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Assessment of biological effects of environmental pollution in Mersin Bay (Turkey, northeastern Mediterranean Sea) using *Mullus barbatus* and *Liza ramada as* target organisms

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ABSTRACT

The increasing emphasis on the assessment and monitoring of marine ecosystems has revealed the need to use appropriate biological indicators for these areas. Enzyme activities and histopathology are increasingly being used as indicators of environmental stress since they provide a definite biological endpoint of pollutant exposure. As part of an ecotoxicological assessment of Mersin Bay, EROD enzyme activity and histopathological response in selected organs and tissues of two species of fish, *Mullus barbatus* (red mullet) *and Liza ramada* (thinlip grey mullet), captured from area were examined. Pollutant (Organochlorines (OC), alkylphenols (APs) and BPA) levels and biomarker responses in tissue samples were evaluated together for their potential to alter the metabolism and cellular aspects in liver and gonad. Elevated induction of EROD activity and histopathological alterations in contaminated samples from Mersin Bay was observed compared to reference site indicating the exposure to potential pollutants.

1. Introduction

Coastal area of Mersin Bay, situated in northeastern Mediterranean Sea, is the recipient of organic pollutants from a variety of sources such as sewage outfalls, industrial wastes, maritime shipping, oil transportation and agricultural activity from adjacent urban areas. Pollution originating from Mersin Bay may not cause common acute effects in aquatic organisms, however, long term exposure to these pollutants can lead to chronic effects and accumulation in food chain by the time.

The OC compounds persist in the environment for a long period of times. They can accumulate in fat tissue and pass through the food chain and they tend to cause wide range of toxicity. Due to their tendency of bioaccumulate and biomagnify, the effects of these chemicals on animals are a matter of concern. Fish living in coastal areas have often been used as target organism for monitoring the pollution as they concentrate in their tissues. Several studies and biomonitoring programs conducted in the area have revealed the presence of OCs (PCBs, DDT and its metabolites, OC pesticides) in Mersin Bay. These pollutants referenced in the literature have been reported in fish tissues (*Mullus barbatus*, *Mugil cephalus*) at various concentrations (MEDPOL reports of Tugrul et al., 2005, 2007, 2008, 2009; Yemenicioglu, 2003; Yemenicioglu et al., 2004, 2006).

In their studies, Gedik and Imamoğlu (2011) reported that low levels of PCBs and Aroclors in sediment indicated that there is no ecotoxicological risk for marine environment in Mersin Bay. But the integration of pollutant analysis together with biochemical and cellular responses should be considered for evaluating both the fate of pollutants and their impact on the biota. However, endocrine disrupting effects of these OC compounds on fish is not reported in Mersin Bay. Beside OCs, information on the levels of APs and BPA in biota are not also available.

An effective monitoring system using biochemical markers has been established to demonstrate the xenobiotics in the environment. The cytochrome P450 system has proved to be a very suitable tool for biochemical and environmental monitoring. It is particularly sensitive to a broad spectrum of industrial contaminants (e.g

Abbreviations: OP, 4-t-octylphenol; NP, 4-n-nonylphenol; PCB, polychlorinated biphenyls; BPA, bisphenol-A; PAH, polycyclic aromatic hidrocarbon; EROD, ethoxyresurufin-o-deethylase; OC, organochlorine; AP, alkylphenol; BSTFA, Bis(-trimethylsilyl)trifluoroacetamide; TMCS, trimethylsilyl chloride; SMI, Scaled Mass Index; FCF, Fulton's Condition Factor.

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dioxins, PCB, PAH) (Havelkova et al., 2007). Ethoxyresorufin-O-deethylase (EROD) is functionally linked to cytochrome P450 system. This enzyme transforms substrates into products emitting a measurable fluorescent signal and is more sensitive than the determination of CYP 450. Regarding the biochemical responses of organisms to most aquatic pollutants, EROD has a major role in oxidative metabolism and is recognized as a biomarker of exposure of contaminants. Among toxicity mechanisms, oxidative stress, defined as an injuries effects due to cytotoxic reactive oxygen species, causes oxidative damage to tissues. This occurs mainly in the endoplasmic reticulum of liver cells, where cytochrome P450 (CYP) activities may generate reactive oxygen species (ROS) as byproducts of detoxification processes (van der Oost et al., 2003)). ROS can inflict irreversible damage to cell constituents, either lethal or carcinogenic. In OC-contaminated organisms, CYP1A expression is increased, which tends to elevate ROS levels in cells (Schlezinger et al., 2006). This ROS production adds on to that of the mitochondrial electron transport chain (Cadenas and Davies, 2000). Liver cells showed increasing EROD levels, probably reflecting the induction of CYP1A by some of the PCB congeners. An increased ROS production by CYP1A activity would require higher activities of antioxidant enzymes such as catalase, superoxide dismutases and glutathione peroxidases (Lemaire et al., 2010).

Numerous field studies demonstrated significant and strong increase of hepatic EROD activity in *M. barbatus* (Porte et al., 2002) and *Liza ramada* (Mihailovic et al., 2006) from PCB-polluted marine environment. They have found good relation between PCB and EROD activity in fish tissue.

Histopathological alterations in liver and gonad tissues induced by OCs and APs have been used as supporting parameters in several studies. Ovaries of red mullet from Ionian areas (Gallipoli and Porto Cesareo) exhibited an abnormal space between cytoplasm and oocyte envelope (Corsi et al., 2002). Melanomacrophage centers in testes and disturbances in gonad and connective tissue in red mullet (*M. barbatus)* of Cortiou area from French coast were reported (Zorita et al., 2008; Martin-Skilton et al., 2006). In carp (*Cyprinus carpio*), BPA caused severe alterations in gonad structure such as lost of typical lobular appearance in testis and oocyte atresia (Mandich et al., 2007). Fibrosis in testis, increase in picnotic nucleus and ovarium atresia in 4-NP exposed *Danio rerio* were reported (Weber et al., 2003).

The purpose of this study was an assessment of biomarker responses in two fish species. Considering OCs, APs and BPA have a potential to induce alterations in fish tissues, liver and gonad ultrastructure of contaminated *M. barbatus* and *L. ramada* was also observed and compared with fish collected from reference site.

2. Materials and methods

2.1. Sample collection and preparation

Red mullet (*M. barbatus*) and thin lip mullet (*L. ramada*) were collected between January 2010 and February 2012 at 10–20 m depth by local fisherman using trawl and seine. Specimens from the same species were also collected from the reference area, 100 km away from the center of Mersin, where sediment OCs, APs and BPA were typically low (Fig. 1).

Mature individuals were chosen for analysis and their genders were determined by macroscopic observations of gonads. Once caught fish were anesthetized with phenoxyethanol (0.4 ml/L) and their length and weight were measured. These morphometric measurements were used to calculate individual body condition using the scaled mass index (M) reported by Peig and Green (2009). This index is based on the equation $M = M_i(L_a/L_i)^{bsma}$, where M_i and L_i are the individual weight and length, respectively, L_a is the average length of all individuals, and b_{sma} is the quotient between the slope of the regression of the Ln-transformed body weight on the Ln-transformed length of each individual. Fulton's condition factor (K) was calculated according to the equation (body weight)/(body length)^3 × 100, where W is the weight expressed in g and L the length in cm (Nash et al., 2006). Morphometric measurements and condition indices were given in Table 1. Livers and gonads were quickly dissected and hepatosomatic index [HSI = (liver weight/body weight) × 100] was calculated. Subsamples of tissues were taken for electron microscopic examination and analysis of EROD and pollutants.

2.2. Extraction and clean-up

Extraction and derivatization were performed according to the procedure found elsewhere with small modifications (Khim et al., 1999; Li et al., 2001). Briefly, liver samples of individuals per location were pooled and freeze-dried. Samples of approximately 5 g were then homogenized by grinding in a mortar and soxhlet-extracted with 300 ml hexane:dichloromethane (1:1) for 18 h. Solvent extract was evaporated to 1 ml under gentle stream of N_2 and cleaned up by adding 1–2 ml concentrated H_2SO_4. Resultant hexane layer was collected and dried with Na_2SO_4 for removal of excessive water. Hexane extract was then evaporated to 1 ml for fractionation.

Extracts of 1 ml were passed through 15 g activated florisil (60–100 mesh, Sigma–Aldrich, 46,385) column for cleanup and fractionation. First fraction, eluted with 75 ml of 100% hexane, contained PCBs and DDE. DDT and DDD in second fraction were eluted with 100 ml of hexane:dichloromethane (4:1). Remaining APs, NP, OP and BPA were eluted in third fraction using 100 ml of dichloromethane:methanol(4:1). Fractions, contain OCs and APs and BPA, were evaporated to 1 ml. 100 μl aliquot of BSTFA (with 1% TMCS) was added to third fraction followed by vigorous shaking for 60 s at room temperature for derivatization. 100 μl of water was then added to hydrolyze excess unreacted BSTFA. 1 g Na_2SO_4 was added to remove water. Solution was transferred to another vial and remaining residue was collected by successive rinsing with dichloromethane. Solution was again evaporated to 1 ml under gentle stream of nitrogen.

2.3. Instrumental analysis

Individual PCB congeners (18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 170, 180, 194, 209) and DDT and its metabolites, DDD and DDE were quantified using gas chromatography-electron capture detector. A fused capillary column with HP5 phase (Agilent J&W, 19091J-413), 30 m × 0.32 mm in diameter, with a thickness of 0.25 μm, was used. The oven temperature was held at 70 °C for 2 min, then elevated to 260 °C at 3 °C/min and held at 260 °C for 30 min. The recoveries of surrogate standard 2,4,5,6 tetrachloro-m-xylene were 92 ± 12%. Quantitation was performed using external standard calibration mixture (Accustandard, AE-00061). For each Aroclor (1254 and 1260), nine peaks were selected to quantify the amount of that Aroclor.

Liver alkylphenols (NP and OP) and BPA were determined using a Agilent 7890 GC interfaced to Agilent 5975C mass spectrometer with HP5-MS column (Agilent J&W). The column oven temperature was programmed from 60 to 290 °C at a rate of 3 °C/min, with a final holding time of 24 min. The ion source and the analyzer were maintained at 250 °C. The selected ions used for monitoring were 179 and 292 for NP, 135 and 206 for OP and 357 and 358 for bisphenol-A. NP, OP and bisphenol-A were identified and quantified by comparison of retention times and spectra of standard compounds (Accustandard, M-1626). Recoveries were obtained

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