal concentration (mg/kg w.w.) and BW is the body weight.

Risk factors were calculated according to the guideline of the United State Environmental Protection Agency (US-EPA, 1989, 2000). Additionally, based on the US-EPA Guidance (1989), we assumed that the ingestion dose is equal to the adsorbed contaminant dose and that cooking has no effect on the contaminants (Chien et al., 2002).

Target hazard quotient (THQ), indicate the ratio between exposure and the reference dose, and calculations were made using the standard assumption for an integrate US-EPA risk analysis.

When THQ risk is above 1, considered by the US-EPA (1989), systemic effects may occur, and it means that THQ is higher than the reference dose.

$$THQ = (EF \times ED \times MS \times C)/(RfDo \times BW \times AT).$$

Lifetime cancer risk (CR) for As was obtained by using the cancer slope factor (CSF), provided by EPA only for this metal. If CR risk is above the acceptable lifetime risk – ARL – of 10^{-5}, value considered by the US-EPA (2000), and applied in this study, it indicates a probability greater than 1 chance over 100,000 of an individual of developing cancer. The equation is expressed in the following equation (US-EPA, 1989):

$$CR = (EF \times ED \times MS \times C \times CSF)/(BW \times AT)$$

EF is the exposure frequency, or number of exposure events per year of exposure (from 365 days/year for people who eat fish seven times a week to 52 days/year for people who eat fish one time a week); ED is the exposure duration (adults, 70 years; children, 6 years); MS is the food meal size (adults, 0.227 kg/day; children, 0.114 kg/day); *C* is the metal concentration in fish (μg/g, wet weight); RfDo is the oral reference dose (μg/g/day); BW is the body weight (adults 70 kg; children 16 kg); AT is the averaging time (it is equal to EF × ED); CSF is the cancer slope factor (μg/g/day) set by US-EPA only for inorganic As. EF, ED, MS, BW and AT, are default data provided by US-EPA, 1989, 2000) for consumption limits calculation. RfDo and CSF for single contaminant are provided by EPA's Integrated Risk Information System (IRIS, 2012) online database.

The allowable number of fish meals of a specific meal size that may be consumed over a given period of time was also evaluated.

For non-carcinogenic effects, we obtained the maximum allowable fish consumption rate meals/week (CRmw) (US-EPA, 2000) that would not be expected to cause any chronic systemic effects.

3. Results

Mean concentrations and standard deviations of all analyzed metals are shown in Table 2.

Arsenic concentration was found significantly higher in muscle tissue of *M. barbatus* compared to the other analyzed species ($p < 0.001$). The same was found for Cd, Cr, Pb, Mn, Ni ($p < 0.001$) and V ($p < 0.01$) concentrations in *D. trunculus* compared to the other fish species. *A. laterna* had also V ($p < 0.05$) and Mn ($p < 0.01$) concentrations significantly higher than the other fish species. Zn presented the major concentrations in all analyzed species, but only in *E. encrasicolus* and in *D. trunculus* values were significantly high ($p < 0.05$). Values of estimated daily intake per meal size of seafood for humans (Table 3) were higher than those suggested by JECFA for inorganic As in *M. barbatus* for children age class, and lower for Zn for both adults and children age classes.