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Analysis and occurrence of some phenol endocrine disruptors in two marine sites of the northern coast of Sicily (Italy)

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ABSTRACT

This study reports the occurrence of some endocrine disrupting chemicals in red mullet samples and sediments collected in two representative sites of the northern Sicilian coast (Italy). For this purpose, an improved method, using solid extraction and high-performance liquid chromatography analyses for the simultaneous determination of bisphenol A (BPA), 4-nonylphenol (4-NP) and 4-t-octylphenol (4-t-OP) in fish tissues and sediments, has been developed and validated. Method performance was demonstrated over the concentration range 0.1–200 ng/mL, with detection limits from 0.06 to 0.1 ng/mL. Recoveries ranged from 83.4% to 102.6%, with relative standard deviations of 7.7–14.0% for the entire procedure.

Results showed that BPA, 4-t-OP and 4-NP were detected in all fish samples and sediments from two sampling sites, indicating that these chemicals have contaminated Mediterranean aquatic ecosystem and have accumulated in fish. The study provided more comprehensive fundamental data for risk assessment and contamination control of phenolic EDCs in aquatic environment.

1. Introduction

Bisphenol A (BPA), 4-t-octylphenol (4-t-OP) and 4-nonylphenol (4-NP) are environmental chemical contaminants, which interfere with endocrine system function (Markey et al., 2001). In the last years, they are one of the major topics of the European and International Research on risk assessment in food and environmental safety, especially in aquatic environment. As xenoestrogen compounds, they bind to the estrogen receptors (ERs) and act competitively toward natural hormones (*i.e.* 17β-estradiol). These pollutants belong to category 1 of Endocrine Disruptors Chemicals (EDCs), in relation to their mechanism of action (clear evidence of endocrine disrupting activity). Several studies showed potential harmful effects on reproduction and development of environmentally exposed aquatic animals (Canesi and Fabbri, 2015; Chen et al., 2014; Guerranti et al., 2016; Lindholst et al., 2003; Liu et al., 2011; Mortazavi et al., 2013). Therefore, the investigation of these chemicals in surface water, sediments and fish has been followed out in many regions of the world (Belfroid et al., 2002; Corrales et al., 2015; Fries and Puttmann, 2003; Loos et al., 2007a; Viganò et al., 2015; Ying et al., 2002).

Due to extensive anthropization and industrialization, NW Mediterranean coastal areas are the main recipients of phenolic EDCs, especially of BPA, 4-t-OP and 4-NP, with adverse effects on reproduction and viability of fish populations, as well as on the ecological status of the marine environment. The hydrographic features of the Mediterranean Sea, which is a semi-enclosed basin with a microtidal regime, reduce the potential for dilution and dispersion of dissolved and particulate wastes (EEA, 2006).

In the present study, in the framework of a multi-disciplinary research project funded by the Italian Ministry for Health (RF-2009-1536185), we investigated the occurrence of BPA, 4-t-OP and 4-NP in red mullets from two sites, characterized by different degrees of pollution, in the Tyrrhenian Sea (NW Mediterranean). Red mullet (*Mullus barbatus*) is considered an environmental marker of contamination in monitoring programmes (UNEP/RAMOGE, 1999; Lionetto et al., 2003; Martin-Skilton et al., 2006; Zorita et al., 2008), because of non-migratory behaviour and a feeding strategy based mainly on organisms living in close association with sediments, where most of contaminants accumulate. Red mullet constitutes a basic food of the Mediterranean diet, since it is a cheap fish (Di Lena et al., 2016). Therefore, in addition

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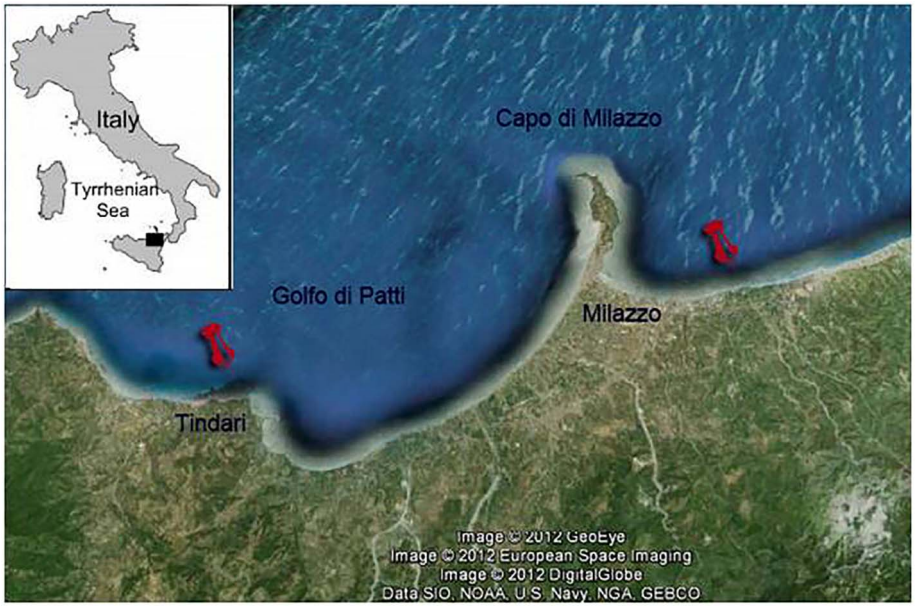

Fig. 1. Sampling areas located in Sicily (Italy) along the Tyrrhenian coast: Milazzo (Latitude 38°12′73″N, Longitude 15°17′92″E Average Depth 31 m); Mongiove-Patti (Latitude 38°09′77″N, Longitude 15°00′03″E, Average Depth 53 m).

to the environmental concern, the aquatic contamination due to BPA, 4-t-OP and 4-NP may represent a potential risk to human health (Jeng, 2014).

In literature, there are a lot of studies on the contamination of red mullet due to heavy metals (Miramand et al., 1991; Kontas, 2006; Filipović Marijić and Raspor, 2007a, 2007b), polycyclic aromatic hydrocarbons (PAHs) (Escartin and Porte, 1999; Martínez-Gómez et al., 2012; Martínez-Gómez et al., 2013), and organochlorine residues (Giouranovits-Psyllidou et al., 1994; Kucuksezgin et al., 2001; Pastor et al., 1996; Porte et al., 2002). On the contrary, the contamination due to alkylphenols and bisphenols has received less attention (Ferrara et al., 2008; Martínez-Gómez et al., 2013).

In this paper the simultaneous levels of BPA, 4-NP and 4-t-OP in fish tissues (liver and muscle) and sediments have been determined by a new analytical procedure, developed and validated *ad hoc*.

Recently, liquid chromatography coupled to mass spectrometry has become a standard in environmental analysis (Gallart-Ayala et al., 2011; Petrovic et al., 2002a; Petrovic et al., 2002b). However, many alkylphenolic compounds (octyl and nonylphenol) are not amenable to analysis by mass spectrometer as a result of different alkyl-chain isomers, which produce different MS-MS fragment ions (Loos et al., 2007b). The analytical standard is not a pure single isomer; its mass spectrum is characterized by different peaks, whose values are difficult to be determined. For this reason, we used fluorescence spectrophotometer as detector. In order to increase method sensitivity and accuracy, we optimized a rapid and simultaneous sample clean-up and concentration procedure, by solid phase extraction (SPE) on molecularly imprinted polymer (MIP) cartridges. MIP cartridges show high selectivity, due to the presence of specific sites for binding of the analyte, improving the crucial clean-up step, thus reducing the presence of matrix components, and consequently increasing method sensitivity.

The method was validated by evaluating the linearity of detector response, the limit of quantification, accuracy, precision and specificity. Improved analytical methods are needed to plan a large scale monitoring, in order to provide new evidence and more comprehensive fundamental data for risk assessment and contamination control of these two alkylphenols and BPA in aquatic environments. These data are crucial for the development of interpretative models able to correlate the presence of these environmental pollutants to physiological alterations in organisms exposed to them.

2. Materials and methods

2.1. Materials

BPA (native and d_{16}), 4-Nonylphenol mixture of chain isomers [4-(2,4-dimethylheptan-3-yl)-phenol], 4-n-nonylphenol-2,3,5,6-d4 and 4-t-Octylphenol [4-(1,1,3,3-tetramethylbutyl)-phenol] were purchased from Sigma Aldrich (Sigma-Aldrich, Milano, Italy). HPLC grade reagents, including ultrapure water, methanol (MeOH) and acetonitrile (ACN) were purchased from Romil (ROMIL Ltd., UK). MIP cartridges (catalog N. FS106-02G) were purchased from Polyntell (Polyntell SA, Paris, France). Frozen livers and muscles from 10 red mullets were shipped in glassware and stored at − 20 °C. These tissue samples were used only for preparation of quality control samples during the validation of the method.

2.2. Preparation of standard reference materials

Standard stock solutions of a single analyte at 1.0 mg/mL were prepared in MeOH. Similarly, stock standard solutions of d_{16}-BPA and d_4-NP, used as internal standards (IS) within each sample to monitor method performance, were prepared at 1 mg/mL in MeOH. Standard stock solutions were stored in dark glass vials at − 20 °C until their use. The mixture of standard solutions at intermediate concentrations was prepared from the stock solutions by serial dilution with MeOH/water (1:1, v/v).

2.3. Sampling

In consideration of anthropogenic activities, red mullets (*Mullus barbatus*) were collected by bottom trawling in April and October 2012, from two different sites along the northern coast of Sicily (Fig. 1). The first site (Site 1), chosen as Impact Site, recognized as a pollution hot spot by the Strategic Action Programme (SAP) of UNEP (UNEP/WHO, 2003), was located in a dense shipping traffic area, close to the city and harbour of Milazzo, in front of an oil refinery and a thermal power plant. The second site (Site 2), chosen as Reference Site, was located in a leisure area, where both industrial activity and commercial fishing are forbidden. Once on board, fish were euthanized by a lethal dose of MS-222. The entire liver and a piece of dorsal muscle were dissected and stored at − 20 °C.