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Risk characterization for polycyclic aromatic hydrocarbons and toxic metals associated with fish consumption

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ABSTRACT

Concentrations of 11 polycyclic aromatic hydrocarbons (PAHs) and toxic metals (Hg, Cd and Pb) were measured in three fish species [(*Raja miraletus* (brown ray), *Lepidorhombus whiffiagonis* (megrim) and *Lophius piscatorius* (angler)] from Mediterranean Sea (Adriatic Sea). No significant difference in PAHs and metal concentrations was encountered among the fish species examined. Total PAH concentrations ranged from 209.9 ng g^{-1} wet weight to 227.2 ng g^{-1} wet weight. Low-molecular weight (two and three rings) PAHs were observed dominating over the high molecular weight (from four to six rings) PAHs. With regard to metals, Hg exhibited the highest concentrations (0.68–0.98 μg g^{-1} weight wet), followed by Pb (0.08–0.12 μg g^{-1} weight wet) and Cd (0.05–0.10 μg g^{-1} weight wet). The health risks by consumption of these species were assessed and did not present threat to public health concerning PAH, Pb and Cd intakes. However, the estimated exposure from Hg illustrates the importance of limiting the dietary consumption of larger-sized fish.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the most ubiquitous organic pollutants in the marine environment (Ramalhosa et al., 2012). They enter the aquatic environment primarily from oil spills, atmospheric pollution resulting from incomplete combustion of fuels, and industrial and urban effluents (Ramalhosa et al., 2012). Owing to their low aqueous solubility and strong hydrophobic nature, these contaminants tend to associate with suspended material with the underlying sediments as their ultimate sink (Patrolecco et al., 2010). Also toxic metals, such as Hg, Cd, and Pb, once released in the marine environment, can be rapidly adsorbed on particulate material and precipitate on the bottom (Ney and Van Hassel, 1983). Consequently fish living in the bottom waters with recurrent contact with the sediments are more exposed to sediment-associated contamination than fish living in the upper zone of the water column (Yilmaz et al., 2010). *Raja miraletus* (brown ray), *Lepidorhombus whiffiagonis* (megrim) and *Lophius piscatorius* (angler), important commercial species of the Mediterranean Sea, besides depending on the bottom to feed, have carnivorous feeding habits in higher trophic levels (Stergiou and Karpouzi, 2002), a property that favours accumulation of toxic compounds. Information about PAHs and toxic metal concentrations in these edible fish are, therefore, essential for evaluating possibly risks for human health. In fact, these substances express toxicity, so several of them are classified as probable or possible carcinogens by the International Agency for Research on Cancer (IARC). In addition, it is a well-known fact that the general population is most commonly exposed to metals, especially to Hg, through the consumption of fish. In this context the present study examines the extent of accumulation of PAHs and some toxic metals, namely Hg, Pb and Cd in three different fish species above mentioned commonly consumed in Italy, and evaluates whether such contamination levels may pose risks to human health.

2. Materials and methods

2.1. Sample collection

Specimens of *R. miraletus* (brown ray: no. 35), *L. whiffiagonis* (megrim: no. 28) and *L. piscatorius* (angler: no. 30) were collected in the South Adriatic Sea (Mediterranean Sea) from May to July

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2011. The fish lengths (brown ray: 35.0–85.7 cm, average: 66.9 ± 1.8 cm; megrim: 18.2–32.3 cm, average: 23.5 ± 1.9 cm; angler: 40.0–120.0 cm, average: 101.7 ± 2.2 cm) and weights (brown ray: 241.5–1057.6 g, average: 915.6 ± 11.3 g; megrim: 32.0–275.3 g, average: 150.2 ± 8.5 g; angler: 250.9–3069.0 g, average: 1015.7 ± 232.0 g) were individually measured before they are pooled (brown ray: 11 pools; megrim: 8 pools; angler: 12 pools) for analysis. From fish of each pool, muscle tissue was taken, homogenized and kept in a deep freeze at −20 °C until chemical analysis. Length has been chosen as basic measure to reflect fish age, because it is less subject to fluctuation than body weight (Diaz et al., 1994).

2.2. Sample preparation

For PAH analyses [naphthalene (NA), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), chrysene (CHR), benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), dibenzo[*a*,*h*]anthracene (DBahA), benzo[*g*,*h*,*i*]perylene (BghiP)], 20 g of the sample homogenates were weighed and digested in an alkaline mixture (100 mL ethanol, 1 M KOH) for 2 h by mechanical shaking extracted three times by water *n*-hexane (15 mL). Then, *n*-hexane combined extracts were dried over anhydrous sodium sulphate and concentrated by evaporation. The extract was purified on a column with a 15 mL hexane dichloromethane (3:1, v/v) eluent. The column was packed with 5 g of Florisil (mesh 60/100, Supelco, USA) and a top layer of anhydrous sodium sulphate and was washed with 10 mL of hexane before use. The extract collected was concentrated under a gentle nitrogen stream (Hwang et al., 2011) and dissolved in dichloromethane before the analysis.

The analysis was carried out using a GC Thermo Trace Ultra coupled with a Thermo PolarisQ mass spectrometer. A 30 m long column model DB 5-MS of 0.25 μm I.D. was used. Helium was used as the carrier gas and the column head pressure was maintained at 10 psi to give an approximate flow rate of 1 mL/min. The injector was maintained at 280 °C, while the transfer line was maintained at 250 °C. All injection volumes were 1 μL in the splitless mode. The column temperature was initially held at 80 °C for 1 min, ramped to 300 °C at a rate of 5 °C/min, and then the temperature was held at 300 °C for 20 min. The mass spectrometer was used in electron ionisation (70 eV) mode and operated in the selected ion monitoring (SIM) mode using the typical PAH molecular ions (M+) *m*/*z*: 128, 166, 178, 202, 228, 252, 276 and 278.

The most abundant ion was used for quantification and two other ions were additionally used for confirmation. The quantification and confirmation ion abundances were determined by injection of standards under the same chromatographic conditions using full-scan (mass/charge ratio ranging *m*/*z* 50–600). PAHs were confirmed by their retention times, the identification of quantification and confirmation ions and the determination of ion ratios. The extractive analytical procedures and the instrumental conditions to determine metal concentrations have been described elsewhere (Storelli, 2008).

In detail, for Pb and 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 °C. Additional aliquots of nitric acid (maximum of 0.2 mL) 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 the Official Italian Agencies (GURI, 1994). The content of toxic metals was determined by atomic absorption spectrophotometer (AAS) (Perkin Elmer Analyst 800). Cd and Pb was analyzed by graphite furnace technique (THGA-800 P.E.) and Hg by a hydride system (FIMS 100) after reduction by $SnCl_2$.

2.3. Quality control and assurance

Prior to the sample analysis, PAH standards (98–99% purity) (Absolute Standards, Analytical Tecnology S.r.l., Milano) were used to make multipoint calibration curves (1, 10, 100 and 1000 ng g^{-1}) whose correlation coefficients ranging from 0.993 to 0.999. The limit of detection (LOD) and the limit of quantification (LOQ) for the individual PAHs ranged from 0.10 to 2.0 ng g^{-1} and 0.30 to 4.0 ng g^{-1}, respectively. For metals, 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; Pb: 0.32 ± 0.18 μg g^{-1} dry weight) were in good agreement with the certified values (Hg: 0.27 ± 0.06; Cd: 26.7 ± 0.60; Pb: 0.35 ± 0.13 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; Cd: 0.10; Pb: 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) are the following: Hg: 13; Cd: 0.38; Pb: 40 ng g^{-1} wet weight. Two empty samples blank were analysed together with each sample batch to detect contamination during the sample handling, preparation and or analysis. Metal and PAH concentrations in blanks were below the detection limits in all the analyses. Blanks and calibration standard solutions were similarly analysed as the samples 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 and PAHs in selected samples by spiking analysed samples with aliquots of the metal and PAH standards (50 ng g^{-1}). The recovery percentages ranged from 96% to 99% for metals and from 85% to 110% for PAHs, and the concentrations were not corrected for percent recovery. Metal and PAH concentrations were determined on a μg g^{-1} wet weight basis and ng g^{-1} wet weight basis, respectively. Fig. 1 shows the chromatograms of a PAH standard (a) and of a fish sample (b).

3. Results and discussion

3.1. Content of PAHs and heavy metals (Hg, Cd and Pb) in fish muscle tissue

The mean values, standard deviations and range of concentrations of PAHs and Hg, Cd and Pb in studied fish species are given in Table 1. The mean total concentrations of PAHs were 227.2, 209.9 and 214.1 ng g^{-1} wet weight for brown ray, megrim and angler, respectively. Statistical comparison revealed that total PAH concentrations were not significantly different ($p > 0.05$) among fish species investigated. Also PAHs composition pattern was similar, being dominated by the presence of PAHs with 3 rings (Fl, PHE, AN) (59.8–65.4%), followed from those with 4-rings (FA, PY,CHR, BaA) (30.3–35.5%) and 2-rings (NA) (3.8–4.7%), while BaP, DBahA, and BghiP showed levels below the limit of quantification (LOQ) in all samples.

This common pattern with a larger amount of lower molecular weight contaminants, could probably depend from the metabolic transformations of heavier PAHs occurring in fish liver (Meador et al., 1995). In addition, fish converting up to 99% of the PAHs to